

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

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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.



Fig1: A young female spotted hyena with erected "pseudo-penis" [1].

### 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.

## 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 processing pipeline using amplified sequencing variants (ASVs) [2] is available as an R package [3].

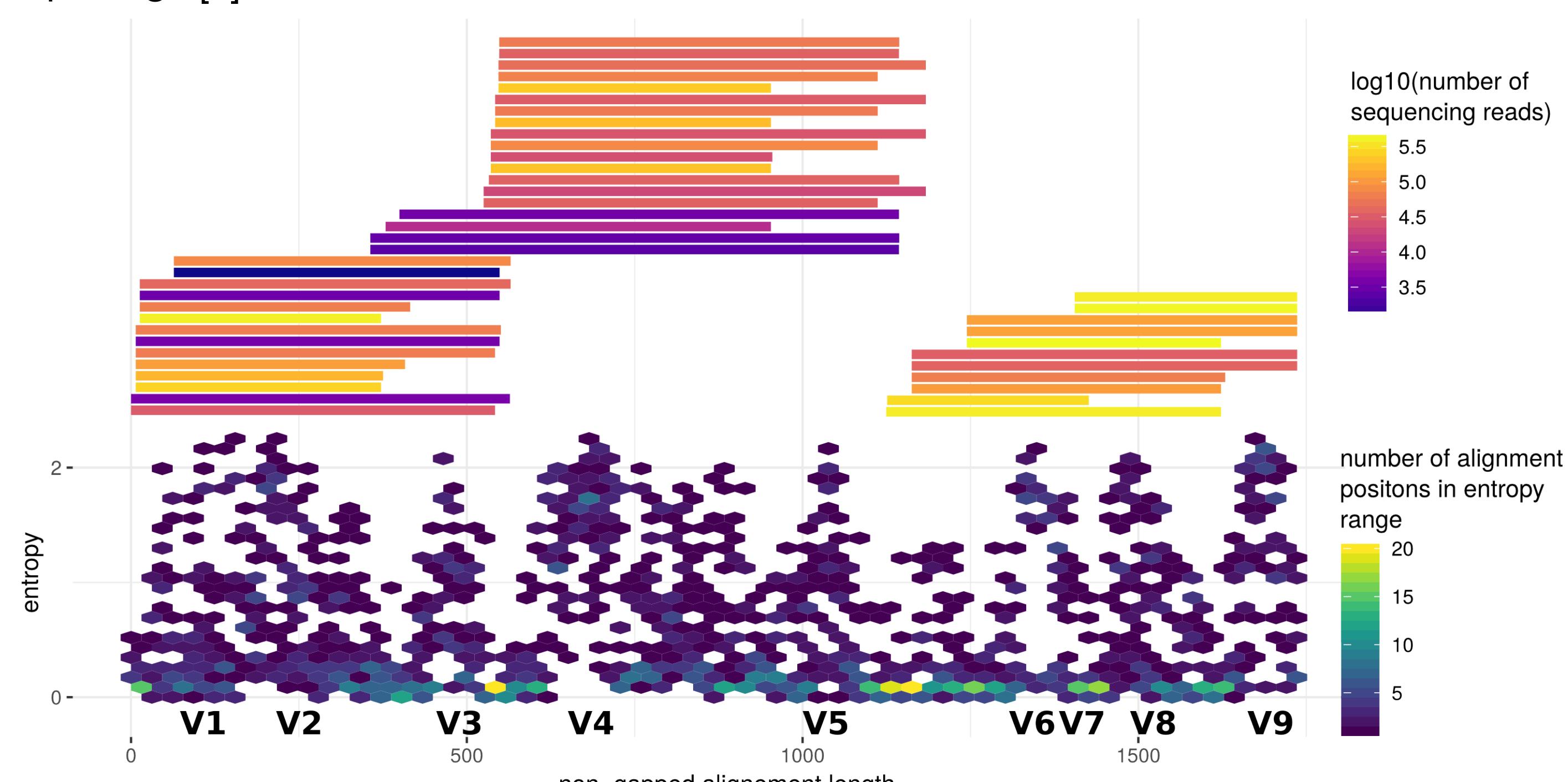
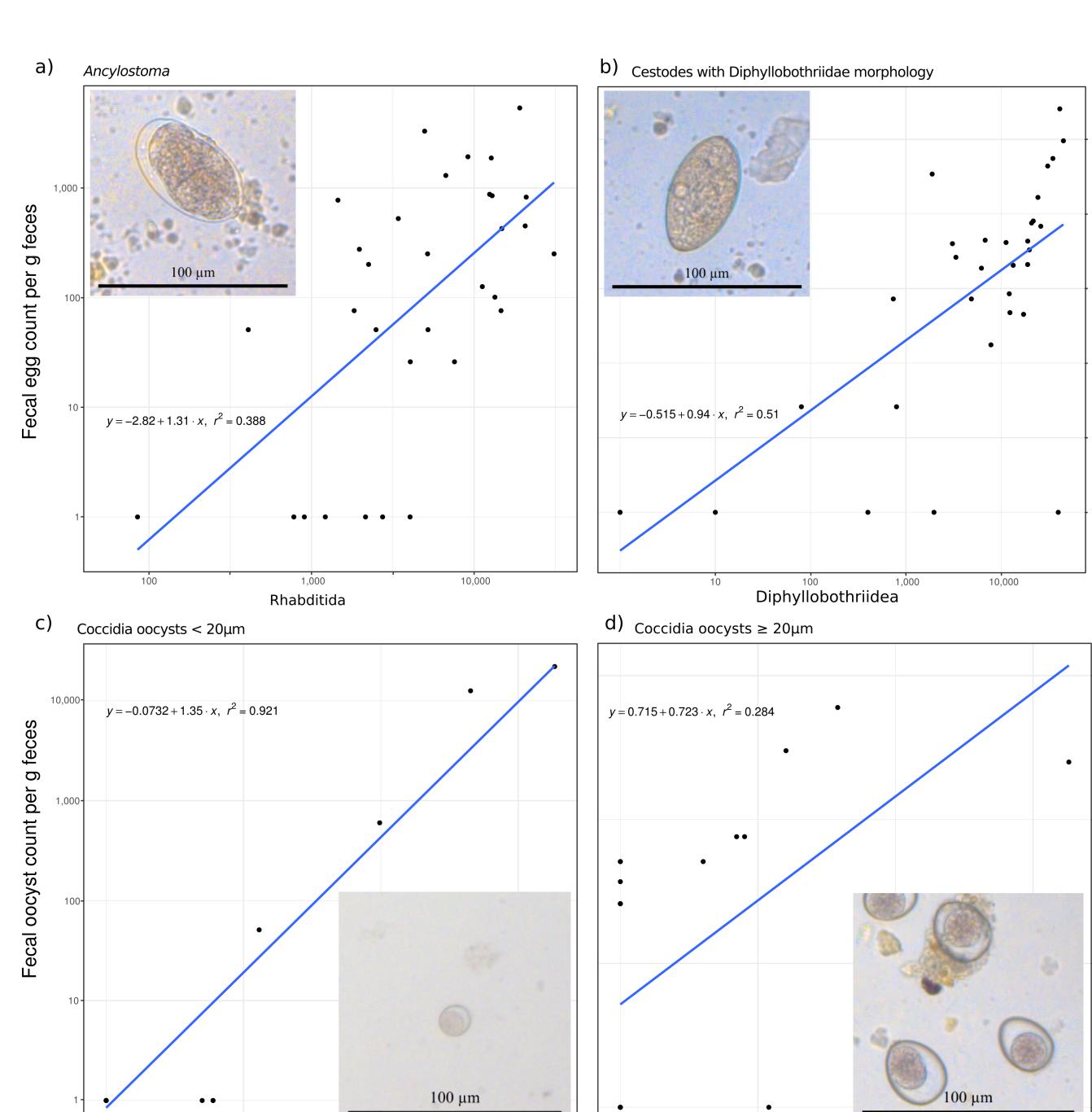


Fig 2: Amplicons used in this study in the context of entropy (evolutionary conservation and information content) of the eukaryotic 18S gene. Shannon entropy is displayed for the Silva reference alignment [4] of all eukaryotes. Primers were mapped against the same alignment. Positions along the gene are expressed as mean length of sequences (excluding gaps) for each column in the alignment.

## Results: Multi-amplicon sequencing predicts coprological counts



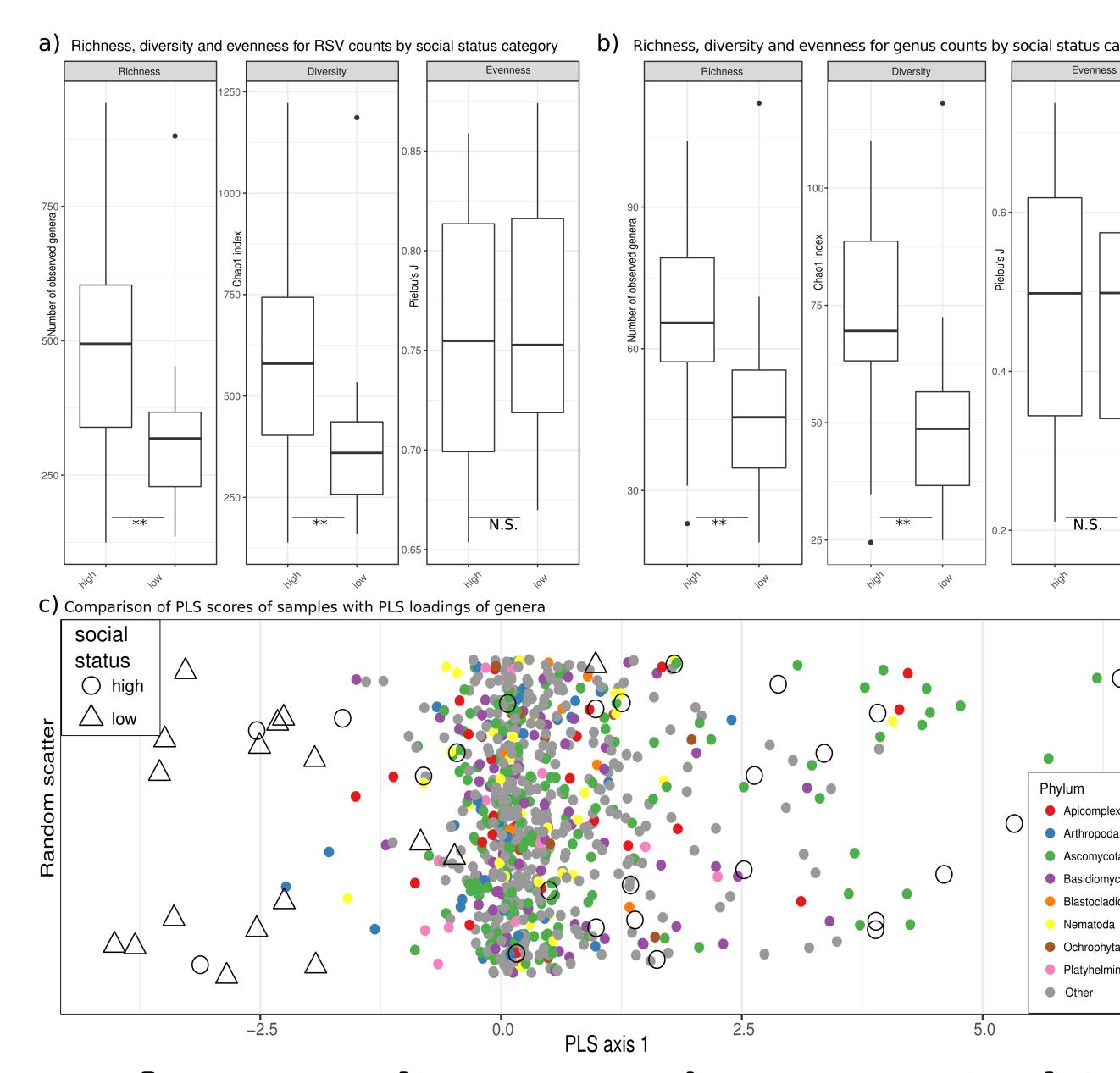
⇒ Quantitative assessment of eukaryotes with high sensitivity.

## Results: Bacterial microbiome

**Fig 4:** Bacterial genera richness, diversity and microbiome composition in different age categories. (A) Distributions of observed rarefied genera counts, diversity (Chao1 index) and evenness (Pielou's J) for juveniles and adults. (B) NMDS ordination based on pairwise Bray-Curtis dissimilarities partially separate juveniles from adults. (C) Comparison of scores (for samples) and loadings (for genera) from the first two axes of a partial least squares (PLS) model, demonstrating a clear separation of adults and juveniles. 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.

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

## Results: Eukaryome



⇒ A more diverse eukaryome in high-ranking than low-ranking hyenas.

## 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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