# Morphological and Molecular Techniques for the Diagnosis of Nematodes

Jon Eisenback
Professor of Plant Nematology
Virginia Tech

# Half the world's natural history specimens may have the WRONG name: Lack of time and too many discoveries are to blame

- **Researchers at the University of Oxford and the Royal Botanic Garden Edinburgh used different approaches to study the number of mistakes**
- **Examined records for tropical plant and genus containing sweet potatoes**
- **The researchers believe that as many as half of all natural history specimens held in the world's museums could have the wrong names**
- **Accuracy could be improved by remote access and DNA sequencing**

By SARAH GRIFFITHS FOR MAILONLINE

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Meloidogyne fruglia - Wikipedia, the free encyclopedia

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# *Meloidogyne fruglia*

From Wikipedia, the free encyclopedia

***Meloidogyne fruglia*** is a plant pathogenic nematode infecting African daisies.

| ***Meloidogyne fruglia*** | |
|---|---|
| **Scientific classification** | |
| Kingdom: | Animalia |
| Phylum: | Nematoda |
| Class: | Secernentea |
| Subclass: | Diplogasteria |
| Order: | Tylenchida |
| Superfamily: | Tylenchoidea |
| Family: | Heteroderidae |
| Subfamily: | Meloidogyninae |
| Genus: | *Meloidogyne* |
| Species: | ***M. fruglia*** |
| **Binomial name** | |
| ***Meloidogyne fruglia*** | |

## See also [ edit ]

- List of African daisy diseases

## References [ edit ]

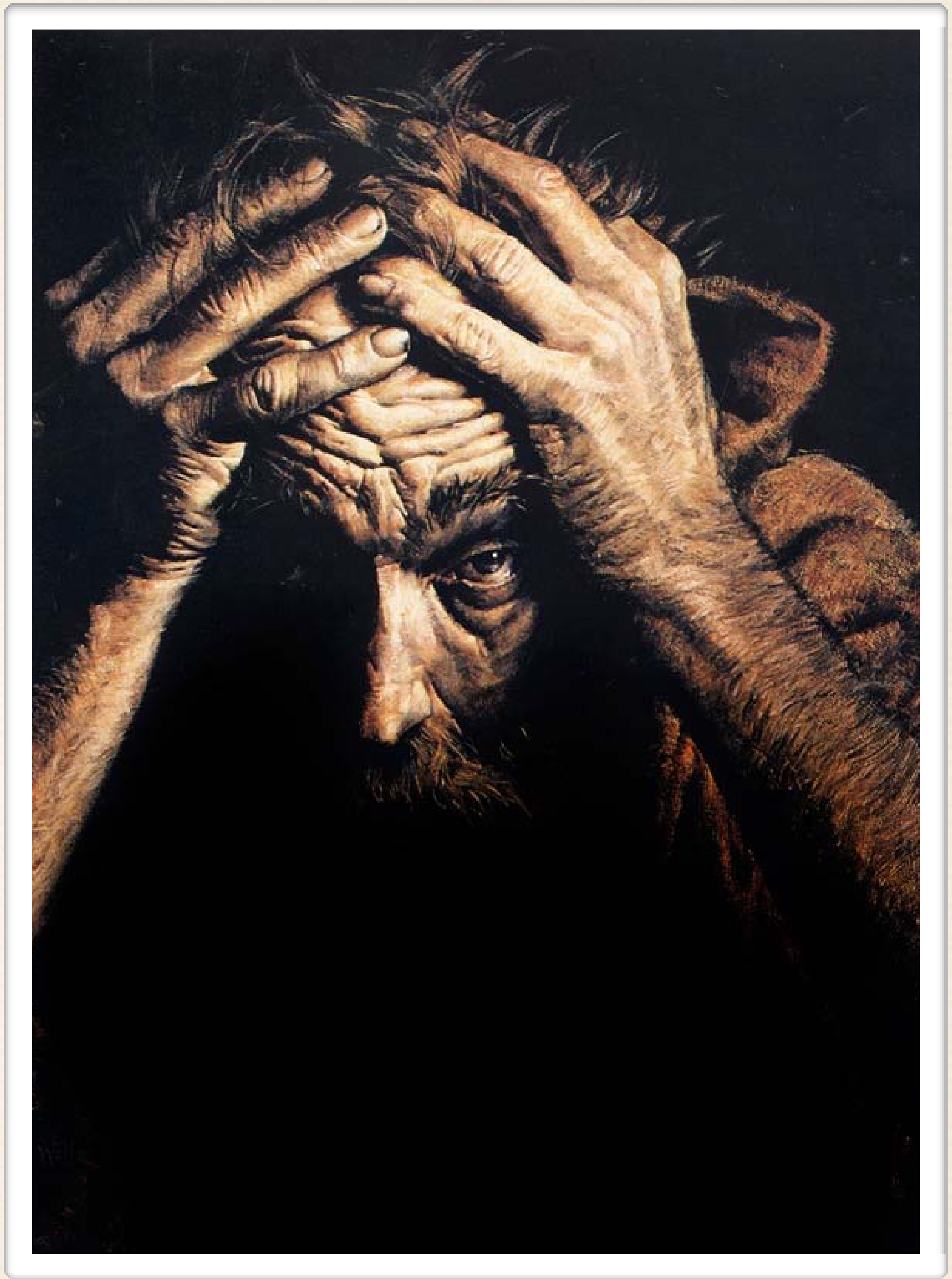
*This nematode- (or roundworm-) related article is a stub. You can help Wikipedia by expanding it.*

*This plant disease article is a stub. You can help Wikipedia by expanding it.*

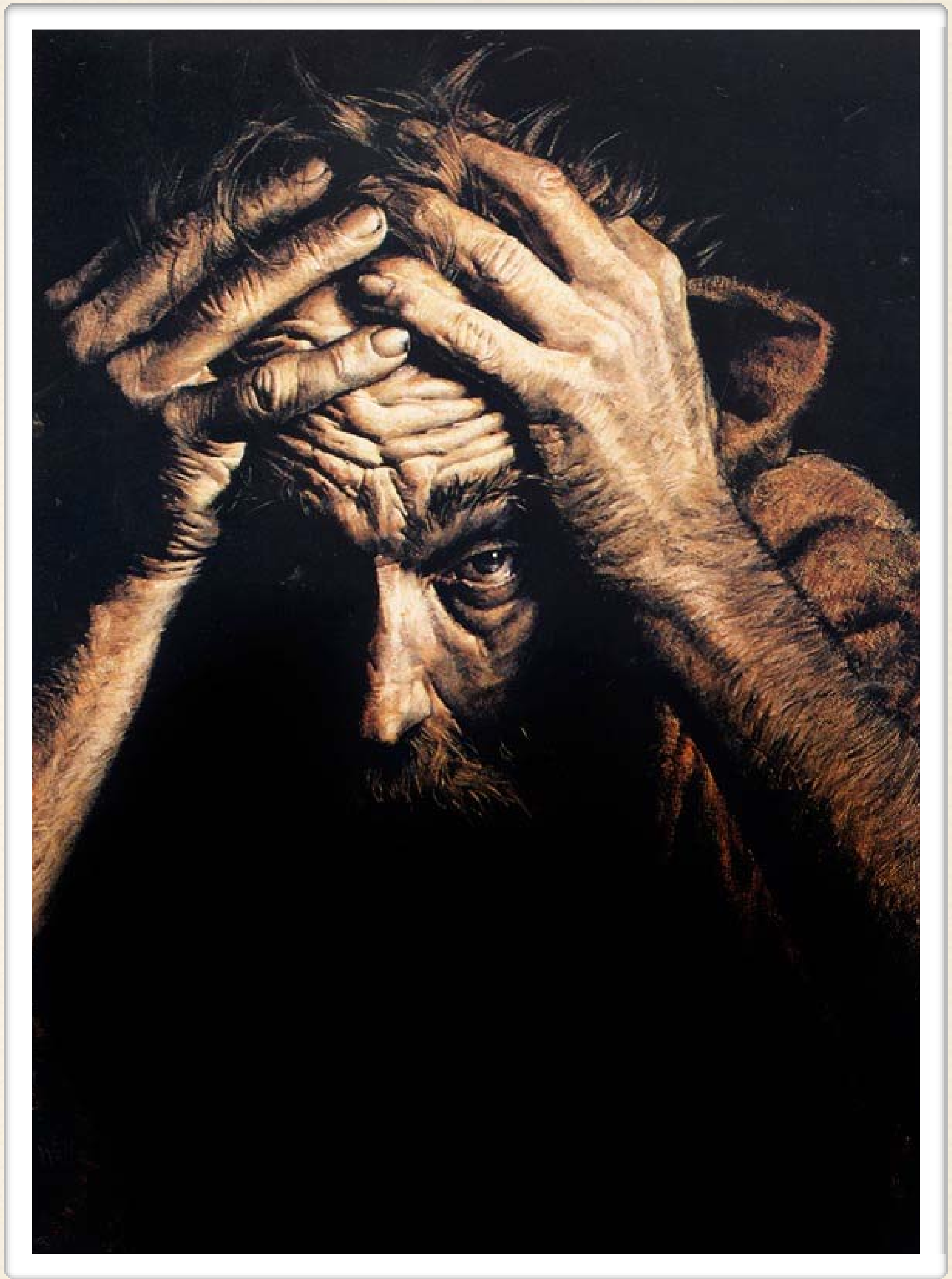
Categories: Tylenchida | Plant pathogenic nematodes | Ornamental plant pathogens and diseases | Nematode stubs | Plant disease stubs

The internet may contain incorrect information regarding species

- What is a nematode taxonomist to do?
- 25,000+ species have been described during the last 250 years
- An average of 100/year
- Descriptions are from all over the world



- Each researcher has to collect the species descriptions
- Not all libraries subscribe to all journals
- Some journals are not open access
- Identification of species and taxonomy in general can only occur with the original descriptions



# **Project:** Nematoda Rudolphi, 1808

- The goal is to collect every description of every nematode species
- Make them available for all on the Internet
- Use this resource to enhance nematode taxonomy

# I. INTESTINA.

Animalia *ſimplicia*,

275. GORDIUS. *Cor*

aquaticus. 1. G. pallidus extre
*It. gotl.* 282. G
*Geſn. aqu.* 463.
*Aldr. inſ.* 720. *t.*
*Jonſt. inſ. t.* 25.
*Habitat cum ſequ*
*quam tracat ut*
*auctor.* Mor
*ruſticorum com*
*pta S. Kiumann*

argillaceus. 2. G. flaveſcens ext
2060.*
*Habitat in* Argill
*tranat, adeoqu*

medinenſis. 3. G. totus pallidu
*Sloan. jam.* 2. *p.*
*Kæmph. amæn.* 524. Dracunculus perſarum.
*Velſch. monogr. Vena medinenſis.*
*Habitat in utraque* India, *frequens in rore matutino, inde intrans nudipedes obambulantes ſervos, dolorem febremque excitans, caute extrahendus. Infuſo Mercurii ſublimati corroſivi Swietenii intra 20 dies, qui alias intra 40, educitur.*

marinus. 4. G. plano-ſpirali convolutus filiformis.
*Act. Stockh.* 1760. *p.* 306. Lepra piſcium.
*Strom. ſondm.* 194. Queiſe.
*Habitat in Maris Norvegici &c. piſcibus, frequentiſſime in viſceribus* Clypeæ Halecis, *intra puſtulam, unde* Lepra *Piſcatorum Martini, vid. Diſſ. noſtr. de Lepra.*
Hæret *ſemper in plano ſpirali, albus, lævis, pollicis* $\frac{1}{2}$ *longitudine, apice vix attenuatus.*

5. G.



lacuſtris. 5. G. plano-ſpirali convolutus utrinque attenuatus.
*Habitat in Hepate* Eſocis Lucii, *aquæ dulcis.*
*levis, in ſpiram planam con-*
*ior & utraque extremitate*
*lis.*

es, filiforme utraque
itate attenuatum.

59.
*l.* 1762. *act. t.* 3. Aſcarides.
Radicibus *plantarum putre-*
*llo* Puerorum, Equorum;
*ctus ſalit.*
*is annularibus vix conſpicuis,*
*tice obtuſius; poſtice exqui-*
*picem anteriorem ruga annu-*
*ipſum hujus apicem.* Os

*nis, longitudine Lumbrici*
*levato deſtituitur, adeoque a*
Corpus *teres, utraque extremitate ſubulatum, cauda tamen ſubtriquetra.*

277. LUMBRICUS. *Corpus* teres annulatum, cingulo elevato cinctum, longitudinaliter exaſperatum, poro laterali inſtructum.

terreſtris. 1. L. triſariam retrorſum aculeatus. *Fn. ſvec.* 2073.
*Aldr. inſ.* 693. Lumbricus terreſtris.
*Rhed. exper. quart. t.* 15. *f.* 1.
*Sloan. jam.* 2. *p.* 189. Lumbricus terreſtris minor rubicundus.
*Raj. inſ.* 2. Lumbricus terreſtris minor.
β. *Raj. inſ.* 1. Lumbricus major.
γ. *Raj. inſ.* 2. Lumbricus inteſtinorum teres.
*Valliſ. nat.* 1, *t.* 3. Lumbricus humanus. *Ha-*

Ascaris lumbricoides.

Lives in the intestines of human beings, the length of an earth worm, *Lumbricoides terrestris*, but lacking a raised ring, so a Lumbrico distinctissimus:
Body smooth, both ends awl shaped however the tail sub-triangular.

# I. INTESTINA.

Animalia *simplicia, absque artubus, nuda.*

## 275. GORDIUS. *Corpus* filiforme, æquale, læve.

aquaticus. 1. G. pallidus extremitatibus nigris. *Fn. svec.* 2068.*
*It. gotl.* 282. Gordius.
*Gesn. aqu.* 463. Bitulus aquaticus.
*Aldr. ins.* 720. *t.* 765. Seta s. Vitulus aquaticus.
*Jonst. ins.* *t.* 25. Meerwijn.
*Habitat cum sequenti in* Aquis, & *imprimis in* Argilla, *quam tranat uti piscis aquas, scaturiginum plurium auctor.* Morsuram *hujus excitare* Paronychiam, *rusticorum communi effato, nuper confirmatum auto-pta S. Kiumanno.*

argillaceus. 2. G. flavescens extremitatibus concoloribus. *Fn. svec.* 2069.*
*Habitat in* Argilla, *proprio suo elemento, quam ubique tranat, adeoque ubi illa foditur frequens.*

medinensis. 3. G. totus pallidus. †
*Sloan. jam.* 2. *p.* 190. *t.* 233. *f.* 1. Vena Medina.
*Kæmph. amæn.* 524. Dracunculus persarum.
*Velsch. monogr.* *Vena medinensis.*
*Habitat in utraque* India, *frequens in rore matutino, inde intrans nudipedes obambulantes servos, dolorem febremque excitans, caute extrahendus. Infuso Mercurii sublimati corrosivi Swietenii intra 20 dies, qui alias intra 40, educitur.*

marinus. 4. G. plano-spirali convolutus filiformis.
*Act. Stockh.* 1760. *p.* 306. Lepra piscium.
*Stroem. sondm.* 194. Queise.
*Habitat in Maris Norvegici* &c. *piscibus, frequentissime in visceribus* Clupeæ Halecis, *intra pustulam, unde* Lepra *Piscatorum Martini, vid. Diss. nostr. de Lepra.*
Hæret *semper in plano spirali, albus, lævis, pollicis ¼ longitudine, apice vix attenuatus.*





lacustris. 5. G. plano-spirali convolutus utrinque attenuatus.
*Habitat in Hepate* Elocis Luci, *aquæ dulcis.*
Similis *præcedenti, albus, lævis, in spiram planam convolutus, sed duplo longior & utraque extremitate attenuatus more Ascaridis.*

## 276. ASCARIS. *Corpus* teres, filiforme utraque extremitate attenuatum.

vermicularis. 1. pollicaris. *Fn. svec.* 1269.
*Phelsum. monogr. Leovard.* 1762. *oct. t.* 3. Ascarides.
*Habitat in* Paludibus; *in* Radicibus *plantarum putrescentium; in intestino recto* Puerorum, Equorum; *vesperi inquietus; extractus salit.*
Corpus *lin.* 5, *teres, rugis annularibus vix conspicuis, utrinque attenuatum, antice obtusius; postice exquisite attenuatum; pone apicem anteriorem ruga annularis profundior & ad ipsum hujus apicem.* Os *transversum.*

lumbricoides. 2. A. spithamea.
*Habitat in* Intestinis humanis, *longitudine Lumbrici terrestris, sed annulo elevato destitutus, adeoque a Lumbrico distinctissimus.*
Corpus *teres, utraque extremitate subulatum, cauda tamen subtriquetra.*

## 277. LUMBRICUS. *Corpus* teres annulatum, cingulo elevato cinctum, longitudinaliter exasperatum, poro laterali instructum.

terrestris. 1. L. trifariam retrorsum aculeatus. *Fn. svec.* 2073.
*Aldr. ins.* 693. Lumbricus terrestris.
*Rhed. exper. quart. t.* 15. *f.* 1.
*Sloan. jam.* 2. *p.* 189. Lumbricus terrestris minor rubicundus.
*Raj. ins.* 2. Lumbricus terrestris minor.
β. *Raj. ins.* 1. Lumbricus major.
γ. *Raj. ins.* 2. Lumbricus intestinorum teres.
*Vallis. nat.* 1, *t.* 3. Lumbricus humanus.

# APHELENCHOIDES XYLOPHILUS, N. SP., A NEMATODE ASSOCIATED WITH BLUE-STAIN AND OTHER FUNGI IN TIMBER [1]

By G. Steiner, *senior nematologist, in charge,* and Edna M. Buhrer, *junior nematologist, Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

A new case of unusual ecological conditions to which nematodes have adapted themselves has been found in a nematode species apparently specialized to live in timber affected by blue-stain and other wood fungi. This new species is described herein.

## ECOLOGICAL RELATIONS

The first observation of *Aphelenchoides xylophilus*, n.sp., the timber nema, dates back to 1929, when a small piece of wood that had been cut, in the process of roofing, from the top of a green pole of longleaf Louisiana pine (*Pinus palustris* Mill.) was received from Orange, Tex.[2] This piece of wood had streaks of a bluish color caused by blue-stain fungi. The nemas were found in these streaks and in bordering portions. Larval specimens, males and females, were observed. Although they were not numerous, a dozen or more specimens could be found in a small portion of wood when soaked and dissected properly. Soaking the wood in water activated the nematodes, whereas drying the wood induced dormancy. Some tests showed revival of the nematodes after a dormancy of 1 year but not after 2 years.

Later, through the courtesy of Ross W. Davidson, of the Division of Forest Pathology, Bureau of Plant Industry, there were received four different plate cultures of wood fungi in which nematodes had developed. All of these nematodes proved to be *Aphelenchoides xylophilus*. Three of the cultures were from a sawmill in Bogalusa, La., and were also obtained from blue-stained logs of *Pinus palustris*. These logs had previously been attacked by beetles of the genus *Ips*, which, according to Davidson, usually carry the blue-stain fungus *Ceratostomella ips* Rumbold, but which in these three cases contained a brown fungus belonging probably to the genus *Trichosporium*, of the "Fungi Imperfecti."

The fourth culture on which the same species of nematode developed was obtained from a pine tree (*Pinus echinata* Mill.) that had been recently killed by an attack of the beetle, *Dendroctonus frontalis* Zimm., near Fairfax, Va. In this case the nematodes originated in the interior of unstained wood, one-sixteenth to one-fourth of an inch below the insect galleries. The fungus here associated with this nematode is said by Davidson to be entirely hyaline and also to belong

[1] Received for publication Apr. 4, 1934; issued July 1934.

[2] Received through the courtesy of T. E. Snyder, of the Bureau of Entomology, U.S. Department of Agriculture, who received the wood from C. H. Lyon, chemist of the Texas Creosoting Co. Mr. Lyon wrote: "All such poles came from an area including western Louisiana and southeastern Texas. The climate is hot and, at the time of finding that specimen, was very humid. The average annual humidity is given by the Government observer of this region as of 83.3 percent. The specimen had not been treated nor come in contact with creosote. It has been at all times exposed to weather."

Journal of Agricultural Research,
Washington, D.C.

Vol. 48, no. 10
May 15, 1934
Key no. G-913





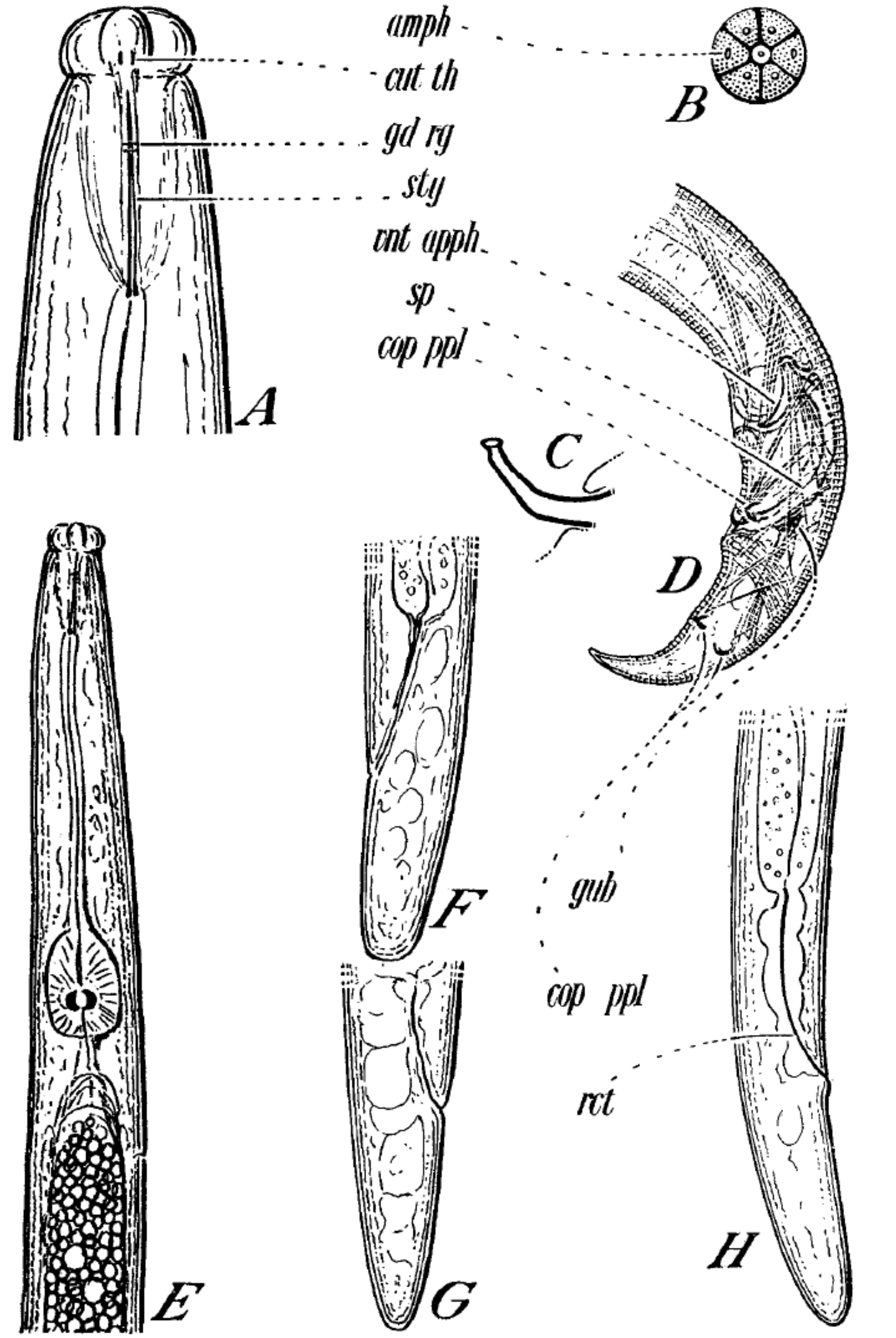
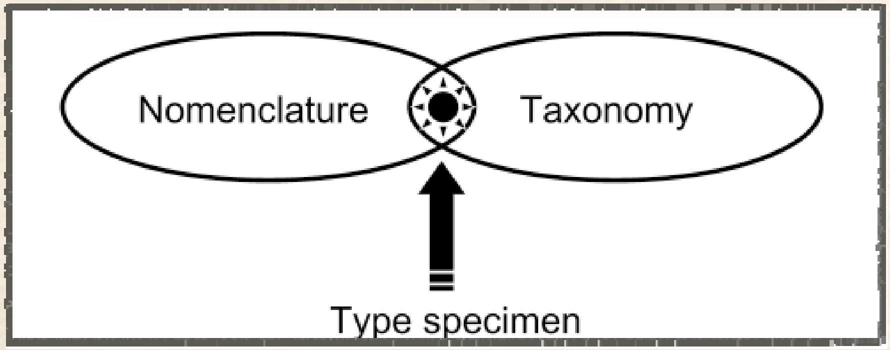
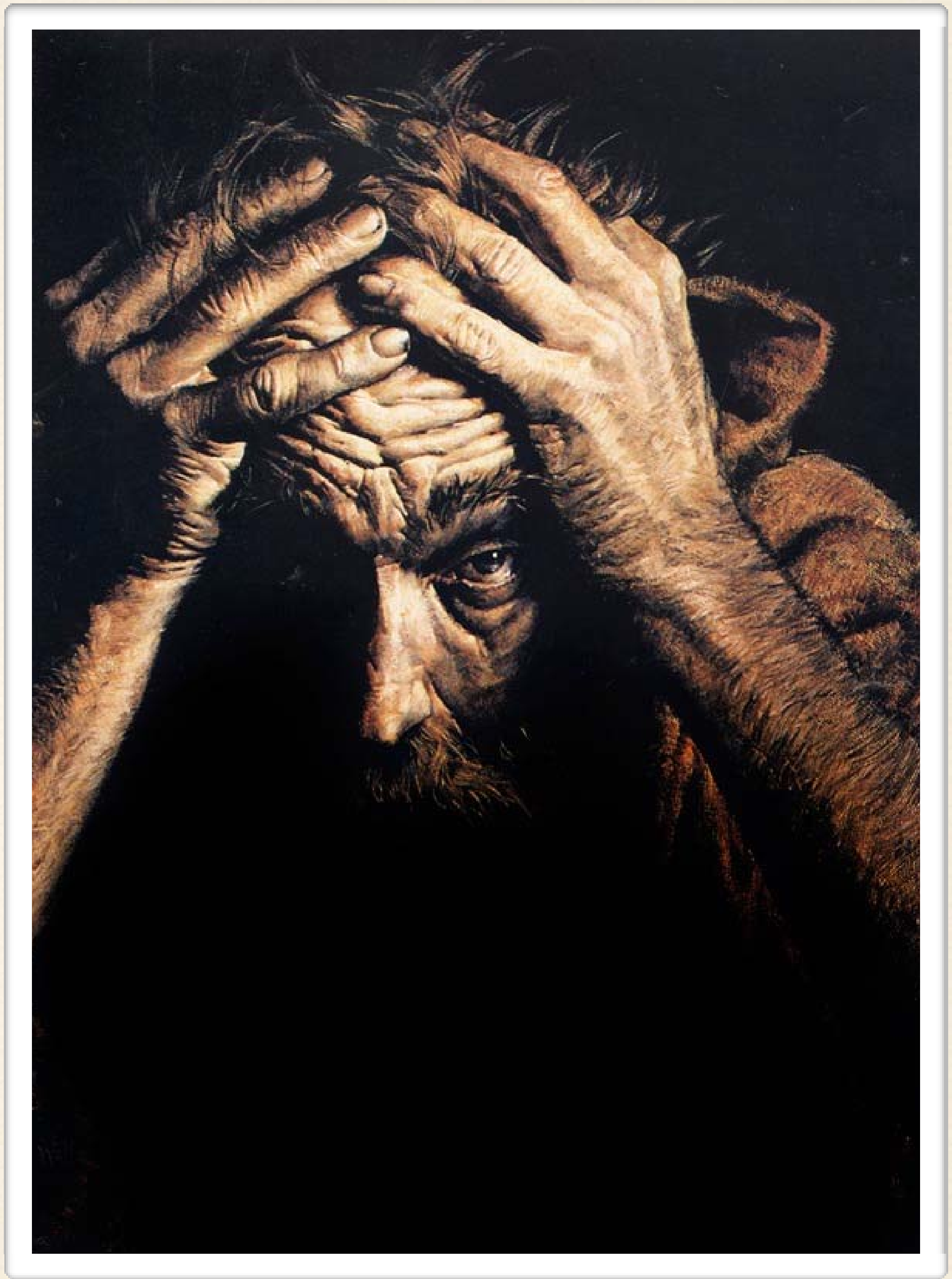


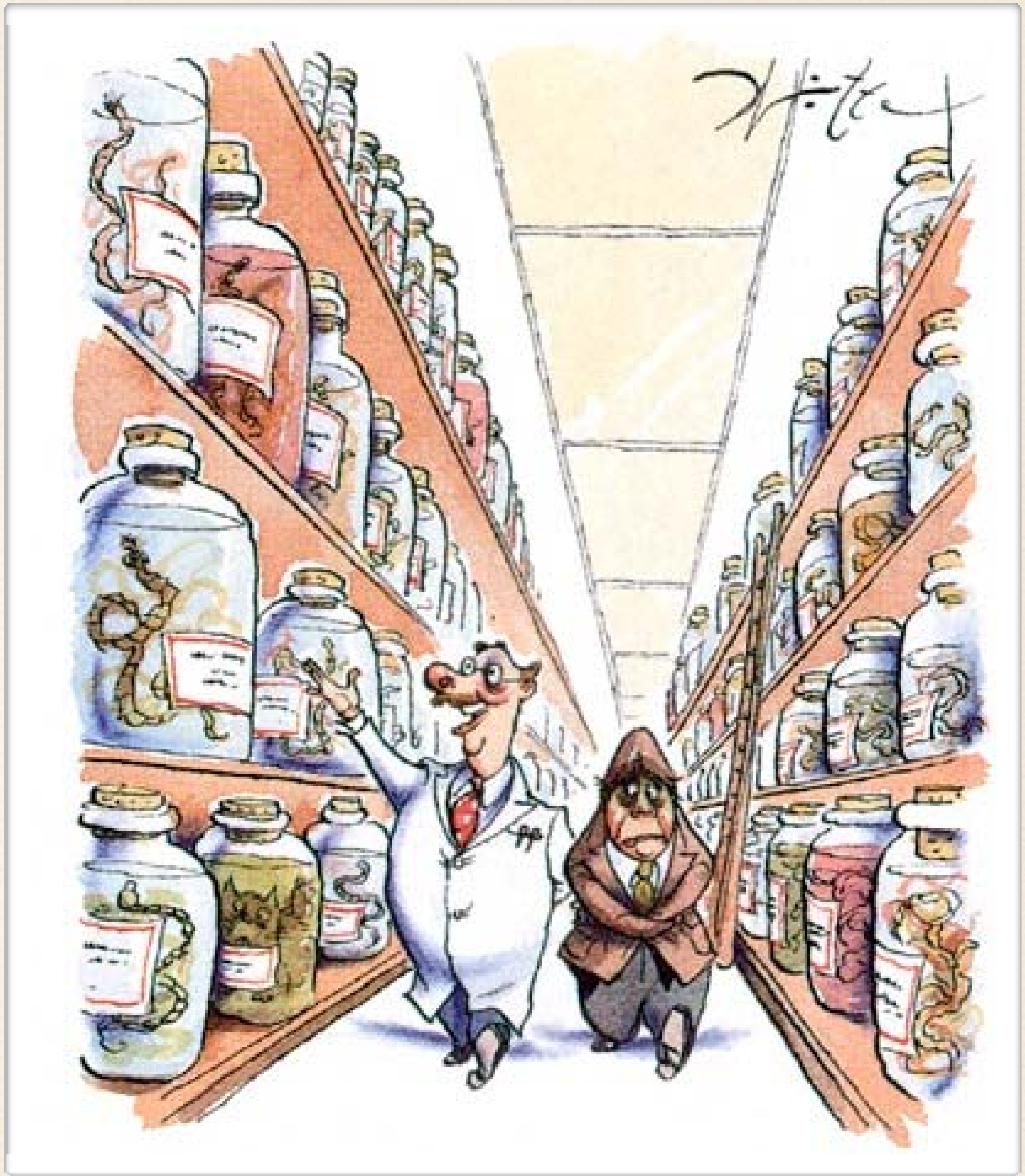

Figure 1.—*Aphelenchoides xylophilus*, n.sp. *A*.—Head of female: *cut th*, Cuticular thickening in cephalic portion of alimentary tract; *gd rg*, guiding rings of stylet; *sty*, stylet. × 2,800. *B*.—Front view of head: *amph*, Amphid. × 1,370. *C*.—Extruded spicula showing circular expansion. × 1,370. *D*.—Tail of male: *vnt apph*, Ventral apophysis; *sp*, spicula; *cop ppl*, copulatory papillae (three pairs); *gub*, gubernaculum. × 1,060. *E*.—Anterior end of larva. × 1,060. *F* and *G*.—Tails of larvae, showing variation in shape. × 1,060. *H*.—Tail of female: *rct*, Rectum. × 1,060.

- Type specimens of nematodes are the backbone of taxonomy
- Descriptions are linked to type specimens

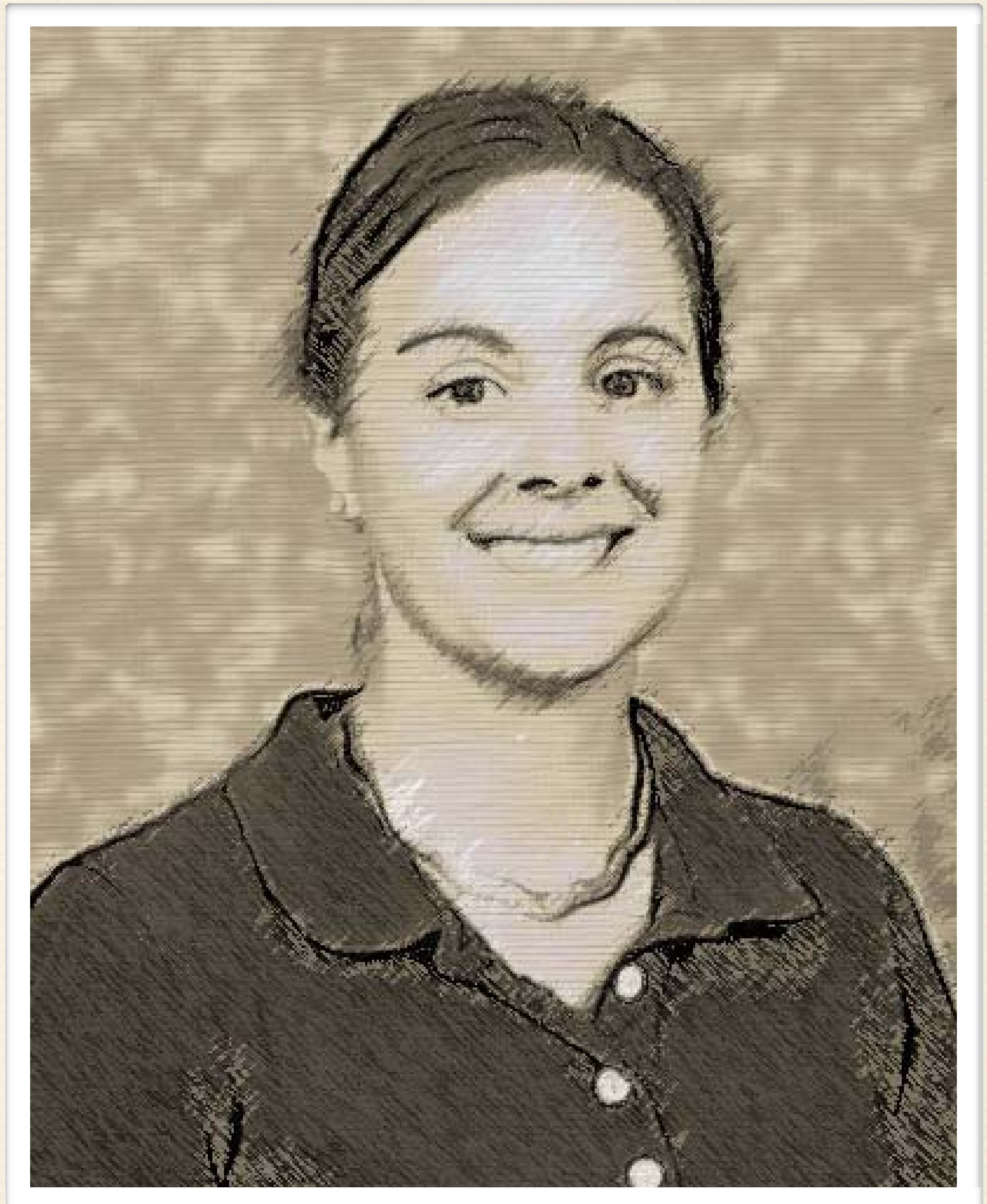


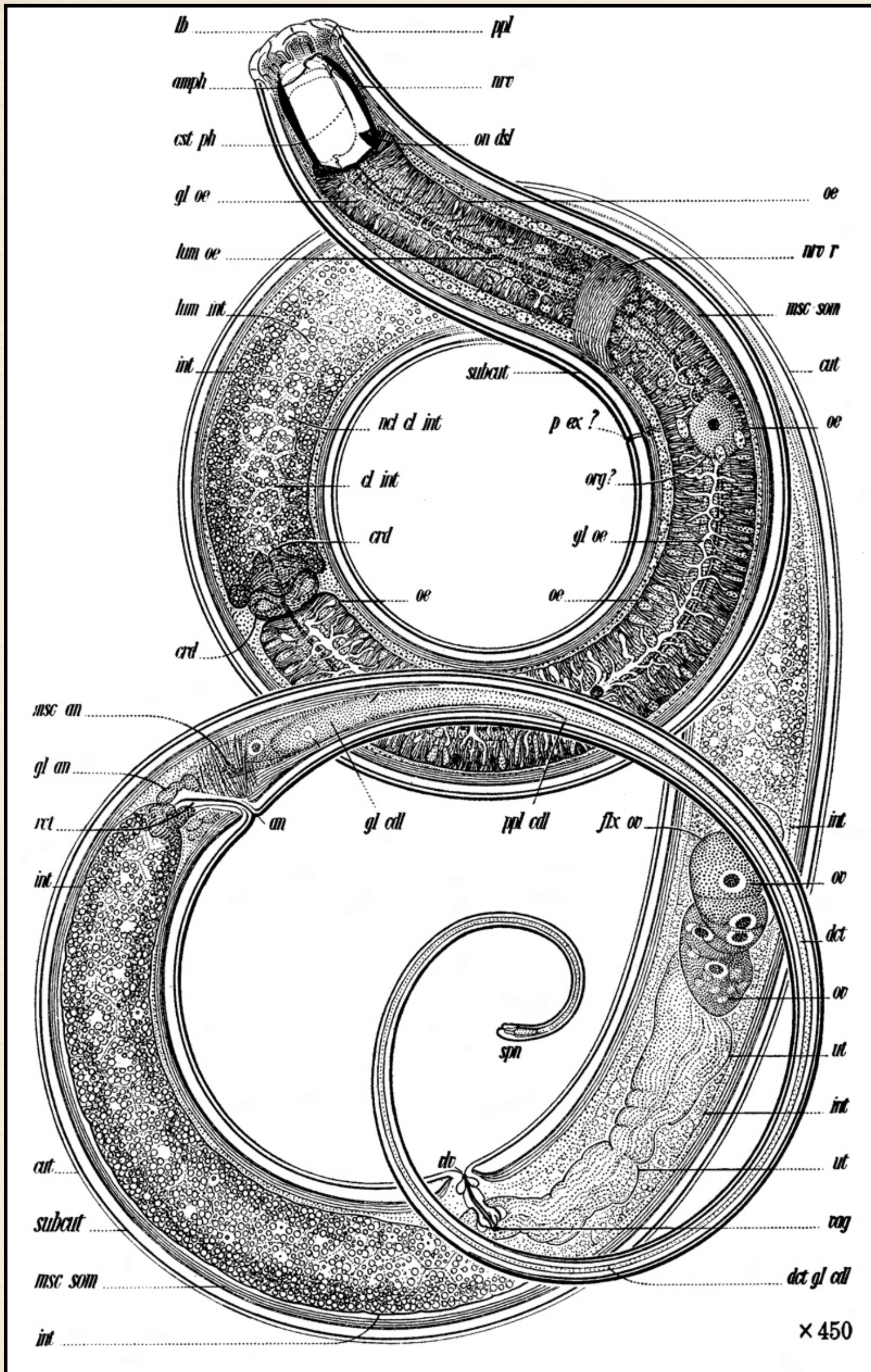



- There is no place like a museum with a collection of real specimens!
- Unfortunately the “permanent” slides are not very permanent
- They are too fragile and valuable to be used as teaching materials
- Drawings often “substitute” for type specimens



- Drawings are useful but vary according to the skill of the artist





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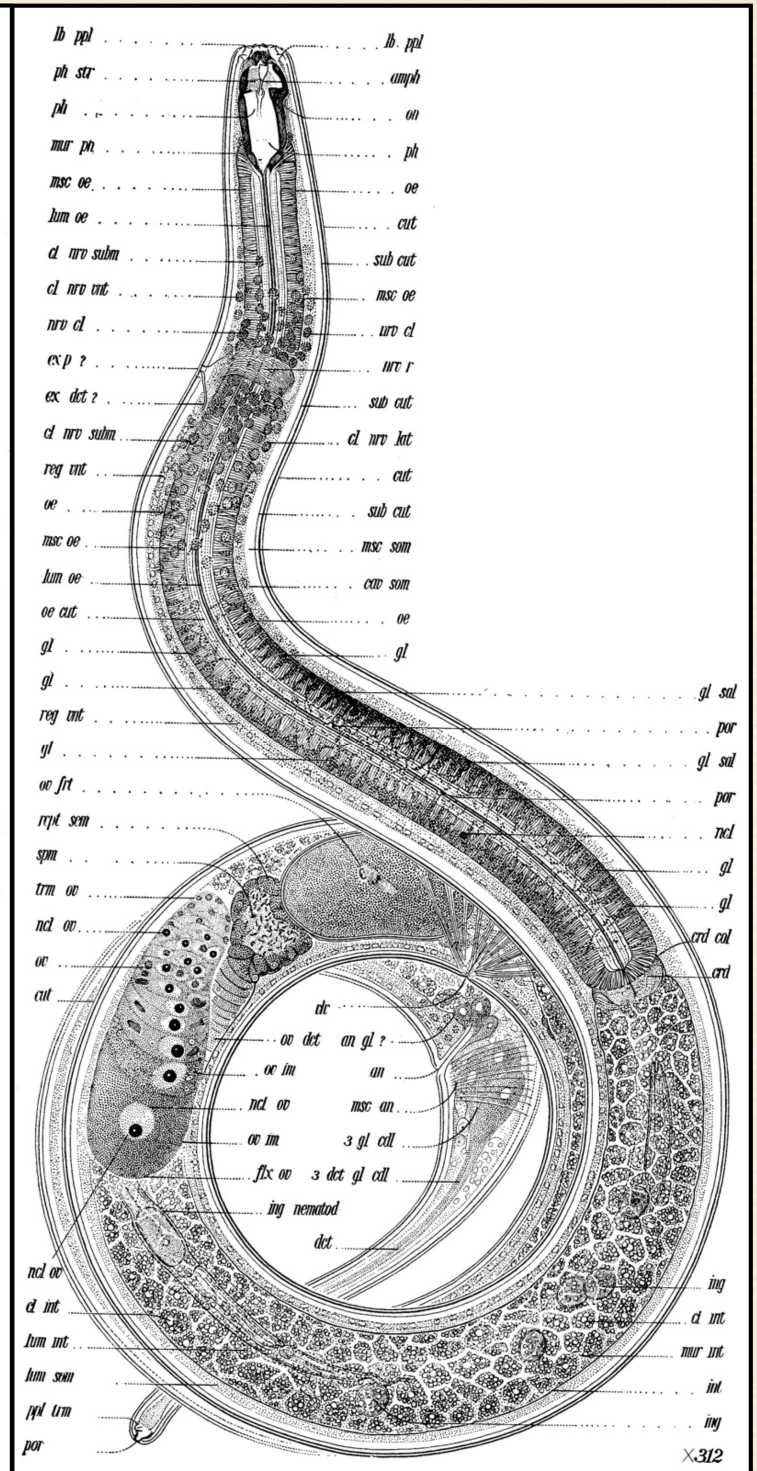

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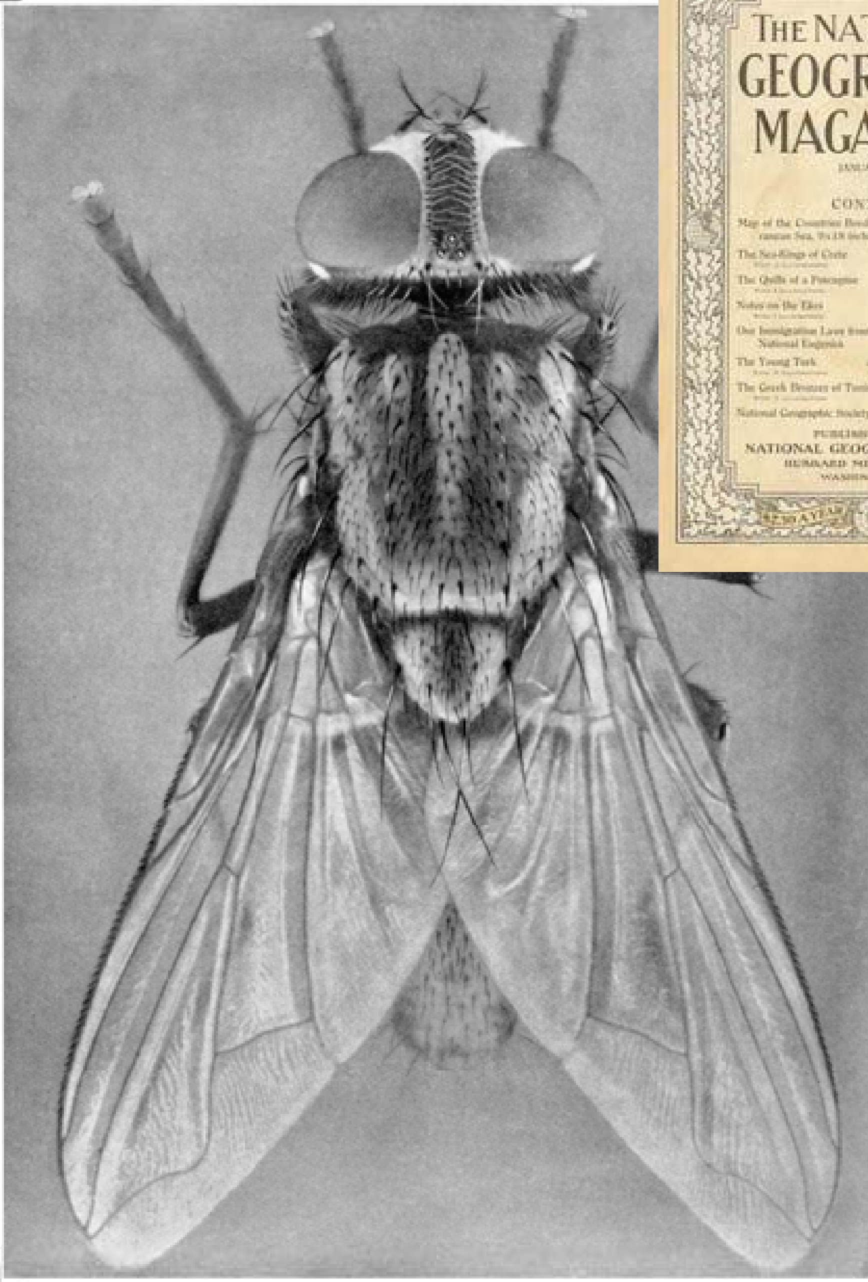

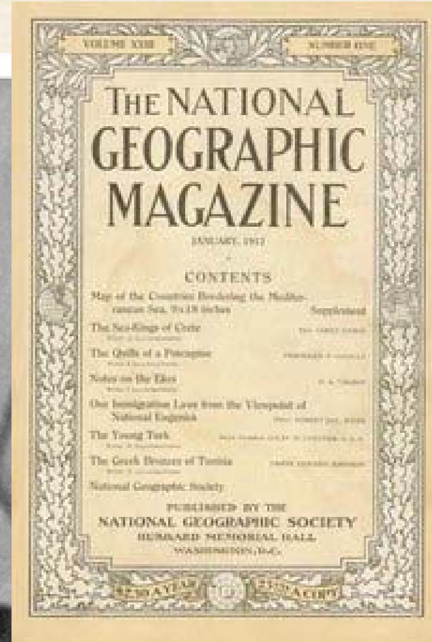

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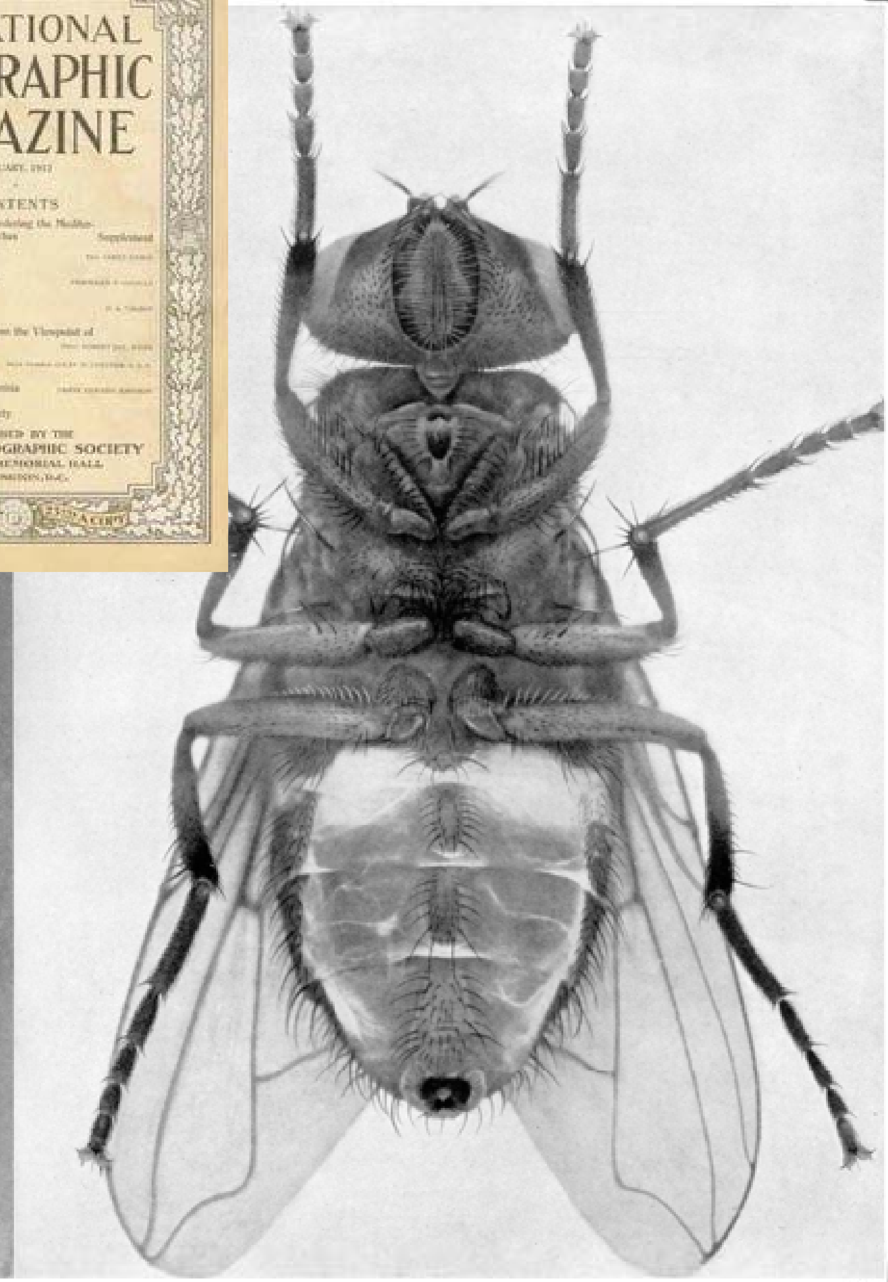
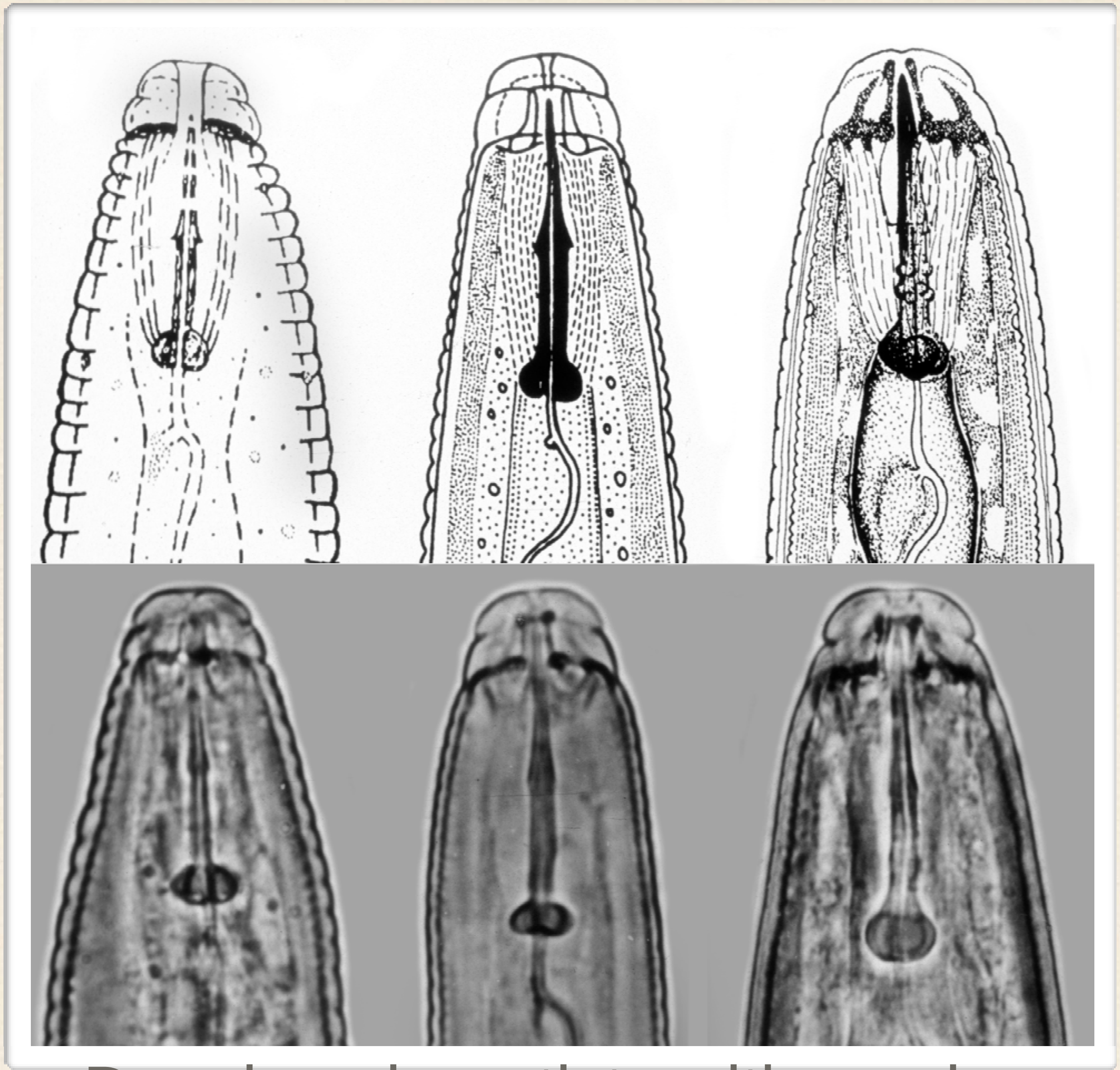




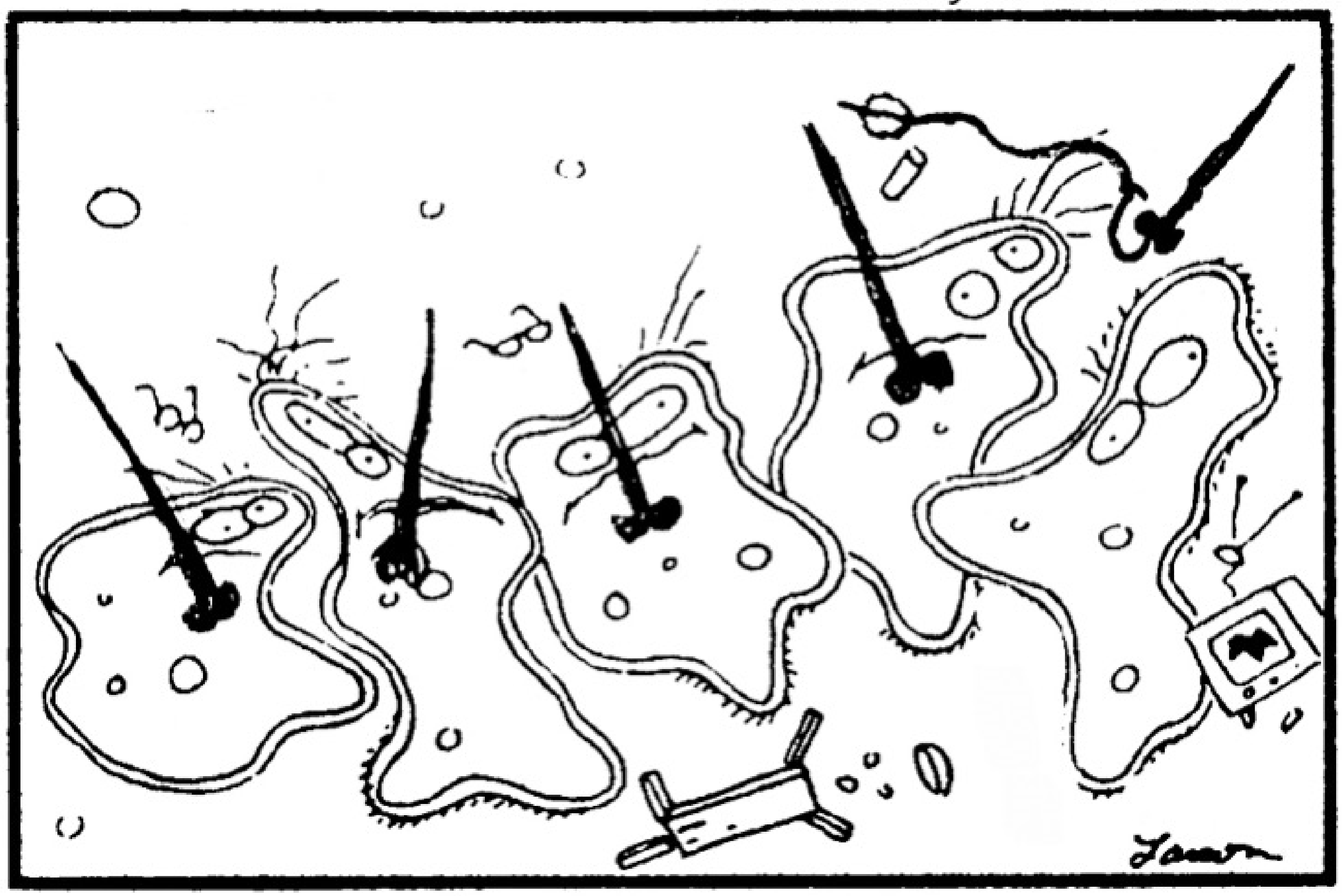
Drawings by artists with varying levels of talent compared to photographs

# Can’t Draw Anymore

Francis Bacon finally says what I’ve been saying

**Far Side** By Gary Larson

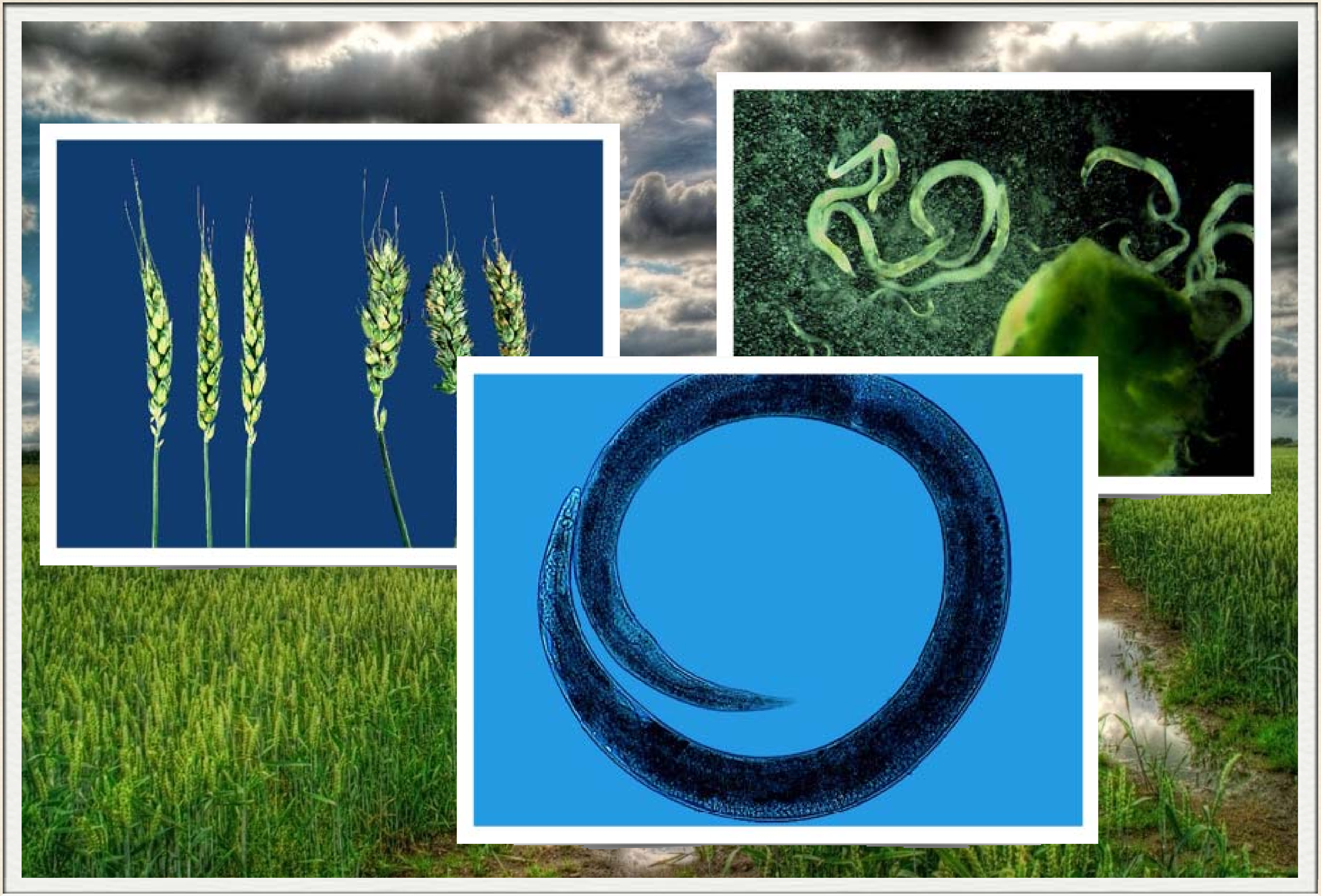
Modified by J. D. Eisenback



**Life on a microscope slide**

Specimen preparation is the first step to successful observation

Dead specimens obviously look similar to live ones; but they quickly fade to just a resemblance



High Dynamic Range (HDR) photography

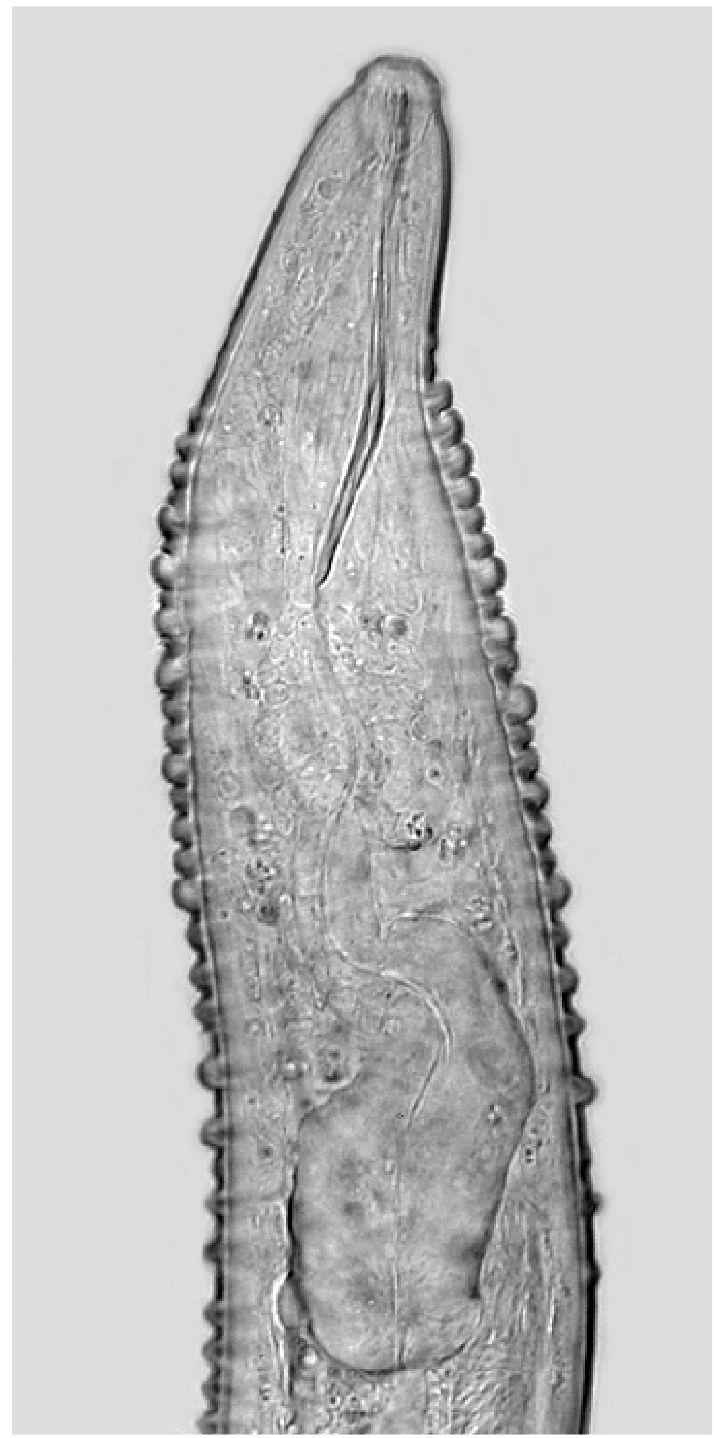
- Drawings are useful but vary according to the skill of the artist.

- Videos are helpful but limited in resolution.

- Megapixel mosaic photomicrographs of live specimens are most like the original.

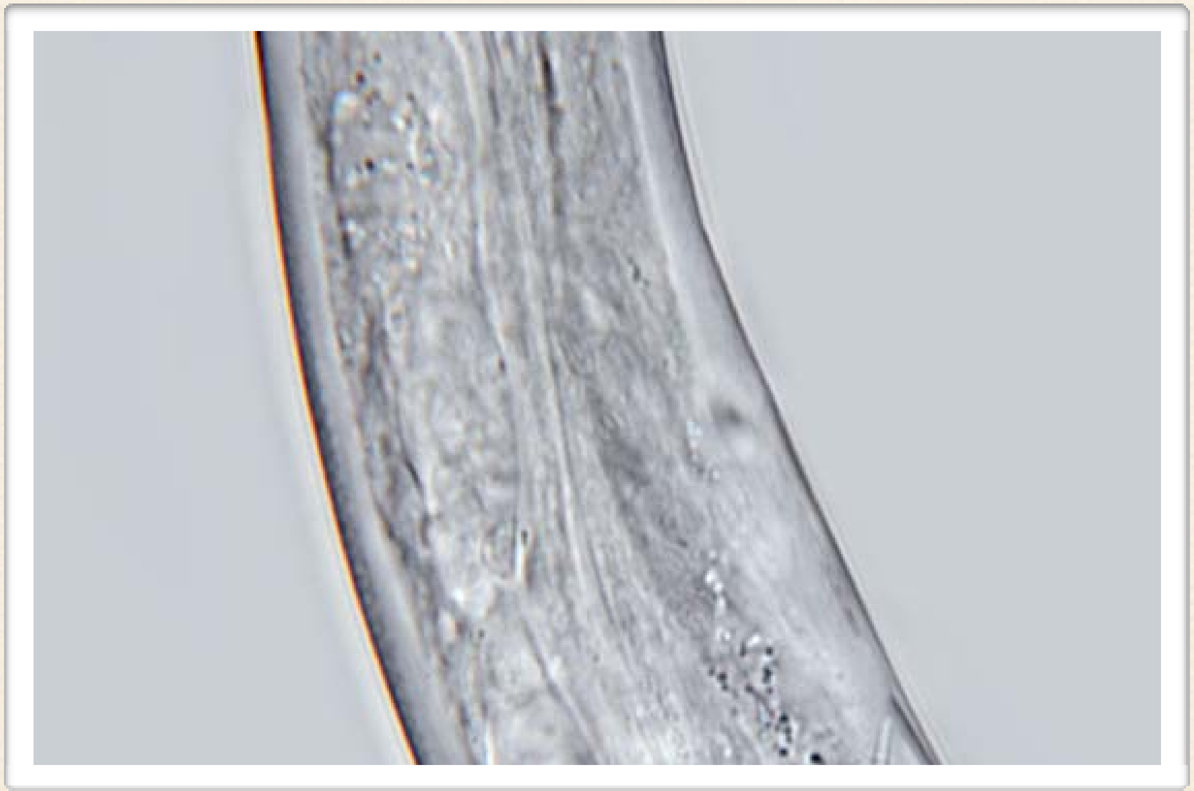
# “Virtual” Type Specimens

- Megapixel mosaic micrographs are most like the real thing
- Photographs of living, albeit paralyzed, specimens need little interpretation
- Additional photos and drawings can contribute to the type
- Perhaps these “virtual type specimens” can substitute for the original types?




Digital images open up a whole new world of possibilities

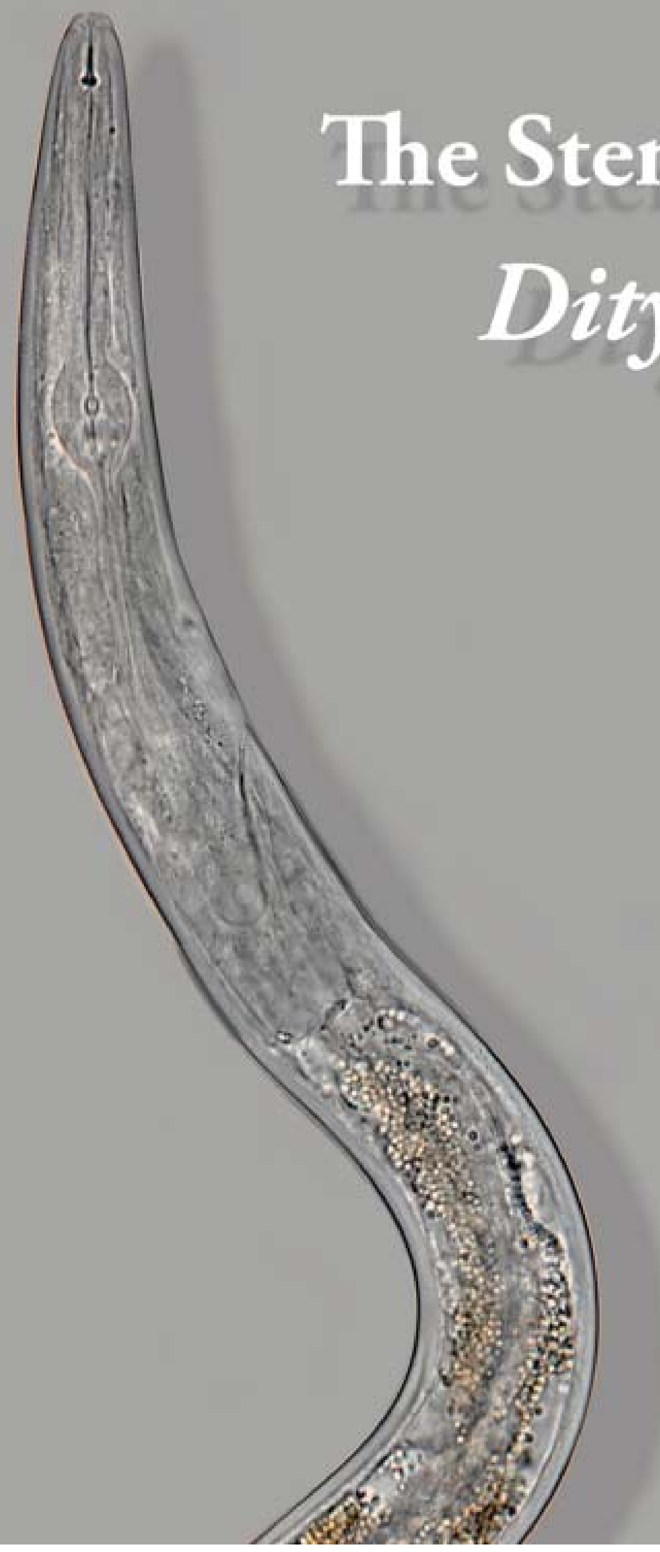
# Megapixel Mosaic Micrographs



Making mosaic pictures are much easier with digital images

# The Stem and Bulb Nematode

## *Ditylenchus dipsaci*

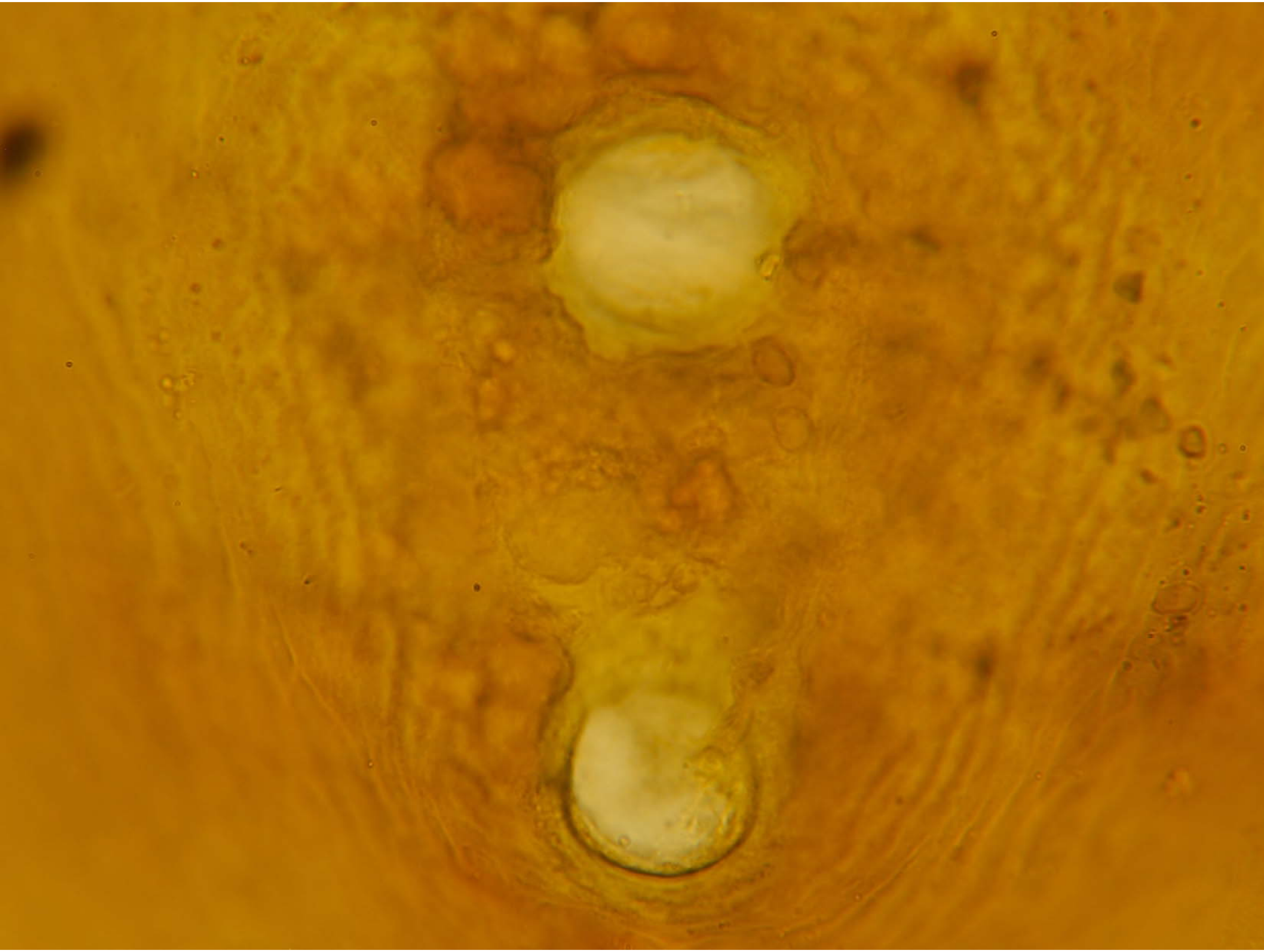


# Mosaic Micrographs

- The resulting image is a 200-600 megapixel mosaic photomicrograph of spectacular resolution and clarity
- Viewed on a computer at a resolution of 72 dpi, they are magnified 10,000 times
- Printed at 300 dpi, they are magnified 3,000 times

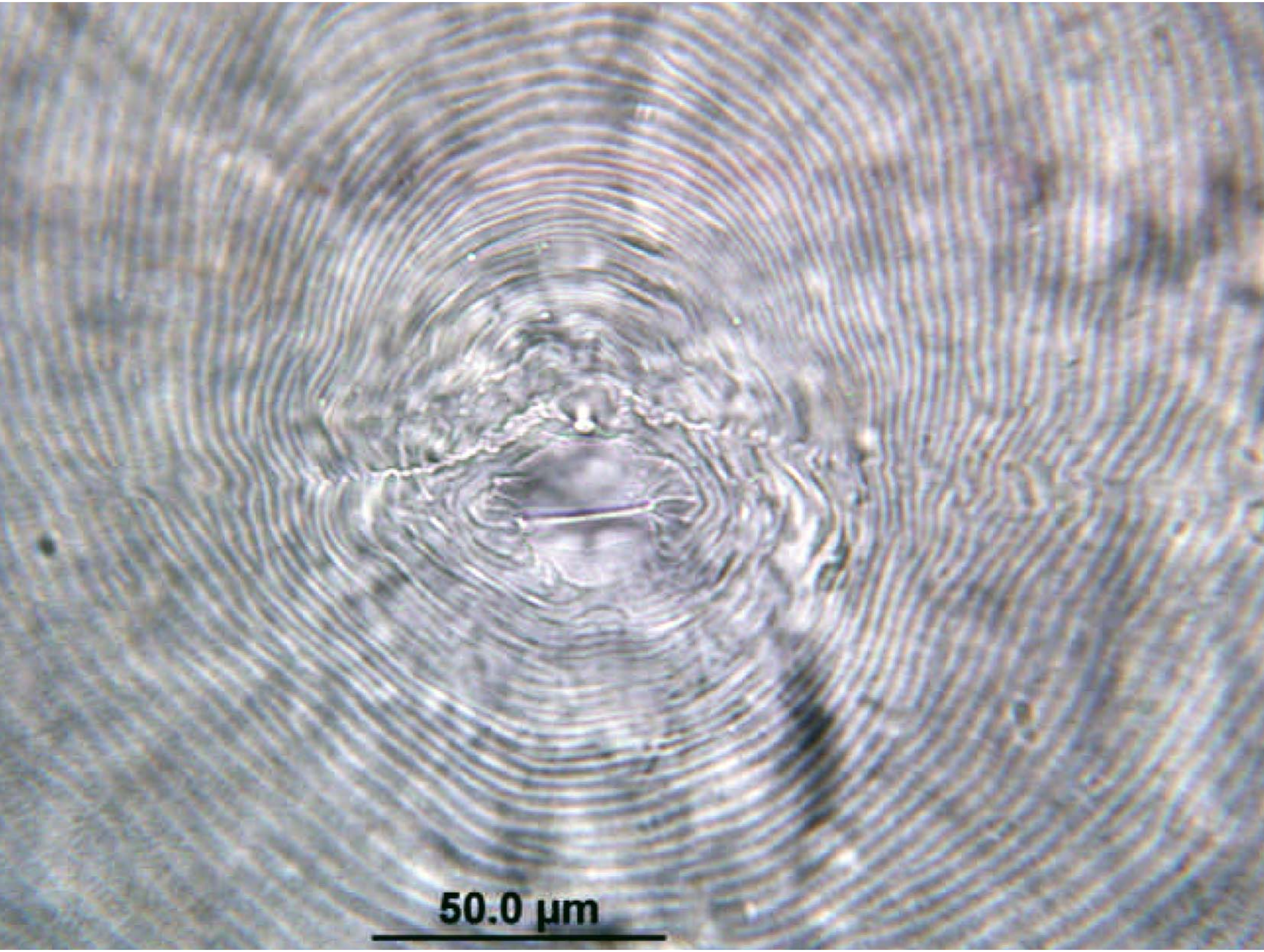
# Layers of Focus

# Layers of Focus




18 images stacked
with Helicon Focus


TOMATO
Rutgers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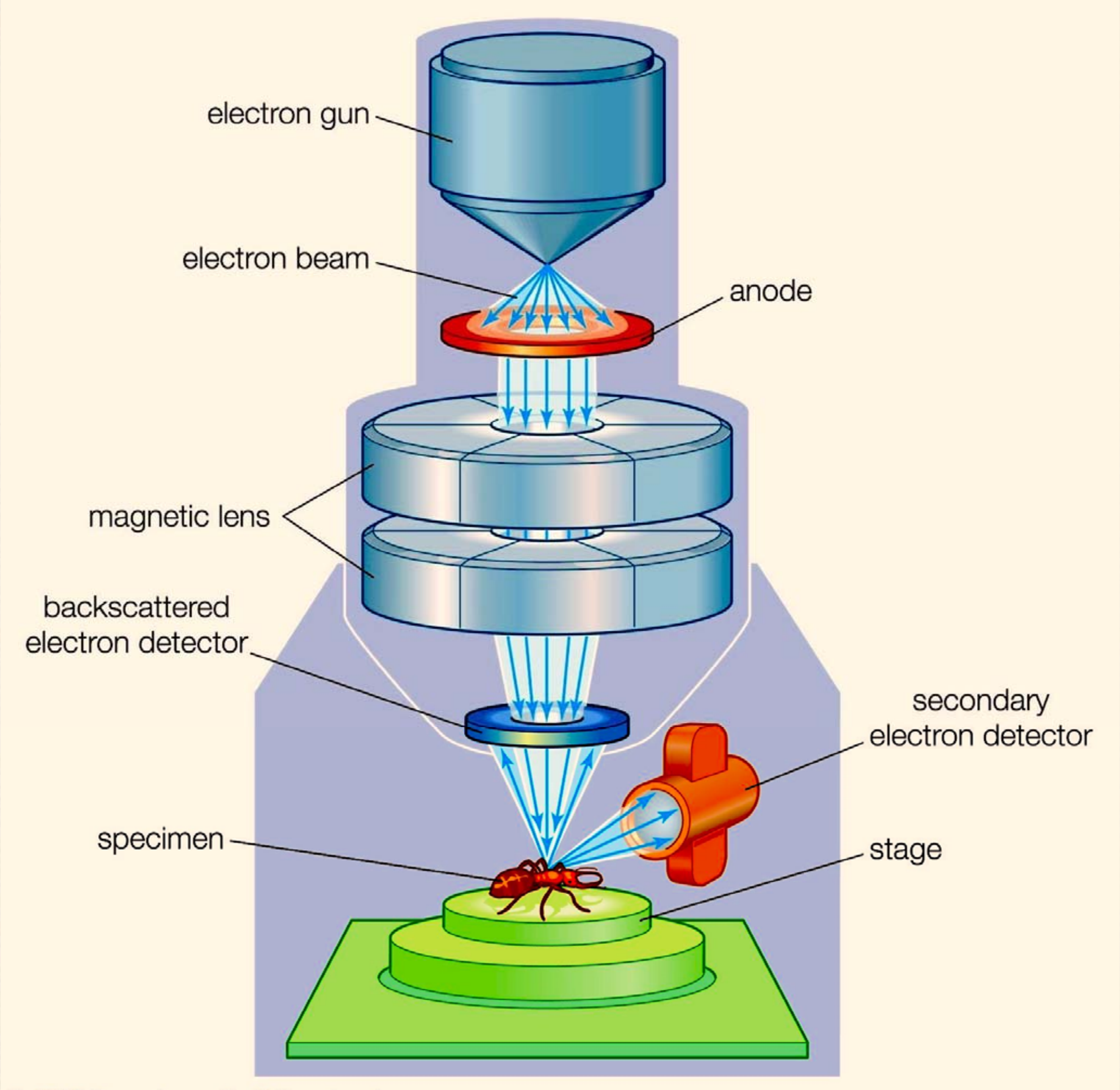
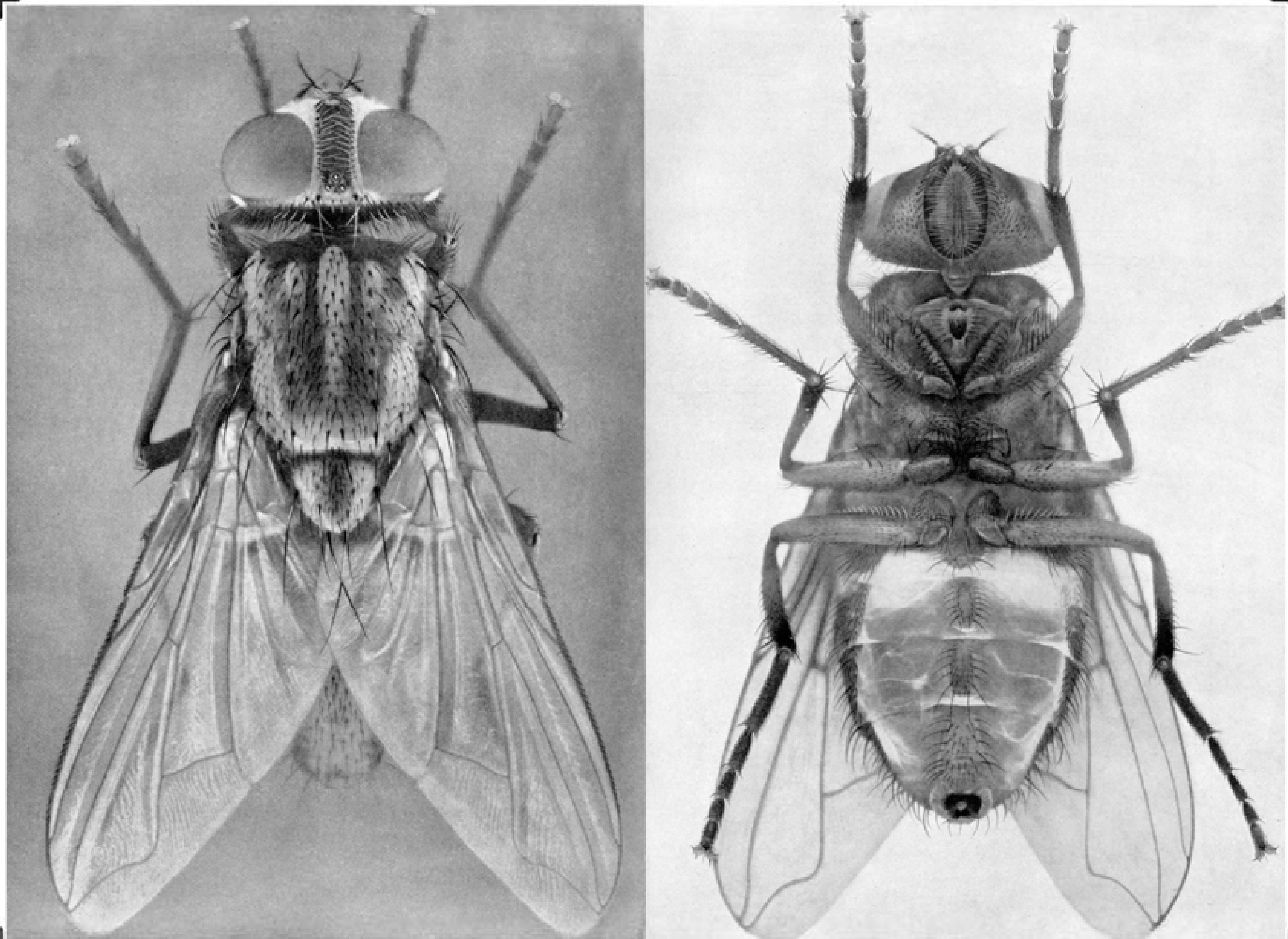
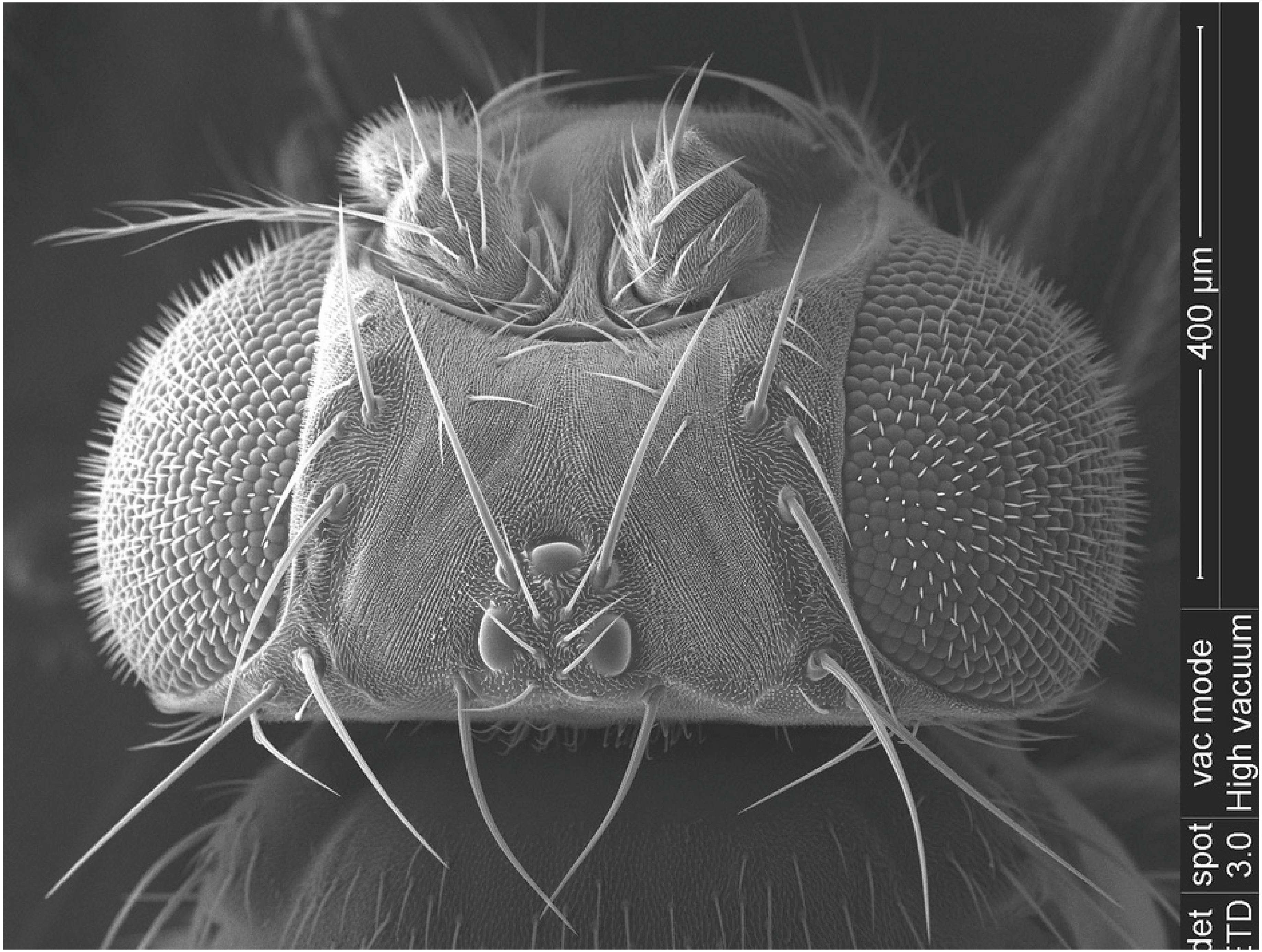



# Scanning Electron Microscope

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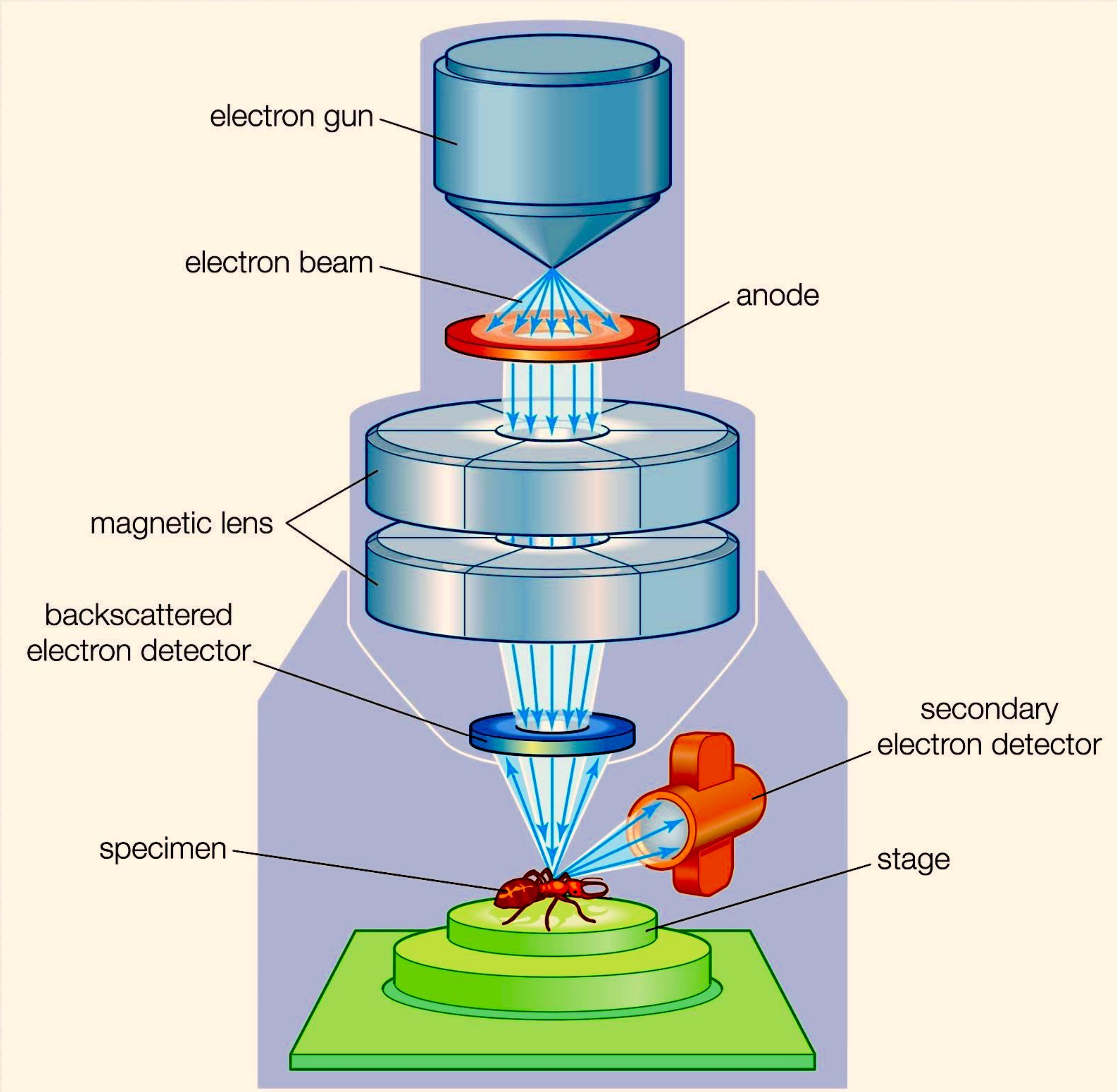
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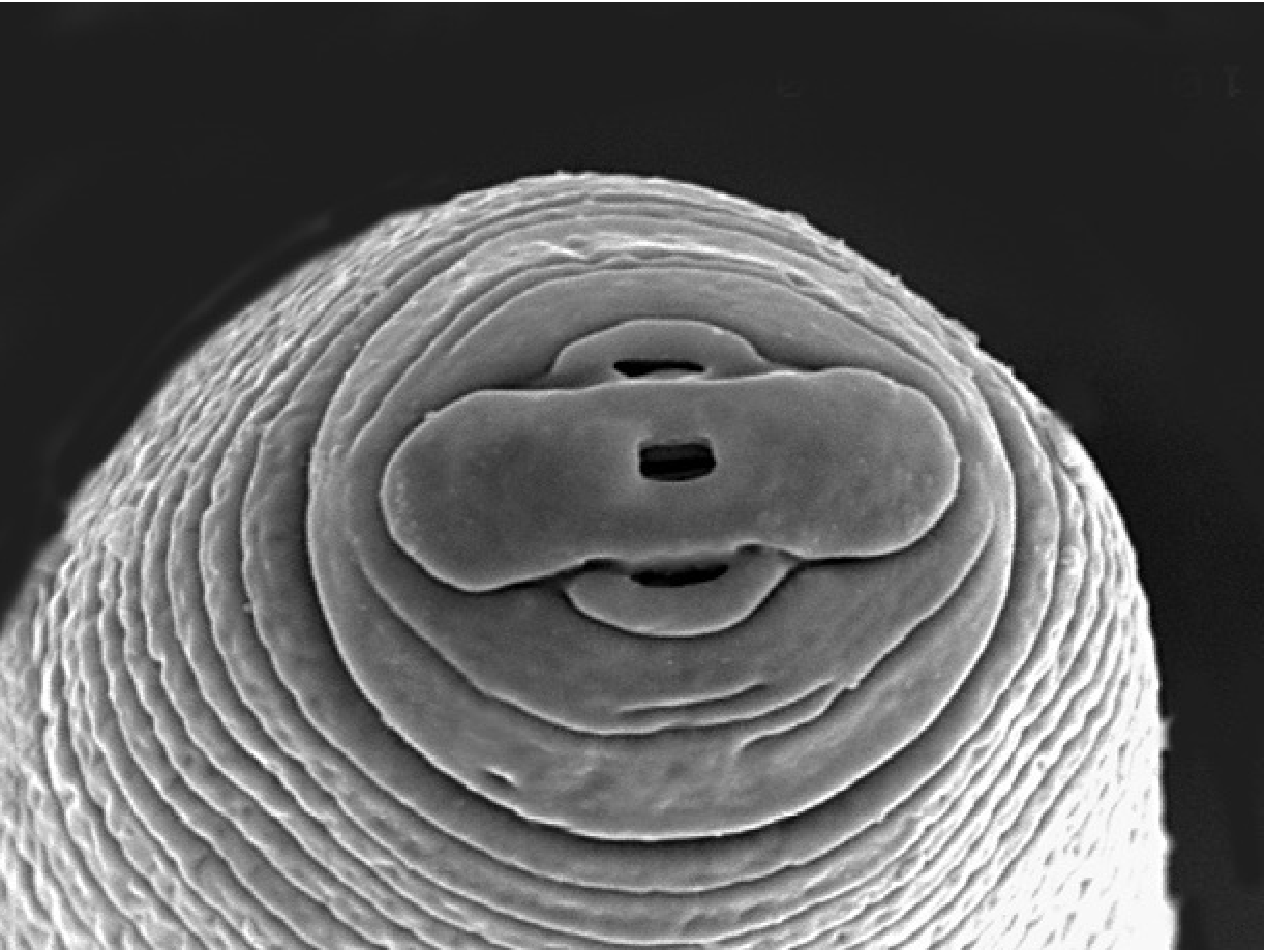
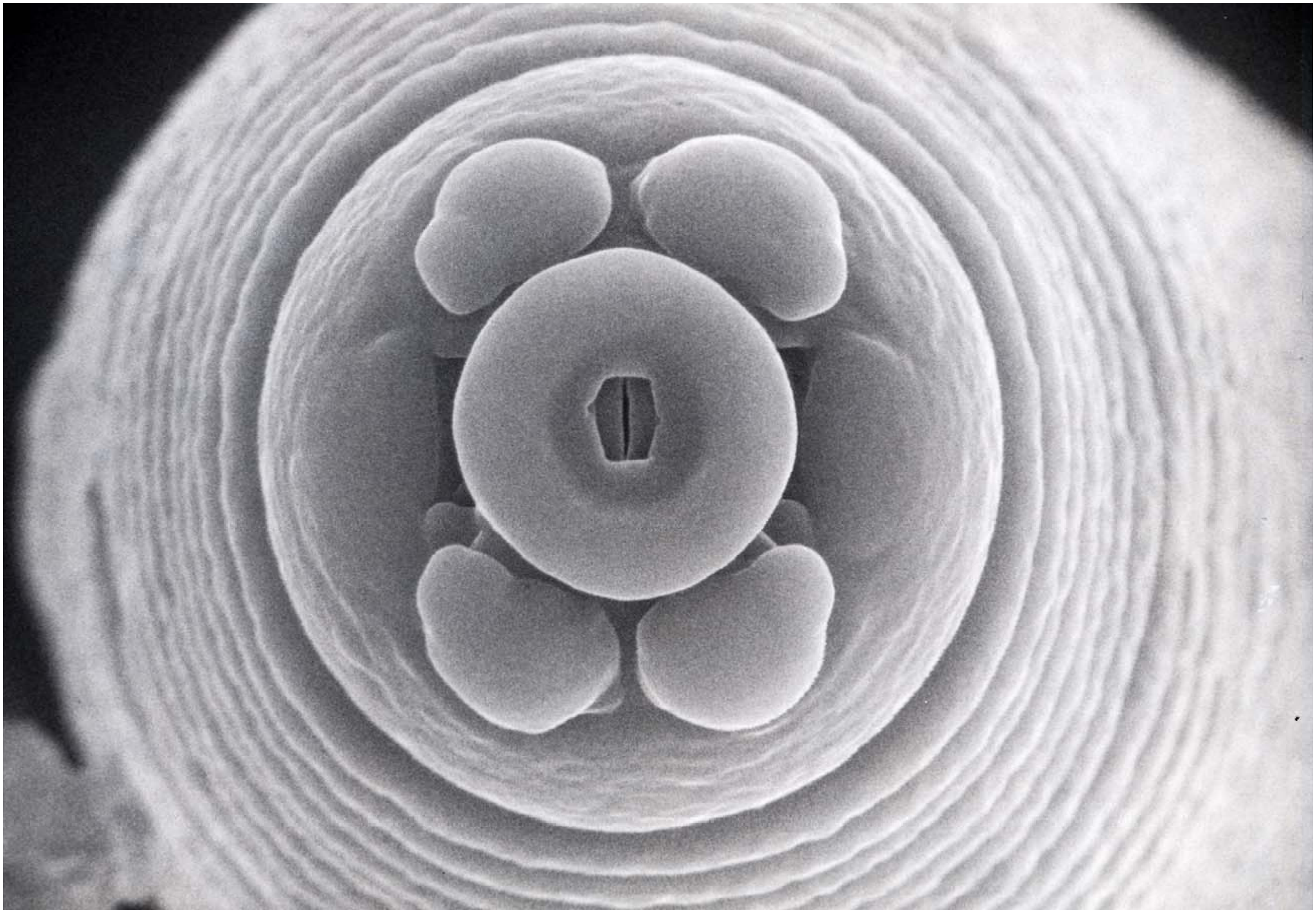


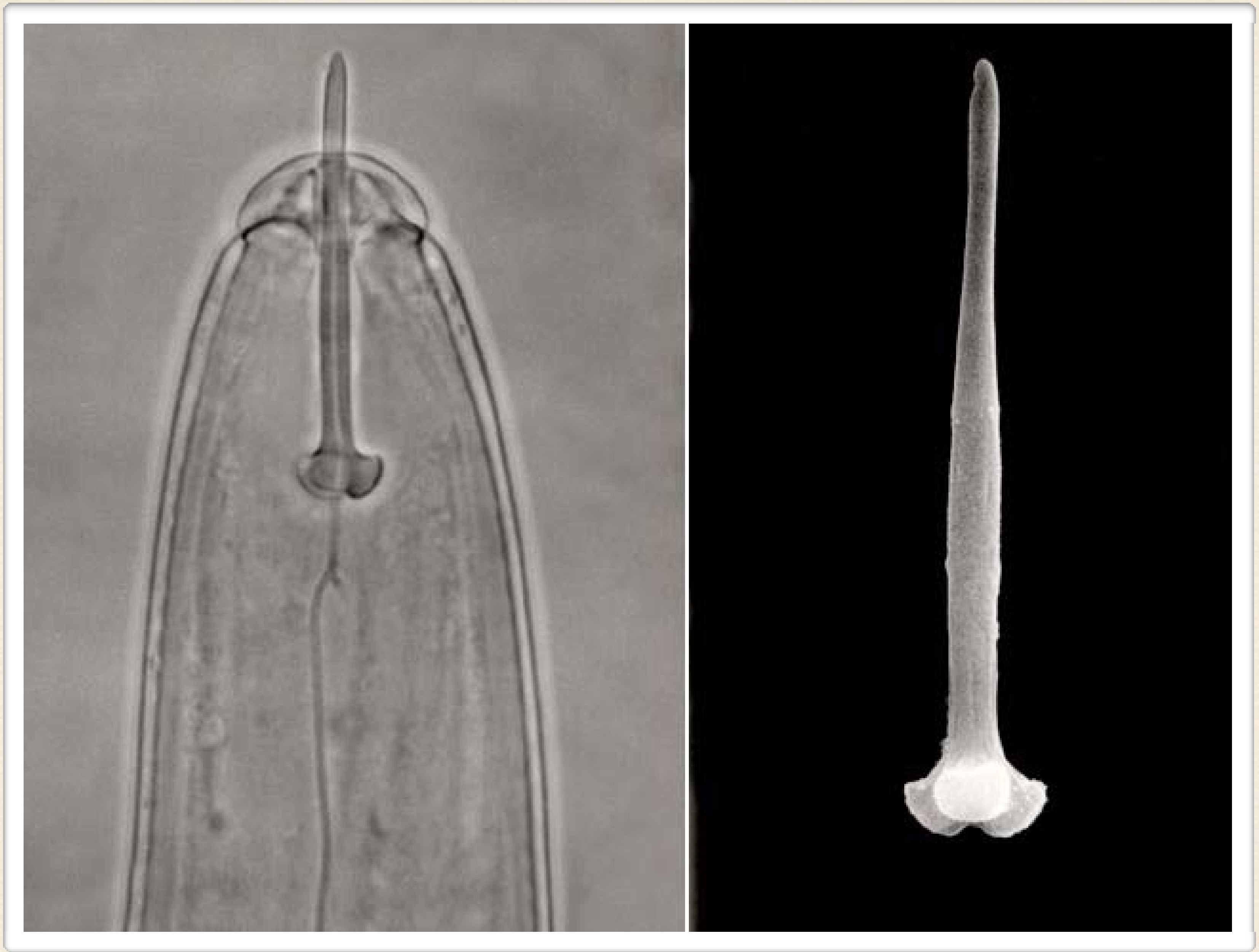
# Scanning Electron Microscopy

- Careful fixation, dehydration, and drying procedures are necessary for adequate observation
- SEM reveals only surface details
- SEM is useful for specimen morphology and nematode/plant interactions


mature female of reniform nematode (*Rotylenchulus reniform*

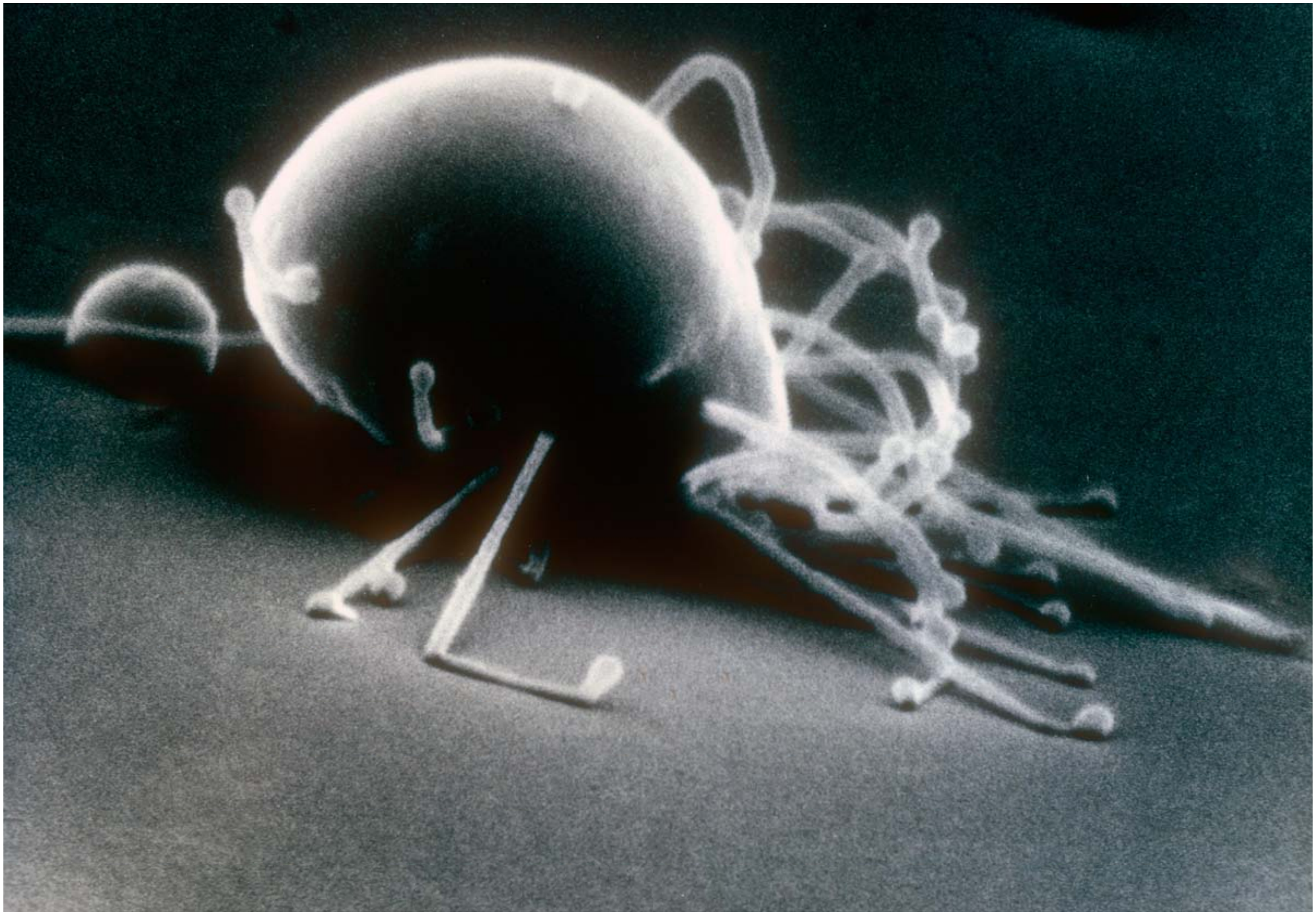


he head of a male root-knot nematode (*Meloidogyne kikuyens*

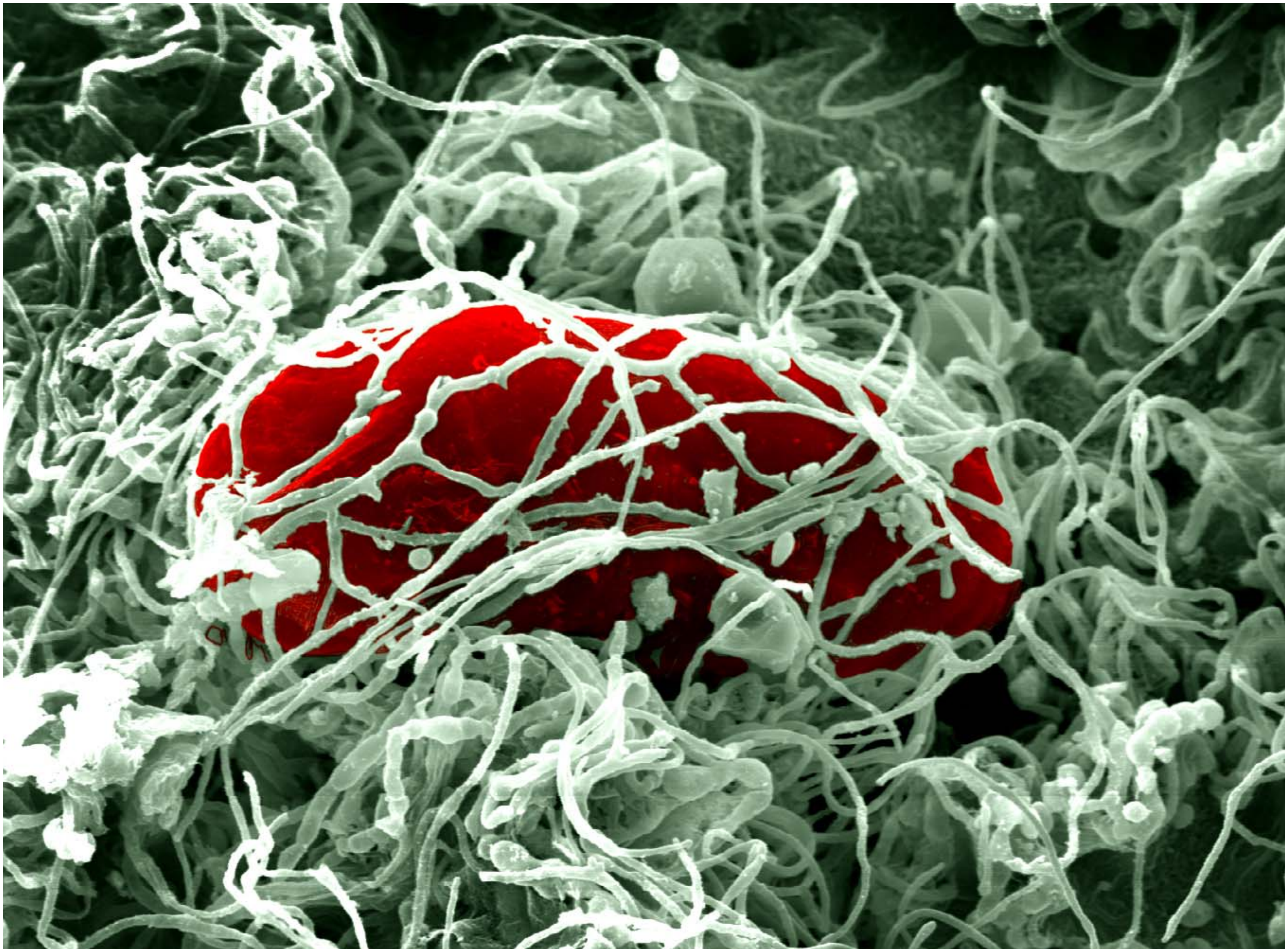


The head of a tobacco cyst juvenile and stylet (*Globodera tabacum*)

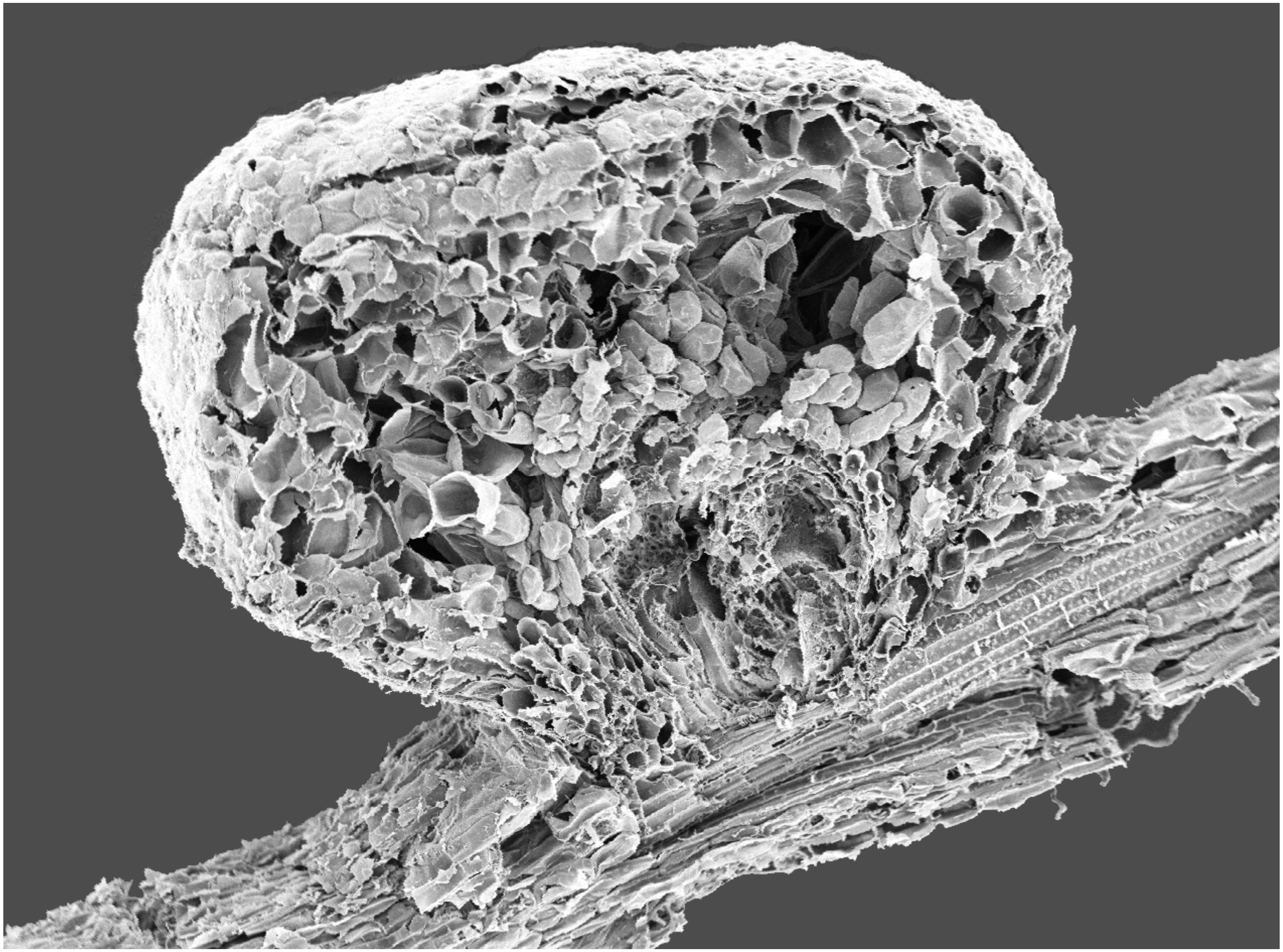

SEM of dissected stylets
from several plant-
parasitic nematodes



A sperm dissected from the spermatheca of a female soybean cyst nematode (*Heterodera glycines*)

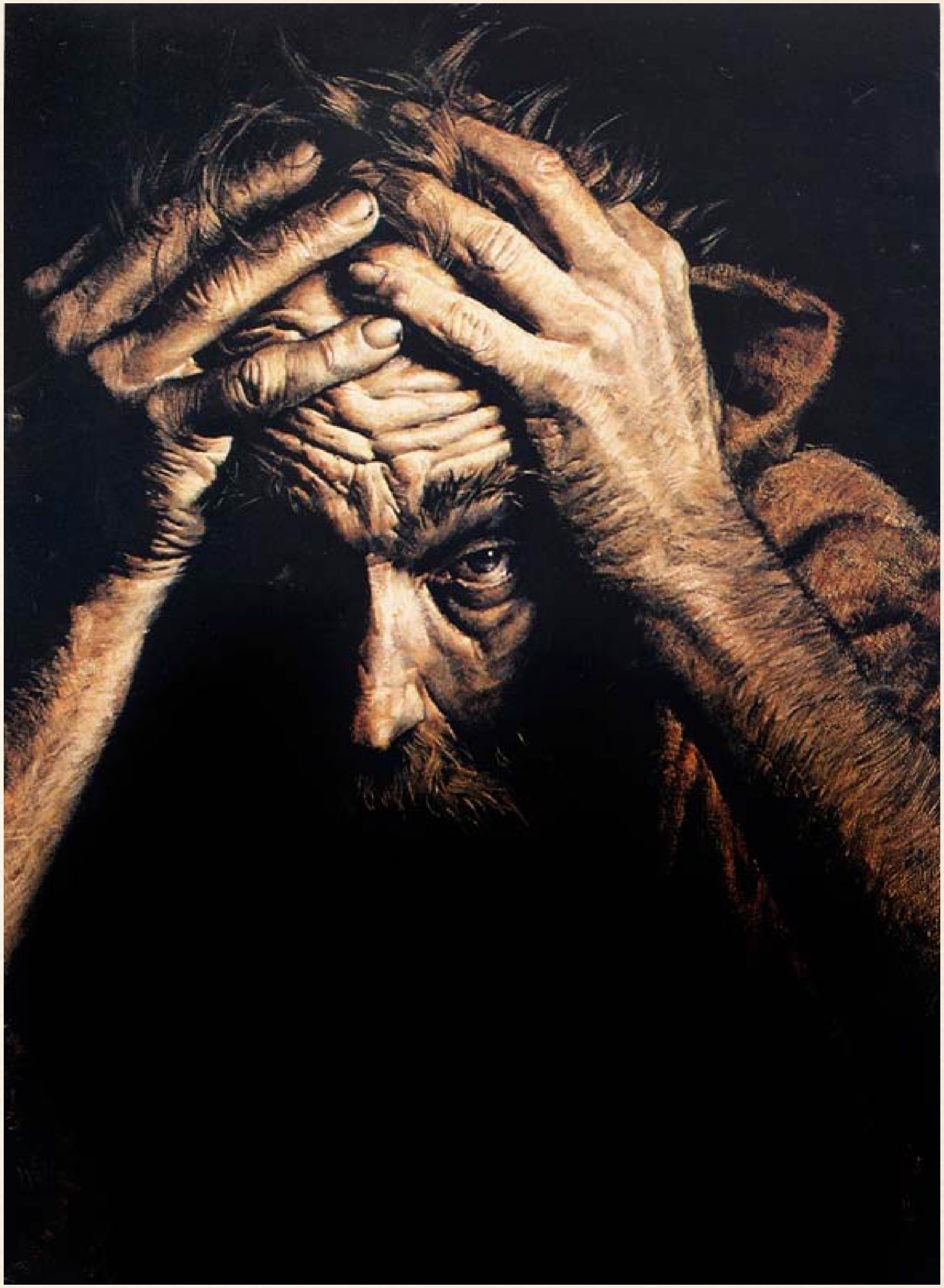


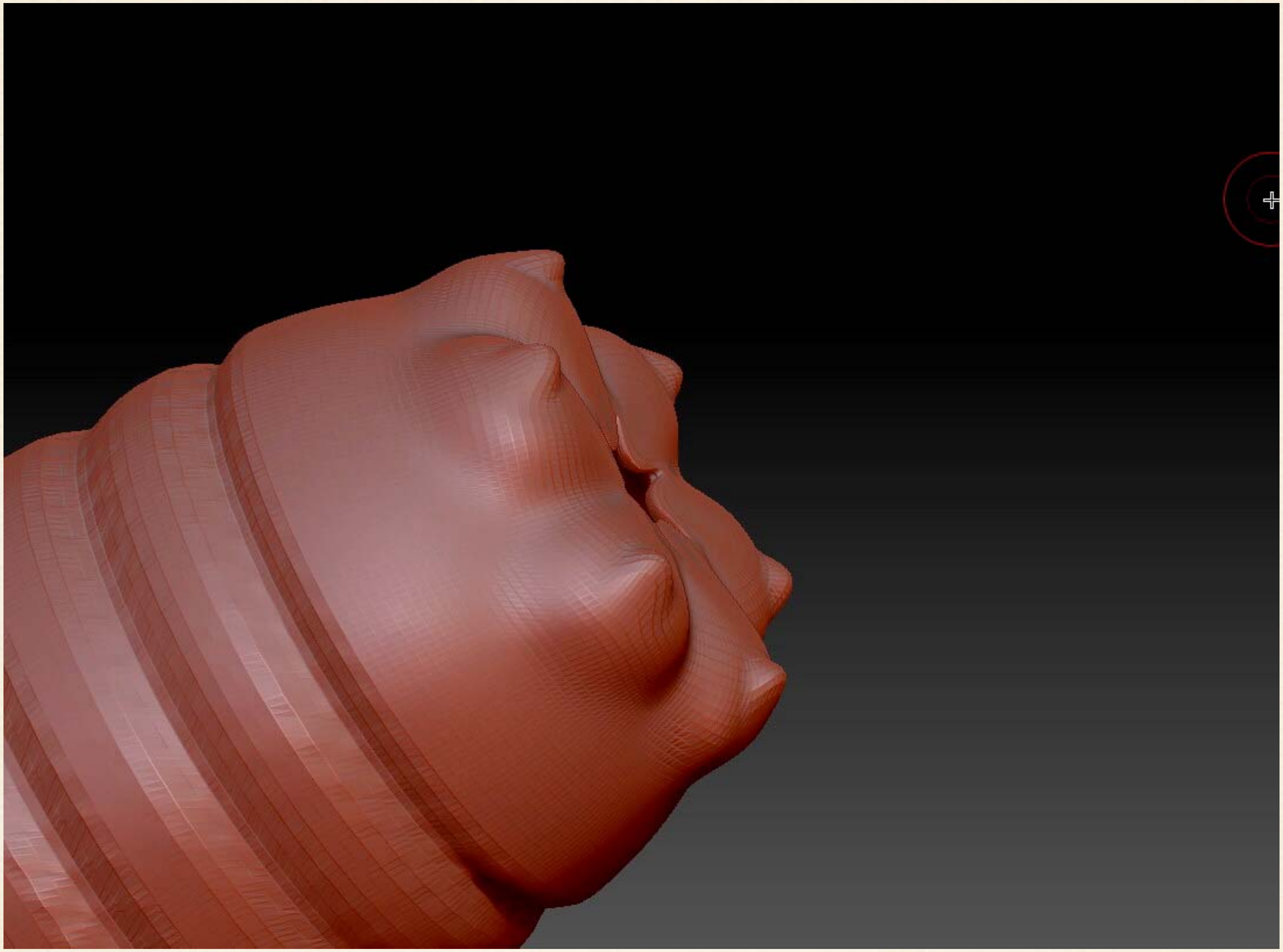
An egg of a root-knot nematode parasitized by a fungus



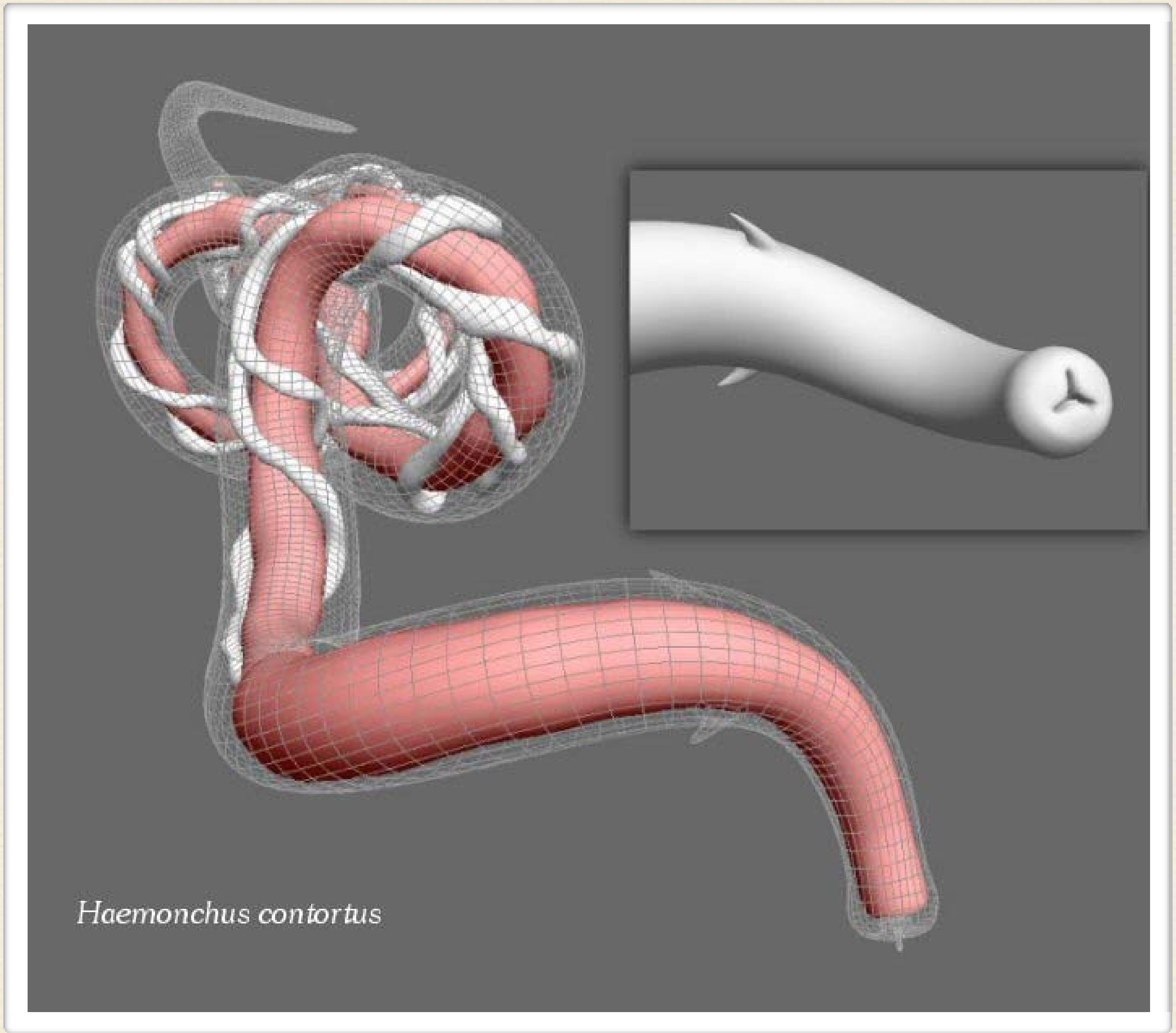
Nodule-like feeding site of *Meloidogyne kikuyensis* from sugarcane

- Help!!!
- My slide collection is deteriorating faster than I am
- How can I provide my students with the best specimens, even though I cannot always find all of the specimens that I need?



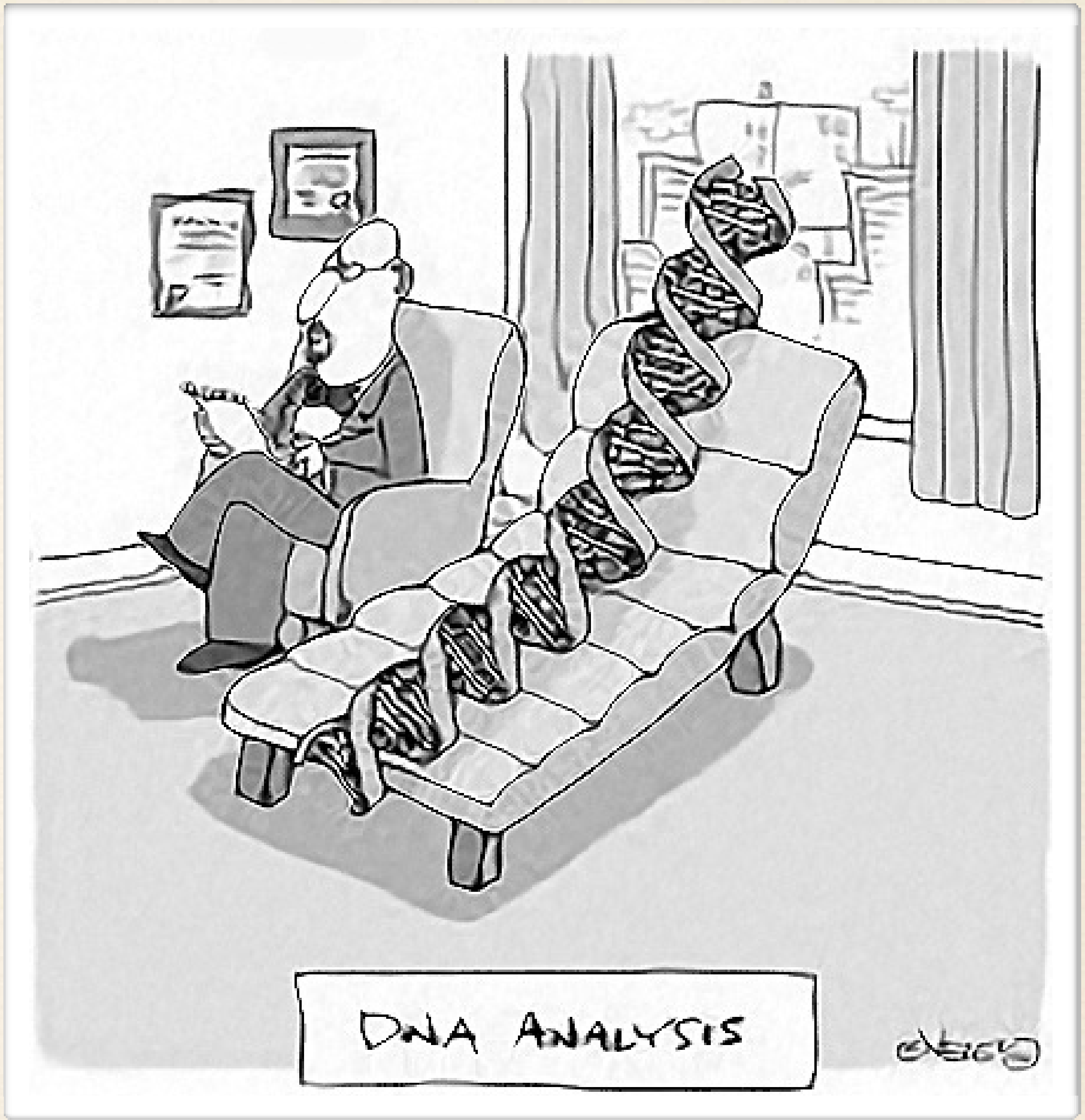


3D model of the head of a free-living nematode



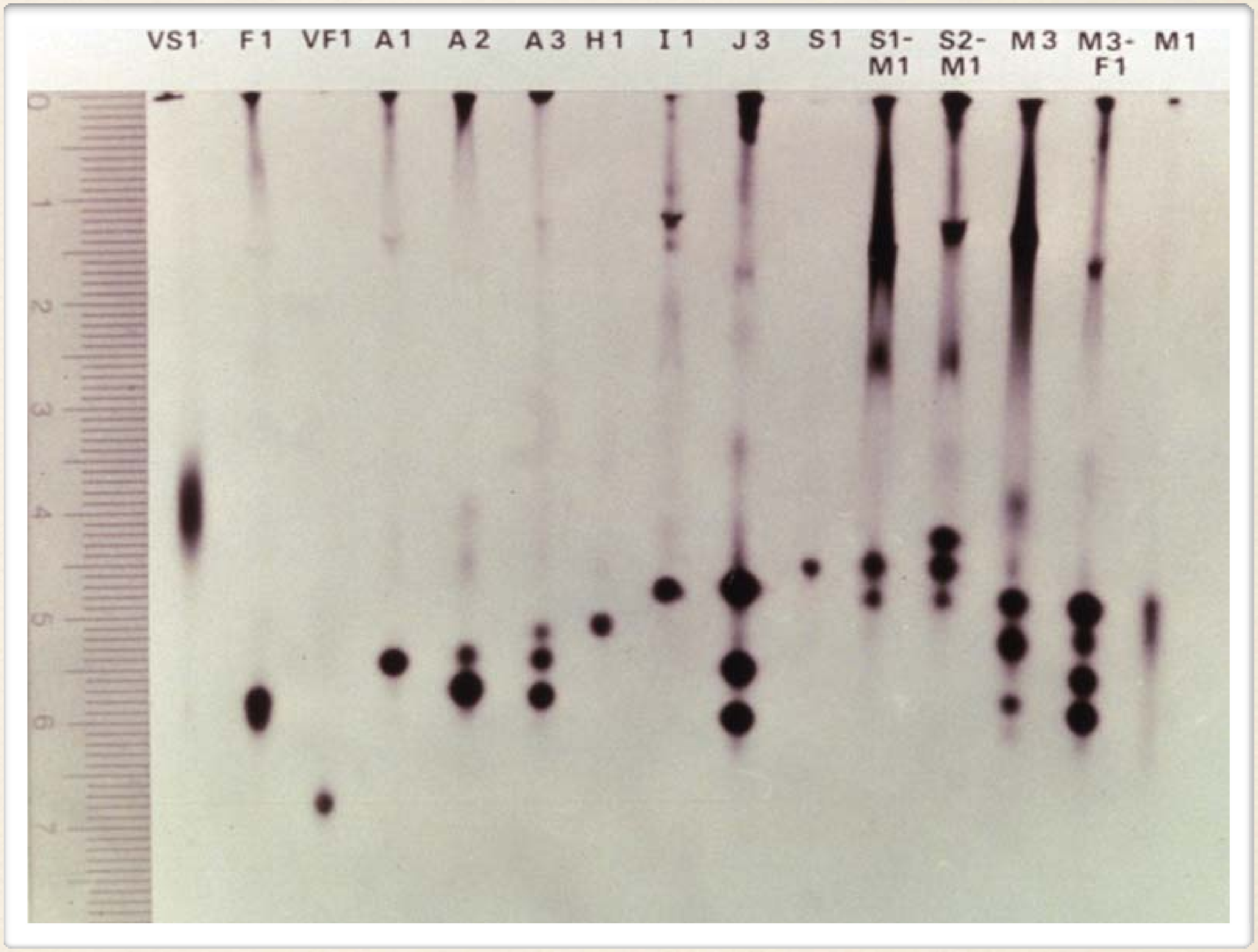

External morphology and internal anatomy can be visualized with 3D modeling techniques

# Molecular Techniques



# Esterases

- Unique banding patterns for many species
- However, since some species have the same esterase pattern, an additional stain for another protein is necessary, i.e. MDH

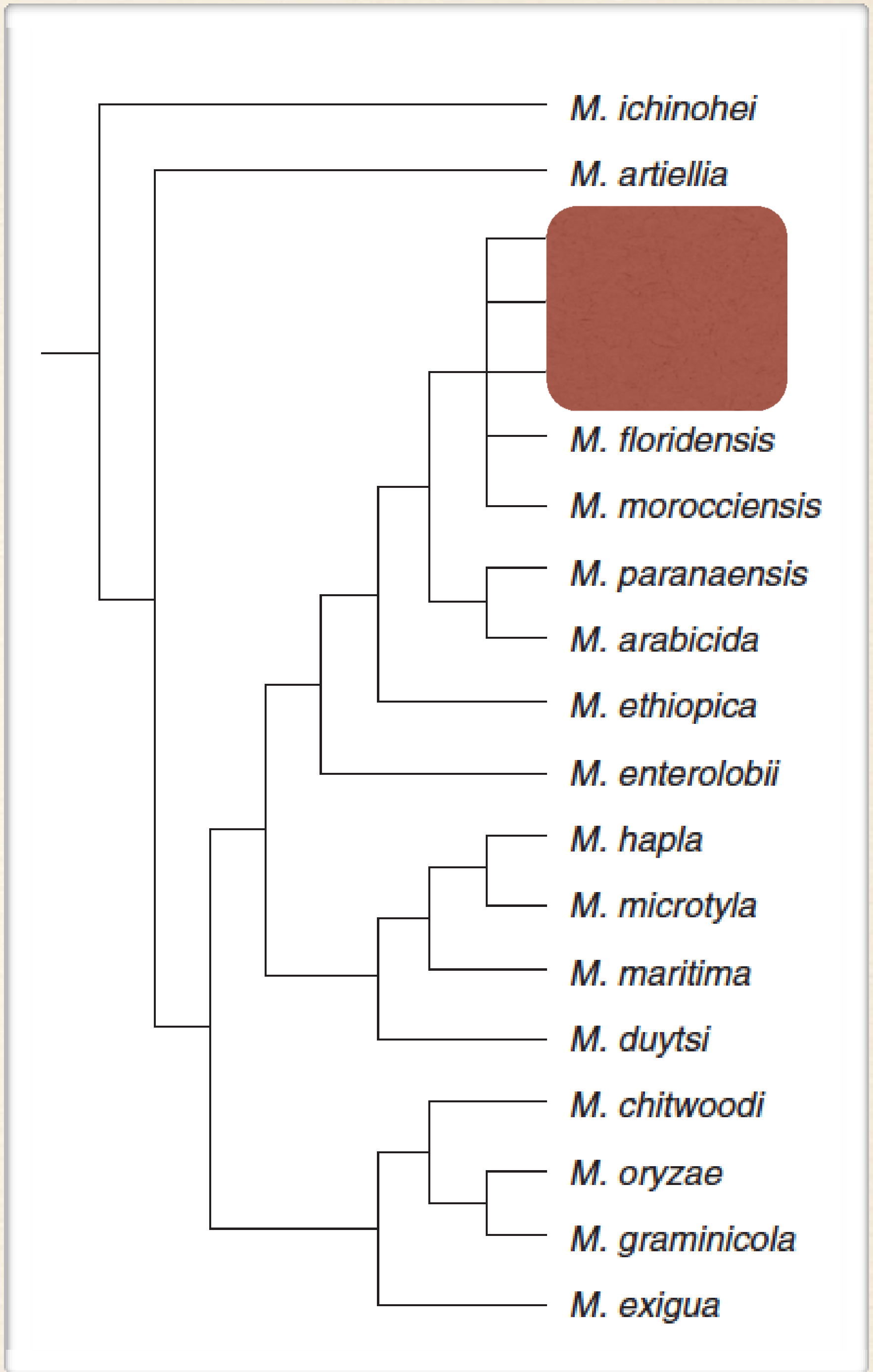


# Gene Sequences

- 18s rDNA
- 28s rDNA
- ITS rDNA
- IGS rDNA
- COII through IRNA mitochondrial DNA
- Elongation factor 1-alpha exon gene
- RNA polymerase 2 exon gene
- Dystrophin exon gene
- Major sperm protein gene
- Satellite DNA probes
- Species specific primers

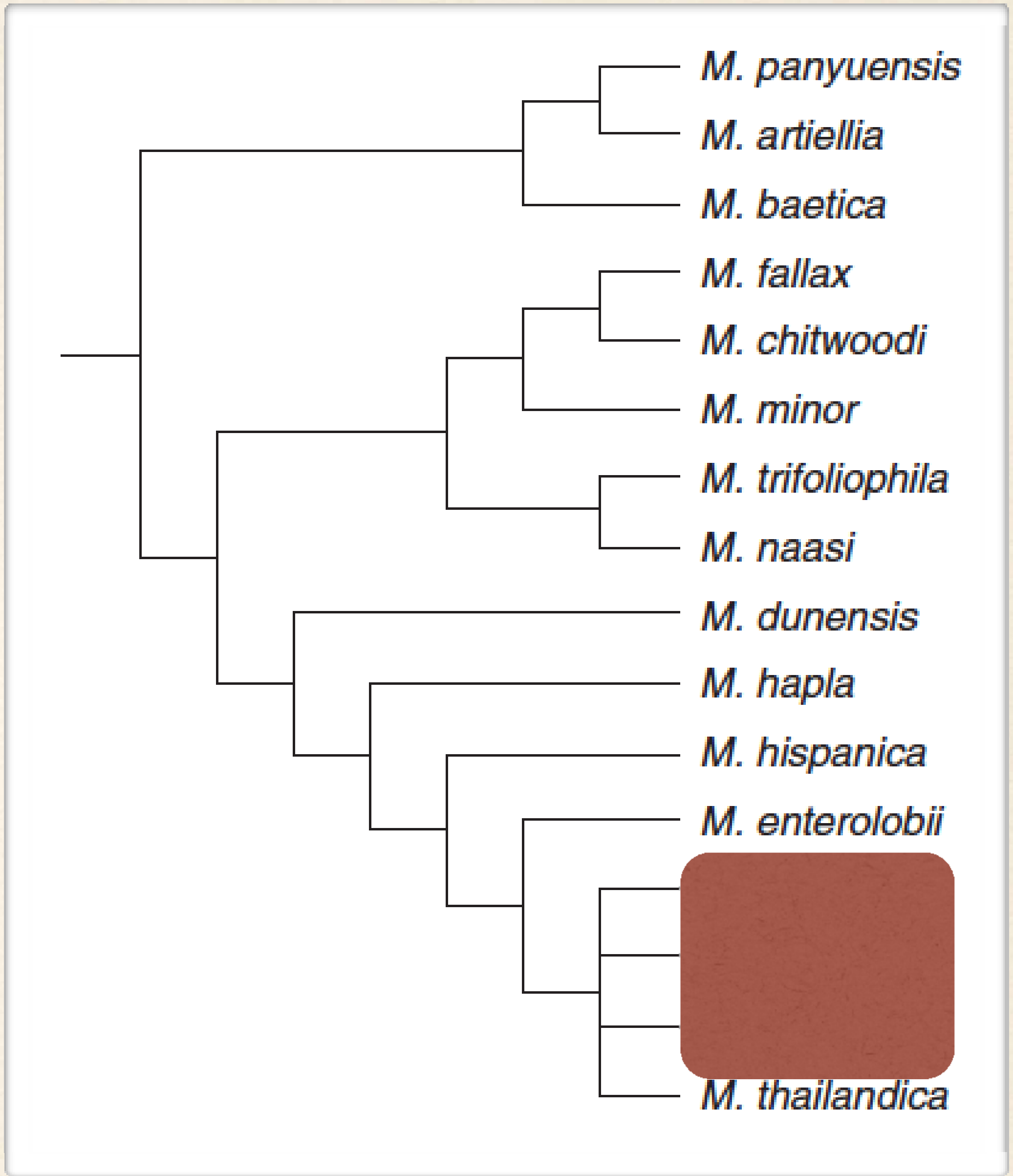
# Gene Sequences

- 18s rDNA phylogeny of *Meloidogyne* species
- The common species are on the same branch and have 100% similarities



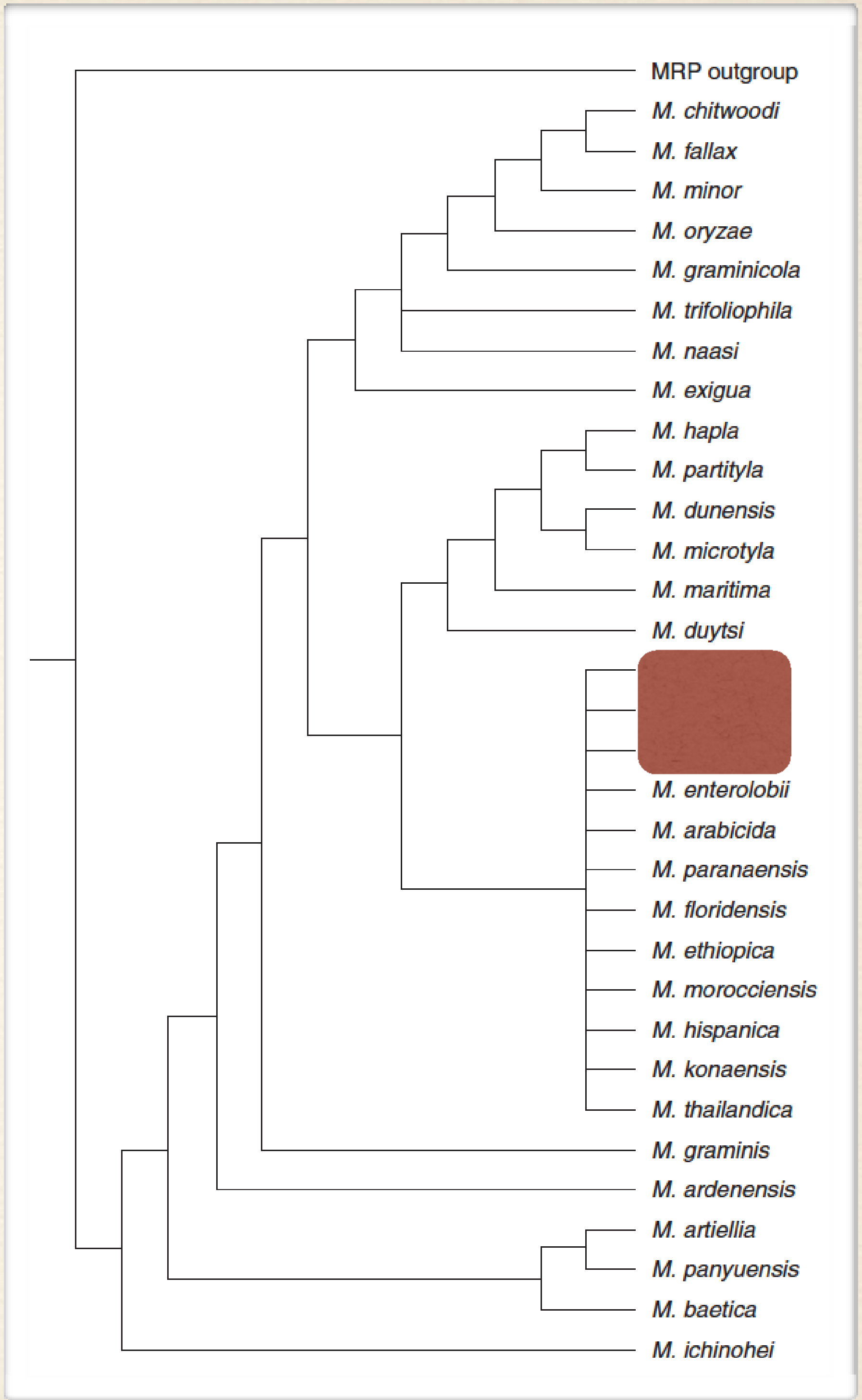
# Gene Sequences

- ITS rDNA phylogeny of *Meloidogyne* species
- The common species are on the same branch and have 100% similarities



# Gene Sequences

- Supertree solutions for seven independent source trees
- The common species are on the same branch and have 100% similarities



# Species specific primers

- From the nuclear or mitochondrial genome
- Useful for 1 second-stage juvenile
- Multiple assays are required

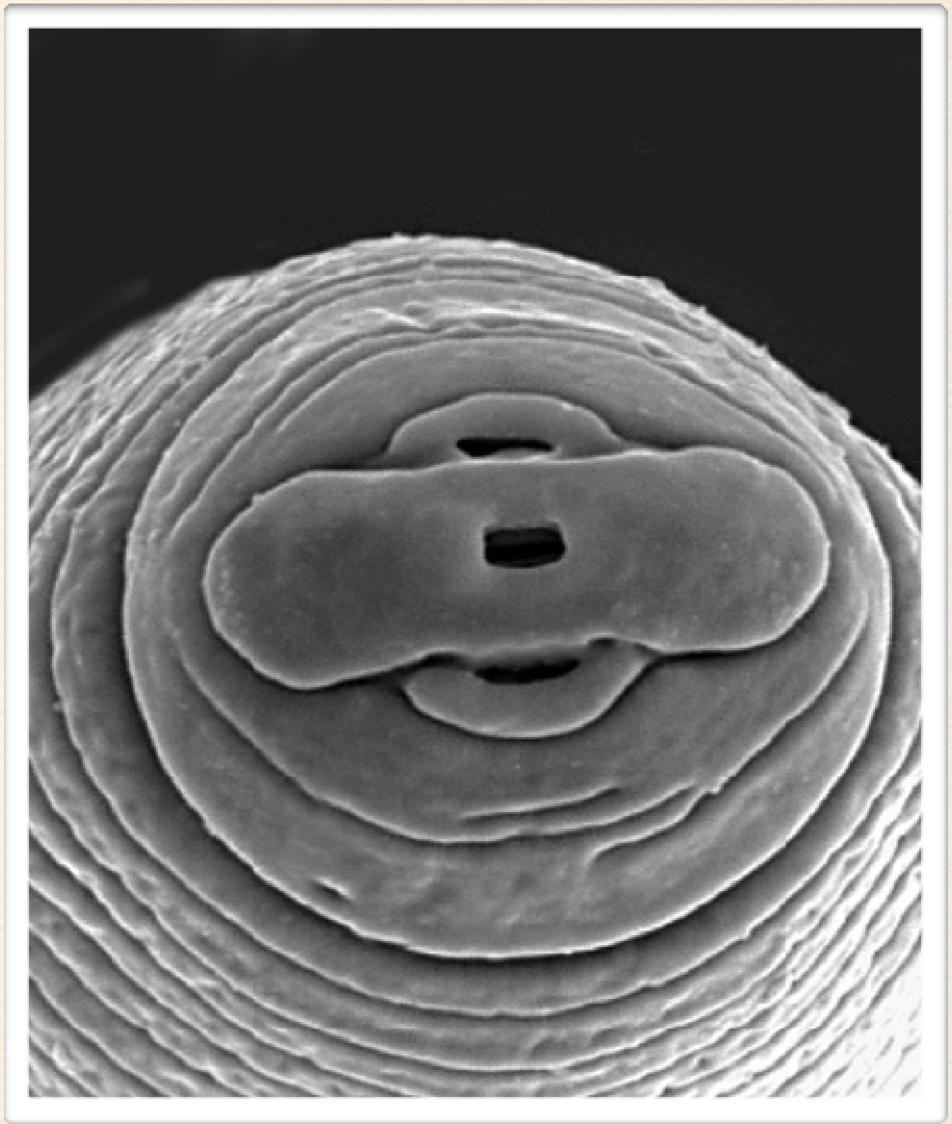
| Species | Primer set (5′–3′) | Amplicon length | Reference |
|---|---|---|---|
| *M. arenaria* | TCGGCGATAGAGGTAAATGAC<br>TCGGCGATAGACACTACAACT | 420 bp | Zijlstra *et al.*, 2000 |
| | TCGAGGGCATCTAATAAAGG<br>GGGCTGAATAATCAAAGGAA | 950 bp | Dong *et al.*, 2001b |
| *M. chitwoodi* | CCAATGATAGAGATAGGAAC<br>CTGGCTTCCTCTTGTCCAAA | 400 bp | Williamson *et al.*, 1997 |
| | GATCTATGGCAGATGGTATGGA<br>AGCCAAAACAGCGACCGTCTAC | 900 bp | Petersen *et al.*, 1997 |
| | TGGAGAGCAGCAGGAGAAAGA<br>GGTCTGAGTGAGGACAAGAGTA | 800 bp | Zijlstra, 2000 |
| *M. exigua* | CATCCGTGCTGTAGCTGCGAG<br>CTCCGTGGGAAGAAAGACTG | 562 bp | Randig *et al.*, 2002a |
| *M. fallax* | TGGGTAGTGGTCCCACTCTG<br>AGCCAAAACAGCGACCGTCTAC | 1100 bp | Petersen *et al.*, 1997 |
| | CCAAACTATCGTAATGCATTATT<br>GGACACAGTAATTCATGAGCTAG | 515 bp | Zijlstra, 2000 |
| *M. hapla* | CAGGCCCTTCCAGCTAAAGA<br>CTTCGTTGGGGAACTGAAGA | 960 bp | Williamson *et al.*, 1997 |
| | TGACGGCGGTGAGTGCGA<br>TGACGGCGGTACCTCATAG | 610 bp | Zijlstra, 2000 |
| | GGCTGAGCATAGTAGATGATGTT<br>ACCCATTAAAGAGGAGTTTTGC | 1500 bp | Dong *et al.*, 2001b |
| | GGATGGCGTGCTTTCAAC<br>AAAAATCCCCTCGAAAAATCCACC | 440 bp | Wishart *et al.*, 2002 |
| *M. incognita* | CTCTGCCCAATGAGCTGTCC<br>CTCTGCCCTCACATTAGG | 1200 bp | Zijlstra *et al.*, 2000 |
| | TAGGCAGTAGGTTGTCGGG<br>CAGATATCTCTGCATTGGTGC | 1350 bp | Dong *et al.*, 2001b |
| | GGGATGTGTAAATGCTCCTG<br>CCCGCTACACCCTCAACTTC | 399 bp | Randig *et al.*, 2002a |
| | GTGAGGATTCAGCTCCCCAG<br>ACGAGGAACATACTTCTCCGTCC | 955 bp | Meng *et al.*, 2004 |
| *M. javanica* | CCTTAATGTCAACACTAGAGCC<br>GGCCTTAACCGACAATTAGA | 1650 bp | Dong *et al.*, 2001b |
| | GGTGCGCGATTGAACTGAGC<br>CAGGCCCTTCAGTGGAACTATAC | 670 bp | Zijlstra *et al.*, 2000 |
| | ACGCTAGAATTCGACCCTGG<br>GGTACCAGAAGCAGCCATGC | 517 bp | Meng *et al.*, 2004 |
| *M. enterolobii* | GAAATTGCTTTATTGTTACTAAG<br>TAGCCACAGCAAAATAGTTTTC | 322 bp | Blok *et al.*, 2002 |
| *M. naasi* | CTCTTTATGGAGAATAATCGT<br>CCTCCGCTTACTGATATG | 433 bp | Zijlstra *et al.*, 2004 |
| *M. paranaensis* | GCCCGACTCCATTTGACGGA<br>CCGTCCAGATCCATCGAAGTC | 208 bp | Randig *et al.*, 2002b |

# Gene Sequences

- The identification of the organism depends completely on the submitter of the sequences
- If our museums are any indication, the Genbank is riddled with errors and mislabeled sequences
- These errors are hard to discover and even harder to stop from propagating
- Misidentifications are a malignancy spreading rapidly throughout the body of science

# In Conclusion

- “The taxonomic community is woefully inadequate for the immense task that is before it” - E.O. Wilson
- Fortunately nematode disease diagnosis is related to the purpose for which is intended
- Root-knot, cyst, and lesion nematodes are the most important for diagnosis



# Any Questions?

# The End