ANCIENT DNA ANALYSIS OF ANCIENT DENTAL CALCULUS: CHALLENGES AND OPPORTUNITIES

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PHOTOGRAPH BY JOE MCNALLY, NATIONAL GEOGRAPHIC

HUMAN EVOLUTION MAY BE TIGHTLY LINKED TO OUR MICROBIAL EVOLUTION

Microbiota:

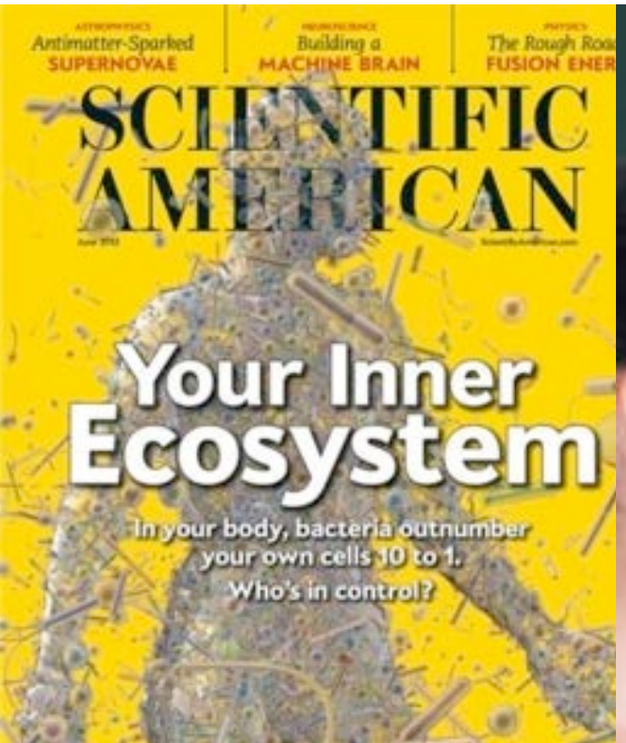
the microorganisms (bacteria, fungi, viruses) that live in your body

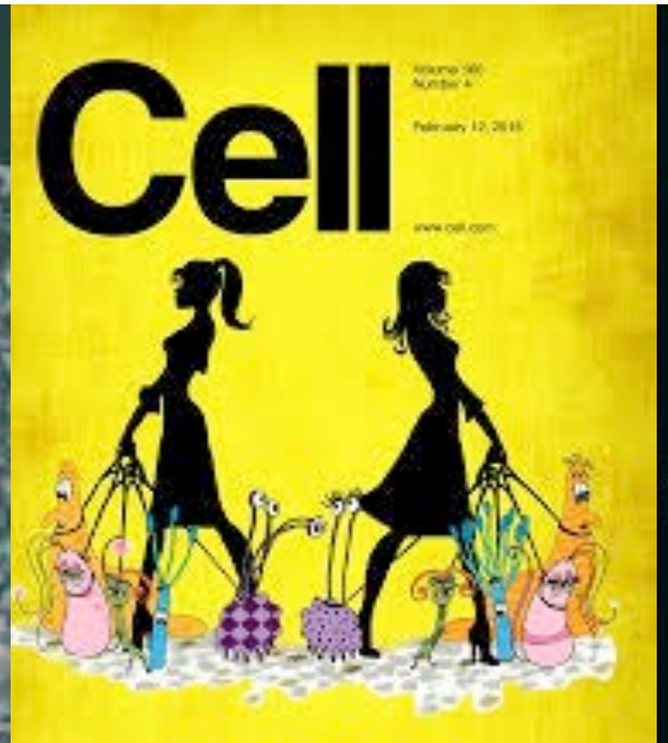
100 trillion bacterial cells

>**50%** of total cells

>**1,000** species

1.4 kg of body weight





MICROBIOTA FUNCTIONS ARE MORE CRITICAL THAN THE SPECIES THEMSELVES

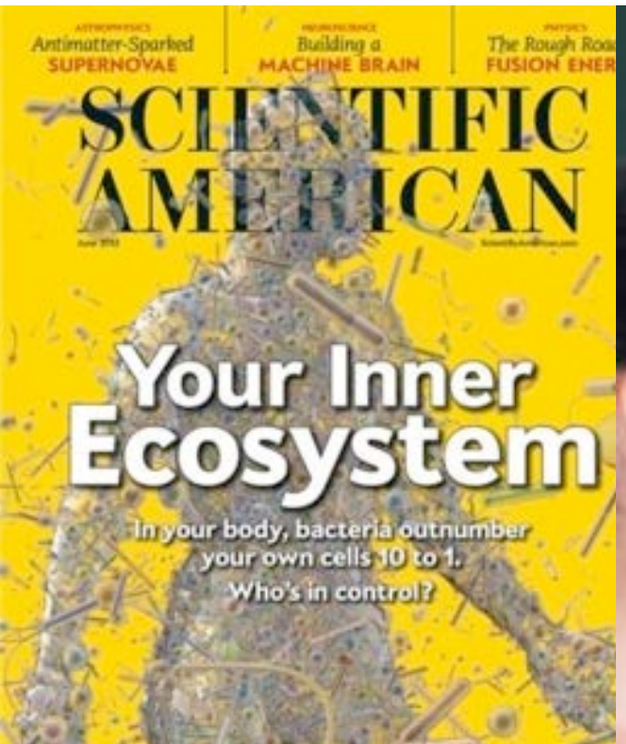
Microbiome:

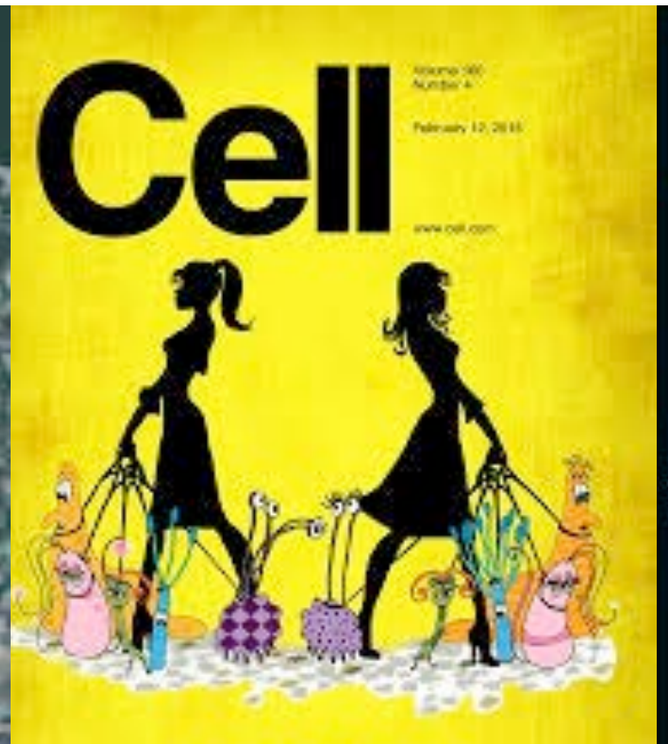
the genetic and environmental content of the microbiota present in the body

Outnumbers human cells **100 to 1**!

2-5 million genes per individual

99% of your genetic makeup!





NUMEROUS FACTORS INFLUENCE THE MICROBIOME

- Diet
- Living Environment
- Job
- Chemical Exposure
- Human Interactions
- Animal or Pet Exposure
- Medical Treatment
- Pollution
- Seasonality

…and now can be used in archaeological contexts.





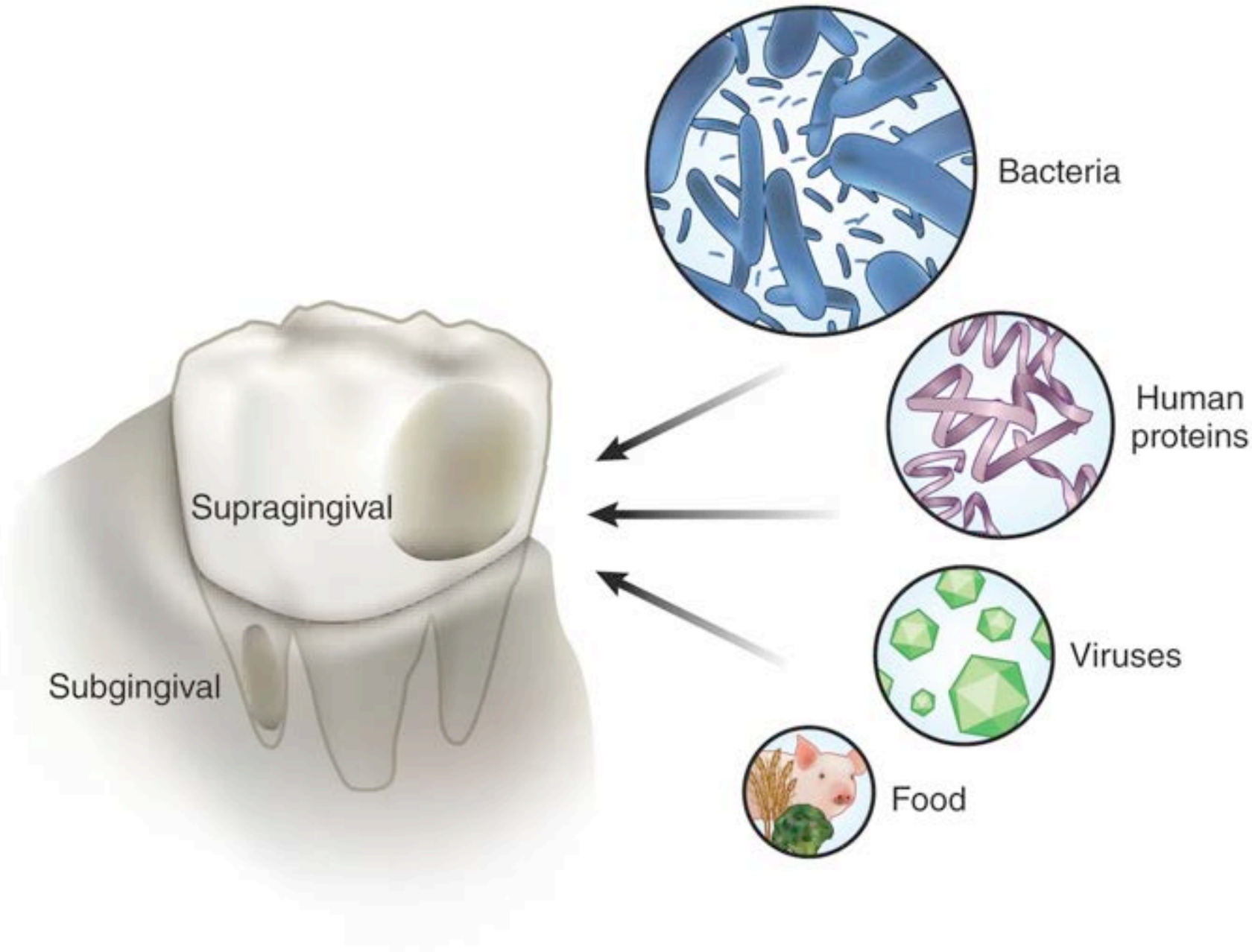
ANALYSIS OF ANCIENT HOMINID MICROBIOTA

Coprolite:
preserved human faeces

ANALYSIS OF ANCIENT HOMINID MICROBIOTA

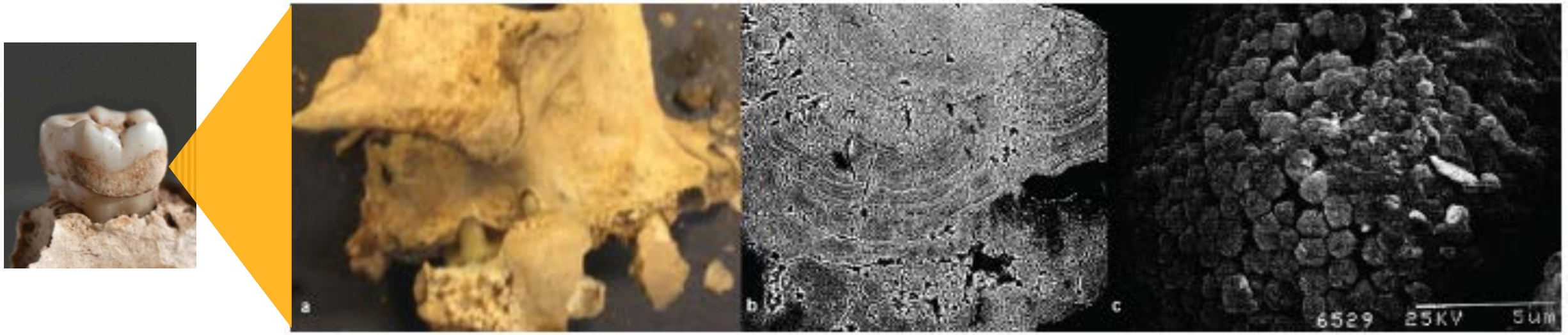
Dental Calculus:
preserved, calcified human oral plaque

ANCIENT DENTAL CALCULUS IS A FOSSILIZED BIOARCHAEOLOGICAL RECORD

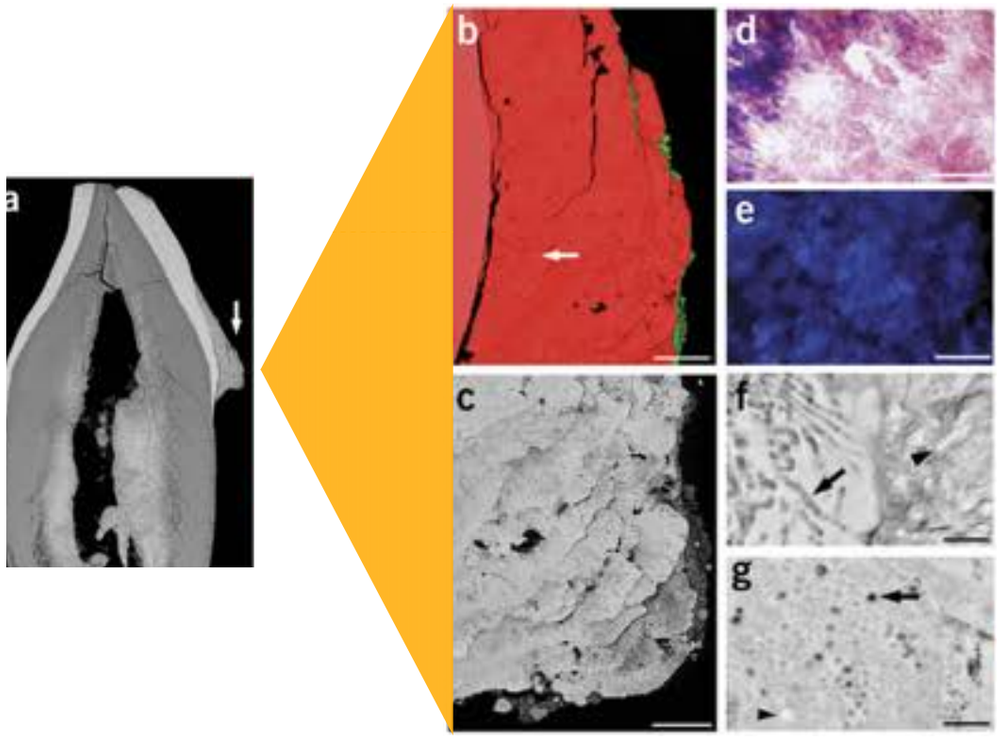


Metcalf, *et al. Nature Genetics*, 2014

INSIDE ANCIENT DENTAL CALCULUS

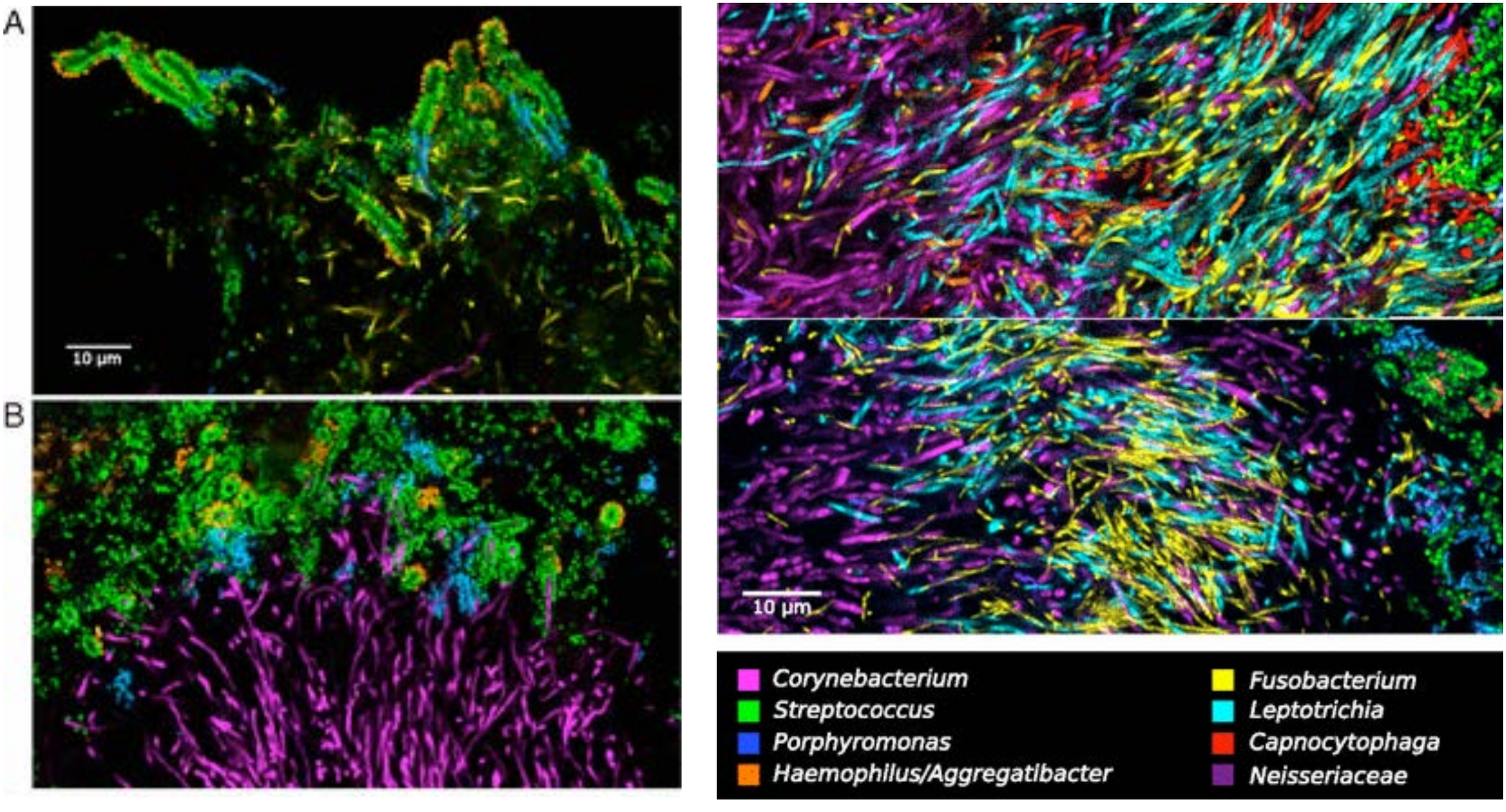


Adler *et al. Nat Gen*, 2013.



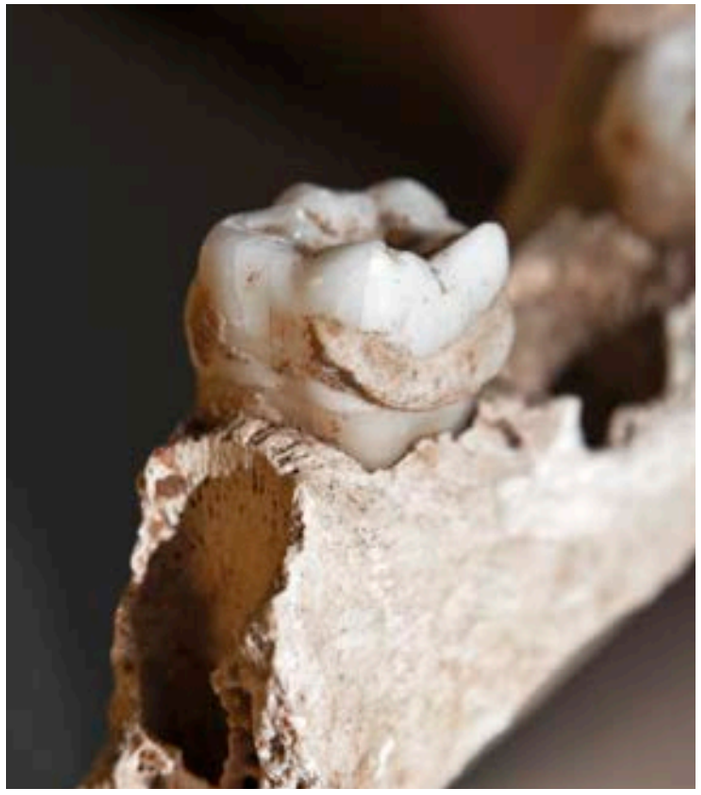
Warinner *et al. Nat Gen*, 2014.

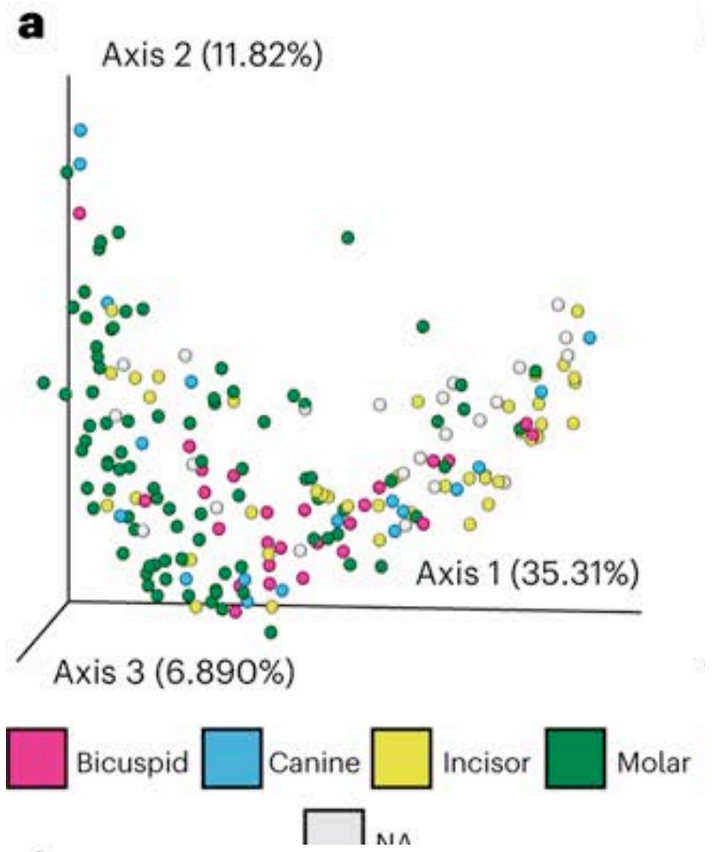
ANCIENT DENTAL CALCULUS IS SIMILAR TO MICROBES FOUND IN DENTAL PLAQUE

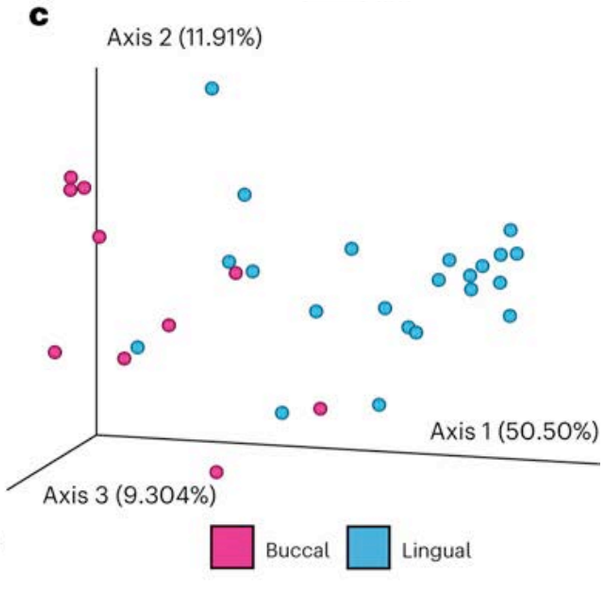


Mark Welch, et al. PNAS, 113:6. 2016.

CRITICAL RECORDING INFORMATION







<u>Oral biogeography:</u>

Tooth (anterior vs. posterior)

Supra or Sub Gingival (CEJ distance?)

Tooth surface (buccal/lingual)

Left or Right Side

Mandibular vs. Maxillary

Gancz, *et al. AJBA*, 2024
Gancz, *et al. Nature Micro*, 2023

CRITICAL RECORDING INFORMATION

<u>Other information:</u>

Oral health (caries, PD, abscess, tooth loss, LEH)

Systemic health

Sex

Age at death

Archaeological age

Cultural affiliation

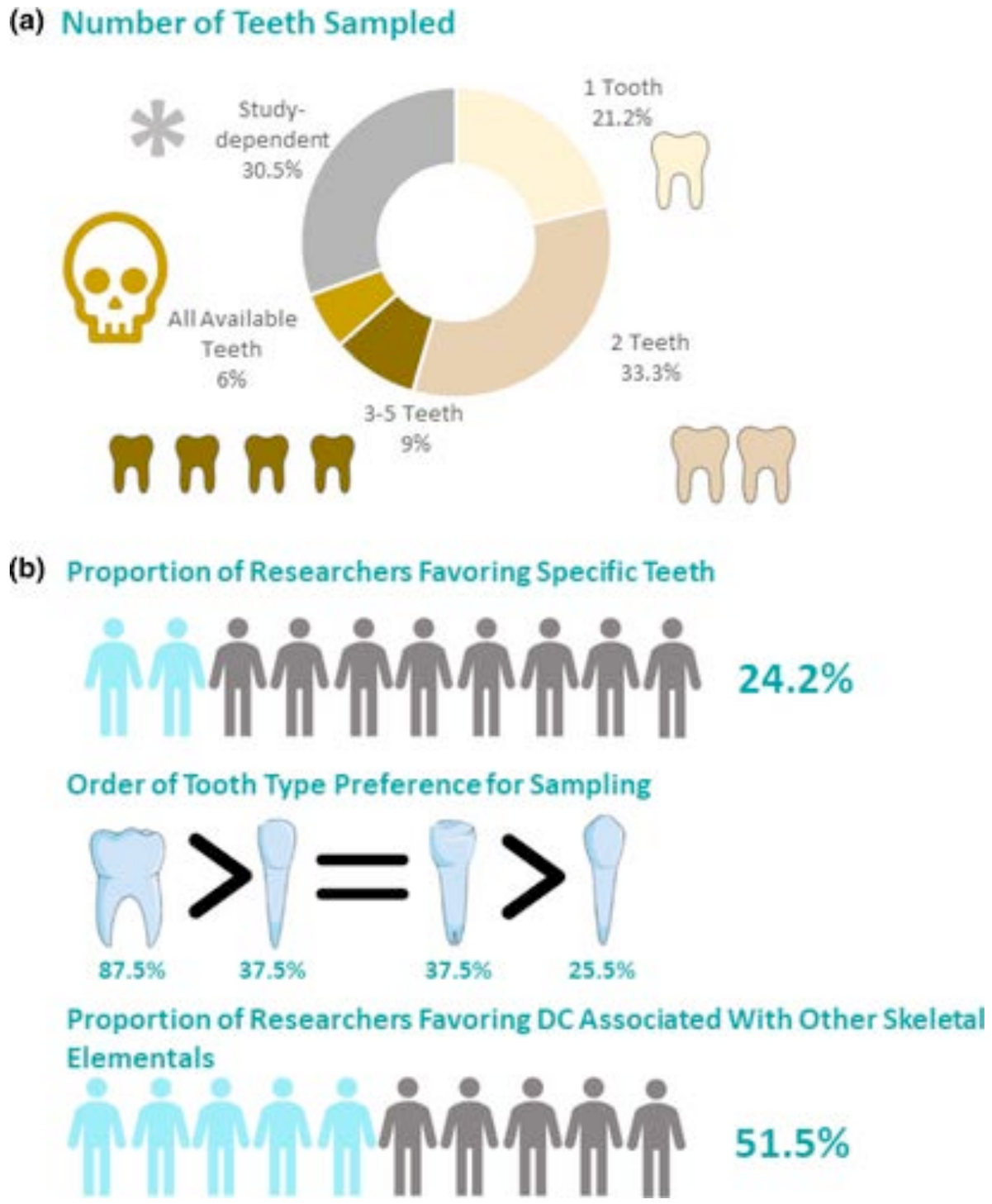
Burial context

Burial goods

Soil quality

Etc……

***Methods of collection



Gancz, *et al. AJBA*, 2024

CRITICAL RECORDING INFORMATION

Archaeological Dental Calculus Recording Guidelines

Introduction:

The inherent tradeoff between detailed sample documentation and sampling speed presents a dilemma to researchers whose work entails destructive sampling. While one researcher may spend hours meticulously recording features of a skeletal individual, another may jot down a few notes and move on. Neither approach is inherently faulty so long as the project leaders have taken the impacts of destructive sampling, future sample access, and accuracy into account.

To assist researchers in planning data collection approaches for future projects, we provide the following in these guidelines:

(1) An overview of different dental calculus recording systems and their respective benefits and drawbacks

(2) A synopsis of skeletal and dental metadata traits, their respective recording systems, and ways in which these traits can be applied to improve population-level inferences

Dental Calculus Recording Systems:

A number of different dental calculus recording systems have been historically utilized by researchers. In this section, we briefly review some of the most widely-used and cited of these approaches in chronological publication order. The first of these is the calculus surface index of Ennever, Sturzenberger, and Radike (1961), which has 91 citations on Google Scholar. The next is Green and Vermillion's (1964) simplified Oral Hygiene Index, which has 2,484 citations. The third is the method devised by Dobney and Brothwell in 1987, which has 83 citations. Fourth is Buikstra and Ubelaker's (1994) standards method of data collection, for which we cannot provide an exact citation count as it is one part of a larger text. The most recent method we discuss is that of Greene, Kuba, and Irish (2005), which has 51 citations on Google Scholar. These recording systems were selected from those referenced in survey responses.

Ennever, Sturzenberger, and Radike 1961

In 1961, a number of clinical researchers devised a method for the evaluation of dental calculus formation (Ennever, Sturzenberger, and Radike 1961). This evaluation system was devised for each of the

Gancz, *et al. AJBA*, 2024

Data Collection Key	
Sample_ID	Specific sample name assigned by research group to dental calculus sample
Individual_ID	The individual identification code given by research group or musem to the individual
Continent	Continent
Country	Modern country of origin
Specific_Location	Detailed location of sample origin such as town or province
Specific_Burial	Which burial an individual originated from (there could be multiple individuals per burial)
Site	Specific stie name and/or code
Culture	Which culture the individual originated from (if relevant)
Museum	Name of museum the sample came from (if relevant)
Latitude	Latitude of burial/site
Longitude	Longitude of burial/site
Sample_Date	The date the sample was collected
Collector_Name	The name of the person who sampled the dental calculus
Collection_Date	The date during which the sample collection occurred
Analyses_Type	The type of Analyses this sample is intended for
Total_Teeth	The total number of observable teeth associated with the individual
Total_Teeth_With_Calculus	The total number of teeth on which calculus is observable
Detailed_Calculus_Record_YN	Whether or not a detailed dental calculus record was collected
Sample_Tooth_Type	Which tooth (molar, premolar, incisor, canine) did the sample come from?
Sample_Tooth_Number	The number of the tooth the sample came from (1-32)
Sample_Tooth_Surface	Which surface (buccal/lingual/interproximal) did the sample come from?
Sample_Proportion_Calculus	What proportion of the sampled tooth is covered by calculus? (0, .2, .4, .6, .8. 1)
Sample_Sub_or_Supra	Is the sample subgingival, supragingival, or unclear?
Sample_Maxilla_or_Mandible	Did the sample originate from the mandible (lower jaw) or maxilla (upper jaw)?
Sample_Left_or_Right	Did the sample originate from the left or right sides of the mouth?
Sample_In_Situ_or_Loose	Did the sample come from a loose or in situ tooth?
Photo Numbers	IDs of scans or photographs associated with this sample
Sampling_Notes	Sampling notes
Individual_Sex	What is the sex of the individual?
Individual_Sex_Method	Which method was used for sex estimation? (specific publication or method)
Individuals_Age	What is the age of the individual?
Individuals_Age_Category	Individual age category (if applicable) (subadult, young adult, middle adult, old adult)
Age_Estimation_Technique	Methods of sex estimation (publication or detailed method)
Periodontal_disease_YN	Is there evidence of periodontal disease?
Abscess_YN	Is there evidence of abcesses?
Caries_Y_N	Is there evidence of caries?
Caries_Description	On which teeth are caries present? How many carious lesions are there?
Antemortem_Tooth_Loss_Y_N	Is there antemortem tooth loss?
Antemortem_Fractures_YN	Are there antemortem fractures?
Decontamination_Method	What methods were used to decontaminate the area/sample (gloves, face mask, etc)?
Other_Description	

SAMPLING DENTAL CALCULUS

Tools:

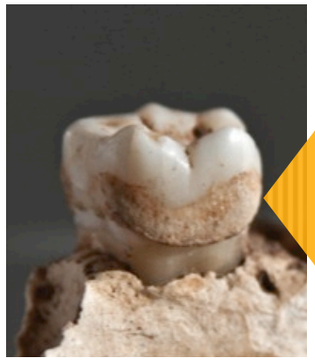
Aluminum Foil
Dental pick/screwdriver
PPE (minimal: gloves/mask)
3% Bleach
90% Ethanol
Sterile bag
Marker
Towels

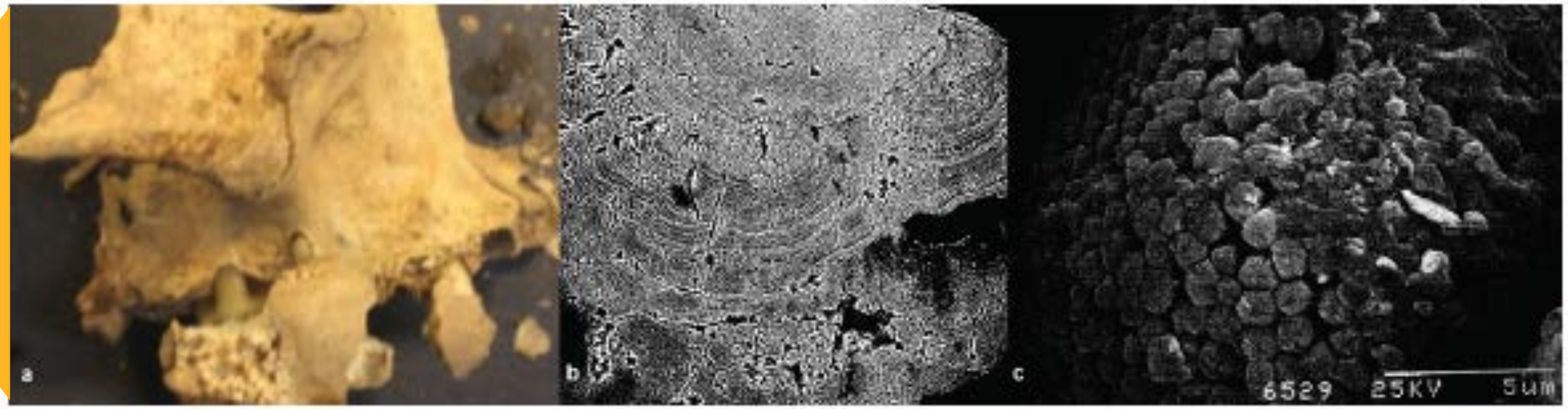
Process:

Clean area.
Identify single tooth.
Photography/record.
Wrap skeletal elements in foil.
'Pop' calculus from tooth.
Pour into sterile bag.
Label with critical information.
Clean again before next sampling.

Llamas, et al. *STAR*, 2017
Moore and Weyrich, *Methods Mol Biol*, 2021

ANCIENT DENTAL CALCULUS IS A FOSSILISED BACTERIAL RECORD





Adler *et al. Nat Gen*, 2013.

ANCIENT DNA ANALYSIS OF MICROBES IS... DIFFICULT.

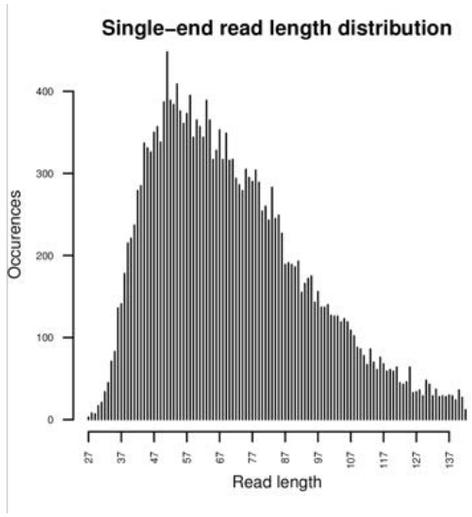
Ancient DNA is subject to:

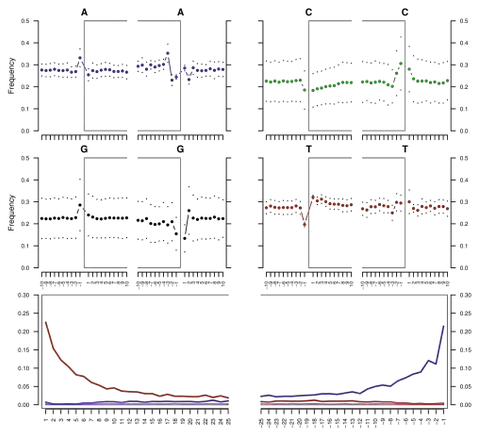
DNA Fragmentation

DNA Damage

Contamination

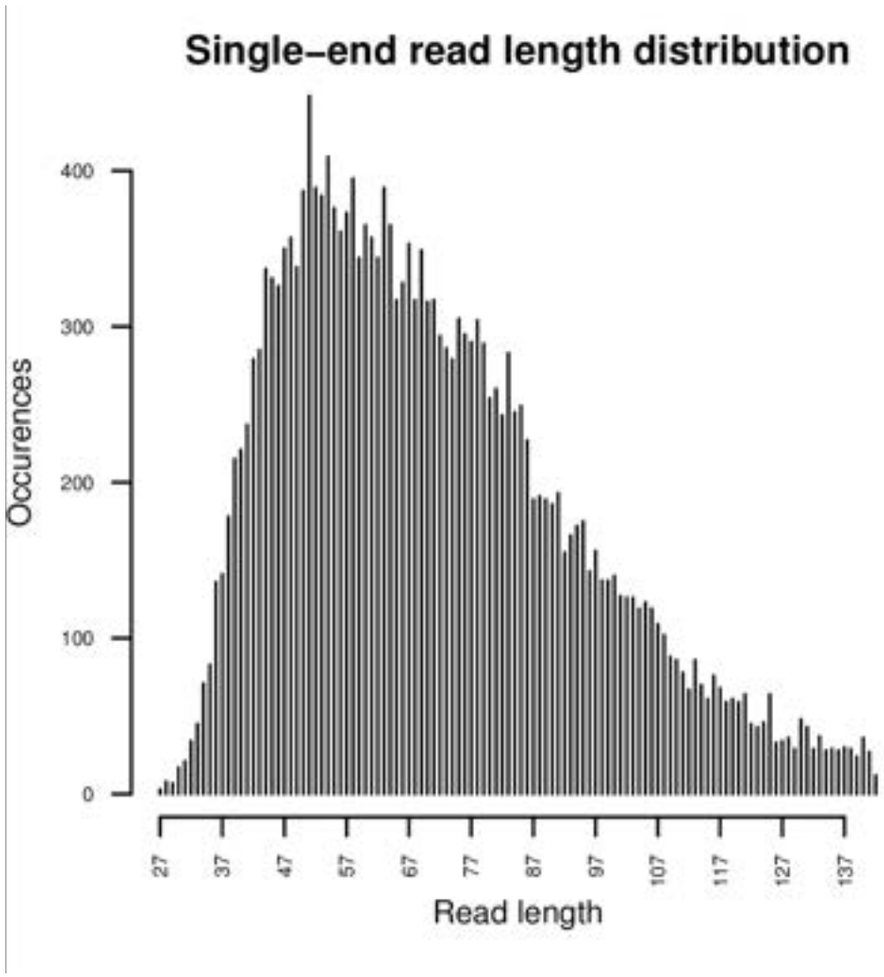
Taphonomy

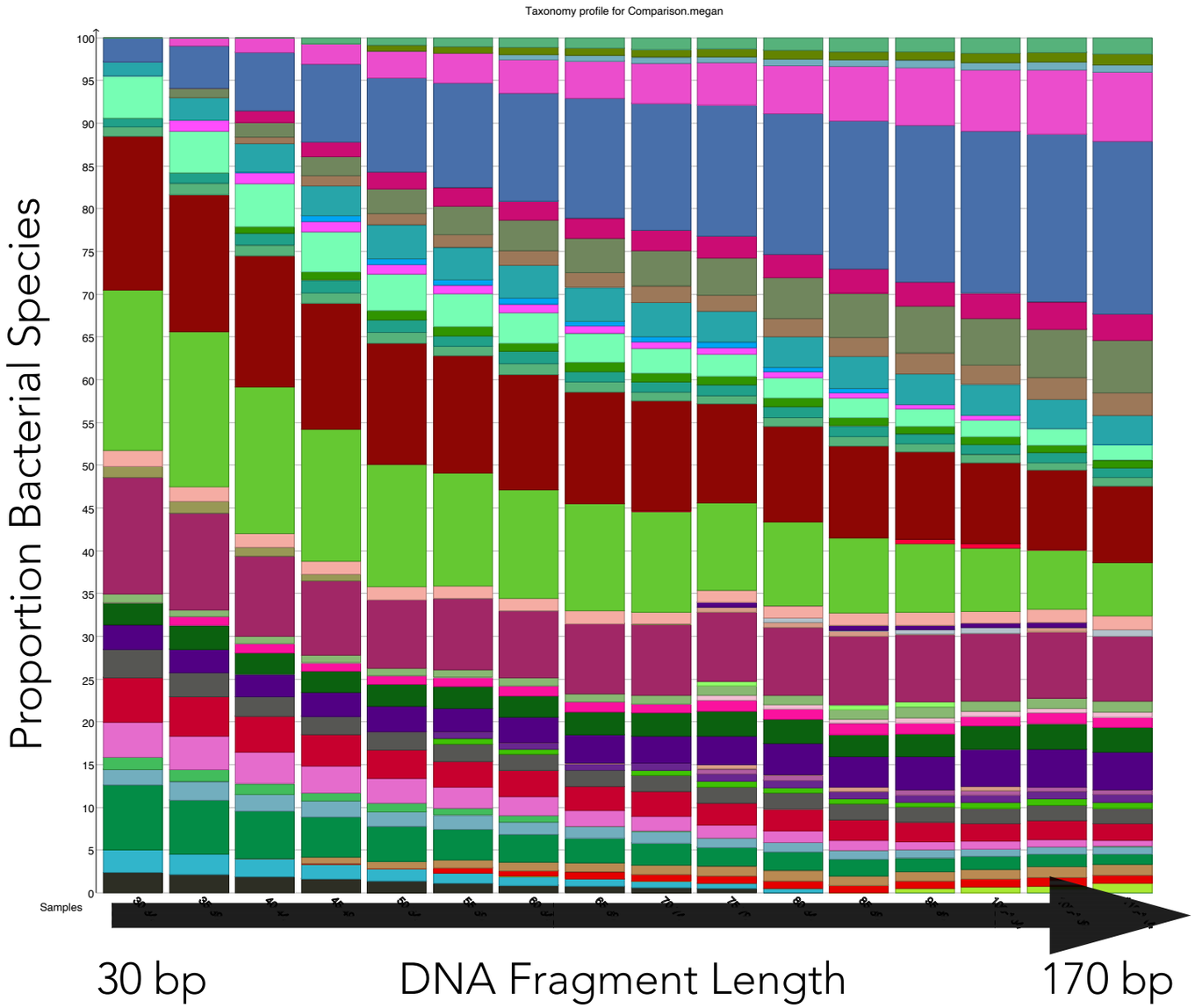




Ginolhac, *et al*, *Bioinformtics*, 2011

IMPACTS OF DNA FRAGMENTATION





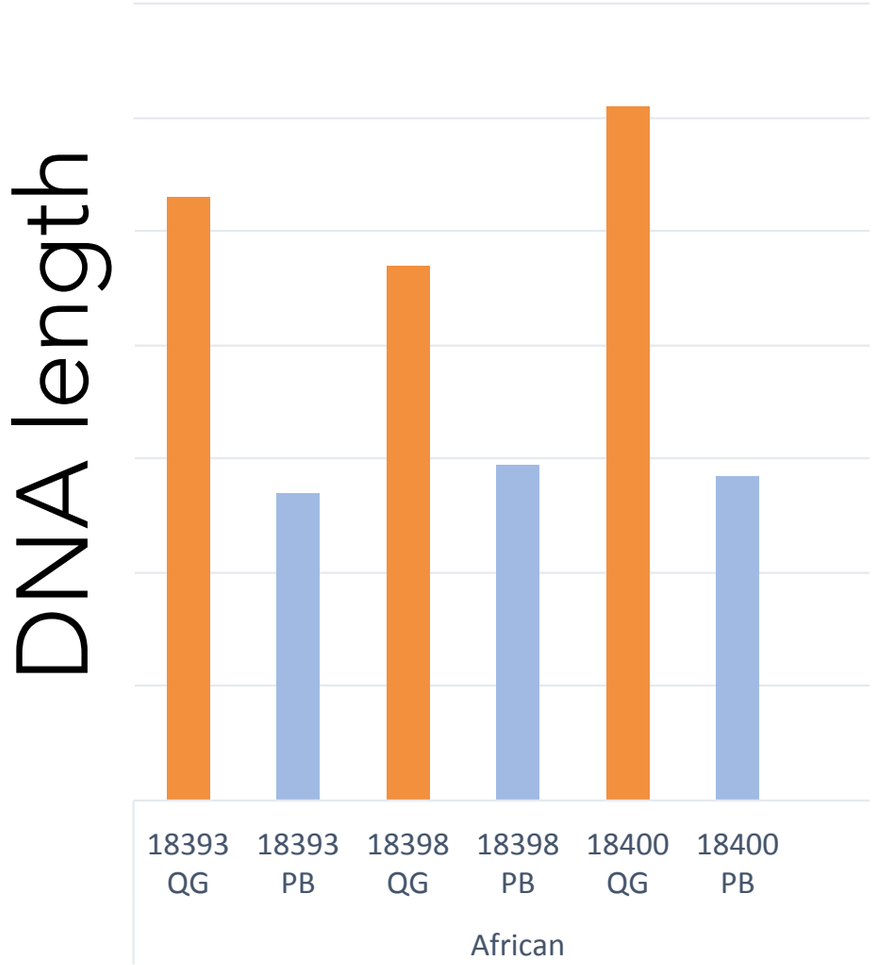
Solutions:

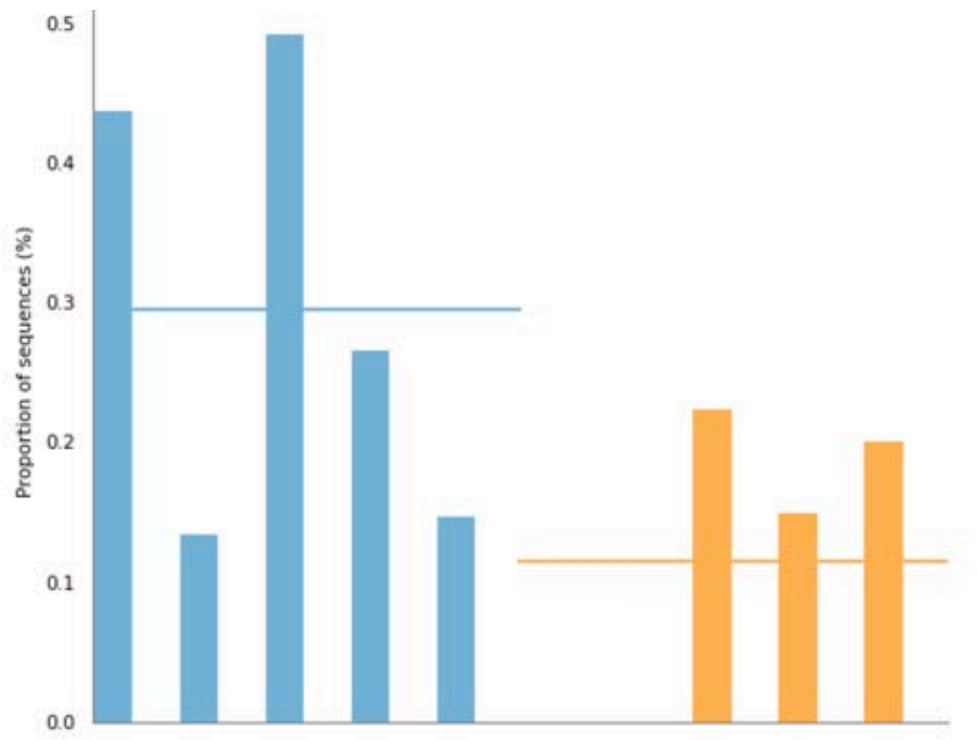
-Modeling effects of fragment size

-Understanding sample age

Eiseinhofer, et al. *PeerJ*, 2019.

IMPACTS OF DNA FRAGMENTATION





Solutions:

-Modeling effects of fragment size

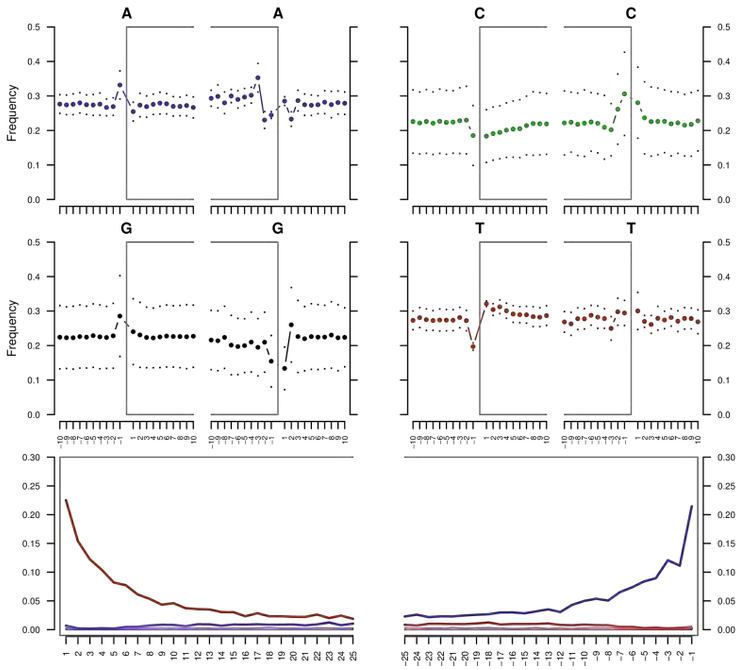
-Understanding sample age

-Applying new extraction/library preparation methodologies to counteract

Wright, *Mol. Ecol. Res.* 2025

Dabney, et al, PNAS, 2013.

IMPACTS OF DNA DAMAGE



Ginolhac, *et al*, *Bioinformtics*, 2011

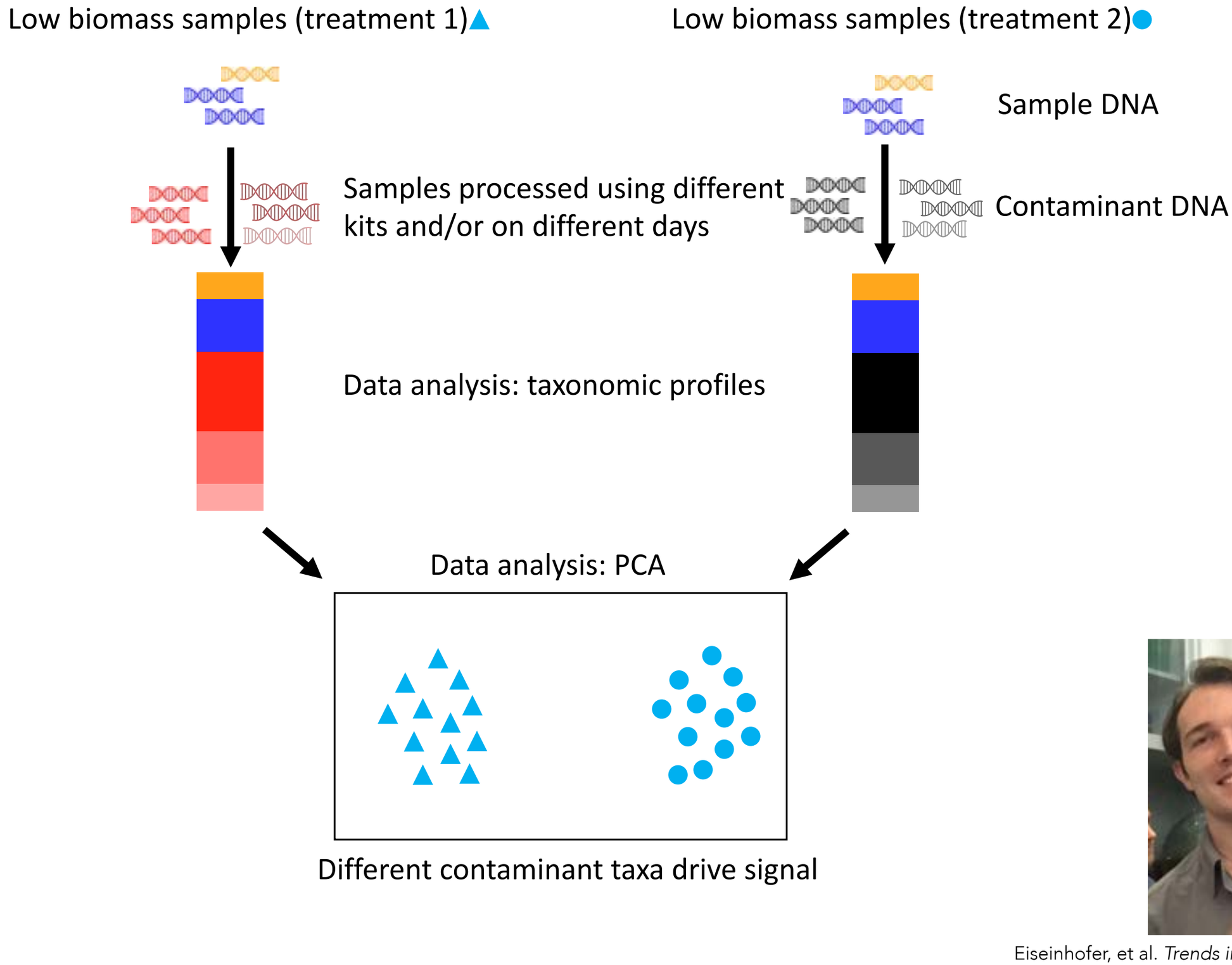
DNA DAMAGE	% READS ASSIGNED TOTAL	% READS ASSIGNED GENUS	% READS ASSIGNED SPECIES
0%ΔS	98.6%	98.4%	96.6%
10%ΔS	98.4%	98.2%	96.5%
20%ΔS (LABRANA)	98.5%	98.3%	96.5%
50%ΔS	97.7%	97.5%	95.7%

Solutions:

-Not really needed

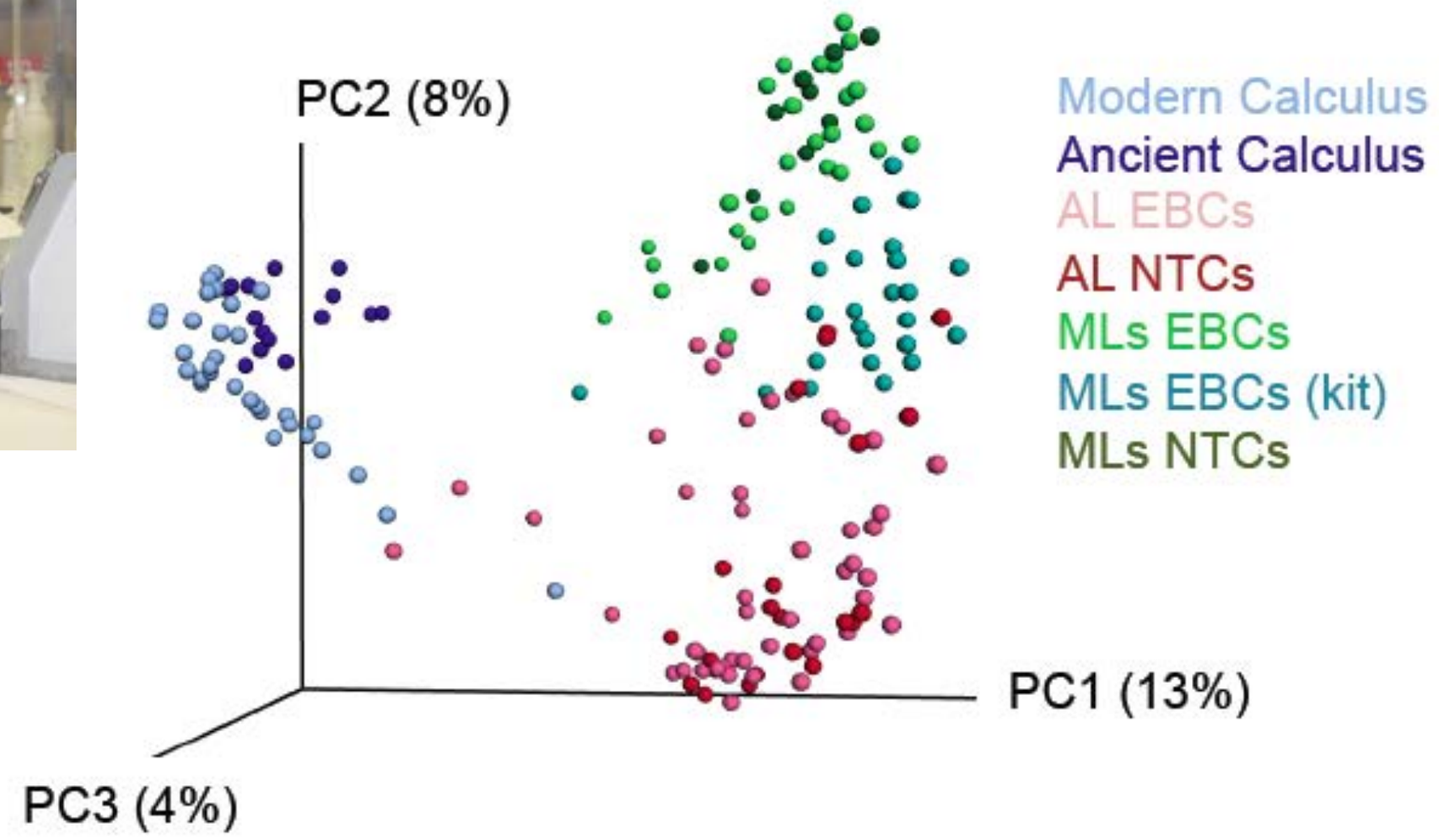
Eiseinhofer, et al. *PeerJ*, 2019.

IMPACTS OF CONTAMINATION



Eiseinhofer, et al. *Trends in Micro*, 2019.

IMPACTS OF CONTAMINATION: CLEAN LAB



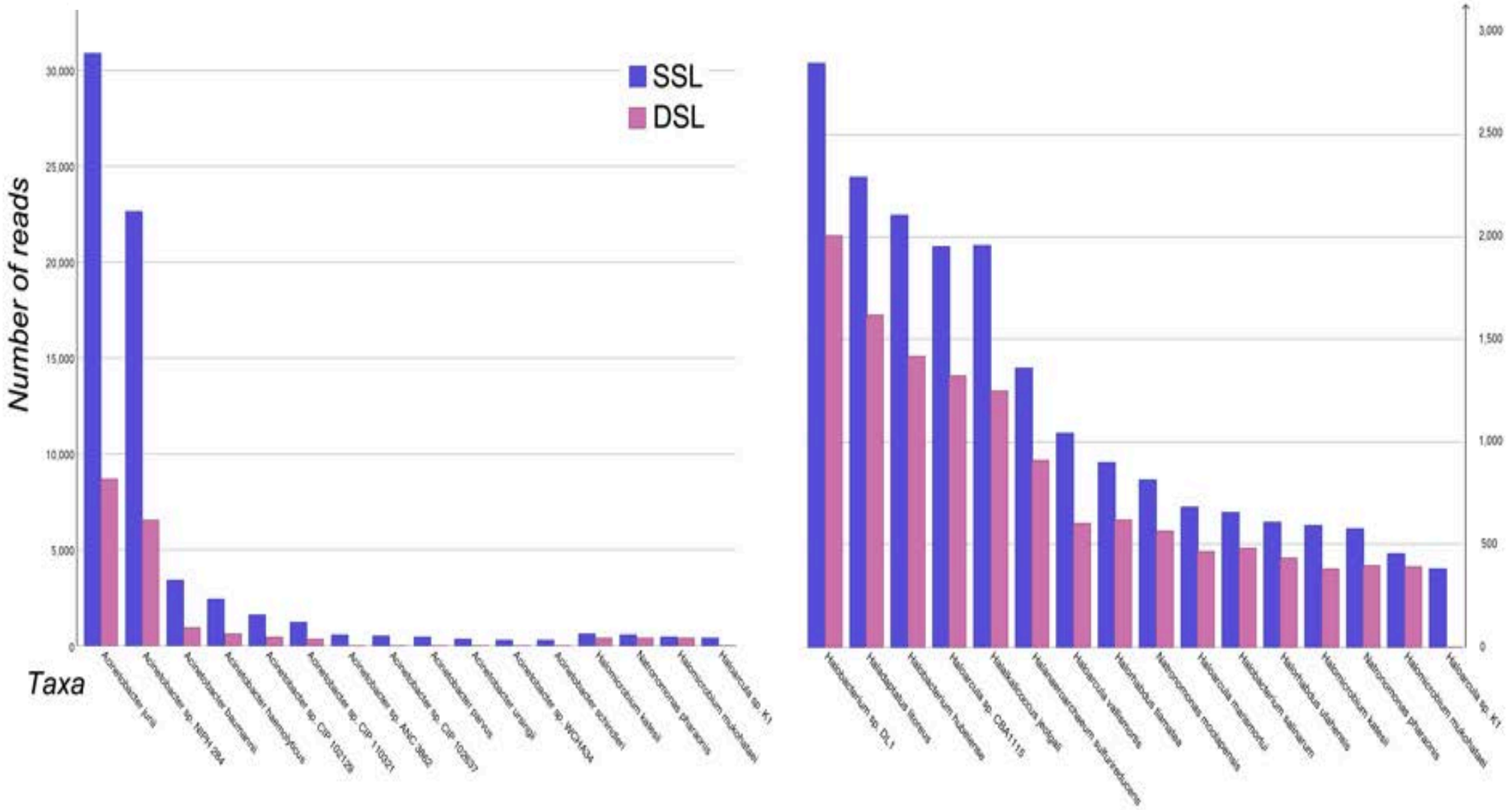
Solutions:

Work in a clean lab space

Monitor controls consistently

Weyrich, et al. *Mol. Ecol. Res.*, 2019.

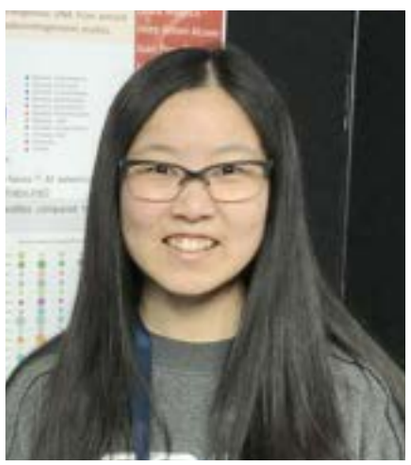
IMPACTS OF CONTAMINATION: MONITOR CONTROLS REGULARLY



Solutions:

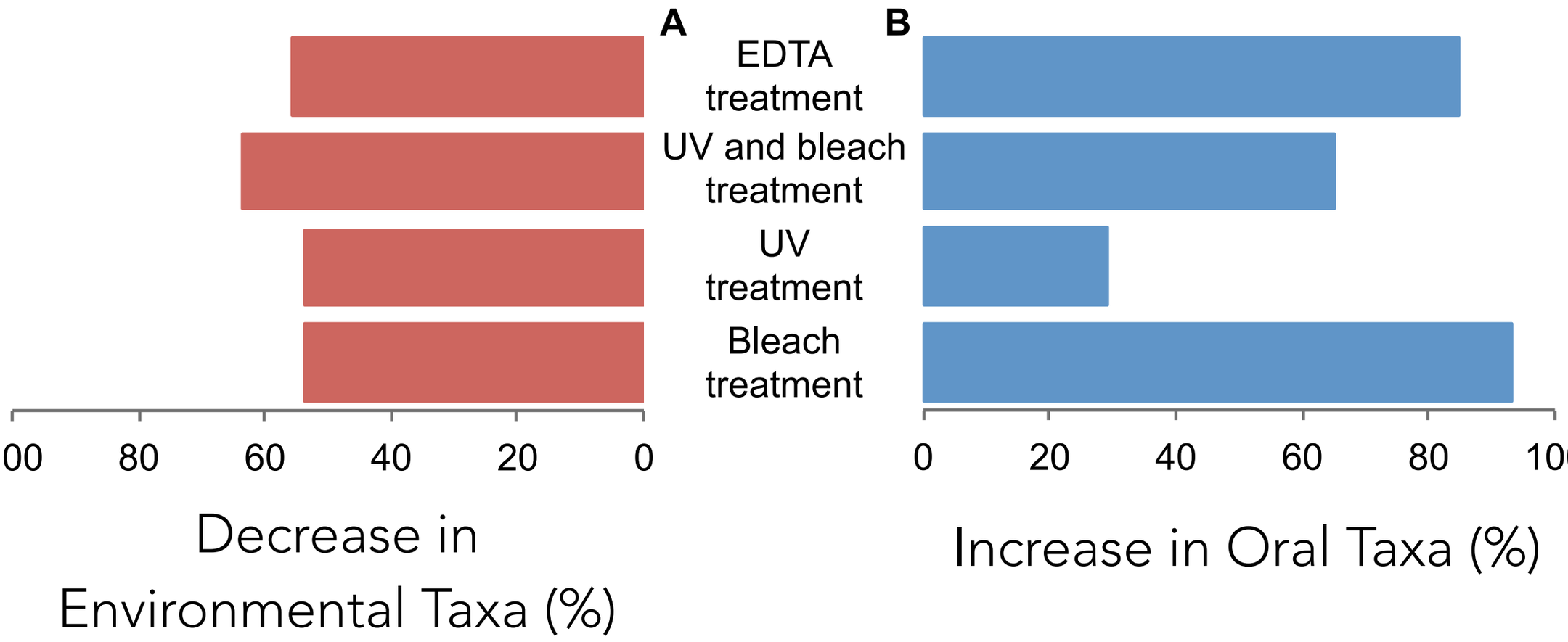
Work in a clean lab space

Monitor controls consistently



Liu, et al. In preparation.

IMPACTS OF CONTAMINATION: DECONTAMINATE



Solutions:

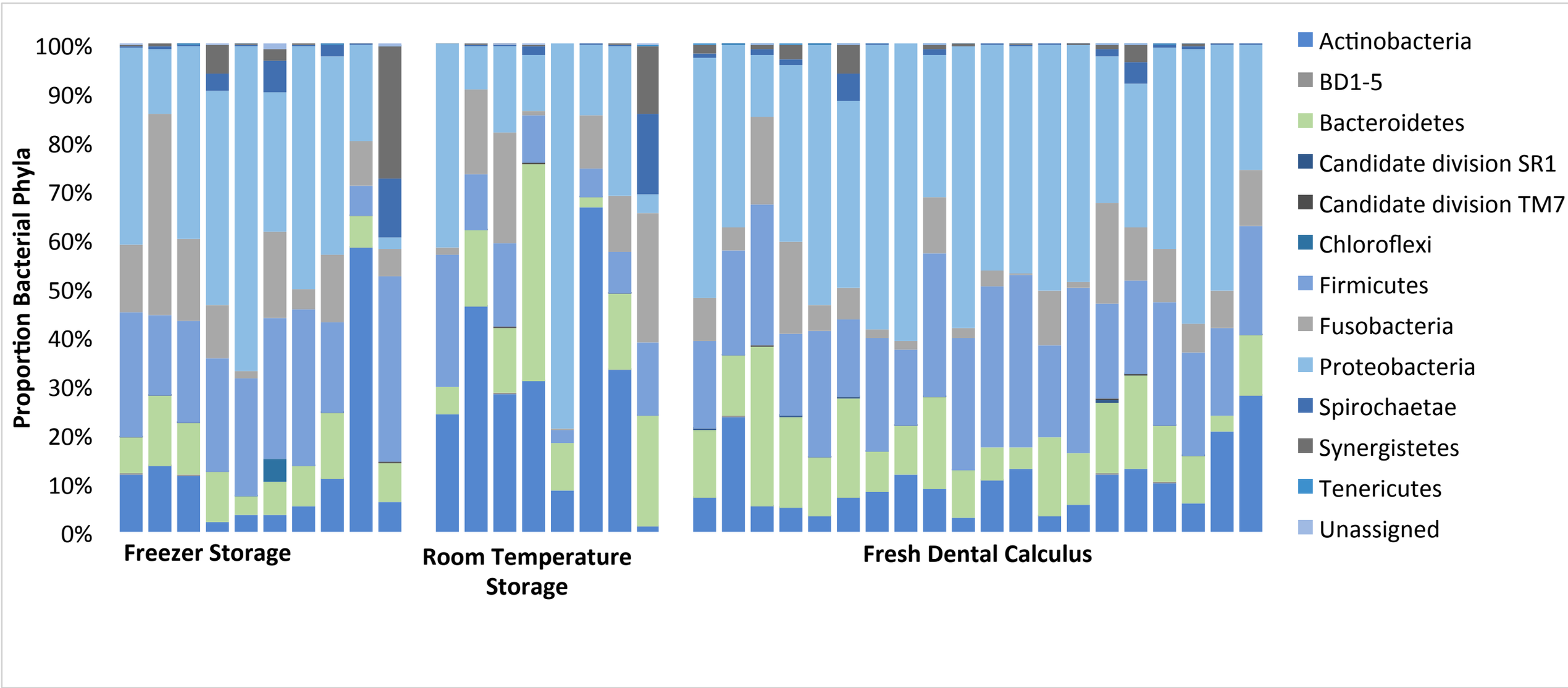
Work in a clean lab space

Monitor controls consistently

De-contamination

Farrer et al., *Sci Rep*. 2021

IMPACTS OF TAPHONOMY



Solutions:

-In vitro and modern studies

-Better programs to model effects

Skelly and Ta et al., In prep

How might microbiome science add to your archaeological research?

(diet, migrations, health, lifestyle, etc.)

What pitfalls might you encounter during sampling or paleomicrobiome analysis?

(fragmentation, contamination, taphonomy, etc.)

Calculus is an amazing resource for many areas of scientific inquiry!

WE'RE HIRING!!

Australian Government
National Health and Medical Research Council

Australian Government
Australian Research Council

The Wenner-Gren Foundation
For Anthropological Research, Inc.

PennState

Adelaide University

NSF

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NIH