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Further Investigation of a Novel Rhabditid Nematode

Evan Hudgens
Ohio Wesleyan University

Nathan Schmidt
Ohio Wesleyan University

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Evan Hudgens, Nathan Schmidt, and Danielle Hamill

Department of Zoology, Ohio Wesleyan University, Delaware, OH

Abstract:

Nematodes are among the most numerous and widespread animals on earth. The nematode *Caenorhabditis elegans* is a well-established model organism used for a wide-variety of studies ranging from biomedical to behavioral to ecological, and more. While *C. elegans* is well characterized, there are thousands of different species of nematodes, many of which have not been studied. We are characterizing several Rhabditid nematodes isolated in association with millipedes from both Ohio and Florida. Sequence analysis of rDNA genes supports that one of the species of worms we isolated is *Oscheius myriophila*. Other worms we isolated are similar, but we believe may be a distinct species that for now we are calling *Rhabditis sp.*. We are using a combination of molecular, phenotypic and genetic approaches to characterize these worms and to establish if they represent a previously undescribed species. One of our primary approaches is to use scanning electron microscopy (SEM) to visualize key structural features. We developed a method for SEM and have gotten detailed images to compare *C. elegans* to *O. myriophila* to our *Rhabditis sp*. worms. We are also using light microscopy to capture images and do measurements critical for species identification. Finally, we are conducting crosses. Previous work in the lab has resulted in conflicting evidence about whether *O. myriophila* and *Rhabditis sp*. can produce normal offspring, so we are extending these studies. Together with examining embryonic development in these worms, we believe our studies will help us to better understand this diverse phylum of animals.

Introduction:

- Nematodes are one of the most common phyla of animals.
- Within nematodes, *rhabditids*, include a large number of diverse species.
- *C. elegans* is the most well known member of the *rhabditids*.
- *C. elegans* development has been studied extensively.
- But how similar is *C. elegans* development to that of related nematodes?

We isolated several *Rhabditid* nematodes in association with millipedes in Delaware, Ohio and the Florida Everglades.

We present our studies comparing *C. elegans* to two of the nematodes we collected: *Oscheius myriophila* and *Rhabditis sp.* focusing on morphological features observed by light and Scanning Electron Microscopy.

We believe *Rhabditis sp.* Is a novel species that has not yet been described.

Results:

DNA Sequencing

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R CTCTTCGGATATCCTTTATGGATAACTGCGGAAATTCTGGAGCTAATACATGCAACAAAACCCCG
O CTCTTCGGATATCCTTTATGGATAACTGCGGAAATTCTGGAGCTAATACATGCAACAAAACCCCG
R ACTTTTGAAGGGGTGCAGATATTAGTACAAACCAATCGTCTTCGGACGTTGTTTGTTGACTCTGA
O AC-TTTGAAGAGGTGCAGATATTAGTACAAACCAATCGTCTTCGGACGTTGTTTGTTGACTCTGA
R ATATCGCAGTTTACGGCAGGTAATCTACCGTAACCGAAAGAAGTGTCTGCCCTATCAACCAGA
O ATATCGCAGTTTACGGCAGGTAATCTACCGATAACCGAAAGAAGTGTCTGCCCTATCAACCAGA
R TGGTAGTCTATTAGTCTACCATCGGTTATTACGGGTAACGGAGAATAAGGGTTCGACTCCGGAGAG
O TGGTAGTCTATTAGTCTACCATCGGTTATTACGGGTAACGGAGAATAAGGGTTCGACTCCGGAGAG
R GGAGCCTTAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGGTAACTTATCCACTACTTCA
O GGAGCCTTAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGGTAACTTATCCACTACTTCA
R GTGAGATAGTGACTAAAAATAAAAAGACCAATCCTCACGGATCGGTTATTTCAATGAGTTGAGCT
O GTGAGATAGTGACTAAAAATAAAAAGACCAATCCTCACGGATCGGTTATTTCAATGAGTTGAGCT
R TAAATAGCTCTTCGAGGACTCTAGTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCT
O TAAATAGCTCTTCGAGGACTCTAGTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCT
R CCACTAGTGTAAATCGTCATTGCTGCGGTTAAAAAGCTCGTAGTTGGATCTGAGTTGCATGCGTT
O CCACTAGTGTAAATCGTCATTGCTGCGGTTAAAAAGCTCGTAGTTGGATCTGAGTTGCATGCGTT
R GGTTCCTCTCTGAGGTTAATCTTCGCTGCAACTATTTGCTGGTTTCTGCAGATAGCTTCGGCT
O GGTTCCTCTCTGAGGTTAATCTTCGCTGCAACTATTTGCTGGTTTCTGCAGATAGCTTCGGCT
R CCTGTAGTGGCTAGCGAGATTACTTTGAATAAAACAGAGTGTTCAAAACAAGCGATAGCTTGAAT
O TCTGTAGTGGCTAGCGAGATTACTTTGAATAAAACAGAGTGTTCAAAACAAGCGATTGCTTGAAT
R GCTCGATCATGGAATAATAGAATAGGACCTTCGGTTCTATTTATTGGTTCAAGAACTTGAAGTAATG
O GCTCGATCATGGAATAATGGAATAGGACCTTCGGTTCTATTTATTGGTTCAAGAACTTGAAGTAATG
R ATTAAGAAGAACAATTCGGGGGCATTCGTATCCCTGCGCGAGAGGTGAAATTCGTGGACCGCAGG
O ATTAAGAAGAACAATTCGGGGGCATTCGTATCCCTGCGCGAGAGGTGAAATTCGTGGACCGCAGG
R GGGACGCCCAAAAGCGAAAGCATTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGG
O GGGACGCCCAAAAGCGAAAGCATTTGCCAAGAATGTCTTCATTAATCAAGAACGAAAGTCAGAGG
R TTCGAAGGCGATTAGATACCGCCCTAGTTCTGACCGTAAACTATGCCATCTAGCGATCCGATTGG
O TTCGAAGGCGATTAGATACCGCCCTAGTTCTGACCGTAAACTATGCCATCTAGCGATCCGATTGG
R GTTATTCGCCTAGTCGGGGGAGCTTCCCGGAAACGAAAGTCTTTCGGTTCCGGGGGTAGTATGGTT
O GTTATTCGCCTCGCCGGGGGAGCTTCCCGGAAACGAAAGTCTTTCGGTTCCGGGGGTAGTATGGTT
R GCAAAGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATT
O GCAAAGCTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAATT
R TGACTCAACACGGGAAAACTCACCCGGCCCGGACACCGTTAGGATTGACAGATTGAAAGCTCTTT
O TGACTCAACACGGGAAAACTCACCCGGCCCGGACACCGTTAGGATTGACAGATTGAAAGCTCTTT
R CTCGATTTGGTGGTTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTTATTC
O CTCGATTTGGTGGTTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTTATTC
R CGATAACGAGCGAGACTCTAACCTACTAAATAGTTTCACGATTTTCGGGTCGTGTGAACTTCTTA
O CGATAACGAGCGAGACTCTAACCTACTAAATAGTTTCACGATTTTCGGGTCGTGTGAACTTCTTA
R GAGGGATAAGCGGTGTTTAACCGCACGAGATTGAGCGATAACAGGTCTGTGATGCCCTTAGATGT
O GAGGGATAAGCGGTGTTTAACCGCACGAGATTGAGCGATAACAGGTCTGTGATGCCCTTAGATGT
R CCGGGGCTGCACGCGCGCTACACTGGAAGGATCAGCTGGTCGCCCATTGCCGAAAGGTAATGGTA
O CCGGGGCTGCACGCGCGCTACACTGGAAGGATCAGCTGGTCGCCCATTGCCGAAAGGTATTGGTA
R AACCGTTGAAACCCTTCCGTGACCGGGATAGGGAATTGTAATTATTTCCCTTGAACGAGGAATTC
O AACCGTTGAAACCCTTCCGTGACCGGGATAGGGAATTGTAATTATTTCCCTTGAACGAGGAATTC
R CTAGTAAGTGTGAGTCATCAGCTCACGCTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCG
O CTAGTAAGTGTGAGTCATCAGCTCACGCTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCG
R CTGTCCGGGACTGAGCTGTTTCGAGAAGAGTGAGGACTGACGCAAAGGGGCCTTCGGCCTCTTCT
O CTGTCCGGGACTGAGCTGTTTCGAGAAGAGTGAGGACTGACGCAAGGGGCCTTCGGCCTCTTCT
R GGATGGGAATCACTTTAATCGCAATGGCTTGAACCGGGCAAAAGTCGTAACAAGGTACC
O TGATGGGAATCACTTTAATCGCAATGGCTTGAACCGGGCAAAAGTCGTAACAAGGTACC
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Figure 1: Alignment of 18s rDNA sequences.
Rhabditis sp. (R) compared to *Oscheius myriophila* (O). Differences are highlighted. There were 25 differences out of the 1621 bases sequenced.

Crosses

Table 1: Cross results of *O. myriophila* and *Rhabditis sp.*

♀	♂	Number of Plates with Males
O. myriophila	*O. myriophila*	2/2
Rhabditis sp.	*Rhabditis sp.*	3/3
Rhabditis sp.	*O. myriophila*	0/4
O. myriophila	*Rhabditis sp.*	0/4

Light Microscopy

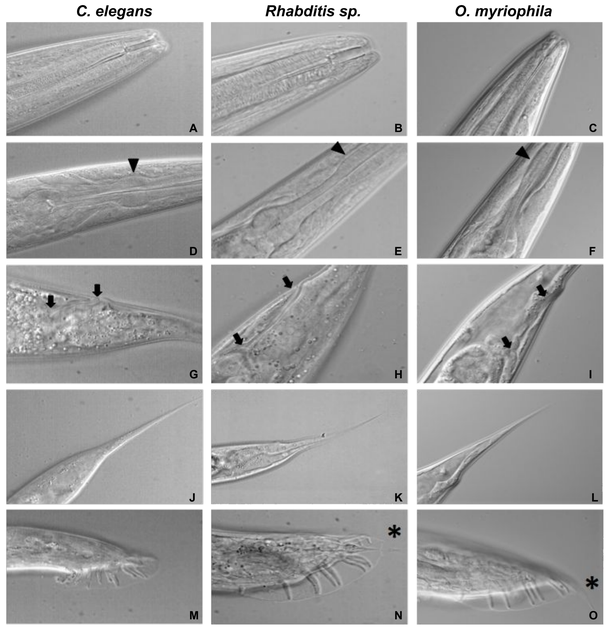

Figure 2: Morphological comparison between *C. elegans*, *Rhabditis sp.* and *O. myriophila*. (A-C) Anterior. (D-F) Pharynx. Arrows point to bulbous anterior pharynx in *C. elegans* vs smoother pharynx in *Rhabditis sp.* and *O. myriophila*. (G-I) Rectum. Longer in *Rhabditis sp.* and *O. myriophila* (arrows show endpoints). (J-L) Hermaphrodite tail - long, sharply tapered. (M-O) Male tail. Unretracted tail (*) in *Rhabditis sp.* and *O. myriophila*.

Table 2. Morphometric data of *Rhabditis sp.* and *Oscheius myriophila*. All measurements are in µm and the form: mean ± s.d.

	Rhabditis sp.		*Oscheius myriophila*	
	Hermaphrodite	Male	Hermaphrodite	Male
n	5	5	4	5
L	1289.8 ± 109.5	984.2 ± 74.4	1481.5 ± 50.6	1006.1 ± 26.3
Pharynx length	172.3 ± 2.3	152.7 ± 7.3	179.7 ± 1.7	170.7 ± 5.2
Greatest width	94.3 ± 6.6	63.0 ± 3.8	100.7 ± 12.9	57.6 ± 4.3
Tail length	105.5 ± 15.7	72.1 ± 8.4	132.8 ± 13.5	98.9 ± 12.2
a (body length ÷ greatest width)	13.7 ± 1.0	15.7 ± 1.8	14.9 ± 1.5	17.5 ± 1.0
b (body length ÷ pharynx)	7.5 ± 0.6	6.4 ± 0.2	8.2 ± 0.4	5.9 ± 0.1

Scanning Electron Microscopy

Methods

Worm samples were:

- Rinsed off plates and transferred into microcentrifuge tubes
- Fixed in 2.5% glutaraldehyde in PBS for 24 hours
- Transferred into porous baskets
- Dehydrated in an ethanol series (10, 25, 35, 50, 75, 95, 100, 100% for at least one hour)
- Dried in Tousamas critical point dryer
- Mounted on stubs and gold-coated

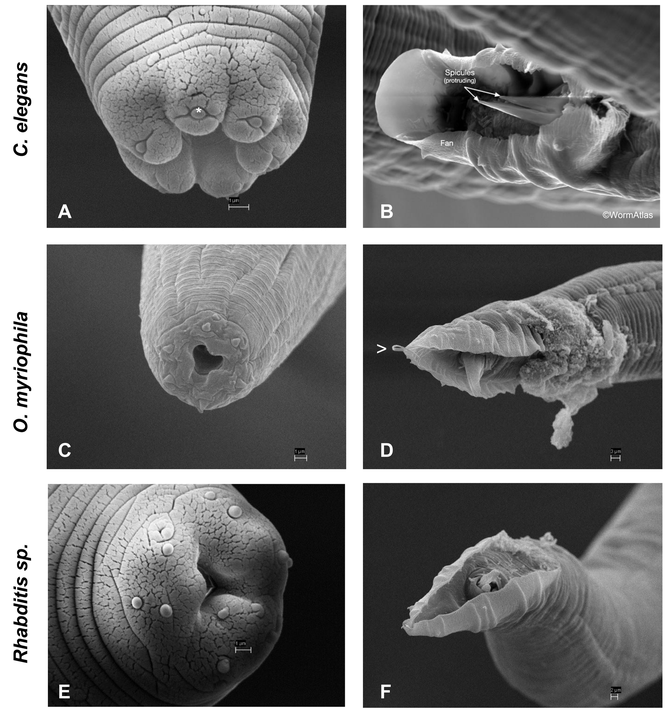

Figure 3: Select SEM images of *C. elegans, O. myrphila,* and *Rhabditis sp.*
Images depict *C. elegans* mouth (A) compared to *O. myriophila* (C) mouth, and *Rhabditis sp.* (E). Asterisk marks sensory papillae, a *C. elegans* feature that is different from *Rhabditis sp* and *O. myriophila.* Male tails (B, D, F) show spicules and fan with sensory rays. Unretracted tail is marked (arrowhead).
Working distance = 10mm, Accelerating voltage = 20 kV, probe current 6 pA.
Image in B: Lints, R. and Hall, D.H. 2009. Wormatlas, SEM [Hall] 434 H07_5.

Summary:

Our recently isolated *Rhabditis sp.* looks similar to *Oscheius myriophila*. However, DNA sequencing and genetic crosses suggest it may be a previously undescribed species. We continue to characterize this new species with additional light and scanning electron microscopy.

Future Directions

- Additional DNA sequencing of rDNA and mitochondrial genes
- More crosses including with *Rhabditis sp.* males
- Additional morphometric measurements for species identification
- Optimize dehydration method for SEM and acquire more images

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