Characterizing the Dermal Microbiome in Biomphalaria Vector Snails

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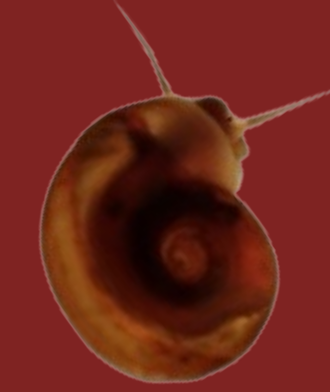


INTRODUCTION

Schistosomes are parasitic blood flukes that utilize intermediate hosts for replication and propagation, including freshwater *Biomphalaria* snails (See Fig. 1). Schistosomiasis remains a global health concern affecting over 200 million people, even despite treatment with Praziquantel, due to the high rate of reinfection[1,2]. Because snails are obligate intermediate hosts, snail location and successful penetration by the larval parasite is key to the parasite's success. This is achieved using chemosensory cues to locate snail hosts. We focused on the dermal microbiome of snails because the dermis secretes signals that attract parasites, and it is also the site of infection. The dermal microbiomes of these snails have also not been characterized. Prior studies have determined, however, that snail species and strains have other unique microbiomes that show strong phylogenomic signals, indicating a long co-evolutionary history between snails and their microbiota[4,5]. One way to disrupt the transmission cycle is through manipulation of the host, parasite, and microbiota relationships. For example, bacteria found in the gastrointestinal tract of a mosquito can reduce the transmission of a disease-causing Flaviviridae by activating the mosquito's immune system[6]. We hypothesize that the dermal microbiome of the snail influences the parasite's ability to locate and penetrate a snail host, perpetuating Schistosomiasis.

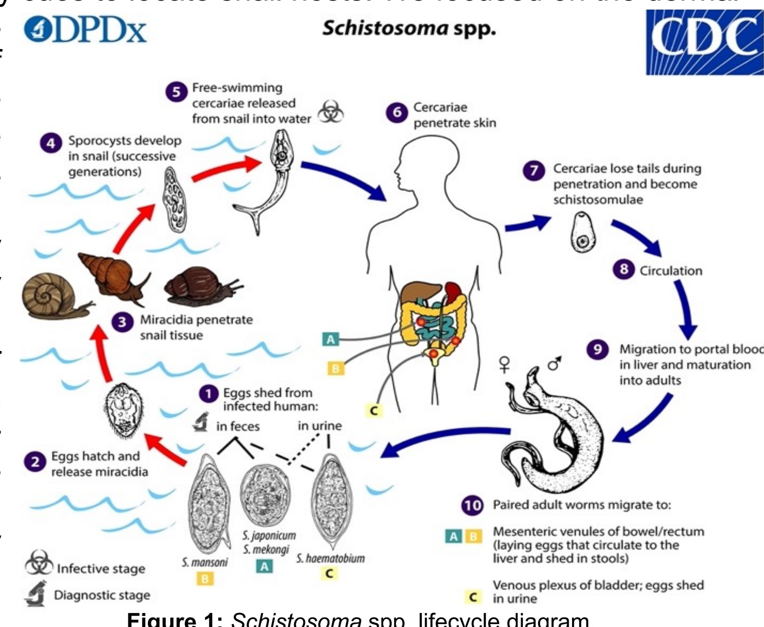


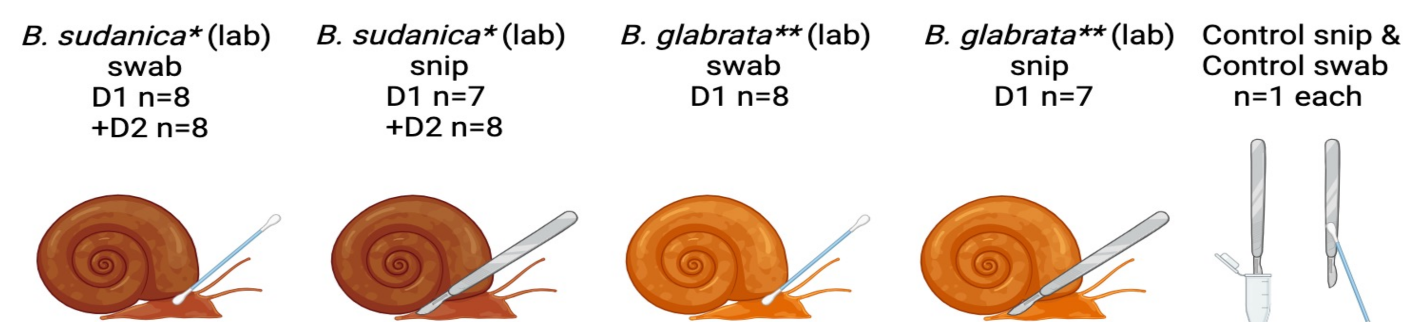
Figure 1: *Schistosoma* spp. lifecycle diagram.

OBJECTIVE

Compare species richness and microbiome composition across dermal head foot swabs and tissue snips of the foot region from laboratory-raised snail (*Biomphalaria sudanica* and *B. glabrata*) lines.

STUDY METHODS

*B. sudanica** (lab) swab	*B. sudanica** (lab) snip	*B. glabrata*** (lab) swab	*B. glabrata*** (lab) snip	Control snip & Control swab
D1 n=8 +D2 n=8	D1 n=7 +D2 n=8	D1 n=8	D1 n=7	n=1 each



*Descendants from snails collected from Lake Victoria, Kenya
**Descendants from 13-16-R1, Brazil and Puerto Rico hybrids
+D2 replicate for *B. sudanica* where snips and swabs taken from the same snail

Sample collection and extraction using ZymoBIOMICS DNA microprep kit

V3-V4 region 16S rRNA gene sequencing and qPCR by Zymo Research (Irvine, CA) for sequencing using Illumina MiSeq

Richness, composition, and associated analyses analyzed with R software

Created in BioRender.com

Figure 2: Schematic of the tissue snips and swabs collection, processing, and analysis across snail species.

Snail dissections: All samples were processed in a sterilized working environment. A stereoscope was used to: collect tissue swab (sterile swab rubbed over the entire headfoot region) and/or a tissue snip (taken from the posterior region of the foot with sterile scissors). Each tissue was placed into a vial of DNA/RNA Shield. For controls, the sterilized dissecting tools were either dipped in the prepared tube (control snip) or swabbed at the end of each dissecting day (control swab).

Statistical analysis: All statistical analysis was performed in the statistical program, R (R Core Team, 2019). Bioinformatic analyses followed the dada2 pipeline[7] with the inclusion of the cutadapt[8] software to remove adapters and primers. Default parameters were used to learn the error rates and chimeras were removed[7]. The silva v.138 databases were used for taxonomy and species assignments. A phylogenetic tree was constructed by aligning sequences using mothur[9] and FastTree (v. 2.1) for nucleotides. See Fig. 2 for simplified diagram.

RESULTS

1. Which sample type produces the most consistent results as determined by PCR amplification?

Key findings:

- Tissue swabs consistently resulted in higher bacterial gene copy numbers post-PCR than tissue snips, suggesting the samples themselves contain higher numbers of bacterial DNA.
- Swab samples were consistently greater than those of the control swab. This suggests samples were amplified successfully.
- Gene copy numbers from snips were highly variable and dataset dependent, particularly in D2, where the resulting gene copy number for tissue snips was not different than controls.
- Inter-sample variability in gene copy number was also much higher in tissue snips than tissue swabs. See Fig. 3.

Statistical analysis: Wilcoxon rank sum test (unpaired data) and Wilcoxon signed rank test (paired data).

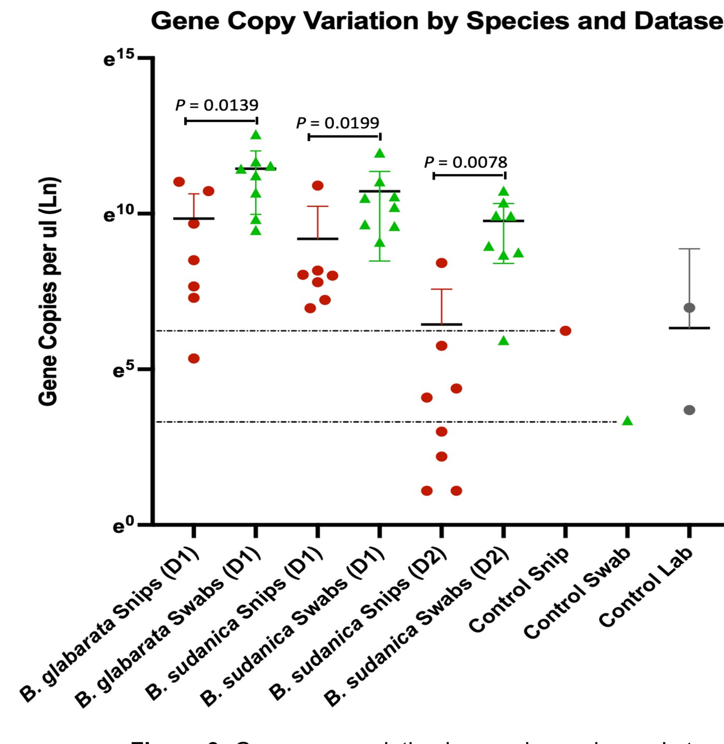


Figure 3: Gene copy variation by species and sample type.

2. Do tissue snips and tissue swabs sample similarly diverse microbial communities?

Key findings:

- We found higher species richness in dermal swabs compared to tissue snips across both laboratory snail lines, *B. glabrata* and *B. sudanica* (Table 1).

Statistical analysis: Wilcoxon rank sum test (unpaired data) and Wilcoxon signed rank test (paired data).

Table 1: Wilcoxon rank sum test analyses for species richness, evenness, and Shannon diversity by sample type (snips vs. swabs) in laboratory snail lines, *B. sudanica* and *B. glabrata*.

	Snips vs. Swabs (Wilcoxon rank sum test)		
Vector Snail	**Richness**	**Evenness**	**Shannon**
***B. sudanica* KEMRI (dataset 1)**	*P* = 0.0079	*P* = 0.4136	*P* = 0.0593
***B. sudanica* KEMRI (dataset 2)**	*P* = 0.0078	*P* = 0.1953	*P* = 0.0234
B. glabrata 7	*P* = 0.0278	*P* = 0.0939	*P* = 0.3357

3. How do the microbiome compositions between tissue snips and swabs compare?

Key findings:

- *B. sudanica* snails' microbiome species composition was different between tissue swabs and snips in dataset 1 (*P* = 0.0009, Fig. 4A) and in dataset 2 (*P* = 0.0009, Fig. 4B).
- *B. glabrata* snails' microbiome species composition was different between tissue swabs and snips (*P* = 0.0009, Fig. 4C).
- Tissue swabs consistently sampled a greater number of unique taxa compared to snips, including the members of the phyla Acidobacteriota for *B. glabrata*, and Bacteroidota, Firmicutes, Nitropirota, and WPS-2 (Table 2) for *B. sudanica*.

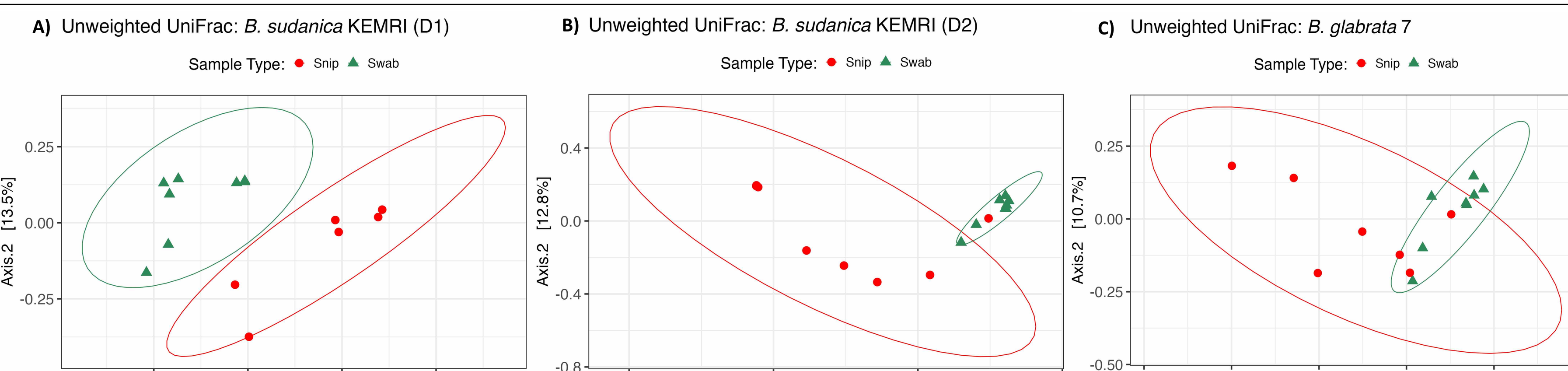


Figure 4: Core taxa determines by a 0.001 detection and 0.50 prevalence threshold and species composition based on unweighted UniFrac distance matrix between tissue swabs (green) and snips (red) for A) *B. sudanica* KEMRI (Dataset 1), B) *B. sudanica* KEMRI (Dataset 2), and C) *B. glabrata* 7 (Dataset 1).

Table 2: Summary of the unique taxa associated with tissue swabs versus snips for each snail species, *B. sudanica* KEMRI (Dataset 1 and Dataset 2) and *B. glabrata* 7 (excluding taxa that were associated with the control samples), as indicated by "x."

Taxa (Phylum_Family_Genus)	***B. sudanica* KEMRI (D1)**		***B. sudanica* KEMRI (D2)**		***B. glabrata* 7 (D1)**	
	Swab	Snip	Swab	Snip	Swab	Snip
Acidobacteriota_Blastocatellaceae					X	
Bacteroidota_Weeksellaceae_*Cloacibacterium*	X					
Bacteroidota_Flavobacteriaceae_*Flavobacterium indicum*	X					
Bacteroidota_Chitinophagaceae_*Dinghuibacter*			X			
Firmicutes_Lachnospiraceae_*Epulopiscium*			X			
Nitrospirota_Nitrospiraceae_*Nitrospira*			X			
WPS-2			X			
Unique Taxa Sampled	20	10	24	3	12	11
Shared Taxa Sampled	33		7		37	

Statistical analysis: PERMANOVA (adonis2 v.2.6-2) with Unweighted UniFrac distance matrixes was used to determine microbiome species composition between tissue samples for each snail species and dataset. LEfSe analysis were used to identify unique taxa associated with sample types across snail species.

RESULTS CONTINUED

4. How do the microbiome compositions compare by snail species?

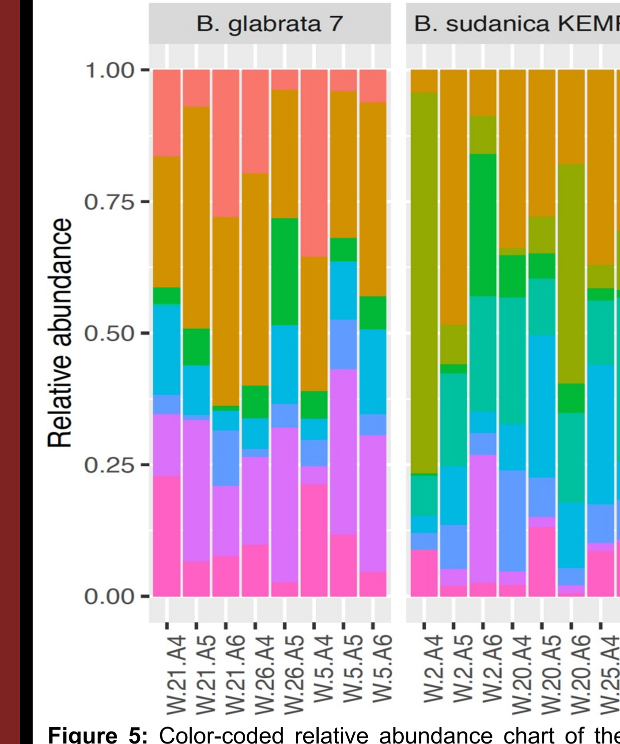


Figure 5: Color-coded relative abundance chart of the top 20 genera for each species by sample, as indicated by "W.XX.XX" code, for dataset 1.

Key findings:

- Abundant *B. glabrata* taxa: See Fig. 5
  - *Aeromonas, Cloacibacterium,* and *Rhodobacter*
- Abundant *B. sudanica* taxa: See Fig. 5
  - Uniquely, the genera *Epulopiscium* and *Gemmobacter,* and similarly, *Cloacibacterium*
- Unique *B. glabrata* taxa: See Table 3
  - *Fluviicola* consistently associated with this snail line in swab and snip samples
- Unique *B. sudanica* taxa: See Table 3
  - Genera belonging to *Firmicutes* and Verrucomicrobiota detected by swabs and snips
  - *Flavobacterium indicum* identified in swabs

Statistical analysis: Relative abundance of top 20 taxa and LEfSe analyses.

Table 3: Summary of the unique taxa associated with *B. sudanica* KEMRI versus *B. glabrata* 7 for each tissue type (excluding taxa that were associated with the control samples), wherein "x" indicates a a greater abundance in that species.

Taxa (Phylum_Family_Genus)	**Swab**		**Snip**	
	B. sudanica KEMRI (D1)	*B. glabrata* 7 (D1)	*B. sudanica* KEMRI (D1)	*B. glabrata* 7 (D1)
Acidobacteriota_Blastocatellaceae		X		
Bacteroidota_Weeksellaceae_Cloacibacterium				X
Bacteroidota_Flavobacteriaceae_Flavobacterium indicum	X			
Bacteroidota_Chitinophagaceae_Fluviicola		X		X
Firmicutes_Lachnospiraceae_Epulopiscium	X		X	
Firmicutes_Peptostreptococcaceae_Romboutsia			X	
Verrucomicrobiota_Parachlamydiaceae	X		X	

DISCUSSION

- **Head foot swabs confer a consistent sampling method** based on amplification success and microbiome variability.
- **Collection by tissue snips was not optimal for dermal microbiome characterization** as performed in this study. Amplified snail DNA had to be removed, with less bacterial DNA amplification seen in snips.
- ***B. sudanica* and *B. glabrata* have distinct dermal microbiomes.**
- ***Cloacibacterium* is a common GI microbe** suspected to be present on the dermis due to snail anatomy and excretory pathways.
- ***Gemmobacter* was a taxa associated with our controls.**
- **Further analysis is necessary** to determine if amplification, particularly for low values in dataset 2, were from too few sampled bacteria or PCR inhibition.
- Our **small sample sizes are a limitation** of this study.
- **Future studies will focus on *Schistosoma mansoni* susceptibility and resistance.**

CONCLUSION

This is the first known study to characterize the dermal microbiome of the primary schistosome vector in the African Great Lakes, *B. sudanica,* which is responsible for the majority of schistosomiasis occurring in sub-Saharan Africa. This foundational work sets the stage for future research aimed at understanding these associations in natural systems and leveraging this portion of the life cycle for schistosomiasis control. Disentangling the mechanisms behind the attraction and successful establishment of schistosomes within their vector host could identify key control targets, leading to novel interventions that not only break the transmission cycle, but actively reduce infections and improve human health.

ACKNOWLEDGEMENTS

Thank you to Western University of Health Sciences for funding both a summer research grant and for the processing tissue samples (National Institutes of Health R01AI141862). Thank you to each of the coauthors for their dedication and contributions, and to the institutions that made this research possible.

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