



# Examining the Effect of Collection Method on the Microbial Communities Detected by Shotgun

## Metagenomics in Elephant Dung

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### Introduction

- The DNA found in dung enables non-invasive monitoring and management of endangered wildlife populations, informing conservation efforts
- Shotgun metagenomic sequencing of dung DNA facilitates high resolution characterization of microbial communities in samples
- However, no studies have been conducted to standardize collection method, or evaluate whether different collection methods can lead to differences in microbial communities detected

**Question:** Does collection method have an effect on microbial communities detected by shotgun metagenomics?

**Hypothesis:** Microbial community variation will differ among sampling methods

**Conclusion:** Species richness is not significantly different throughout collection methods, but species evenness is. The class Gammaproteobacteria is in greater abundance in the swab+BuccalFix method than in the other methods

### Methods

- 10 fresh dung samples were collected from a Bornean Pygmy elephant (*Elephas maximus borneensis*) at the Oregon Zoo using 5 different, commonly used collection methods (n=2 samples per method; Table 1)
- Conducted shotgun metagenomic analyses on the DNA extracted from each dung sample
- Developed a bioinformatic pipeline to analyze the microbial communities detected
- Calculated Species Richness and alpha diversity using Simpson's Diversity Index for all samples
- ANOVA tests were run using R to determine if there were any statistically significant differences between collection methods

### Shotgun Metagenomic Pipeline

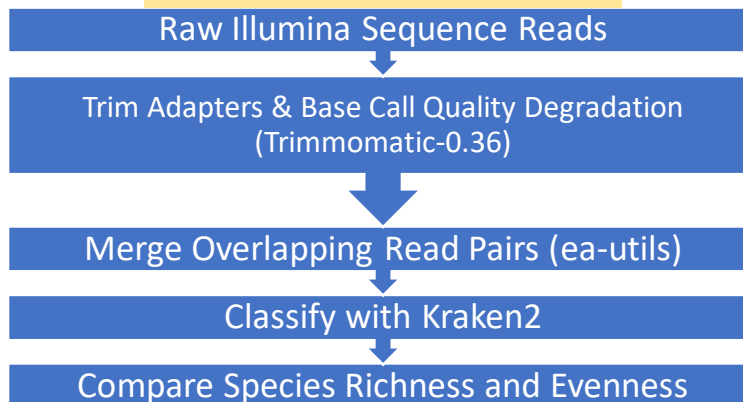


Fig 2. The above bioinformatic pipeline was used to analyze which microbial communities are detected by different sampling methods

### Collection Method

Collection Type	Preservative
Pinch	RNAlater
Pinch	Ethanol + Silica
Isohelix swab	RNAlater
Isohelix swab	BuccalFix
Isohelix swab	DriCapsule

Table 1. Collection methods and preservatives used for samples (n=2 per sample method)

### Results

	Pinch+ RNAlater	Pinch+ Ethanol+Silica	Swab+ RNAlater	Swab+ BuccalFix	Swab+ DriCapsule
Species richness	81	80.5	81	78	79.5
Alpha diversity	0.9074	0.8846	0.9165	0.2576	0.9106

Table 2. This table shows the species richness and alpha diversity across five collection methods. Richness is detected at the same levels throughout collection methods (p-value=0.114, df=4). Alpha diversity is shown using Simpson's Diversity Index. There are differences between communities (p < 0.01, df= 4). (n=2 per collection method).

### Community Composition Detected in Two Samples

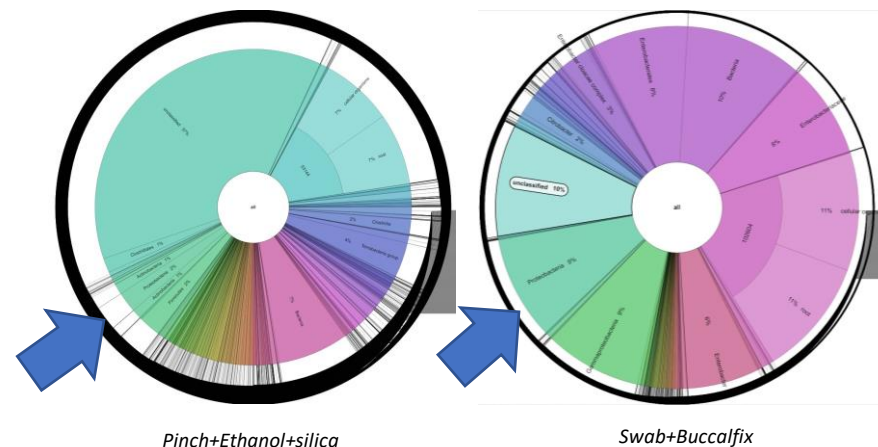


Fig 3. The two hierarchical pie charts above present the distribution of bacterial communities from two of the 10 samples analyzed in this study. Community distribution notably differs across collection method

### Future Direction

- Expand pilot study to include more replicates per method and increase sequencing depth
- Investigating plant DNA in dung, potentially revealing information about diet of animal
- Exploring differences between samples taken from captive and wild elephants



Fig 1. Chendra, a Bornean Pygmy elephant at the Oregon Zoo, who graciously provided generous amounts of dung for samples.