

Microeukaryotic and dietary survey of the human gut by internal transcribed spacer metabarcoding



Allison E. Mann, Cecil M. Lewis Jr.
University of Oklahoma, USA
Laboratories of Molecular Anthropology and Microbiome Research

Introduction

Increasingly integrated into studies of host-associated microbiome communities, microbial eukaryotes impact their prokaryotic neighbors through predation, resource competition, and interaction with the host immune system. Unlike bacteria and archaea, microbial eukaryotes are difficult to identify in these complex microecosystems due to their relatively large genomes, characteristic long stretches of phylogenetically uninformative genomic regions, and comparatively low frequency. While the internal transcribed spacer (ITS) region of the eukaryotic rDNA complex is frequently used to survey fungal inhabitants of microbial ecosystems, it is rarely used to assess the full eukaryotic component. In this study, the ITS region is evaluated for its utility as a target for characterizing microbial eukaryotes in the gut of divergent human populations. Of particular interest are differences in taxonomic profiles generated by the ITS1 and ITS2 regions. We find that while both ITS1 and ITS2 have considerable taxonomic overlap, the ITS2 region performs more poorly when considering specific ecologically important groups in the gut. Importantly, however, both ITS1 and ITS2 document differences in gut microbial ecology among individuals that is likely structured by lifestyle and not geographic distance. In summary, the internal transcribed spacer region may serve as an additional eukaryotic marker for investigating microbial eukaryotes in human associated microbial ecosystems.

Aims

1. Evaluate applicability of ITS metabarcoding for dietary reconstruction from fecal samples.
2. Compare the utility of ITS1 and ITS2 for characterizing microeukaryotic organisms in human and non-human fecal samples.
3. Demonstrate the predictive value of microeukaryotic taxa for subsistence strategy, lifestyle, or environment.
4. Document substrain diversity in members of the common gut protist *Blastocystis*

Methods

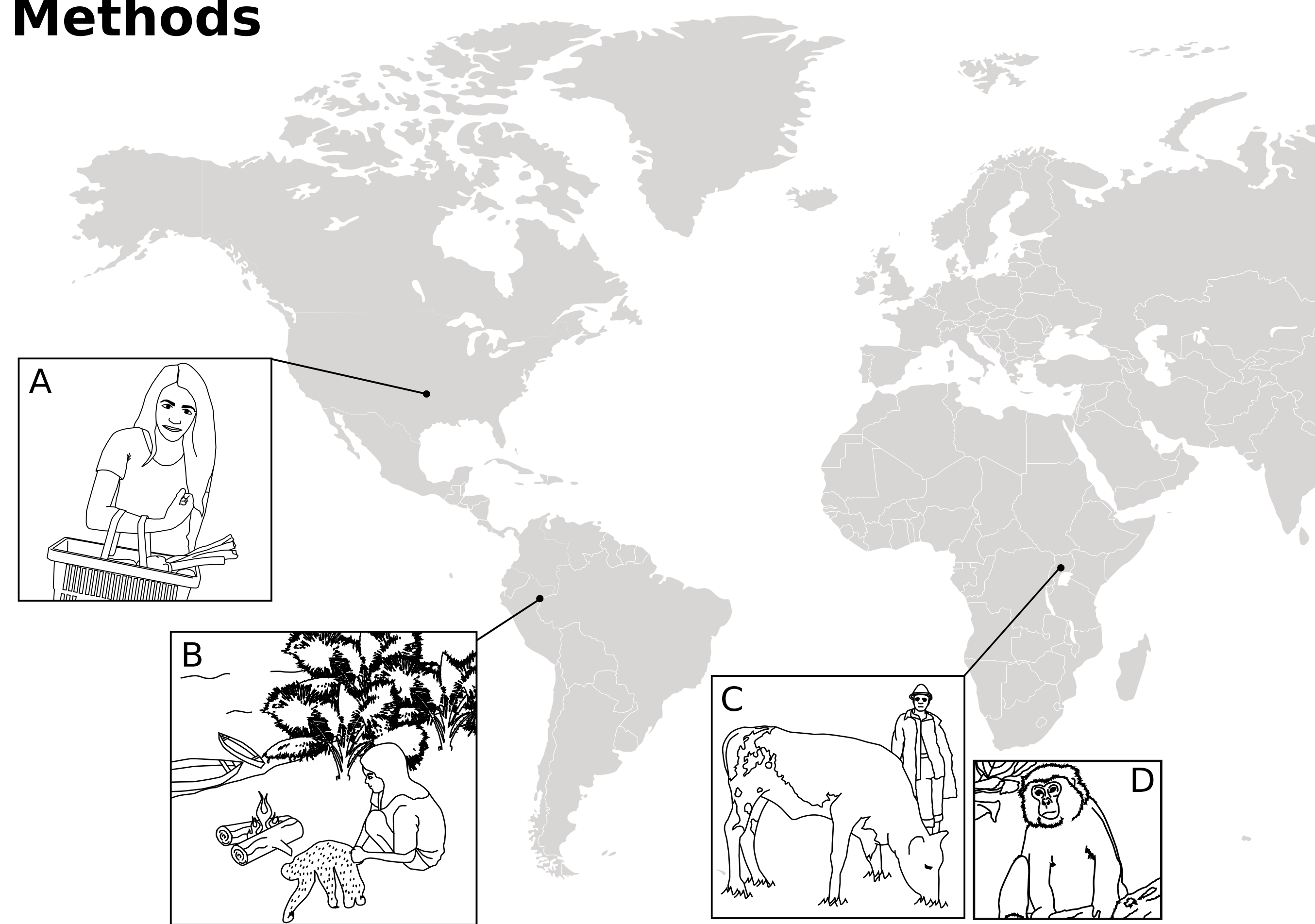


Figure 1: Map of Sampling Locations. (A) Norman, OK, USA (n=4), (B) Matses, Peru (n=6), (C) Kibale national park, Uganda (n=6) and domesticated bovid (n=1), (D) Ugandan Red Colobus (n=1)

- Fecal samples from three human and two non-human groups representing three continents and diverse lifestyles (urban industrialized, rural hunter-gatherer, rural agriculturalist, domesticated herbivore, wild folivore) were collected and extracted.
- ITS1 & ITS2 amplicon¹ libraries sequenced on Illumina MiSeq with 2x250 chemistry.
- OTUs generated at 97% similarity using USEARCH² pipeline and assigned taxonomy with the NCBI NT database and MEGAN v6 Community Edition³

References Cited

- ¹White et al. Amplification and direct sequencing of fungal ribosomal genes form phylogenetics. In: PCR Protocols. San Diego, CA, USA: Academic Press 1990. pp. 315-322
²Edgar, RC. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19), 2460-2461
³Huson et al. 2016. MEGAN Community Edition - Interactive exploration and analysis of large-scale microbiome sequencing data. PLOS Computational Biology 12(6): e1004957

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Results

Table 1: Subset of Dietary Results. OTUs that were assigned to potential dietary sources are consistent with known subsistence patterns of sampled groups.

Taxonomy	Common name	Region	Isolation source
<i>Zea</i>	Corn	1,2	All human populations
<i>Salmonidae</i>	Ray-finned fish	2	Matses
<i>Cecropia peltata</i>	Snakewood	1	Matses
<i>Arachis hypogaea</i>	Peanut	1	Uganda
<i>Sesamum indicum</i>	Sesame	1	Uganda
<i>Solanum pennellii</i>	Wild tomato	2	Uganda
<i>Musa</i>	Banana/Plantain	1,2	Uganda, Matses
<i>Coriandrum sativum</i>	Cilantro/coriander	1,2	Norman
<i>Solanum lycopersicum</i>	Tomato	1	Norman
<i>Spinach oleracea</i>	Spinach	2	Norman
<i>Cynodon</i>	Grasses	1	Bovid
<i>Ficus</i>	Ficus/fig tree	1,2	Colobus

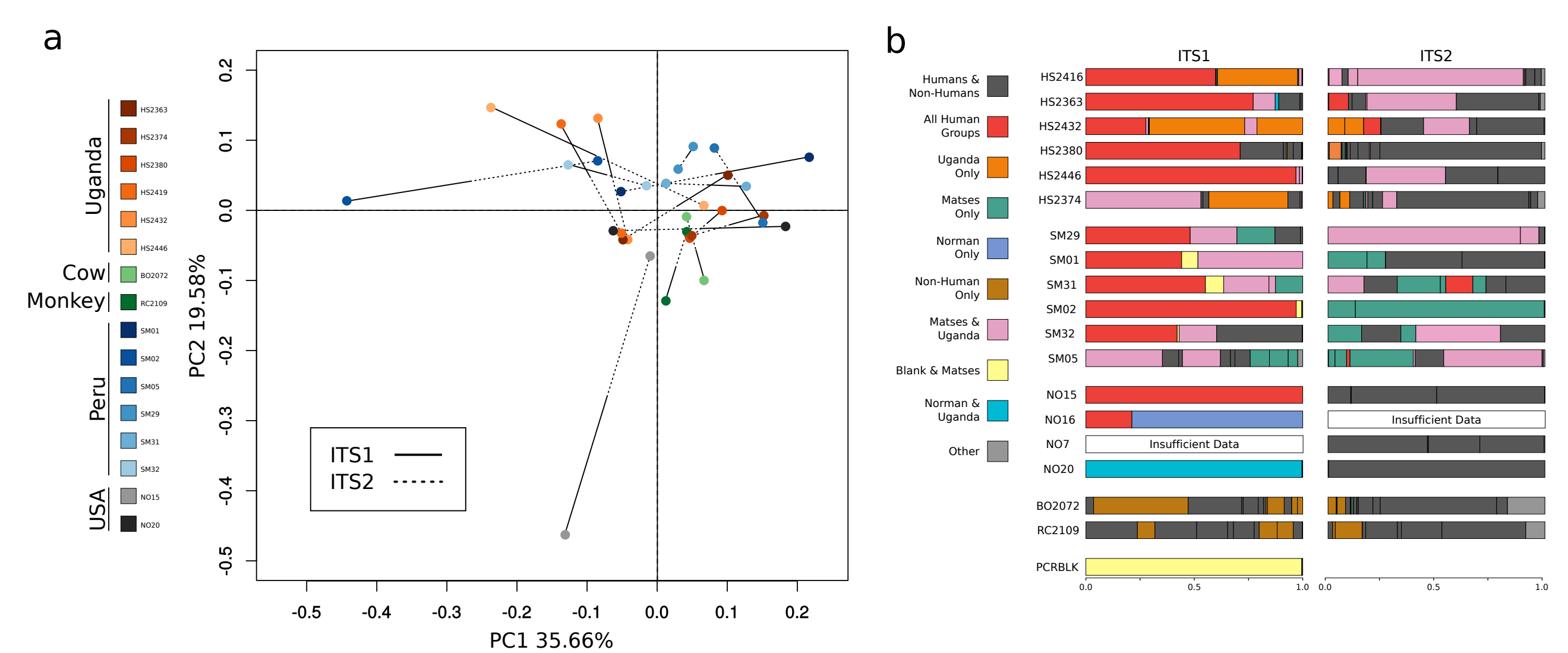


Figure 2: Differences in Detected Microeukaryotic Composition in ITS1 and ITS2 datasets. (a) Procrustes plot of sample ITS1 and ITS2 results. Each pair of dots represents a single individual sequenced by both ITS1 and ITS2. (b) Genus level taxonomic barcharts for each sample split by targeted ITS region. Each section of the total bar represents a single OTU and is colored by group specificity. Despite taxonomic differences, both the ITS1 and ITS2 regions document a higher degree of shared OTUs between the Matses and Ugandan populations than between either rural population and the Norman group

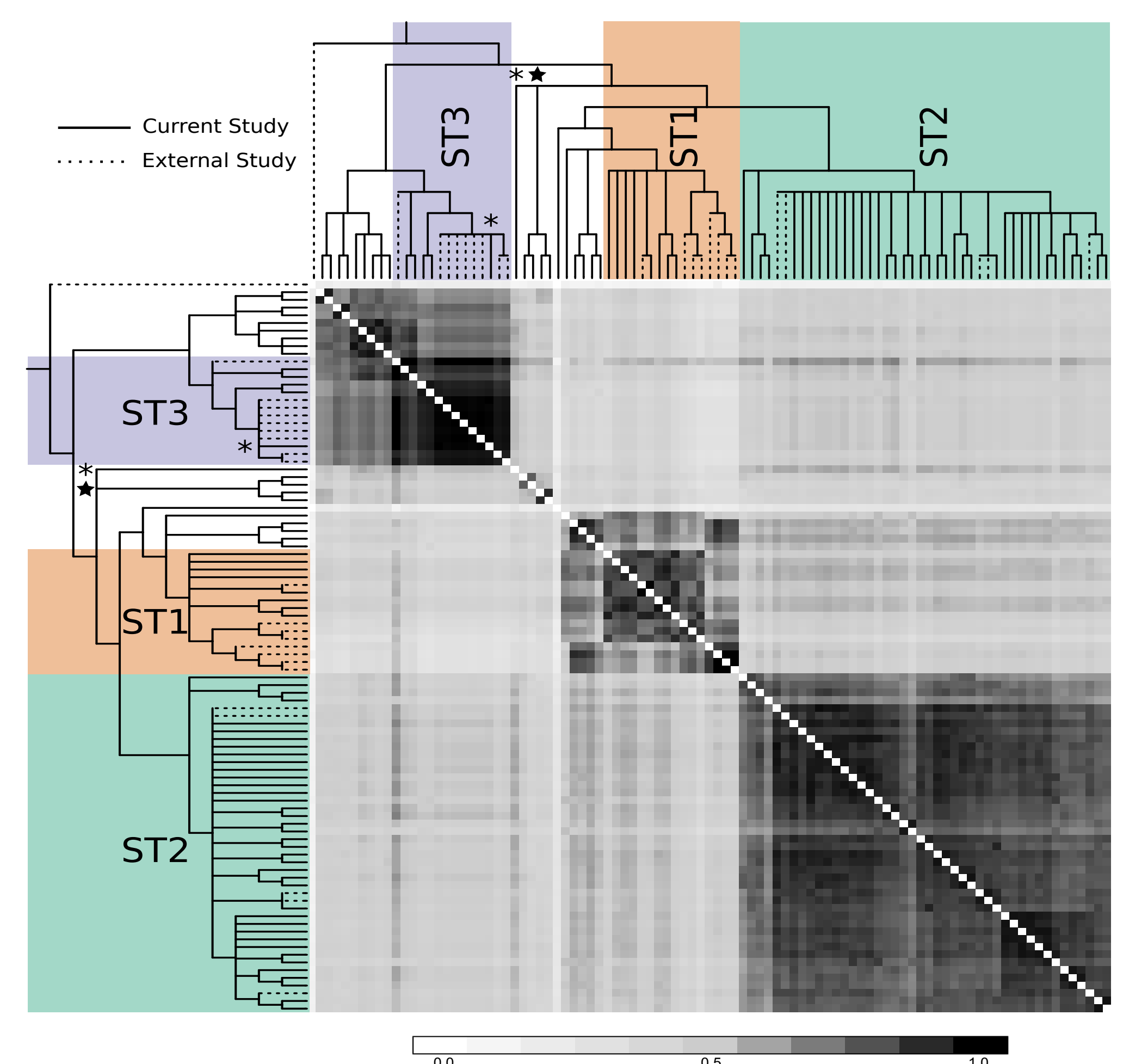


Figure 3: Pairwise Distance and *Blastocystis* Subtype Diversity Among Individuals as Detected by ITS1. Subtypes 1, 2, and 3 are found in the Ugandan and Matses groups with multiple subtypes in a single individual commonplace. Additionally, two Norman individuals (*) were found to carry subtype 3 and a possible alternative subtype not resolved in this tree. Finally, the bovid (*) was positive for a *Blastocystis* strain distinct from the human lineages found here.

Conclusions

- While ITS region provides high-resolution taxonomic survey of dietary and microeukaryotic taxa, ITS1 and ITS2 document different taxonomic profiles. Most strikingly, the ITS2 primers used here will omit the important gut protist *Blastocystis*.
- Non-industrialized groups have more diverse suite of microeukaryotes and have more microeukaryotic taxa in common amongst themselves than between industrialized and non-industrialized groups.
- Members of the genus *Blastocystis* are a highly genetically diverse and cosmopolitan group, detected in all three human groups, irrespective of lifestyle or geography.