

# Parasites and the eukaryotic biome - diversity is associated with social rank in

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## Context, Predictions and Aims

In the spotted hyena (*Crocuta crocuta*), a highly social, female-dominated carnivore social status determines access to resources.



Predictions:

- High species diversity is an index of ecosystem health, the intestinal biome of healthier, socially dominant animals should be more diverse than those of subordinates.
- Gradual colonization of the juvenile intestine after birth predicts lower intestinal biome diversity in juveniles than adults.

We therefore aimed to:

1. Establish an amplicon sequencing approach to assess intestinal eukaryotes (the eukaryome) comprehensively
2. Determine the diversity and composition of both the bacterial microbiome and eukaryome

## Methods

Study animals:

- 35 individually known adult females and 7 juveniles (< 24 months) for three clans of the Serengeti ecosystem
- Social status categorized as above/below the median rank
- Parasite eggs or oocysts counted for 32 individuals

Amplicon sequencing

- 48 different amplicons (4 for bacterial 16S, 44 for eukaryote 18S) in a multi-amplicon sequencing approach
- Processed on a microfluidics PCR system (Fluidigm Access Array)
- Data processed using an amplified sequencing variant (ASV) approach provided by the R package DADA2 [1] and is available as an own R package [2].

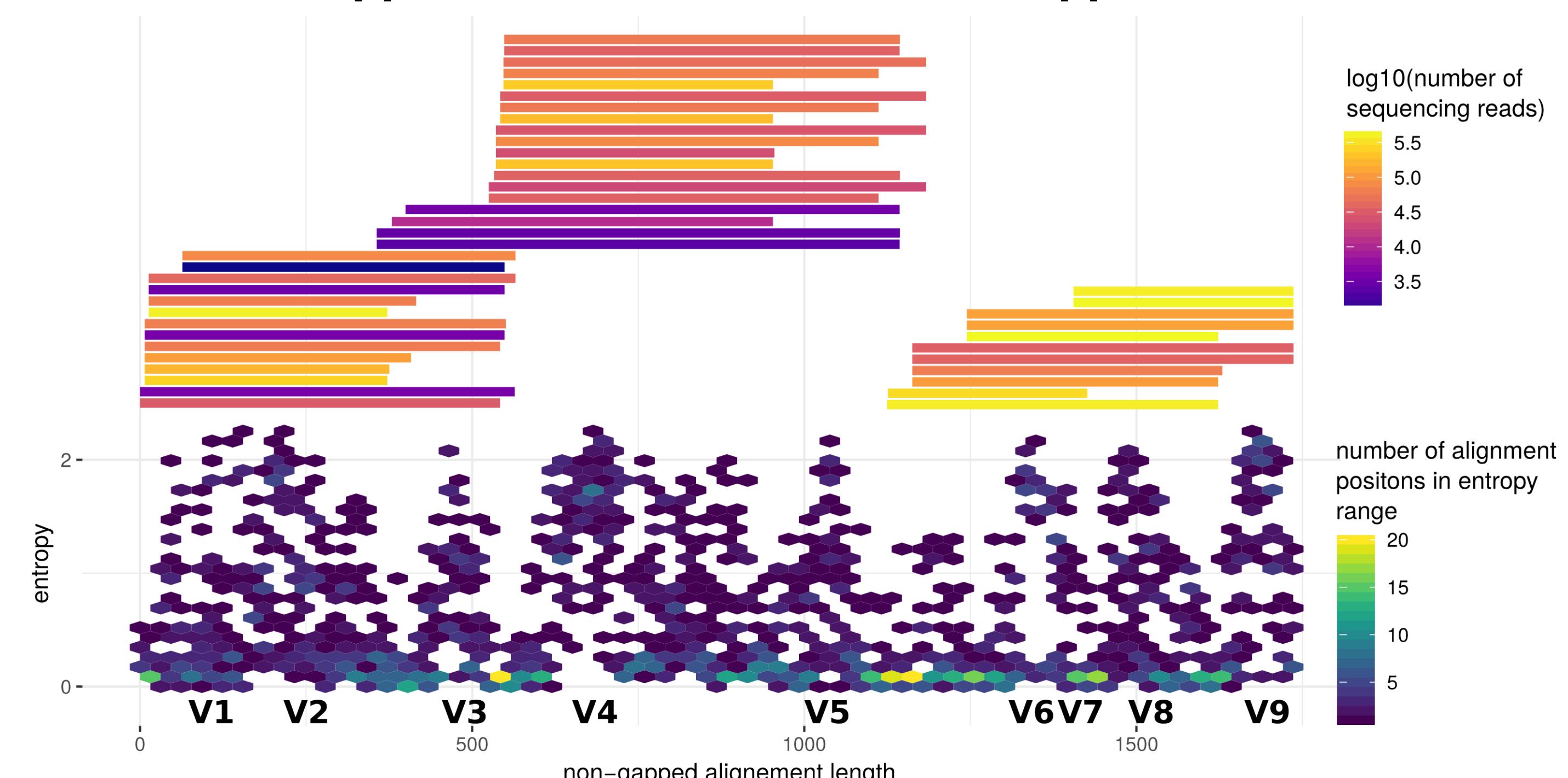


Fig 1: Amplicons used in this study in the context of entropy (evolutionary conservation and information content) of the eukaryotic 18S gene

## Results: Multi-amplicon sequencing predicts coprological counts

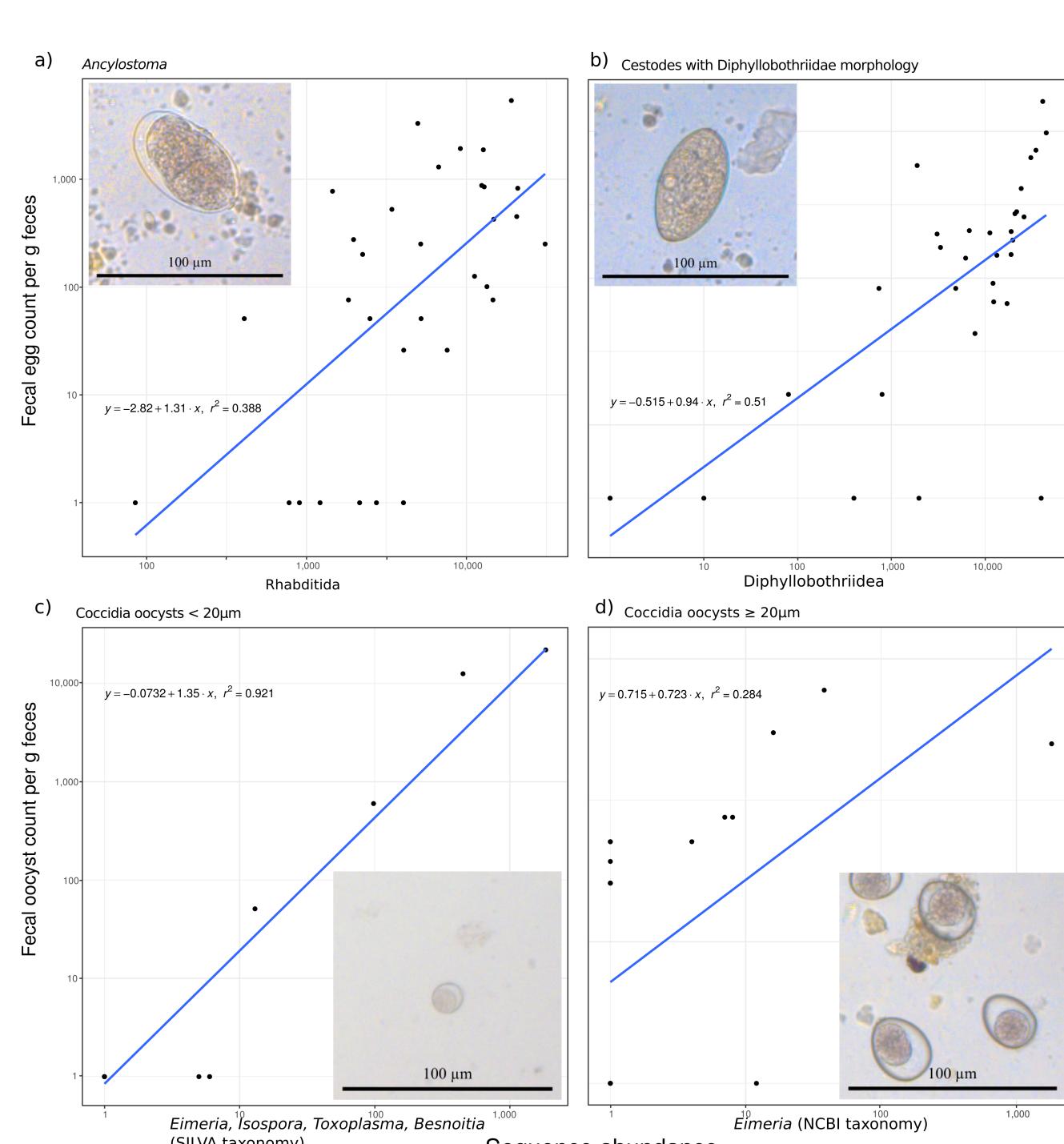
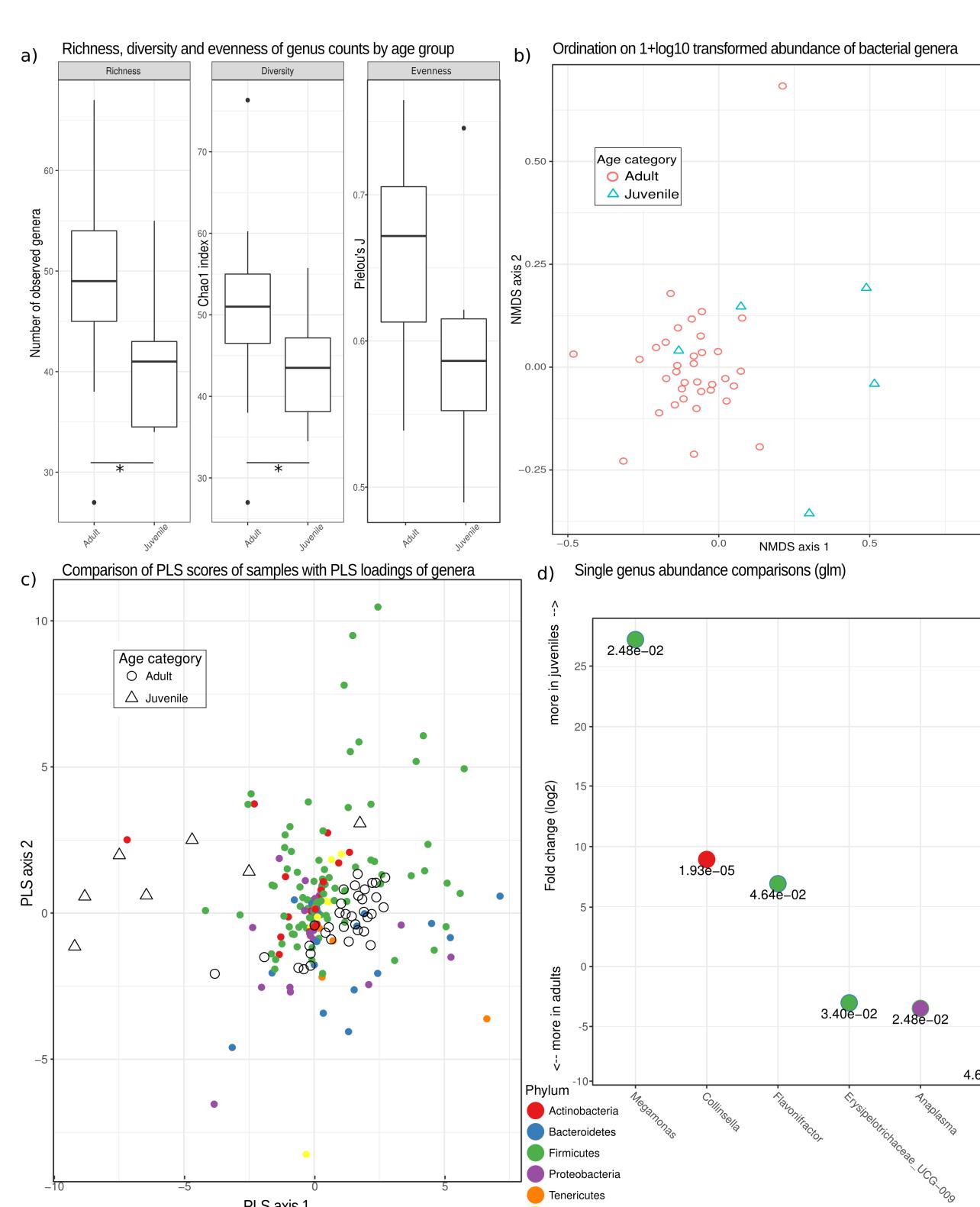


Fig 2: Predicting fecal egg or oocyst counts per g feces from the number of ASV reads. (A) Ancylostoma FEC vs. sequence counts for the order Rhabditida. (B) Diphyllobothriidae FEC vs. sequence counts for the same family. (C) A small size class of oocyst counts vs. added sequence counts for *Eimeria*, *Isospora*, *Besnoitia*, and *Toxoplasma*. (D) A large size class of coccidian oocysts vs. *Eimeria* sequences. All panels contain the formula for the specific linear model on (1+log10) transformed data, R2 as a measure of goodness of fit, and a line representing the predicted relationship. The panels additionally include a representative micrograph depicting the egg or oocyst counted.

⇒ Quantitative assessment of eukaryotes with high sensitivity

## Results: Bacterial microbiome

**Fig 3:** Bacterial genera richness, diversity and microbiome composition in different age categories. (A) Box plots depicting distributions observed counts of genera, diversity (Chao1 index) and evenness (Pielou's J) estimates on rarefied genera counts for juveniles and adults. \* =  $p < 0.05$  in exact Mann-Whitney U tests.

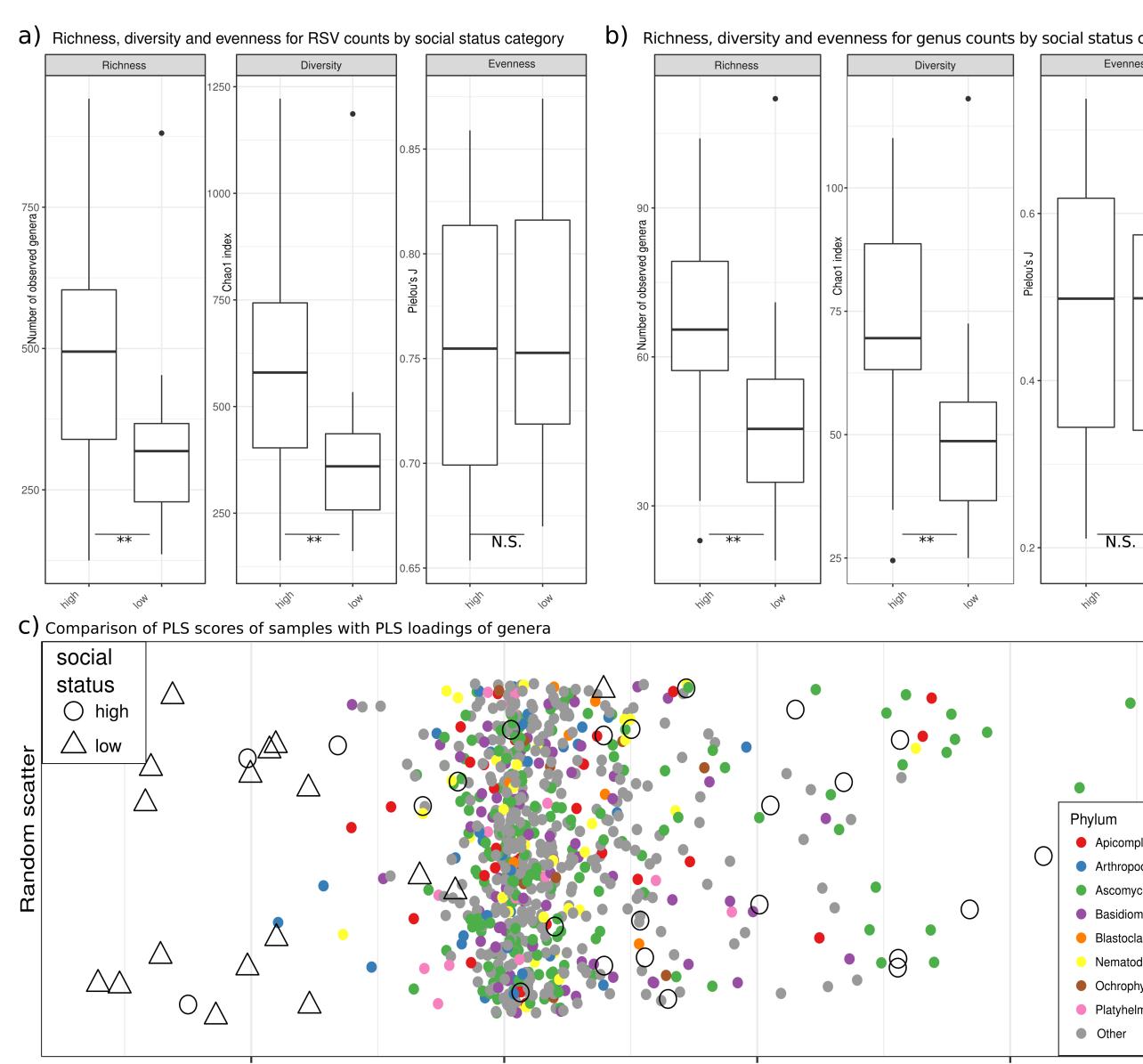


(B) NMDS ordination based on pairwise Bray-Curtis dissimilarities partially separated juvenile from adult. (C) Comparison of scores (for samples) and loadings (for genera) from the first two axes of an optimized partial least squares (PLS) model, demonstrating a clear separation of adult and juvenile samples. Genera colored by phylum can be used to assess the taxa contributing (PLS loading) to the differences underlying this distinction.

(D) Log2-fold change inferred by generalized linear models for differences between adults and juveniles for each genus with a false discovery rate (FDR)  $< 0.05$ . FDR is given below the dot for each genus color-coded for its respective phylum.

⇒ Adult female hyenas have a bacterial microbiome which is more diverse than and differs in composition from that of juveniles.

## Results: A more diverse eukaryome in high-ranking than low-ranking hyenas



On the y-axis random scatter is introduced for visualization. The underlying genera are color-coded for their respective phylum.

## Summary

	Bacterial microbiome	Eukaryome
Adults vs. Cubs	More diversity Higher evenness	More diversity Higher evenness
High vs. low social rank	More diversity Higher evenness	More diversity Higher evenness

Green circles = Results matching predictions Red squares = No confirmatory results

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## References

- [1] Callahan et al. (2016) DADA2: High-resolution sample inference from Illumina amplicon data *Nature Methods* 13, 581–583
- [2] Heitlinger (2017). Derele/MultiAmplicon version 0.01 [R - package]. Zenodo Development version at <https://github.com/derele/MultiAmplicon>