How Bacteria in Snails (Mollusca: Gastropoda) Differ in Relation to Digenean (Platyhelminthes) Infection: An Unfinished Study

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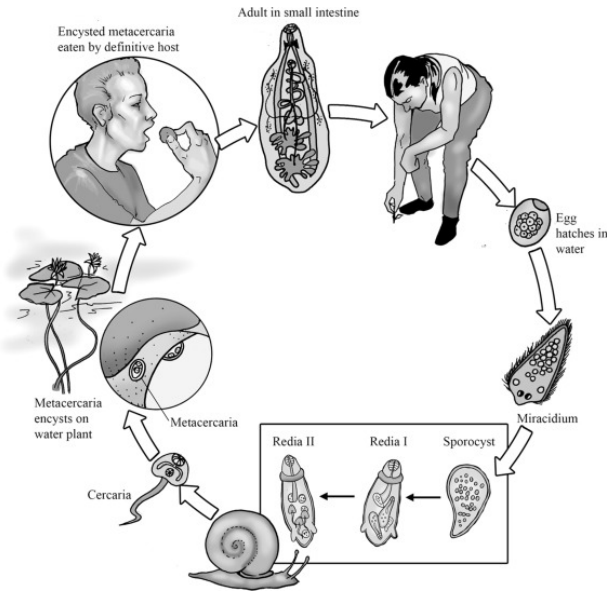
We did not find the information we were looking for. I'm still going to go through everything we did, why it did not work, what could have been done instead, and sometimes, why we chose to do it the way we did knowing the other ways it could have been done.

That being said, let's get into it.

Disclaimer

Background: *Digenea (Platyhelminthes, Trematoda)*

- Parasitic Worms known for their two host life cycle
- Intermediate hosts are very commonly molluscs, particularly snails (Gastropoda)
- Infection is located in the gut of the snail



https://www.sciencedirect.com/topics/immunology-and-microbiology/digenea

Pechenik 2015, Esch and Fernandez 1994

Background: Microbiome

- The community of bacteria associated with an organism
- Help the host organism to digest food and interact with the immune system
- Composition of the microbiome is shaped by the environment inside and around the organism

Carroll et al 2019

Introduction

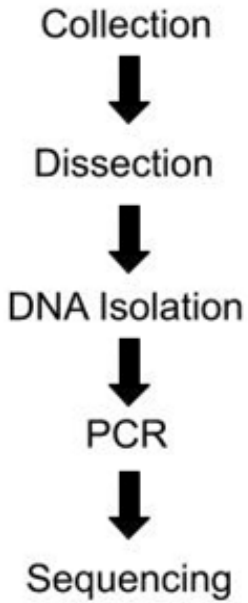
- There are very few studies on the interaction of between bacteria and parasites (at least in gastropods).
- We chose *Digenea* and a freshwater snail population as a model for microbiome/parasite interaction due to local availability. The snails serve as a micro-ecosystem, and the parasites and bacteria should interact to some extent simply due to proximity.
- If parasites and bacteria interact in any capacity , there should be differences in the microbiome between snails with and without parasites.

Hypothesis

- Two main ways the microbiome could have differed:
  - Composition: the same species are present between groups but in different concentrations
    - Ex) Species A composes 95% of microbiome in parasite-free snails, but 16% of infected snails
  - Diversity: different species are present between groups
    - Ex) Species B is present in infected snails, but absent in parasite-free snails
- Null Hypothesis: there are no significant differences between the microbiota

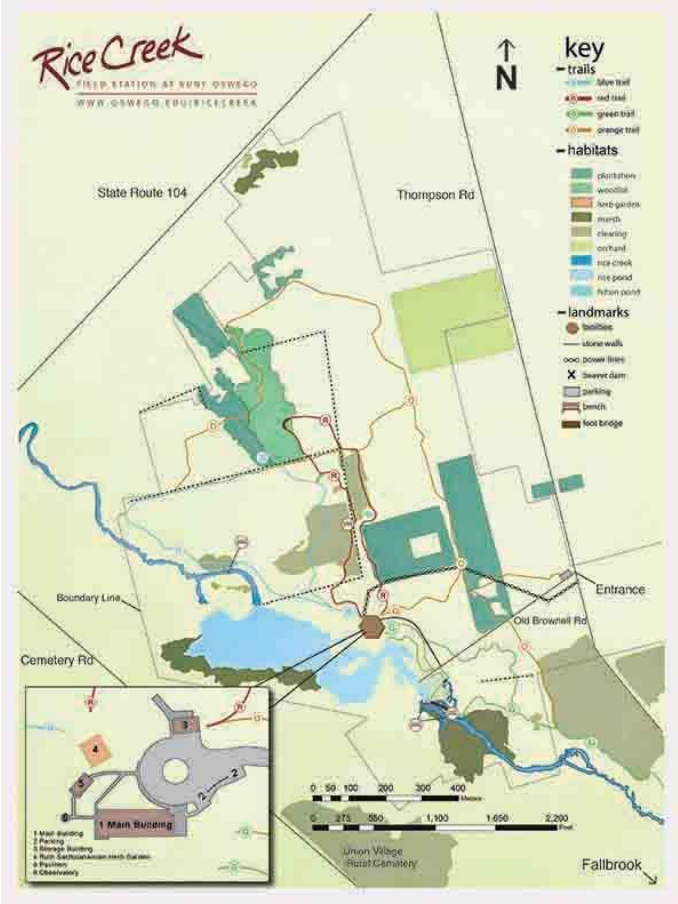
Overarching Study Design

- Two main groups: infected and uninfected snail
- Individuals were dissected, isolated, PCR, and sequenced
- Bacterial analysis was attempted, but ultimately failed
- Parasites were identified genetically



Collection

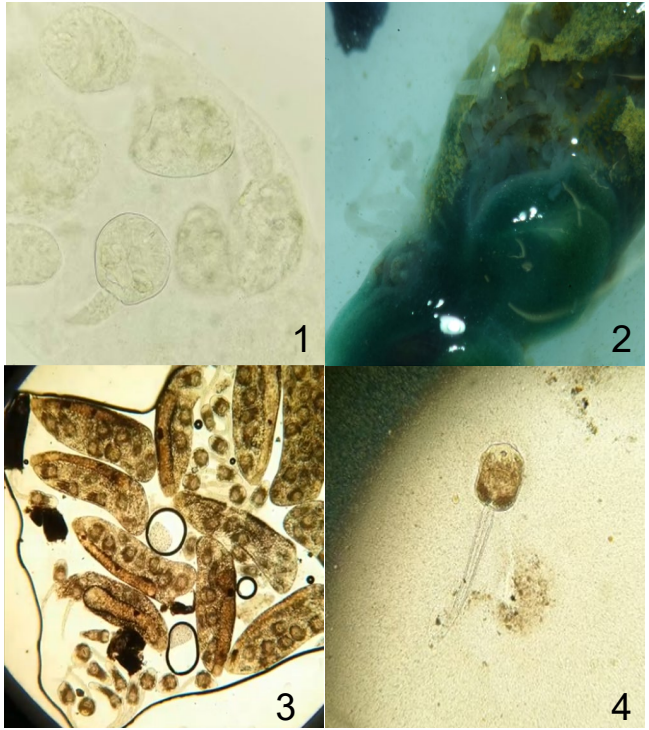
- Two collection locations:
  Fallbrook and Rice Creek Green Trail
- A total of 74 snails were collected (with permit)
- Collection occurred over the summer (Late June - Late August)



https://www.oswego.edu/rice-creek/trails-overview

Dissection

- Individuals were deshelled, and beheaded.
- Parasite infection was identified by type (vermiform, and sporocyst and/or cercaria), most infections were heavy.
- Sporocyst infections were coupled with cercaria. This is just how the life cycle works.
- Spire was used for DNA isolation, as that contained both the bacterial and parasite samples.
- Separate parasite sample were taken when possible for separate genetic analysis.



Picture 2: Vermiform, Pictures 1 and 4: cercaria, Picture 3: sporocyst and cercaria

DNA Isolation of Snail samples

- Phenol-chloroform protocol most efficient to remove proteins and polysaccharides
- Mucopolysaccharides are PCR inhibitors
- CTAB binds to mucopolysaccharides

WORKFLOW

Add T.E., 10%SDS, and homogenization beads to tissue sample. Shake vigorously.

Add NaCl and CTAB and incubate for 10 minutes.

Add proteinase K and incubate for 1 hour.

Alternate between adding chloroform:isoamyl and phenol-chloroform, putting liquid layer into a new tube each time.

Add isopropanol to precipitate DNA, then add ethanol to wash.

Nishiguchi et al 2002, Sokolov 2000, Theodorakis 2014 and Winnepenninckx et al 1993

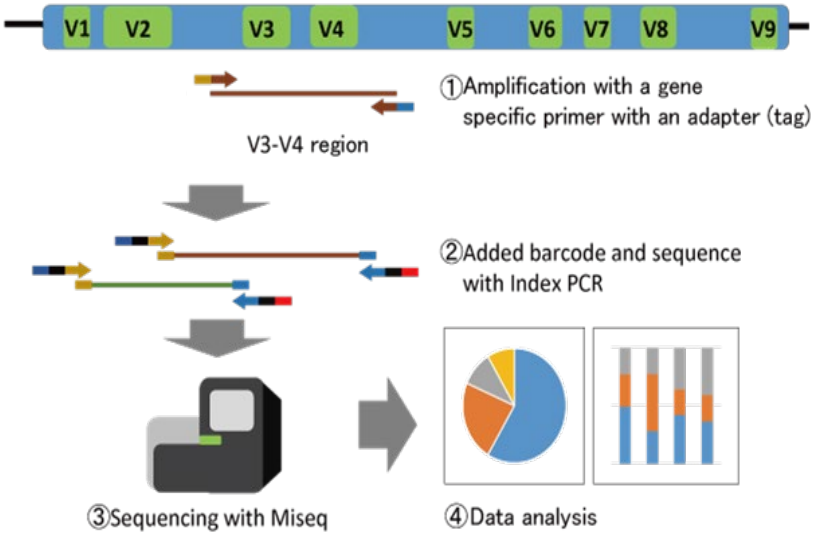
DNA Isolation of Parasite samples

- The separated parasite samples were isolated using Qiagen's DNeasy Blood + Tissue Kit.
- The samples were washed twice with water to remove snail tissue remnants, then the manufacturer's protocol was followed.

https://www.qiagen.com/br/products/top-sellers/dneasy-blood-and-tissue-kit/#orderinginformation

PCR of Bacteria

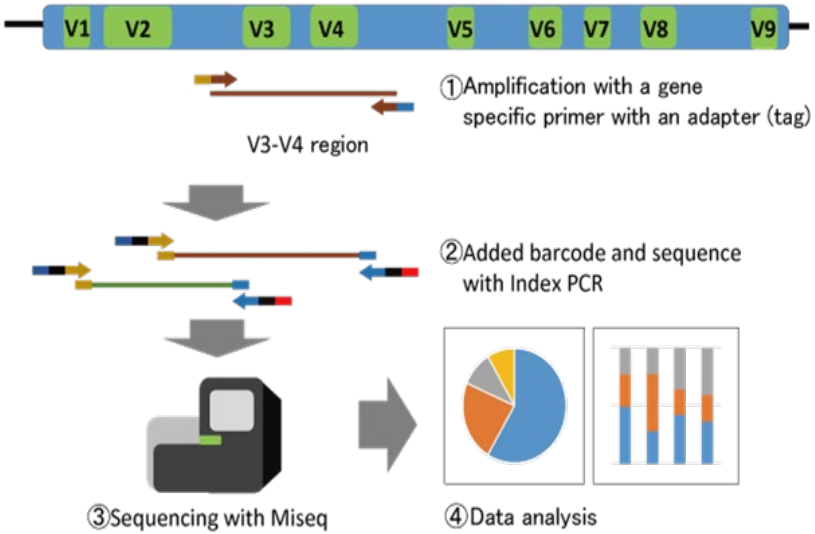
- Uses primers and enzymes to copy specific fragments of DNA
- Used to amplify the 16s gene that is common to most bacterial species
- Was unsuccessful, mostly likely due to proportion



Adapted from https://www.repertoire.co.jp/

PCR of Parasites

- 18s rRNA gene was targeted
- First tested primers for qPCR, this failed
- Used a different primer pair to target whole gene in separated parasites, this was successful



Adapted from https://www.repertoire.co.jp

Sequence Analysis and Identification

- The PCR result were purified using GeneJET PCR Purification Kit.
- Sequences were outsourced
- Used BLASTn default settings to identify the sequences
- Three sequences were analyzed for each sample→ the forward, reverse, and joined

F: 5’- ATGTACAGTAGTAG- 3’
R: 3’- ACTGGACATGATGA- 5’

F: 5’- ATGTACAGT**AGTAG** - 3’
+
RC: 5’ - **AGTAG**TACAGGTCA- 3’

Joined Sequence:
5’-ATGTACAGT**AGTAG**TACAGGTCA- 3’

Results

- Three digeneanswere identified: *Lecithodendriidae* sp. PAFlukeA, *Braunina condiformis*, and *Collyriclum faba*
- Some samples returned with high background, indicative of multiple PCR products
- Some samples returned as *Batillaria cumingi*, a snail species

Top blast hit description	%ID
Lecithodendriidae sp. PAFlukeA 18S ribosom	98.98
Lecithodendriidae sp. PAFlukeA 18S ribo	99.05
Braunina cordiformis voucher UV/Zoo	96.51
Braunina cordiformis voucher UV/Zooma	96.81
Batillaria cumingi isolate LSGB21202 18	98.43
Collyriclum faba 18S ribosomal RNA ger	95.96
Braunina cordiformis voucher UV/Zooma	97.03
Collyriclum faba 18S ribosomal RNA ger	97.46
Batillaria cumingi isolate LSGB21202 18	98.76
Lecithodendriidae sp. PAFlukeA 18S ribo	98.69

The top blast hits of the joined sequences with the percent identity of each individual.

Lecithodendriidae sp. PAFlukeA

- Specimens only identified up to Family
- Mainly parasitize bats and occasionally birds (Lotz, J. M. & Font, W. F., 2008)

Taxonomic Hierarchy

Kingdom	Animalia – Animal, animaux, animals
Subkingdom	Bilateria
Infrakingdom	Protostomia
Superphylum	Platyzoa
Phylum	Platyhelminthes Minot, 1876 – flatworms, plathelminthes, platodes, platelminte
Subphylum	Neodermata
Class	Trematoda Rudolphi, 1808 – flukes, douves, fascíola
Subclass	Digenea Carus, 1863
Order	Plagiorchiida
Suborder	Plagiorchiata
Family	Lecithodendriidae
	Direct Children:
Genus	Acanthatrium Faust, 1919
Genus	Cephalouterina Senger and Macy, 1953
Genus	Echinuscodendrium Skarbilovich, 1943
Genus	Glyptoporus Macy, 1936
Genus	Gyrabascus Macy, 1935
Genus	Langeronia Caballero and Bravo-hollis, 1949
Genus	Lecithodendrium Looss, 1896
Genus	Limatulum Travassos, 1921
Genus	Loxogenes Stafford, 1905
Genus	Loxogenoides Kaw, 1945
Genus	Macyella Neiland, 1951
Genus	Metoliophilus Macy and Bell, 1968
Genus	Mosesia Travassos, 1921
Genus	Ochoterenatrema Caballero, 1943
Genus	Ornithodendrium Oshmarin and Dotsenko, 1951
Genus	Ototrema Font, 1978
Genus	Paralecithodendrium Odhner, 1910
Genus	Prosthodendrium Dollfus, 1931
Genus	Prosthopycoides Martin, 1966
Genus	Pseudosonsinotrema Dollfus, 1951
Genus	Tremajoannes Saoud, 1964

https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=56498#null

Braunina cordiformis

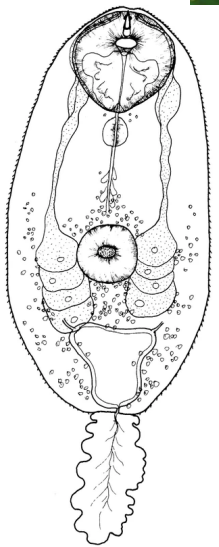
- Observed as the vermiform sporocysts
- Most of the literature involves dolphin hosts
- We most likely found a close relative.

https://www.biolib.cz/en/taxon/id82031/

Collyriclum faba

- Infects birds
- A study by Heneberg et al. (2015) described the complete lifecycle of a Central European population.
- Was observed to have two intermediate hosts, a snail and a mayfly
- In definitive hosts, *C. faba* form cysts that can cause anemia and emaciation when abundant.

https://www.researchgate.net/publication/324601752_Collyriclosis_in_Red-backed_Shrikes_Lanius_Collurio_from_Israel_and_Czech_Republic/figures?lo=1



Cercaria of Collyriclum
(Heneberg et al., 2015)

(Heneberg et al., 2015)

Conclusion

- The bacterial communities of these snails are still unknown.
- The snail species and parasites that were genetically identified are most likely close relatives to the collected specimens as the known attributes of the identified species are inconsistent with the collection environment.

https://taconet.pixnet.net/blog/post/47063644

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If you have any questions, feel free to contact Rebecca Wolff at rwolff2@oswego.edu or Peter Newell at peter.newell@oswego.edu

Literature Citations

Carroll, C., Olsen, K. D., Ricks, N. J., McFarland, K. A., Suen, G., Robinson T. F., Chaston, J.M., (2019) "Bacterial Communities in the Alpaca Gastrointestinal Tract Vary With Diet and Body Site" *Frontier in Microbiology* Vol. 9, pgs. 33-34 https://www.frontiersin.org/article/10.3389/fmicb.2018.03334

Pechenik, J. A. (2015) *Biology of the Invertebrates, Seventh Edition* McGraw-Hill Education pgs. 159-168

Winnepenninckx, B., Backeijau, T., DeWachter, R. (1993) "Extraction of High Molecular Weight DNA from Molluscs" *Trends in Genetics* Vol. 9, Issue 12, pgs. 407

Nishiguchi, M.K. et al. (2002) "DNA isolation Procedures" *Techniques in Molecular Systematics and Evolution* ed. Rob DeSalle et al. Birkhauser Verlag, Basel, Switzerland.

Sokolov, E. P. (2000) "An Improved Method for DNA Isolation from Mucopolysaccharide-rich Molluscan Tissues" *Journal of Molluscan Studies* Vol. 66, pgs. 573 - 575

Literature Citations

Theidorakis, C. W. (2014) Internet forum “What are the effects of carbohydrates in PCR?” *ResearchGate* https://www.researchgate.net/post/What_are_the_effects_of_carbohydrates_in_PCR

Esch, G. W., Fernandez, J. C., (1994) “Snail-trematode Interactions and Parasite Community Dynamics in Aquatic Systems: A Review” *American Midland Naturalist* Vol. 131, No. 2, pgs. 209-237

Lotz, J. M., Font, W. F. (2008) Family Lecithodendriidae Lühe, 1901. In Bray, R. A., Gibson, D. I., Jones, A. (Eds.) *Keys to the Trematoda* (Vol 3, pgs. 527-536). Doi: 10.10799780851995885.0527

Heneberg, P., Faltýnková, A., Bizos, J. et al. Intermediate hosts of the trematode Collyriclum faba (Plagiochiida: Collyriclidae) identified by an integrated morphological and genetic approach. Parasites Vectors 8, 85 (2015). https://doi.org/10.1186/s13071-015-0646-3