Using eDNA to Complete the Horsehair Worm (*C. morgani*) Life Cycle

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Introduction

The horsehair worm, *Chordodes morgani*, resides in Nebraskan waters. Despite playing an important role in ecology, scientific knowledge of its life cycle remains incomplete. Previous work showed that wood roaches (*Parcoblatta* spp.) collected from the field harbored *C. morgani*. Laboratory reared wood roaches that consumed mayflies become infected infected with *C. morgani* cysts also become infected. It is unknown if wild wood roaches become infected in the same way.

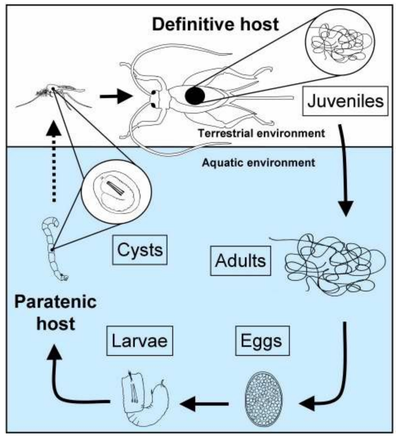


Fig. 1: Life cycle of Nematomorpha in the wild. From Hanelt *et al*. 2012

Fig. 2: Several adult *C. morgani* emerge from a lab reared wood roach after being immersed in a Petri dish of water.

Hypothesis

Field collected wood roaches will contain mayfly eDNA in their gut content as a result of eating them in the wild.

Methods

Mayfly mitochondrial DNA (mtDNA) will be extracted, isolated, and amplified using mitochondrial specific primers and PCR designed from sequences obtained from NCBI using Geneious Bioinformatics package. The isolated mtDNA will then be used to create a positive control by mixing artificial concentrations to find the minimum amount of mayfly mtDNA that can be detected. Lab reared roaches will be fed mayflies while another ten will be served cat food to act as a control. A gut analysis will be performed on all wood roaches. The mtDNA of the mayflies and wood roaches will be isolated. If the mayfly mtDNA can be isolated from lab reared roaches, more wood roaches will be collected from the field and tested through the same technique. If mayfly mtDNA is extracted, then it can be concluded that the roach was eating mayflies in the field.

Results

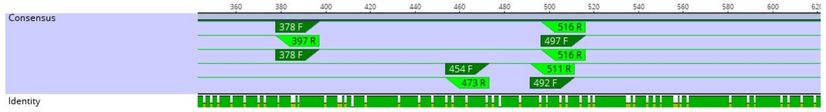


Fig. 3: Genus specific primers. Primers did not bind to mayfly DNA

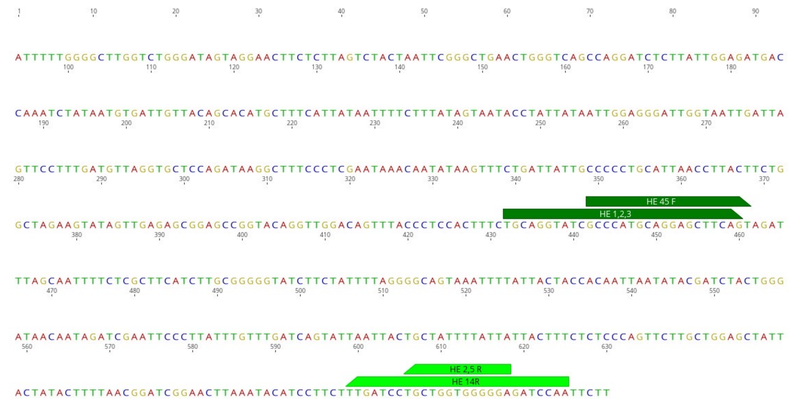


Fig. 4: *Heptagenia elegantula* specific primers. Primers did not bind to mayfly DNA

Primer Name	Primer Sequence (5′ to 3′)	Approximate bp position
28S EP2a	GAGTCGGGTTGCTTGAGAGTG	170
28S EP3a	AGTACCGTGAGGGAAAGTTG	250
28S EP4a	CGTCTTGAAACACGGACCAA	780
28S EP5a	GGTTGCTTAAGACAGCAGGA	1400
28S EP2b	CACTCTCAAGCAACCCGACTC (Reverse compliment of 28S EP2a)	170
28S EP3b	CAACTTTCCCTCACGGTACT (Reverse compliment of 28S EP3a)	250
28S EP4b	TTGGTCCGTGTTTCAAGACG (Reverse compliment of 28S EP4a)	780
28S EP5b	TCCTGCTGTCTTAAGCAACC (Reverse compliment of 28S EP5a)	1400

Fig 5: Ephemeroptera specific primers for 28S rDNA. Designed by Ogden *et al*. 2005

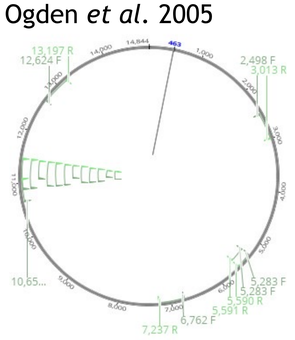


Fig. 6: Wood Roach primers for *Parcoblatta spp*.

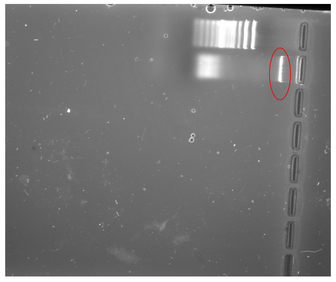
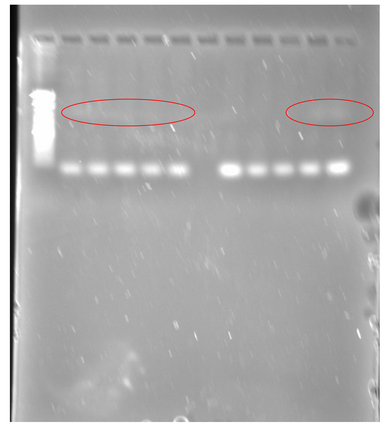


Fig. 8: Gel electrophoresis of DNA extraction. Intact RNA was found.



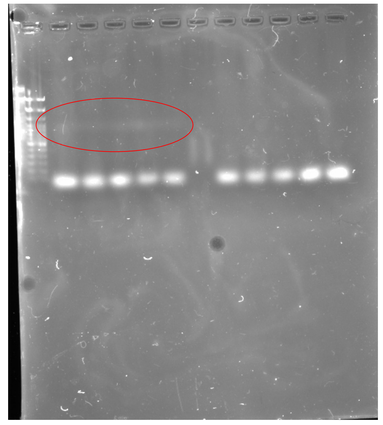


Fig. 6 and 7: Gel electrophoresis of order-specific primers. Faint bands found for primer set EP3, EP4, and EP5 .

Conclusions

Mayfly mtDNA extraction is still a work in progress. Currently, 28S rDNA primers have produced some results, however the bands are very faint. In the past, both species and genus specific mtDNA mayfly primer, Folmer's Filament, was tested but it also did not bind. The next step includes producing clearer results using the rDNA primers. Future projects include using rRNA primers to see if those will bind more conclusively. If this works, the next steps in the project would include trying to isolate mayfly rRNA from wood roach RNA with PCR assay and sequencing.

Citations

Hanelt B, *et al*. 2012. Going Solo: Discovery of the First Parthenogenetic Gordiid (Nematomorpha: Gordiida). *PLOS ONE* 7(4): e34472

Ogden, T. Heath, and Michael F. Whiting. "Phylogeny of Ephemeroptera (Mayflies) Based on Molecular Evidence." *Molecular Phylogenetics and Evolution*, vol. 37, no. 3, 2005, pp. 625-643.

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