Molecular Characterization of the Scorpion Telson Microbiome

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Introduction

Do Scorpion Telsons Contain Bacteria?

- Many organisms, like Antlion larvae, rely on their microbiome for venom functionality and prey capture (1)
- Scorpion venom contains AMPs (2)
- AMPs can be produced by bacteria (3)
- Scorpion stings can result in bacterial infections (4)
- Scorpion telson produces venom but has never been tested for presence of bacteria
- Scorpion gut microbiomes are species-specific, but no studies have investigated the scorpion telson microbiome (5)

Hypothesis: the scorpion telson contains bacteria

1. Scorpion Species and Collection Site

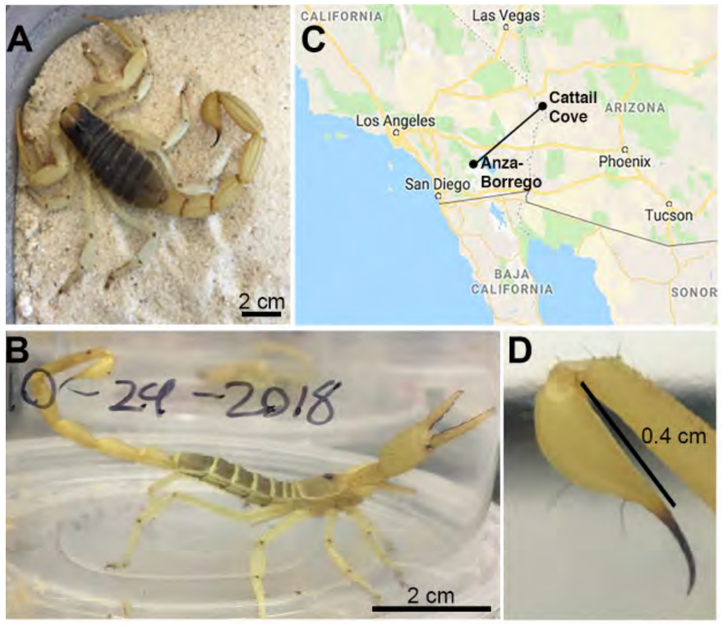
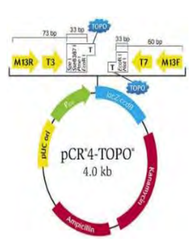


Figure 1. *H. arizonensis* and *S. mesaensis* collection from two distinct geographical locations. A) *H. arizonensis* (n=7) and B) *S. mesaensis* (n=4) scorpions were collected from C) the Cattail Cove State Park, AZ, and Anza-Borrego desert, CA, respectively. Cattail Cove and Borrego Springs, CA are approximately 200 miles apart. D) A close-up image of a *S. mesaensis* telson and stinger. The scale bars in A, B = 2 cm; in D = 0.4 cm.

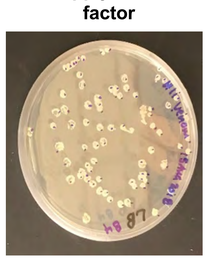
Methodology

1. Isolate scorpion telson tissues and extract DNA

2. Amplify 16S rRNA gene and insert into plasmid



3. Transform plasmid into *E. coli* and apply selective factor



4. Detect clones with correct size insert and send for sequencing

Amplification of bacterial DNA from scorpion telson tissues:

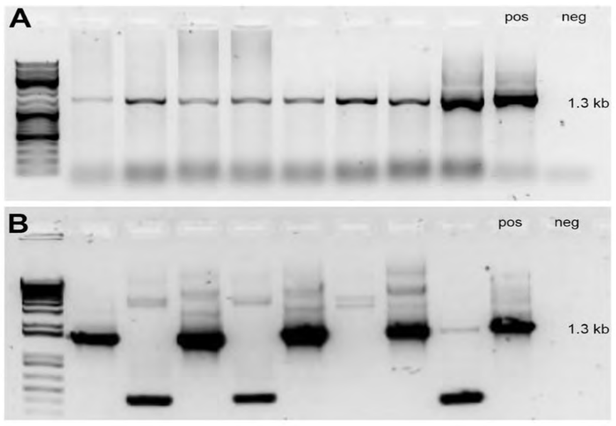
- DNA was isolated from 4 *S. mesaensis* and 7 *H. arizonensis* telson tissues
- 16S rRNA genes were amplified using the 63F and 1387R primer sets

Screening of transformants:

- Amplicons were inserted into TOPO cloning plasmid and transformed into competent *E. coli* grown on LB plates with 50 µg/ mL ampicillin
- Clones were screened and subjected to PCR using M13 F/R primers
- Samples with correct size insert were sent for sequencing

Results

2. Isolating Bacterial DNA from Scorpion Telsons



- 11/11 telsons tested produced amplicons of ~1.3 kb
- 32/96 transformants for *S. mesaensis* and 70/171 for *H. arizonensis* gave correct size insert

Figure 2. PCR Amplification and subcloning of bacterial DNA from scorpion telsons. A) *S. mesaensis* and *H. arizonensis* telson DNA was used as a template for the PCR amplification of bacterial 16S rRNA. Each lane represents a different scorpion telson. B) PCR amplicons were subcloned into TOPO vectors and the DNA from the transformants was screened using PCR with vector-based M13 primers to detect ~1.3kb inserts.

3. Species Specific Microbiome Composition

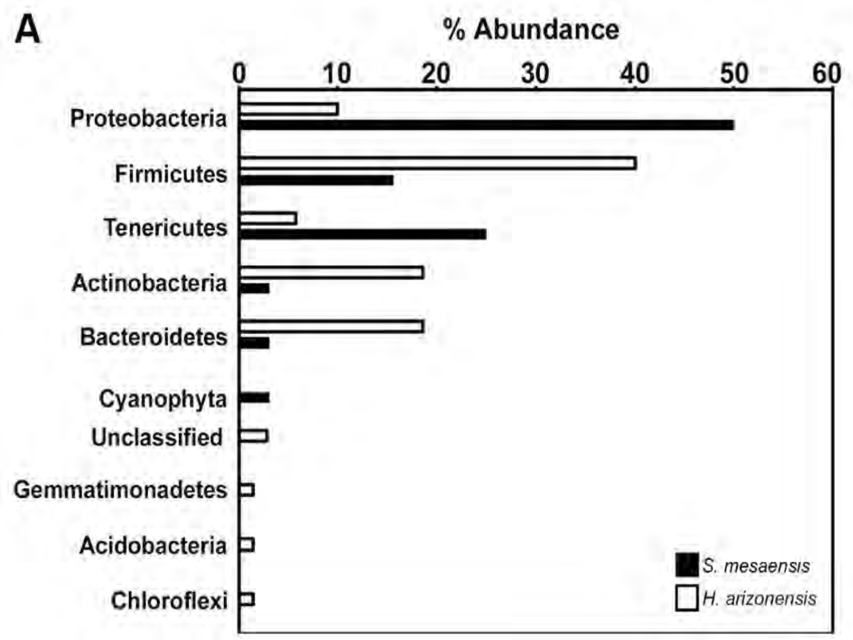


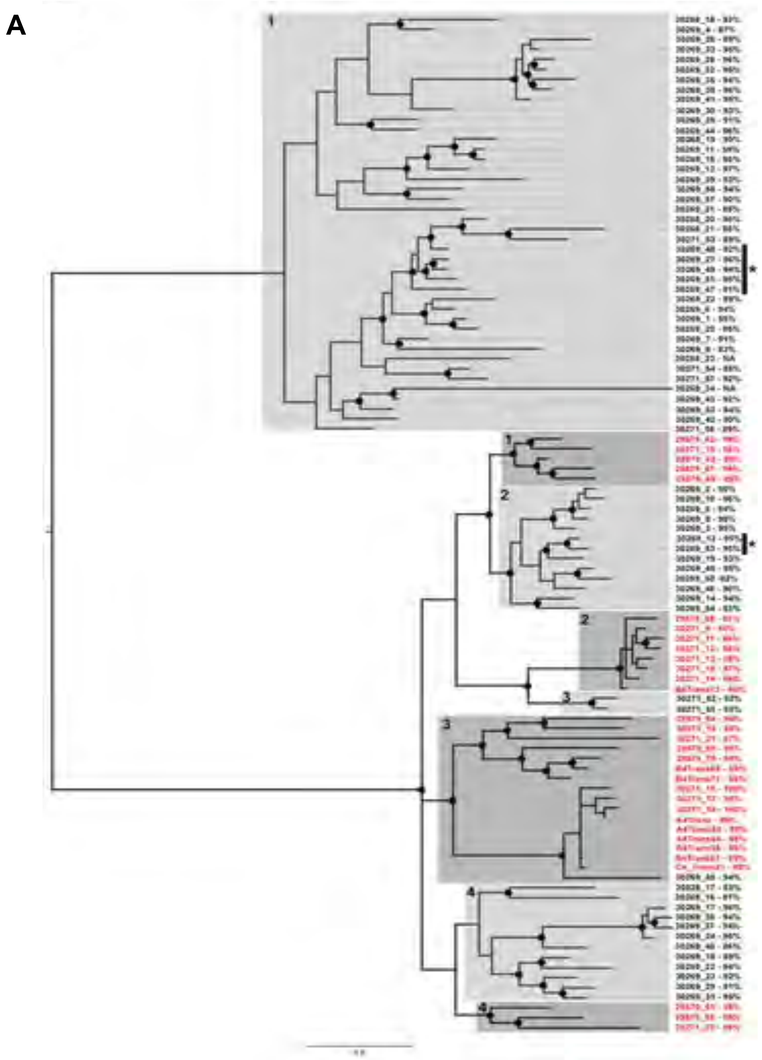
Figure 3. Composition of the *S. mesaensis* and *H. arizonensis* telson microbiota at the phylum level. A) DNA sequences of the ~1.3kb 16S rRNA inserts from the TOPO subclones were compared to the BLAST 16S rRNA database (Bacteria and Archaea) using the Standard Nucleotide BLAST tool. The top BLAST result was used to identify the microorganism's phylum for each sequence. The bars represent *S. mesaensis* (black) and *H. arizonensis* (white). Note for *S. mesaensis,* that there were no phyla for Unclassified, Gemmatimonadetes, Acidobacteria, or Chloroflexi.

- First evidence of bacteria in scorpion telsons

H. arizonensis
- Core group of Firmicutes, Bacteroidetes, and Actinobacteria

S. mesaensis
- Core group of Firmicutes, Tenericutes, and Proteobacteria

4. Sequence Similarity is Species Specific



S. mesaensis

- Sequences are more like one another than *H. arizonensis*
- Low % identity suggests novel species

H. arizonensis

- Forms four distinct clades composed of only *H. arizonensis* sequences
- (⋆) indicates sequences that were identified as *B. paramycoides*
- BLAST presents limitations

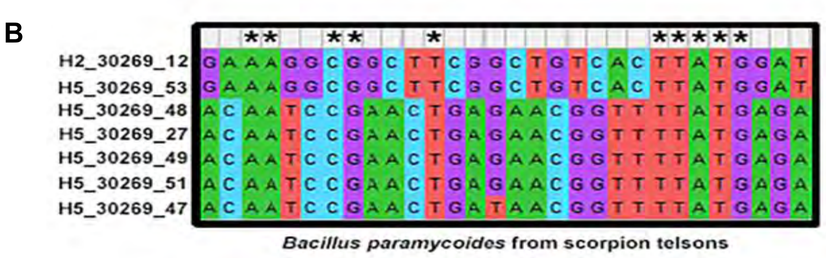


Figure 4. A. Phylogenetic tree of bacteria isolated from four *S. mesaensis* (red text and bar) and seven *H. arizonensis* (black text) telson tissues. MEGA output alignment file was used as an input file for MrBayes and phylogenetic tree was modified in FigTree. **B.** Representative alignment of five ~1.3 kb 16S rRNA sequences that were all assigned *B. paramycoides* based on the top BLAST search result. Sequences are indicated by (⋆) in **A.** Sequences were aligned in MEGA using the ClustalW default settings.

Acknowledgements

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