

# 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.



Fig 1: Hyenas interacting at a den.

### 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.

## Results: Validation

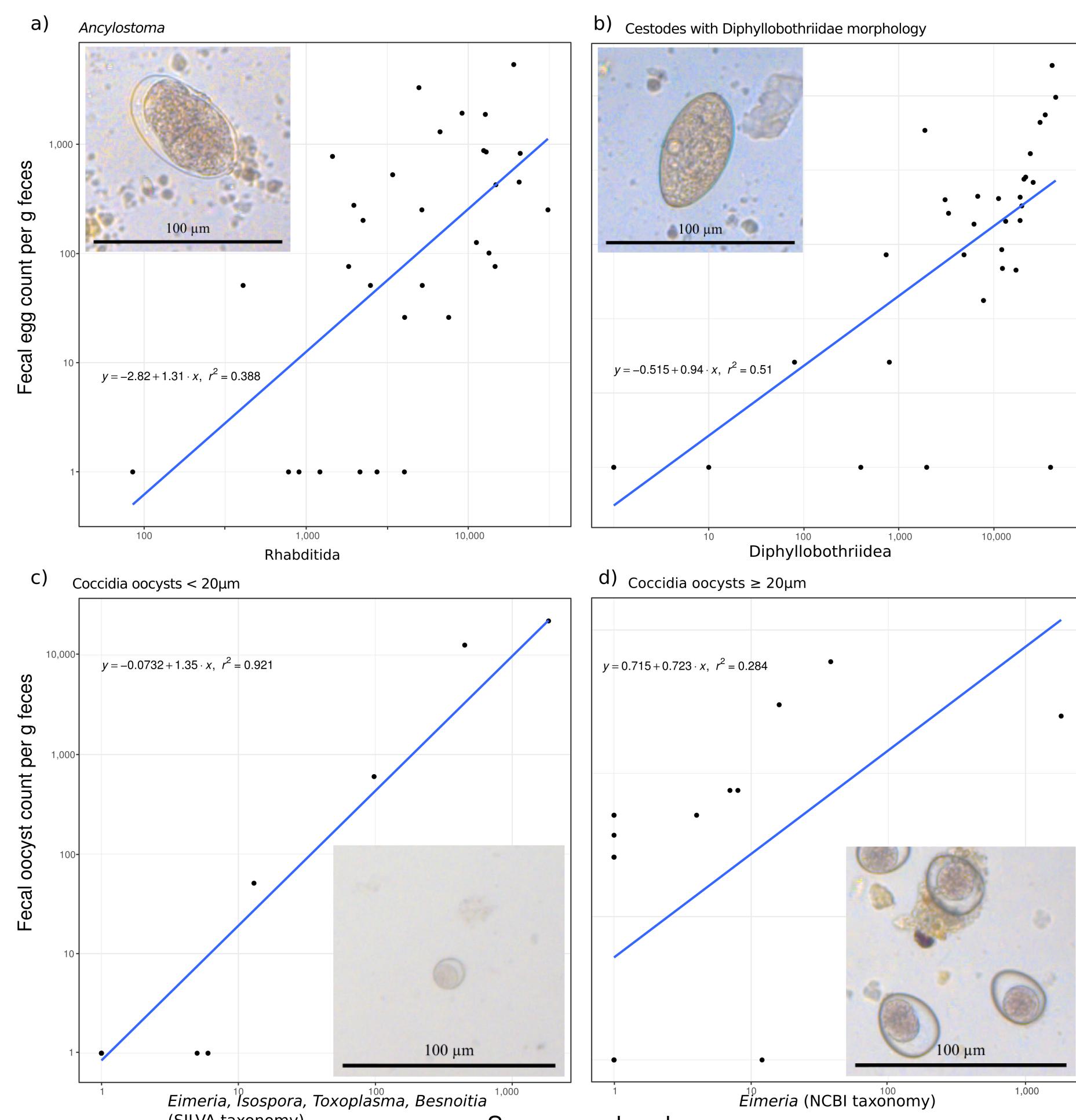


Fig 2: Ribosomal sequence fragments (RSVs) predict fecal egg or oocyst counts for (a) *Ancylostoma* (Rhabditida), (b) *Diphyllobothriidae* and (c and d) small and a large size classes of Coccidia (*Eimeria*, *Isospora*, *Besnoitia*, and *Toxoplasma*).

⇒ Quantitative assessment of eukaryotes with high sensitivity.

## 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 RSVs [1] is available as an R package [2].

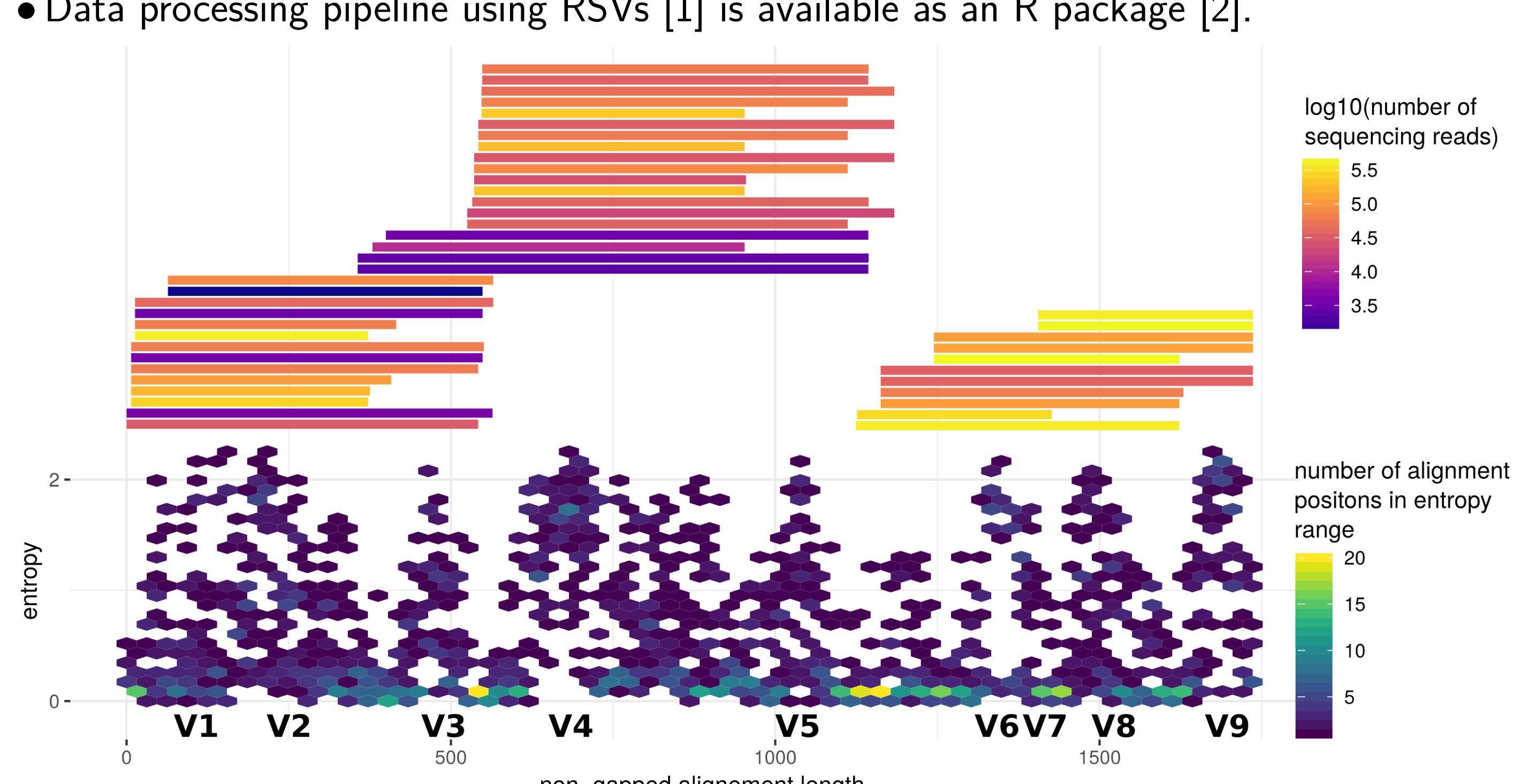


Fig 2: Amplicons used in this study in the context of entropy (evolutionary conservation and information content) of the eukaryotic 18S gene. Shannon entropy is displayed for the Silva reference alignment [3] of all eukaryotes. Primers were mapped against the same alignment. Positions along the gene are expressed as mean length of sequences (excluding gaps) for each column in the alignment.

## 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

## Results: Bacterial microbiome

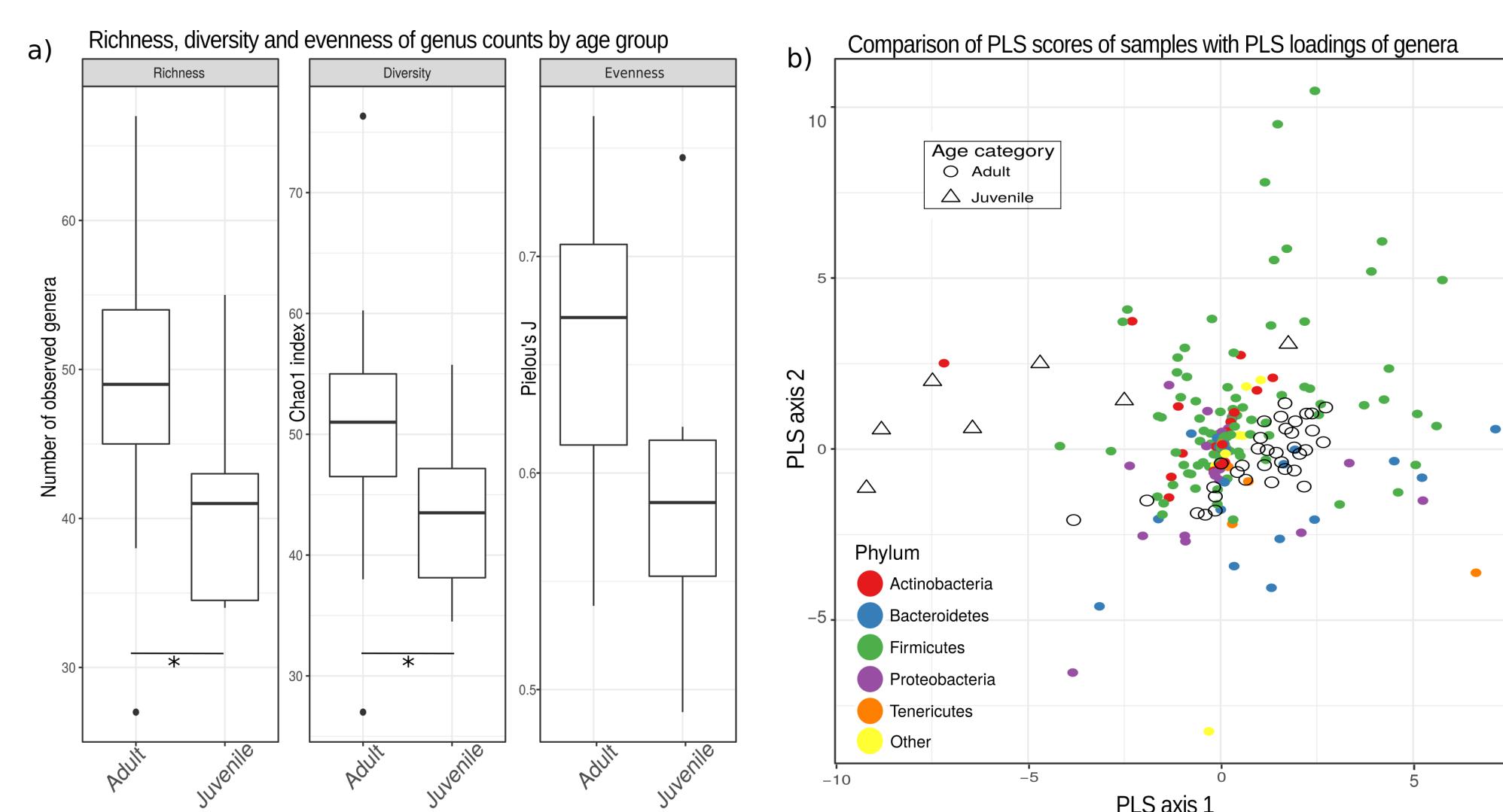


Fig 4: a) Observed counts (richness) and diversity indices for genera are lower in juveniles compared to adults. b) Scores (for samples) and loadings (for genera) in a partial least squares model demonstrate differences in microbiome composition of adults and juveniles and highlight differently abundant taxa.

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

## Results: Eukaryome

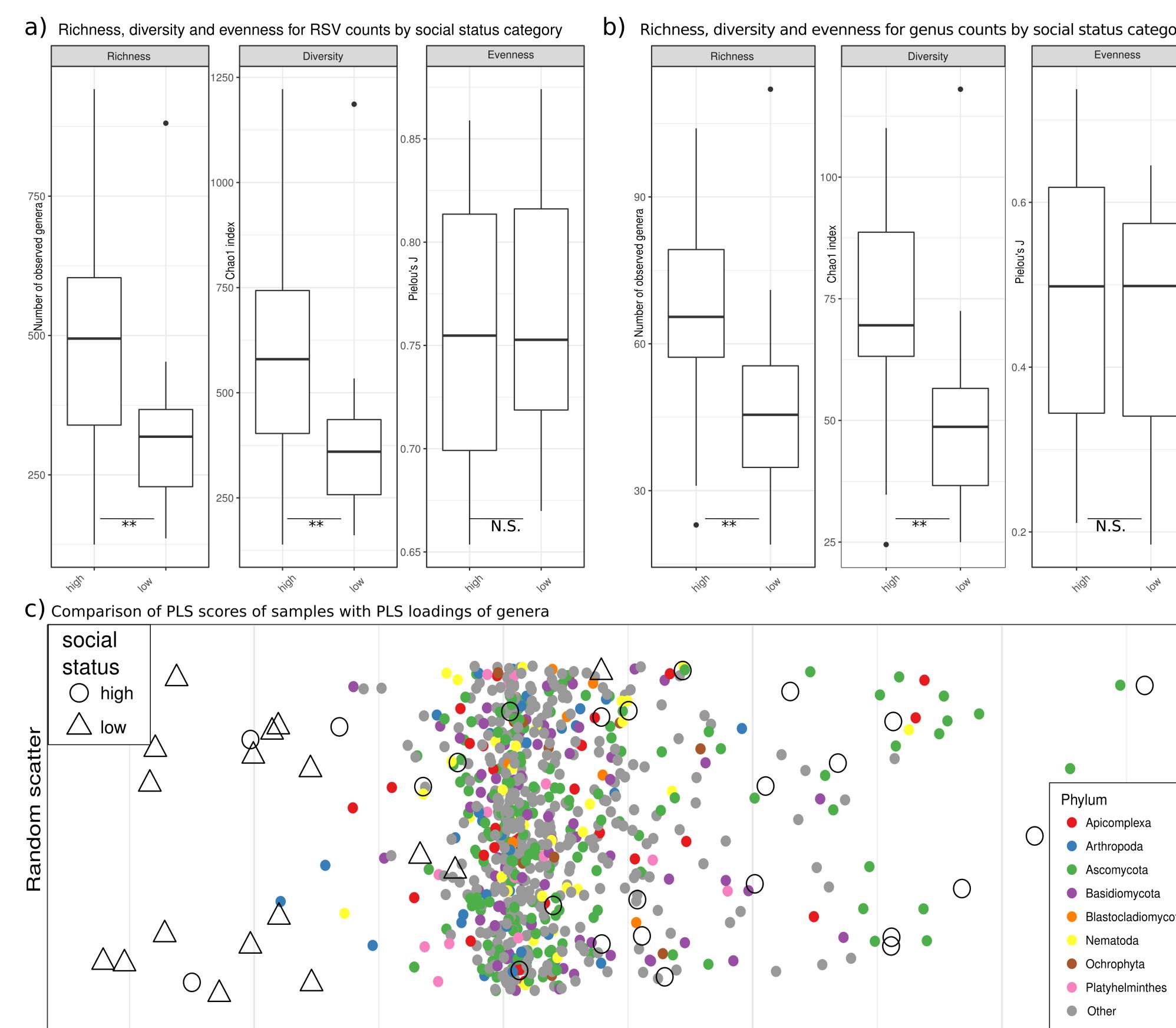


Fig 5: Richness and diversity are higher for socially high compared to low ranking females, both at the level of a) RSVs b) genera. c) PLS scores (for samples) and PLS loadings (for genera) on the single PLS axis of a model, separating the majority of samples from high-ranking individuals from samples from low-ranking animals.

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

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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). MultiAmplicon version 0.01 [R - package]. Zenodo; Development version at <https://github.com/derele/MultiAmplicon>
- [3] Pruesse et al. (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research* 35:7188–7196