

A Diet Analysis of the Hentz Striped Scorpion Using DNA



Metabarcoding

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Abstract

Analyzing the diet of invertebrates is made incredibly difficult by their small size, reclusive habits, and the absence of morphological characteristics present in fecal samples. Due to this nature of invertebrate predators, DNA-based approaches provide us with one of the only concrete methods to analyze their diet. The usage of DNA metabarcoding helps us to explore the dietary composition of these animals by allowing us to extract and analyze samples of prey DNA found in the digestive tract. The Hentz striped scorpion (*Centruroides hentzi*) is a species endemic to the southeastern United States. In this project, we use the process of DNA metabarcoding to analyze the diets of *C. hentzi*. Scorpions were collected and dissected to remove gut tissue. From this, prey DNA was amplified and analyzed to reveal the diet of the predator.

Introduction

To characterize the diet of *C. hentzi*, we use cytochrome c oxidase 1 (COI) metabarcoding. The COI gene is a mitochondrial gene with a sequence unique to each species of arthropod which allows us to determine the species of prey based on the DNA found within the gut of the predator. In this project we use two primer sets, ZBJ-Art (Zeale et al. 2010) and fwh (Vamos et al. 2017). Each of these primer sets amplifies a specific region within the COI gene. Using these amplified regions, we are then able to determine the species of prey found in the gut of the scorpion.



Figure 1: Adult male *C. hentzi*

Methods

- 152 scorpions of the species *C. hentzi* were collected from Apalachicola forest between June and August of 2022 by students of the FSU Young Scholars Program.
- The innards were dissected out of each individual and prepped for PCR.
- PCR was run with two primer sets to amplify arthropod DNA.
- These amplified sequences were then processed using Cutadapt software to remove the primers.
- The sequences of prey DNA with the primers removed are now known as operational taxonomic units or OTUs, sections of amplified DNA that allow us to identify the prey's taxa.
- These OTUs were run through the BOLD database, the Barcode of Life Data System, which contains DNA barcode sequences that can be used to identify which species of prey are present in the diet of the scorpions.

Discussion

The results of this analysis is mostly as expected, with the diet consisting of small insects such as termites, ants, and cockroaches. The most unexpected result however lies in the fact that a small portion of *C. hentzi's* diet is made up of small centipede species. The limitations of this method means that we cannot tell whether these scorpions are cannibalistic, since the scorpion's DNA is also amplified through the primer usage process. A potential way around this problem is to observe the behavior of *C. hentzi* in captivity to determine whether they are a cannibalistic species. Another limit of this method is the fact that the primers used are specific to arthropod cytochrome oxidase I genes and will not amplify the DNA of any other group of animal. The only way to overcome this issue is to use extra primer sets that will amplify the DNA of other groups of prey. A further consideration that may affect the results would be the time of year at which individuals are collected as there may be a difference in diet during different seasons.

Conclusions

In conclusion, further research must be conducted to eliminate the restrictions of the current method used to analyze diet. Further research could elaborate on this diet study by including information on *C. hentzi* cannibalism and non-arthropod prey species. Other research that builds off of this analysis could include researching the diet of other small predators such as spiders, centipedes, or other species of scorpion.

Results

Preliminary results through the analysis of OTUs using the BOLD database shows that the diet of *C. hentzi* primarily consists of small fly species and termites. Another smaller portion of the diet is made up of small ground dwelling spider species and small centipedes.

References

- Martin, M. (2011). CUTADAPT removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, 17(1), 10. <https://doi.org/10.14806/ej.17.1.200>
- Vamos, E., Elbrecht, V., & Leese, F. (2017). Short coi markers for freshwater macroinvertebrate metabarcoding. *Metabarcoding and Metagenomics*, 1. <https://doi.org/10.3897/mbmg.1.14625>
- Zeale, M. R., Butlin, R. K., Barker, G. L., Lees, D. C., & Jones, G. (2010). Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources*, 11(2), 236–244. <https://doi.org/10.1111/j.1755-0998.2010.02920.x>

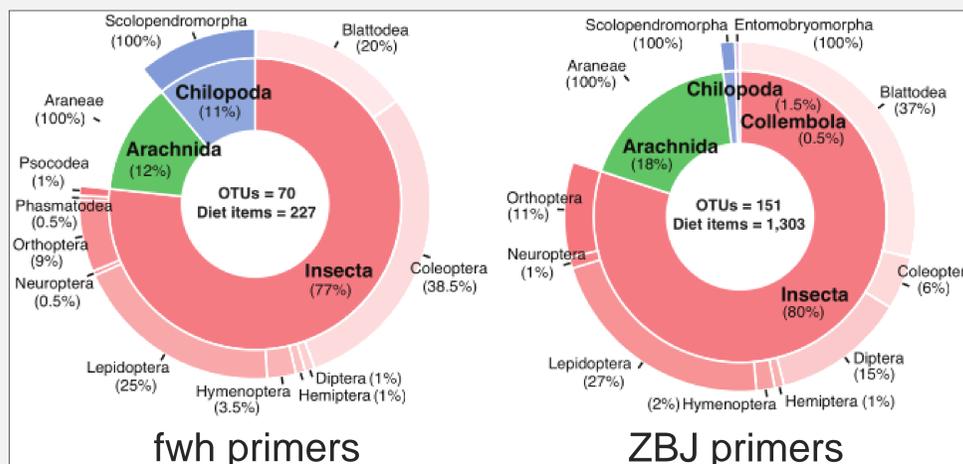


Figure 2: This figure shows the percentage of each taxa found in the diet of *C. hentzi* through the analysis of OTUs extracted using the two different primer sets.