

Next Generation Sequencing of the Scorpion Telson and Tail Microbiome

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Introduction

Evidence for Tissue Bacteria in Scorpions

- Alive for 450 million years, humans 0.2 million years
- Venom has antimicrobial peptides- possibly produced by bacteria (1)
- Scorpion stings can lead to bacterial infections (2)
- Microbes required for production of venom in Pufferfish (3)

Objective: Characterize the diversity of scorpion tissue microbiota

1. Scorpion Sample Sites



Figure 1. Scorpion species and location retrieved.

2. Microbial 16s rRNA in Scorpion Telson

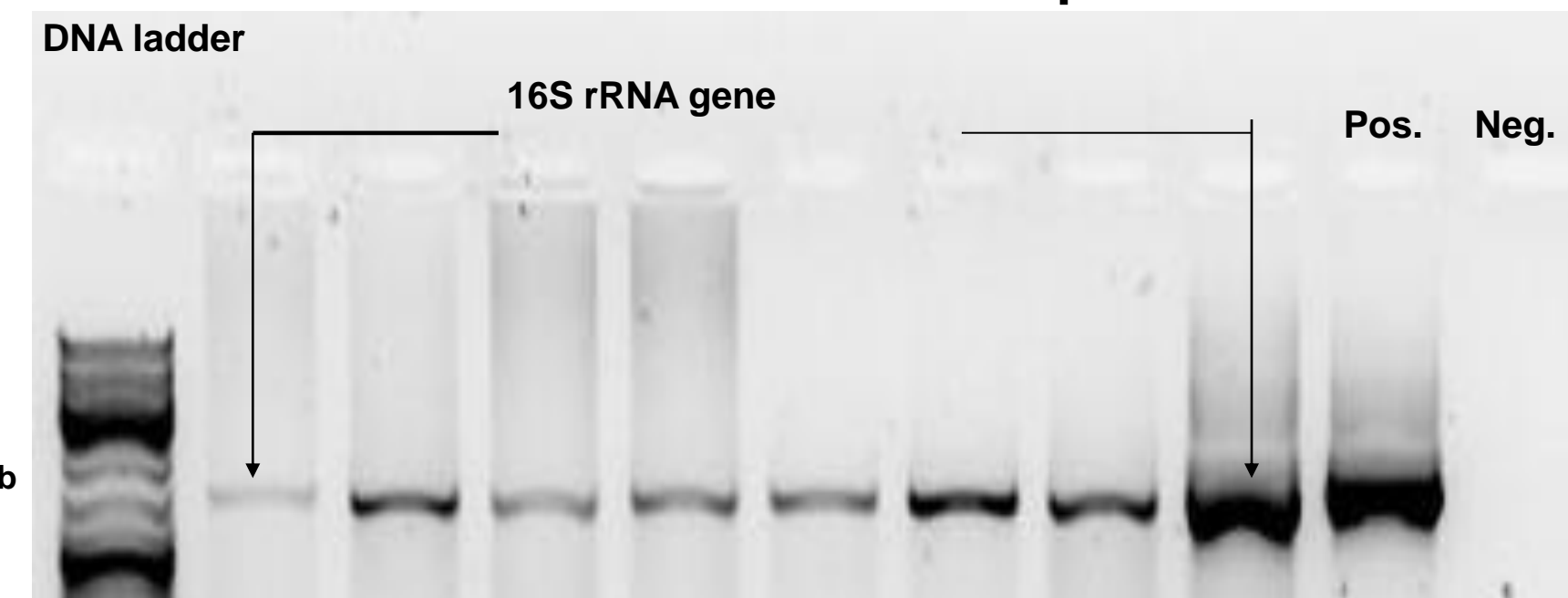


Figure 2. Gel electrophoresis of PCR for 16s rRNA gene. PCR was used to amplify the 16S rRNA gene from microbes in the telson of the scorpions. The 1.4 kb band shows the presence of the 16s rRNA gene.

Materials and Methods

DNA Isolation from Scorpion Tissues

- Isolation of DNA from tail and telson using Epicenter kit
- Amplification of 16S rRNA V4 region

Sequence Analysis

- Samples sent for Illumina sequencing at the MARS sequencing center at UCONN
- OTUs generated in Mothur, using silva.seed database (4)

Statistical Analysis

- Phyloseq created in R using Mothur taxonomy, groups, and shared output files
- Heatmap of OTU abundances
- Shannon Alpha diversity index
- Bray-Curtis Beta diversity index
- Bar plot of abundances at the Phylum level (5)

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- Undergraduate Travel Grant (MP)

Results

3. Alpha Diversity Indicates Individuals Share Diversity Measures across Tails and Telsons

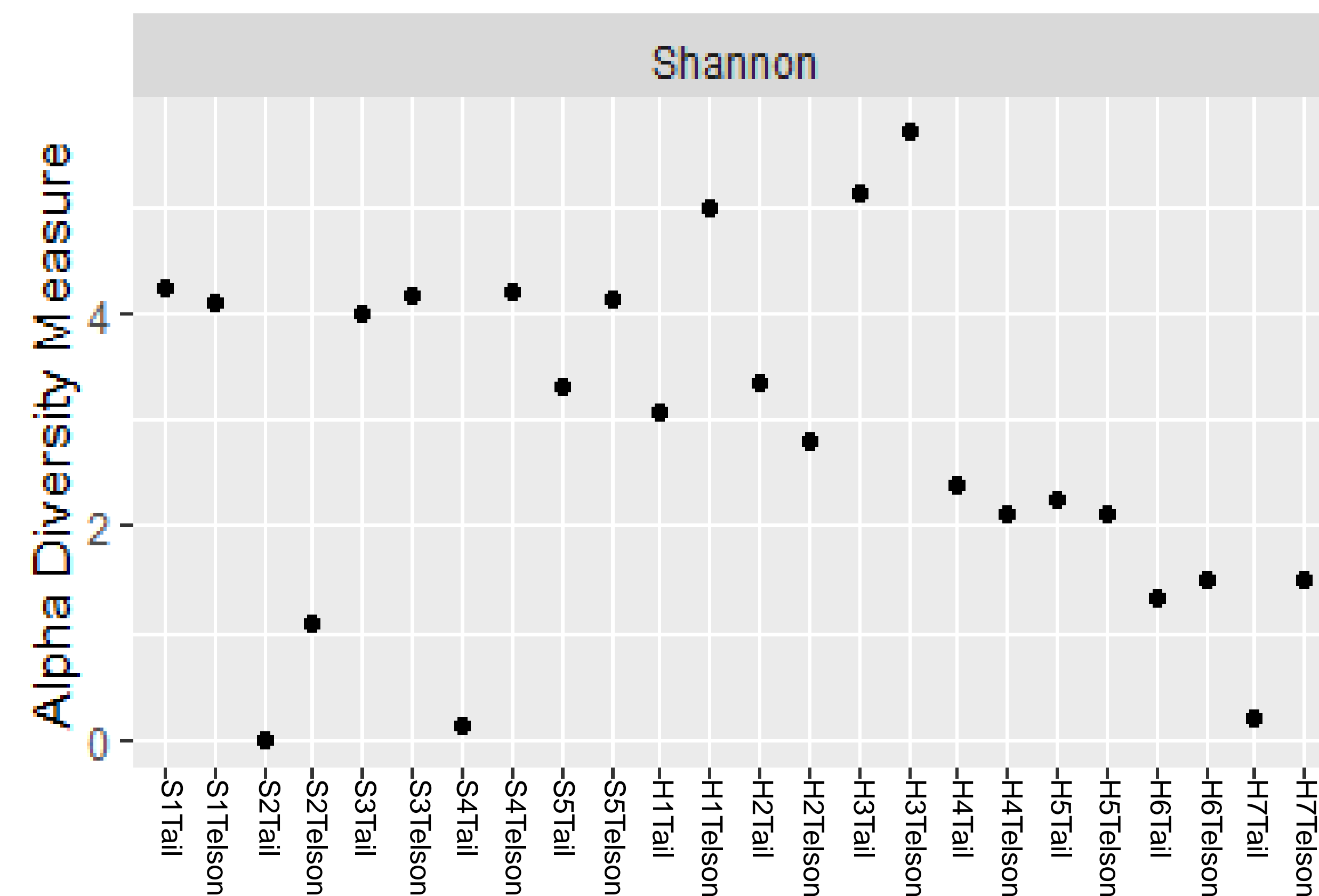


Figure 3. Shannon Alpha Diversity Indices of *S. mesaensis* and *H. arizonensis*. Graph generated in R.

Alpha Diversity Analysis

- Shannon Alpha Diversity Index
- Tail and telson tissues within a species have similar diversity values
- Low diversity individuals have high numbers of unclassified microbes

Beta Diversity Analysis

- *Hadrurus arizonensis* seem to congregate on the right side of the plot
- *Smeringurus mesaensis* congregates on the left side of the plot
- Tails and telsons of individuals generally group together
- S1, S2, S4- greatest tail/telson difference

5. Beta Diversity Index Indicates a Link Between Microbial Compositions of Tails and Telsons of Individual Scorpions



Figure 5. Beta Diversity Plot of *S. mesaensis* and *H. arizonensis* tails and telsons. Bray-Curtis equation for beta diversity. Graph generated in R

4. Heat Map of OTUs Shows Visual Abundance Patterns Within Individuals

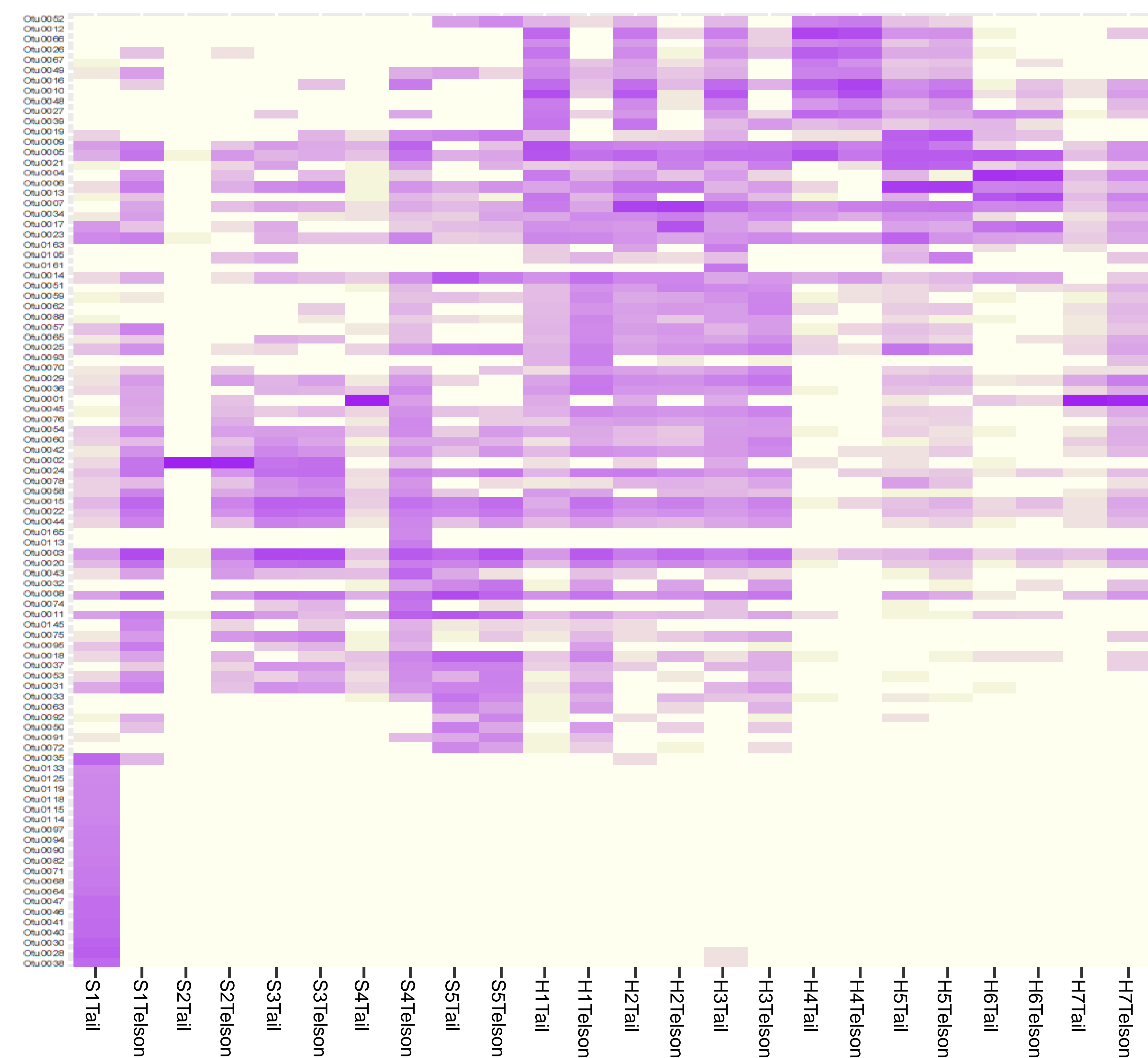


Figure 4. Heat map of OTUs found in scorpion tails and telsons. 93 OTUs present that represent at least 1% of reads in at least one sample. Generated in R.

Heat Map Analysis

- Operational Taxonomic Units (OTU)
- Patterns seen within individuals
- S1 Tail has unique microbial composition
- High abundance of unclassified individuals
- H4, H5, and H6 have nearly identical heatmap images within tail and telson tissues
- S2 tail has very low diversity, supported by Figure 3

Summary

- Tail and Telson tissues have similar microbiomes within individuals – anatomical linkage
- Similar Phylum level abundances within individuals
- Could venom also contain microbes?
- Possible source for novel bacteria or archaea
- Limitations in reference database
- Future studies will examine venom and metabolomic composition of microbes

Bar Plot Analysis

- Analysis completed at the Phylum level
- High numbers of unclassified individuals in S2, S4 tail, and H7
- Within individuals there are similar phylum abundances
- S4 shows the greatest difference in phylum level abundance between tail and telson tissue
- Tail tissues generally have more microbes

6. Bar Plot Shows Phylum Level Abundances of Individual Tail and Telson Tissues

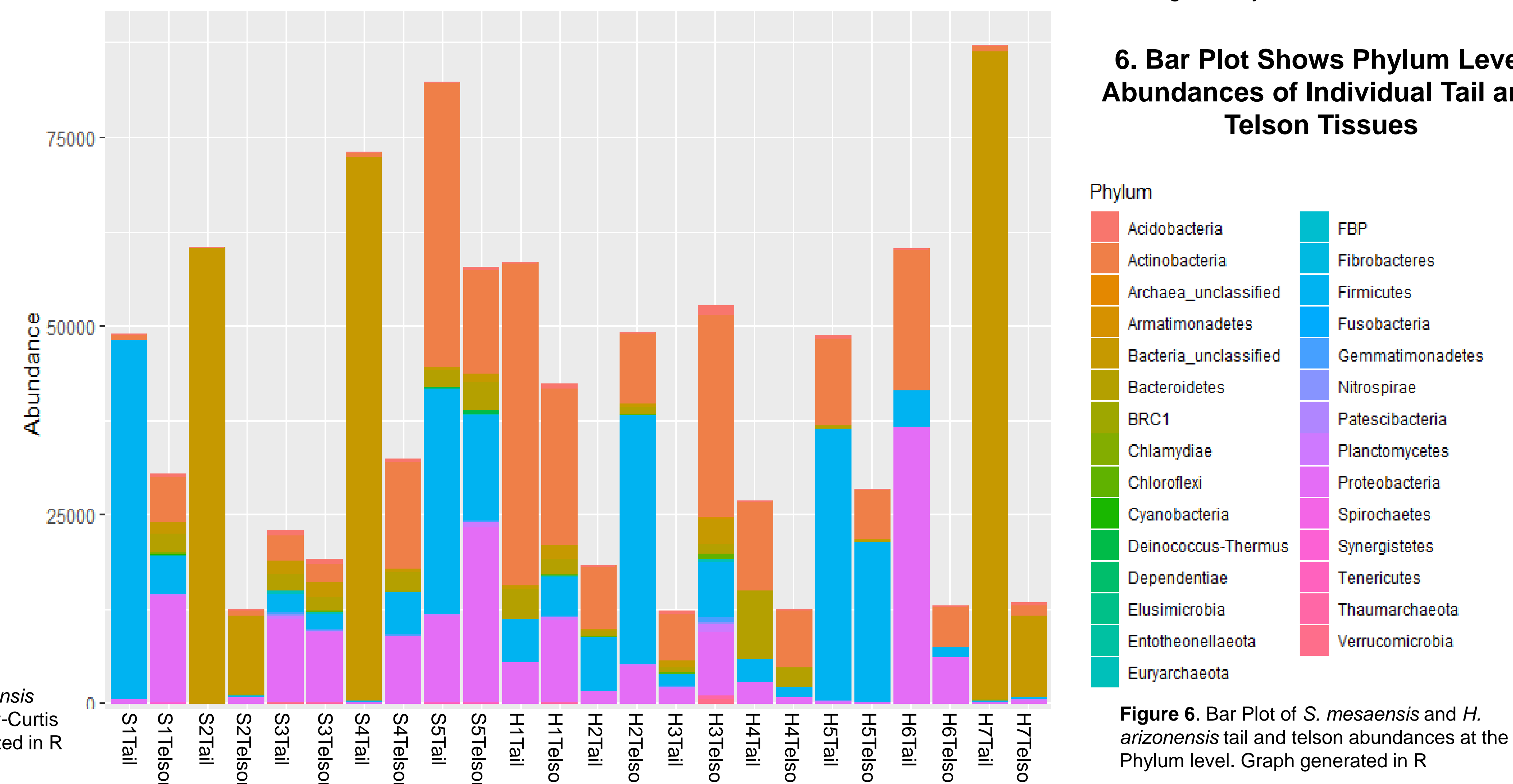


Figure 6. Bar Plot of *S. mesaensis* and *H. arizonensis* tail and telson abundances at the Phylum level. Graph generated in R