

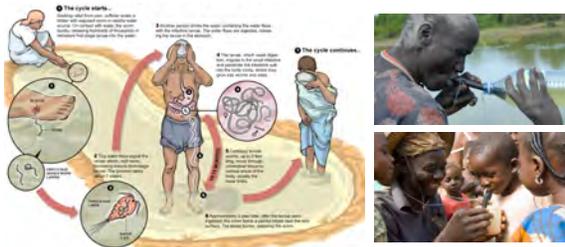
# Natural history and genetic diversity of *Dracunculus* spp.

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## INTRODUCTION

*Dracunculus* spp. are spiruroid nematode parasites that live in the subcutaneous tissues or abdominal cavity of mammals.<sup>1</sup> Female worms release L1 larvae into water when a host enters water and transmission occurs when another host ingests copepods infected with L3 larvae. The best known species is *D. medinensis*, the Guinea Worm. In 1985, over 3.5 million people were infected, but thanks to control efforts by the Carter Center and public health agencies, there were only 22 cases in 2015. Control was primarily through the use of water filters.<sup>2</sup>



In North America, there are two *Dracunculus* species that infect mammals. One, *D. insignis*, is considered a host generalist, raccoons are the primary host but infections have been noted in several wildlife species and domestic dogs and cats. The other species, *D. lutrae*, may be a river otter specialist. These conclusions were primarily based on a single study on wildlife in Canada and limited morphologic identification of adult male worms. It is impossible to identify mature female worms based on morphologic characteristics. Unfortunately, adult males are very rarely found.

## GOALS AND HYPOTHESES

**Determine prevalence of *Dracunculus* in potential vertebrate hosts in Georgia**

- H: Raccoons will be the primary host for *Dracunculus* spp.

**Genetically characterize these worm to identify to species**

- H: We will confirm that *D. insignis* is a host generalist.

**Experimentally evaluate the role of paratenic hosts in the life cycle of *D. insignis***

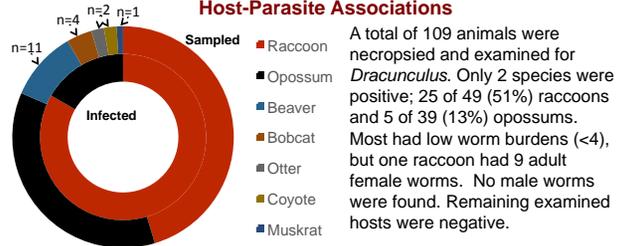
- H: We will confirm that amphibians are suitable paratenic hosts.
- H: Fish have not been proven to be paratenic hosts but will be transport hosts.

## MATERIALS AND METHODS

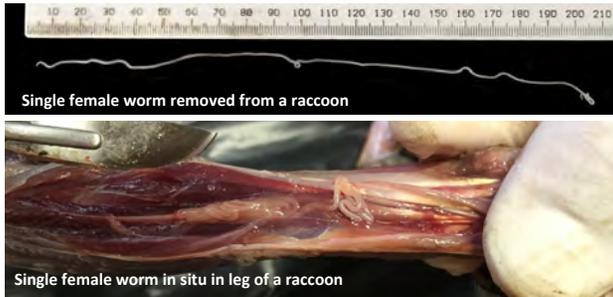
- Necropsy potential hosts and examine for *Dracunculus* spp.
- Extract DNA and perform PCR using primers LCO1490/HCO2198<sup>1</sup>
- Sequence amplicons and conduct phylogenetic analyses
- Conduct experimental infection trials with tadpoles and fish<sup>3</sup>
  - Collect L1 larvae from live *D. insignis* and infect copepods
  - Feed copepods L1 and after 14-18 days, feed to different species of tadpoles and fish
- Assess infection by necropsy and by feeding to ferrets, 6-8 months

## RESULTS

### Host-Parasite Associations

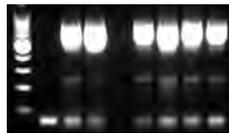


A total of 109 animals were necropsied and examined for *Dracunculus*. Only 2 species were positive; 25 of 49 (51%) raccoons and 5 of 39 (13%) opossums. Most had low worm burdens (<4), but one raccoon had 9 adult female worms. No male worms were found. Remaining examined hosts were negative.



### Molecular Characterization

Amplicons of the appropriate size were observed in numerous worm samples. Positive controls worked and negative controls had no amplification. However, sequencing results were problematic, despite numerous changes to PCR reaction conditions and attempts to isolate DNA from a small portion of worm only. Repeated attempts to directly sequence purified products resulted in mixed products, vertebrate sequences, or a few *Dracunculus* sequences. The four parasite sequences were *D. insignis*.

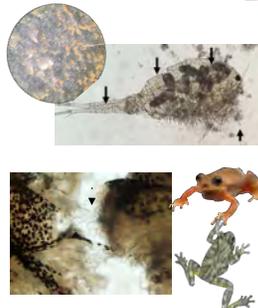


### Experimental Trials

L1 larvae were collected from a single female *Dracunculus* from a raccoon. Copepods were successfully infected as described.<sup>3</sup>

Infections of 2 species of tadpoles (gray tree frog and northern cricket frog) confirmed by necropsy. These parasites and tadpoles were fed to ferrets to confirm infectiousness. Results will take 6-8 months. *Bufo* tadpoles never consumed the infected copepods.

Three species of fish (tilapia, fathead minnows, and gambusia) were exposed to infected copepods and then immediately fed to ferrets. Ferrets will be tested after 6-8 months.



## DISCUSSION

### Host-Parasite Associations

In a previous study in Canada<sup>1</sup>, *D. lutrae* was only found in one host, the river otter, while *D. insignis* was a generalist, infecting raccoons, fishers, and muskrats. There are other studies that have found *Dracunculus* spp. (assumed to be *D. insignis*) in various hosts, but few look at sympatric hosts at the same site to investigate the role of various species in maintaining the parasite. In our study, as expected, raccoons were the most common host. Unexpectedly, we found that opossums were a relatively common host which represents only the 2<sup>nd</sup> report of *Dracunculus* in opossums. Although other hosts were negative, our sample sizes were low so continued surveillance is needed.

### Molecular Characterization

Identification of *Dracunculus* spp. is easily done through the morphological characteristics of the adult male worm. Unfortunately, the male worm is rarely found. Identification of the female worms can only be done through sequencing of DNA. Unfortunately, the primers previously used to identify species<sup>1</sup> were not specific in our study. Many of the amplicons submitted for sequencing showed amplification for the host DNA rather than the parasite DNA. Methods for better specificity and amplification and other gene targets of are currently being researched and results are pending.

### Experimental Trials of Possible Paratenic Hosts

Despite excellent progress, *D. medinensis* is still a public health concern in 4 countries. Recently, the number of infected dogs, particularly in Chad, has increased. A possible explanation is the utilization of a paratenic host. We are conducting experimental trials with *D. insignis* as a model system for *D. medinensis*. Previously, bullfrogs (*Lithobates catesbeianus*) and African clawed frog (*Xenopus laevis*) were experimentally shown to be possible paratenic hosts for *D. insignis*.<sup>4</sup> Although most of our data are pending, we did confirm that two species of Hylidae frogs are susceptible. These frogs/parasites were fed to ferrets to confirm infectiousness. Finally, although previous work on fish indicate they are not likely paratenic hosts<sup>3</sup>, we believe they could serve as transport hosts so we allowed fish to feed on infected copepods and then we immediately fed to ferrets. Due to the long development period of the parasite, results of these experiments will take 6-8 months.



The Rod of Asclepius, a common symbol in healthcare, may reference the most commonly practiced method of removing the Guinea Worm from a patient. Female worms are slowly wound around a stick or gauze until the complete worm has been extracted.

### Acknowledgements

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