

Exploring Nematode Biodiversity using Morphology and DNA

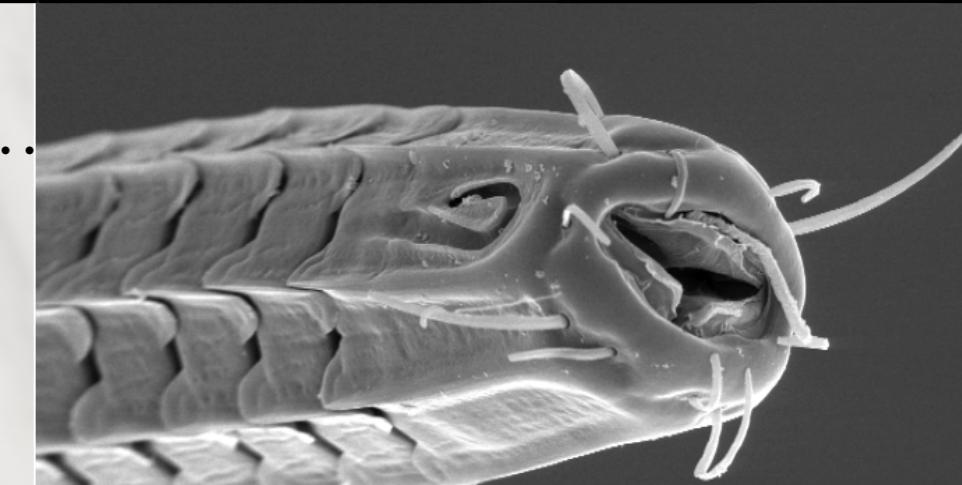
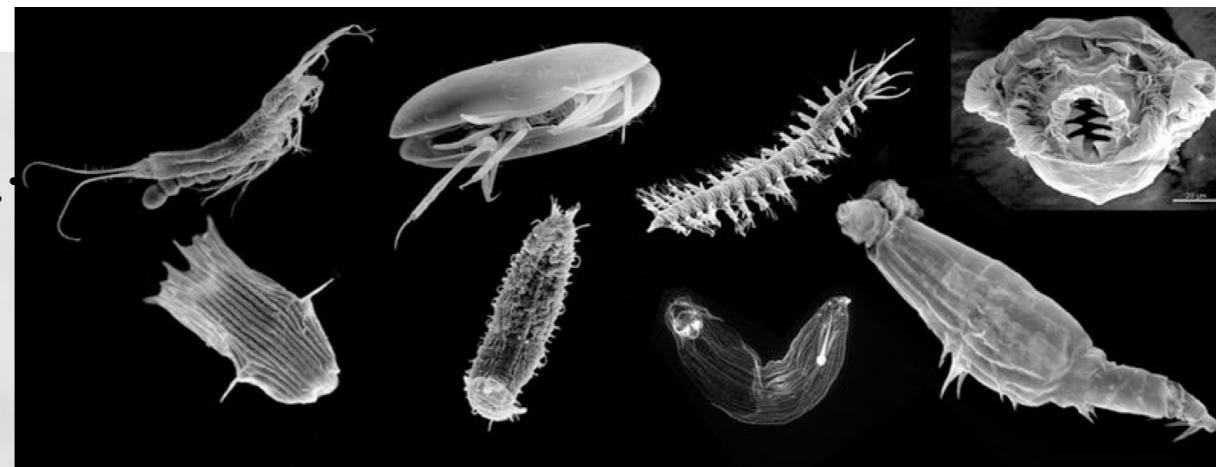
Dr. Holly Bik
Department of Nematology

microbial eukaryotes (metazoa) in marine sediments

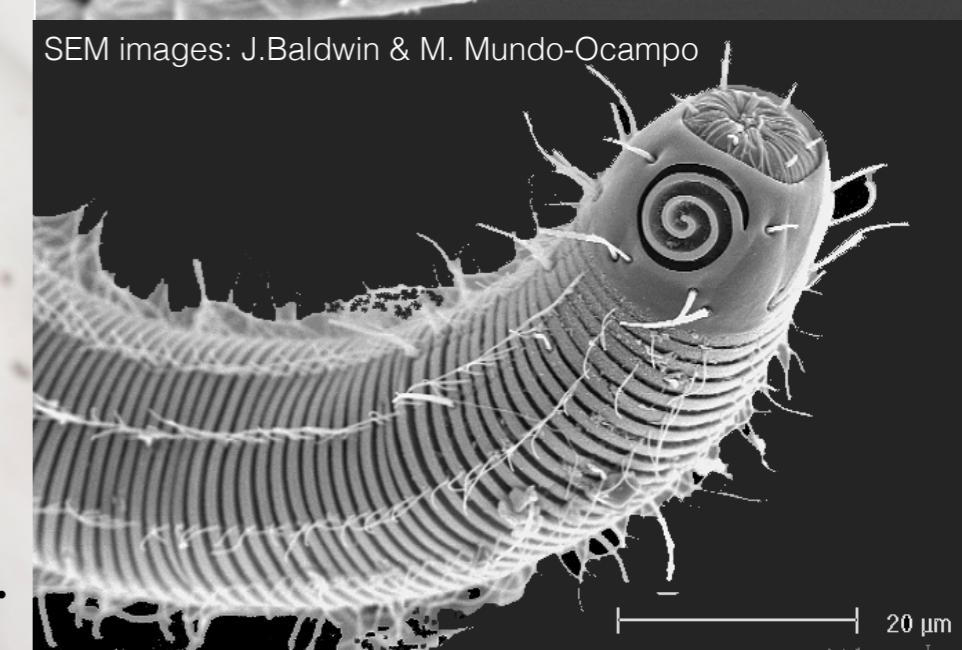
worms, crustaceans, protists, fungi...



<https://flic.kr/p/g6kDzr>

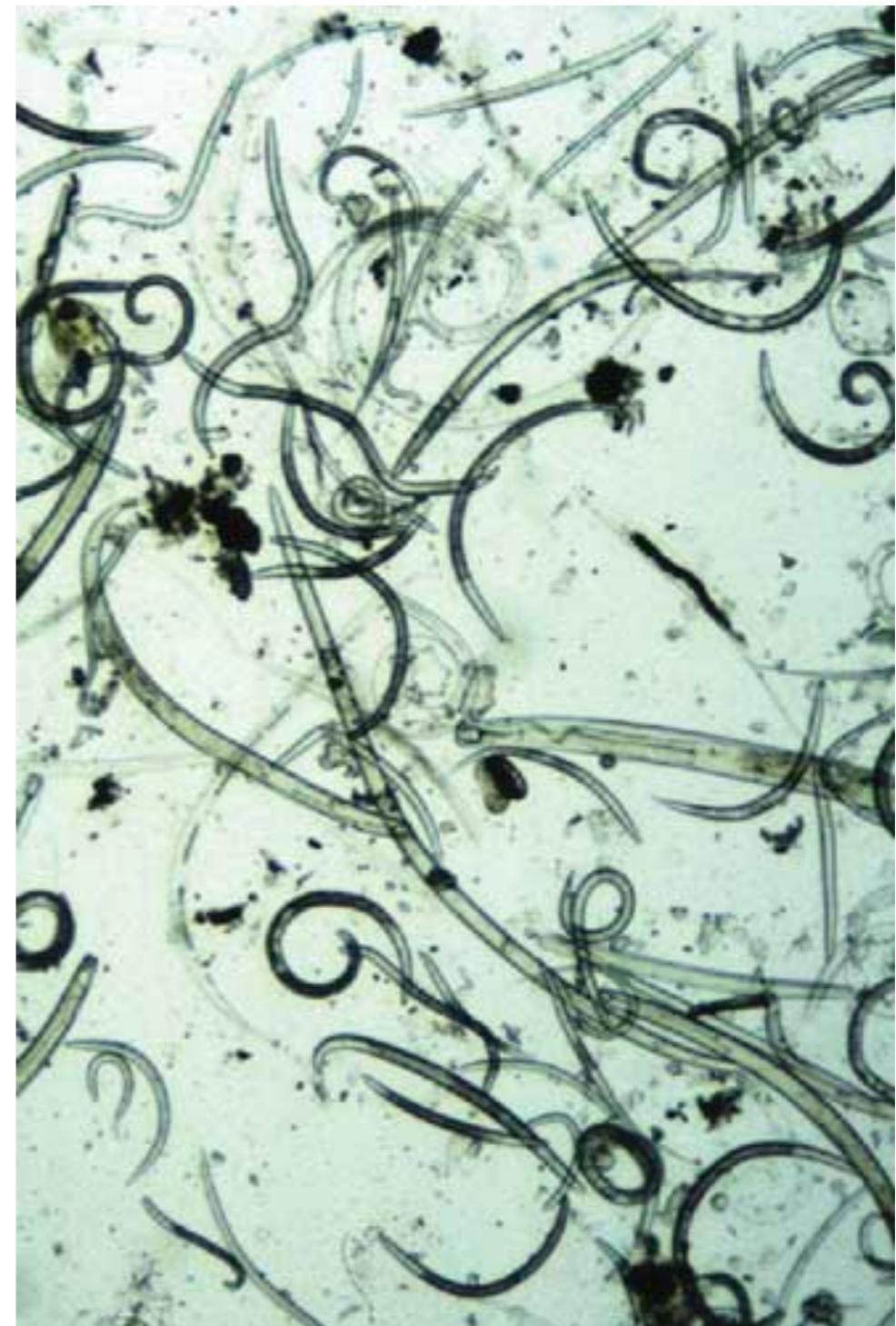


SEM images: J.Baldwin & M. Mundo-Ocampo



Nematode species are globally diverse and abundant

- Free-living assemblages in soils, sediments, sea ice; also parasites
- Meiofauna size class, typically 45µm - 1mm
- 1-100 million estimated nematode species, but only, ~28,00 described species
- Large enough to be physically manipulated, but too small for most genomic interests/efforts (and only a few cultured models)

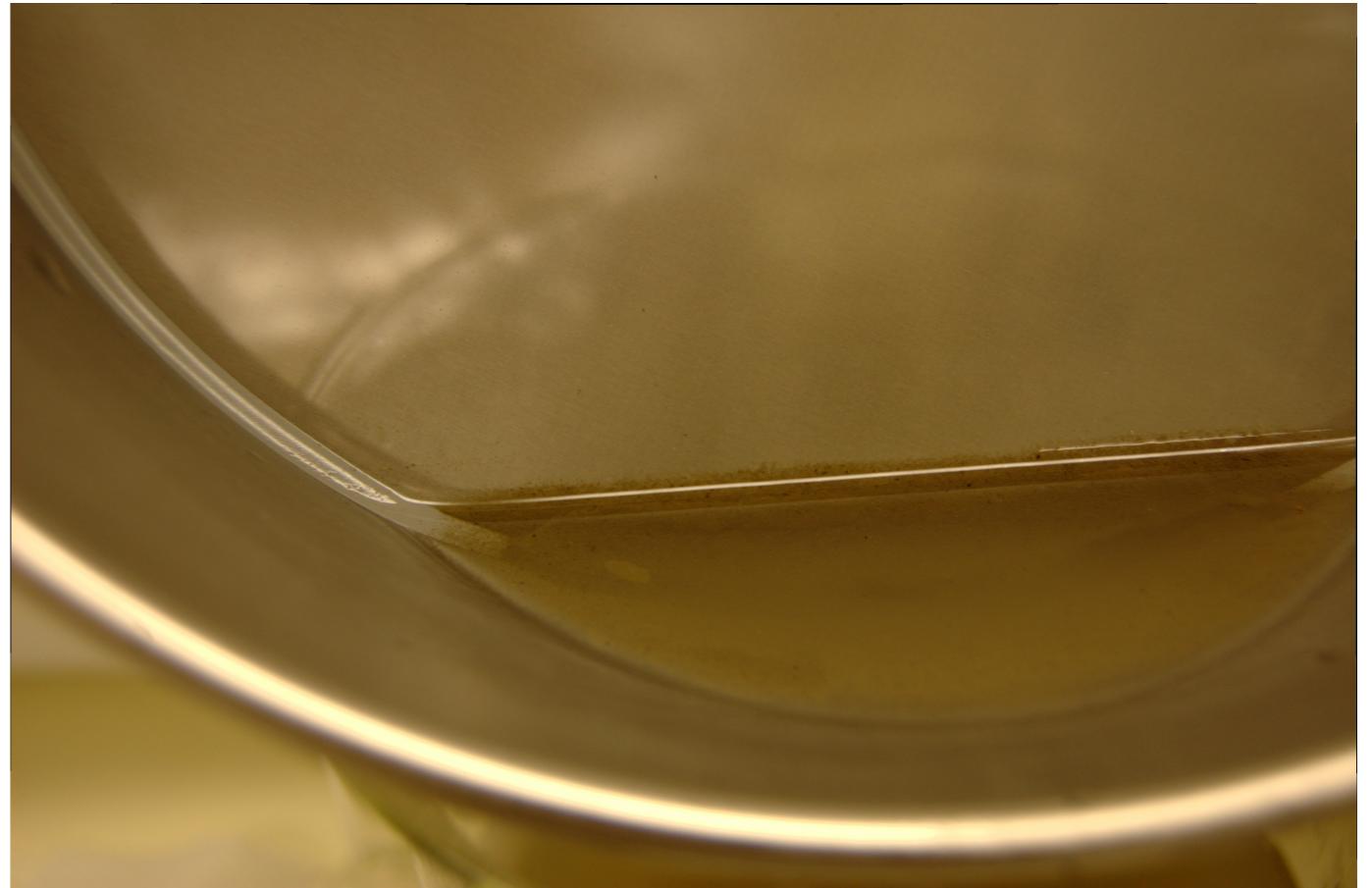


Collecting Samples

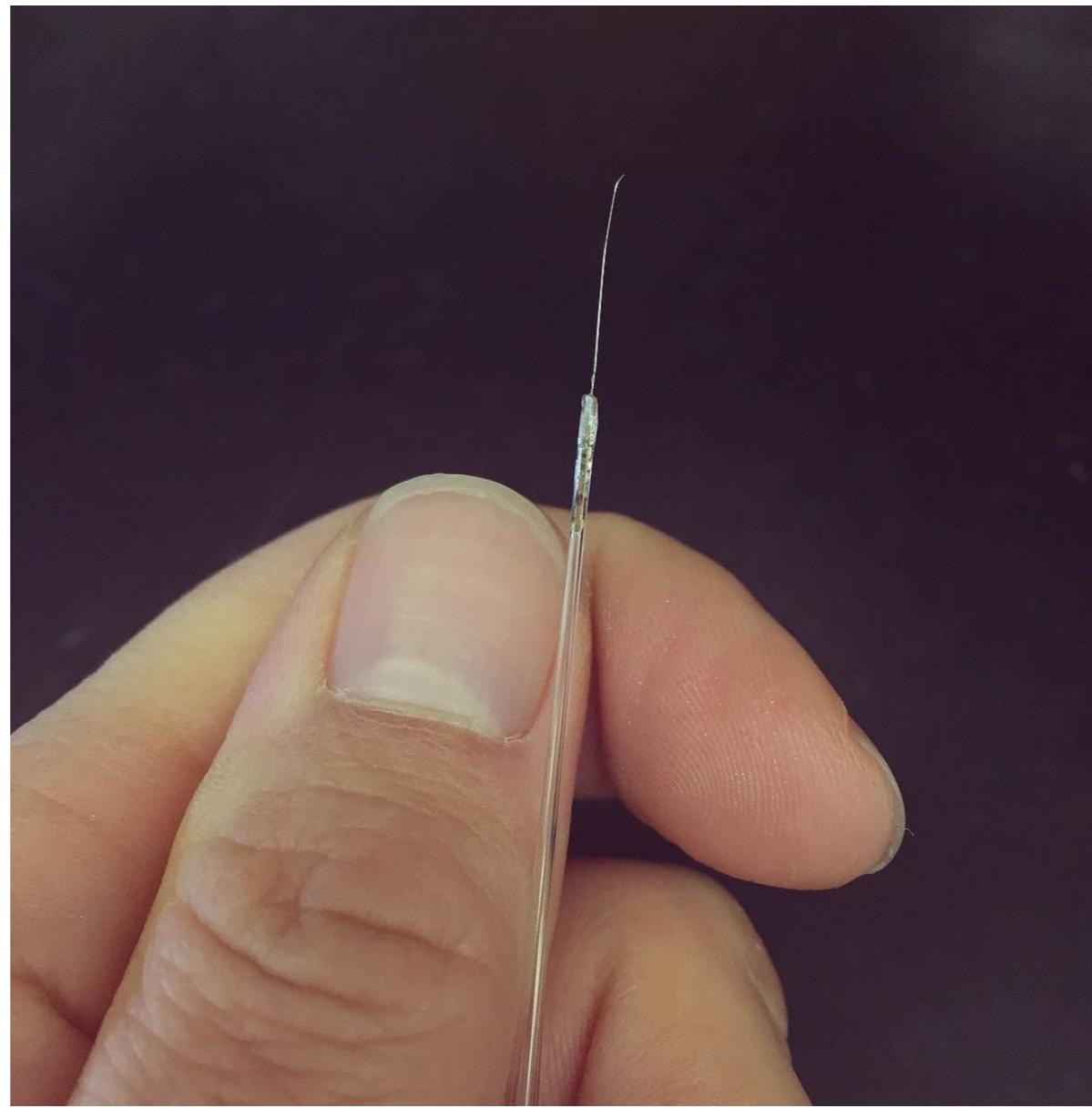


Sediment coring and
depth sectioning

Physical processing of sediments enriches for metazoa

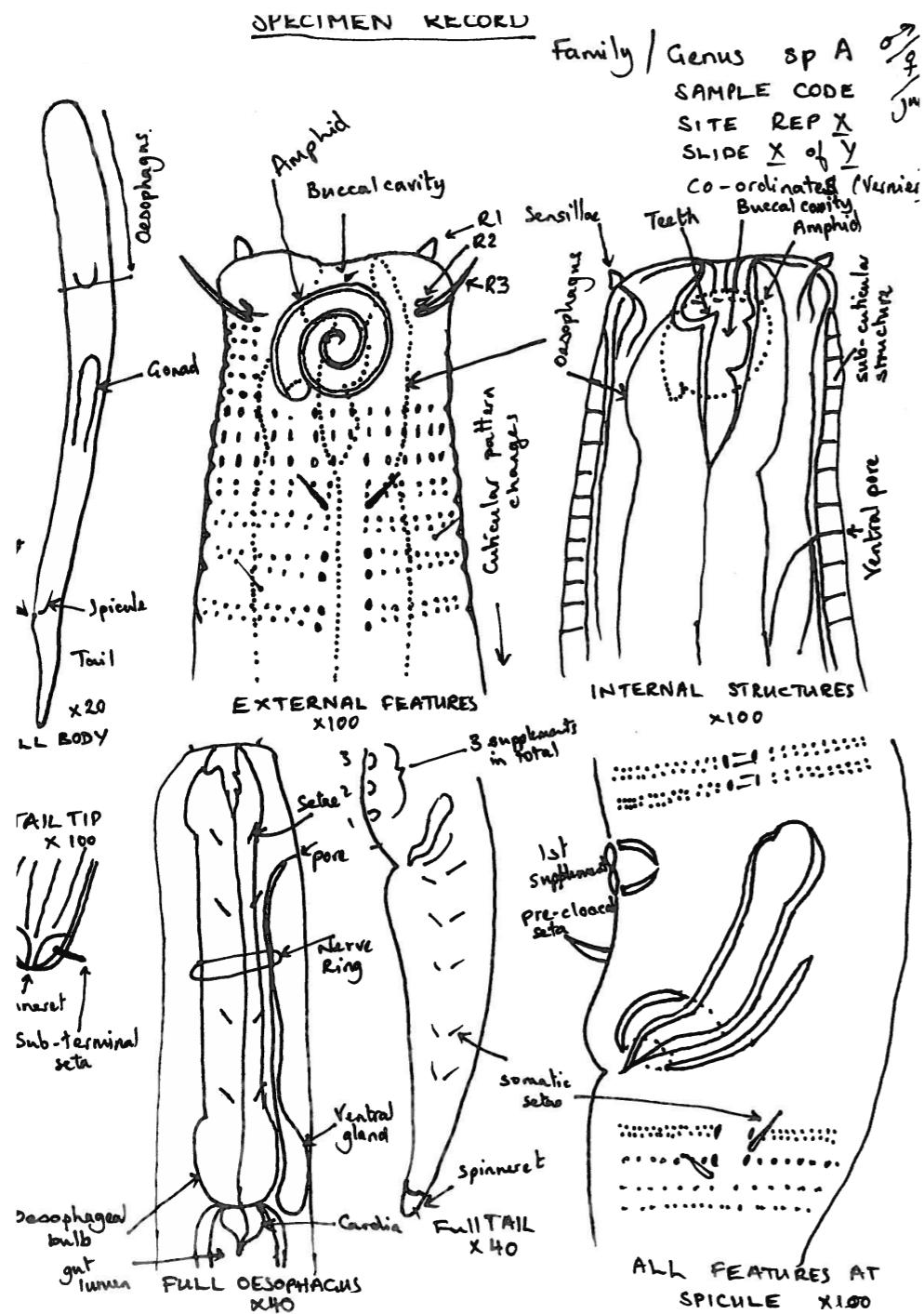


Picking nematodes under the microscope



<https://www.youtube.com/watch?v=Zw1u5TmeHvU>

Morphology DNA



>Nem47

```
ATAGCTCATTACAACAGTCGTAGTTATTAGAAAGTATCTTCTGGATAAC
TGTGGTAATTCTAGAGCTAACATGTTCAAGCCCTGACTAACGAAAGG
GTGCATTATTAGAACAAAGCCAATCAGACTTCGGTCTGTCTCAGGTTGA
CTCTGAATAACTTGCTAATCGCACAGTCTTGCAGTGGCAGTGTATCTT
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AGAAACAGCTACCACATCCAAGGAAGGCAGCAGGCACGCAAATTACCCAC
TCCCAGTTGGGGAGGGTAGTGACGAAAAATAACGAGACGATGCTCTACGA
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GGAGGGCAAGTCTGGTGCCAGCAGCCGGTAATTCCAGCTCCAATAGCG
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GCATGGAATAATGGAATAGGGCCTGGCTATTTGTTGGTTAGGAG
CACGAGGCAATGATTAAGAGAAAACAGACGGGGCATTGTATTGCGGCGT
```

hollybik — bash — 104x33

Last login: Mon Mar 30 08:55:00 on ttys000
Hollys-MacBook-Retina:~ hollybik\$ macqiime

MacQIIME version:
MacQIIME 1.8.0-20140103

Sourcing MacQIIME environment variables...

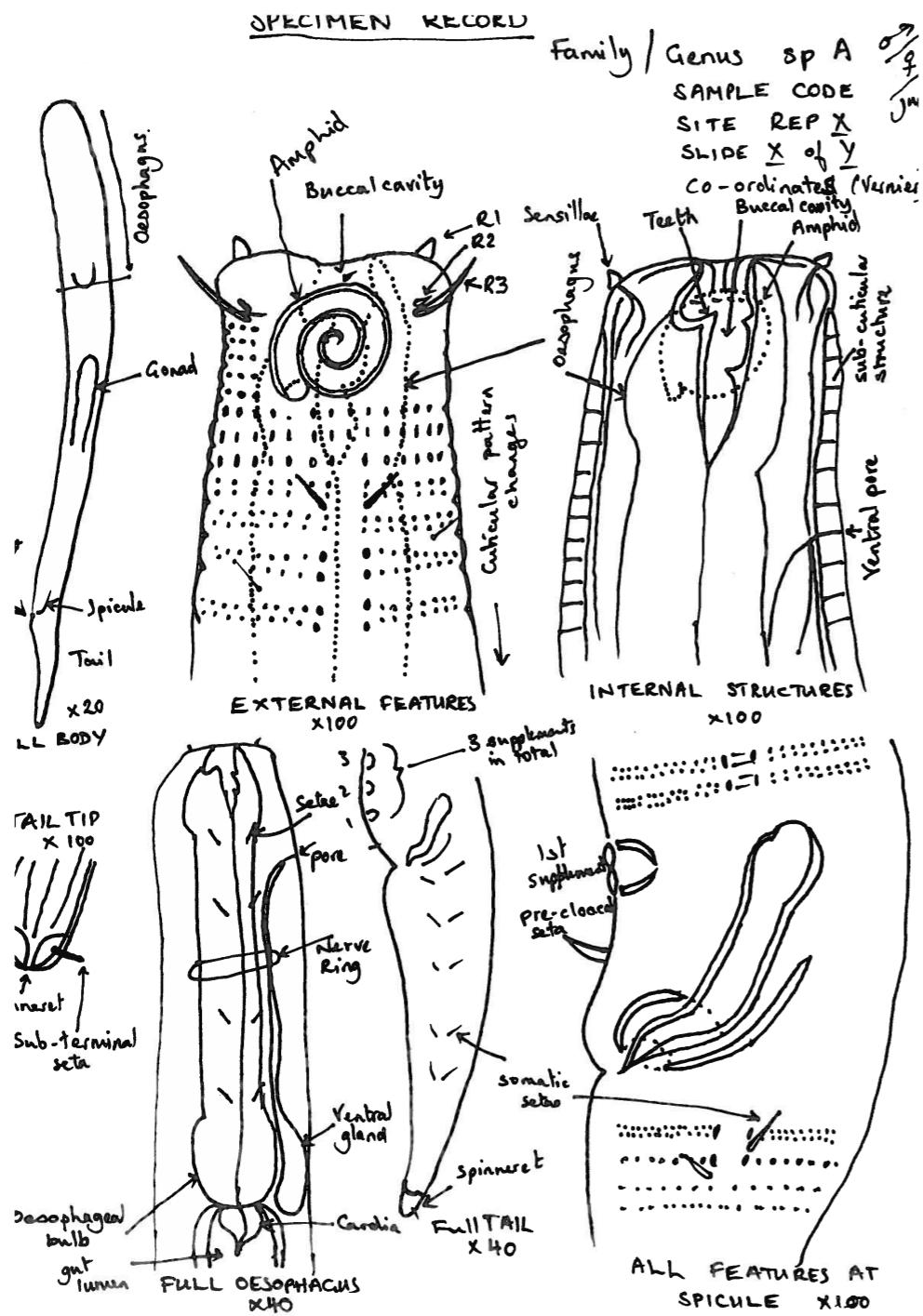
This is the same as a normal terminal shell, except your default python is DIFFERENT (/macqiime/bin/python) and there are other new QIIME-related things in your PATH.

Type "exit" (return) to go back to your normal shell

MacQIIME Hollys-MacBook-Retina:~ \$

-click to edit

Morphology



- Historical tradition - drawings, species records back to 1800s
- Detailed and visual species record (drawings done via microscopy)
- Subjective depending on expert
- Morphological studies not directly comparable
- Convergent evolution in nematode body structures
- Limits of light microscopy - need SEM/TEM for some structures



Visual information from morphological taxonomy



Morphological Character: Amphids

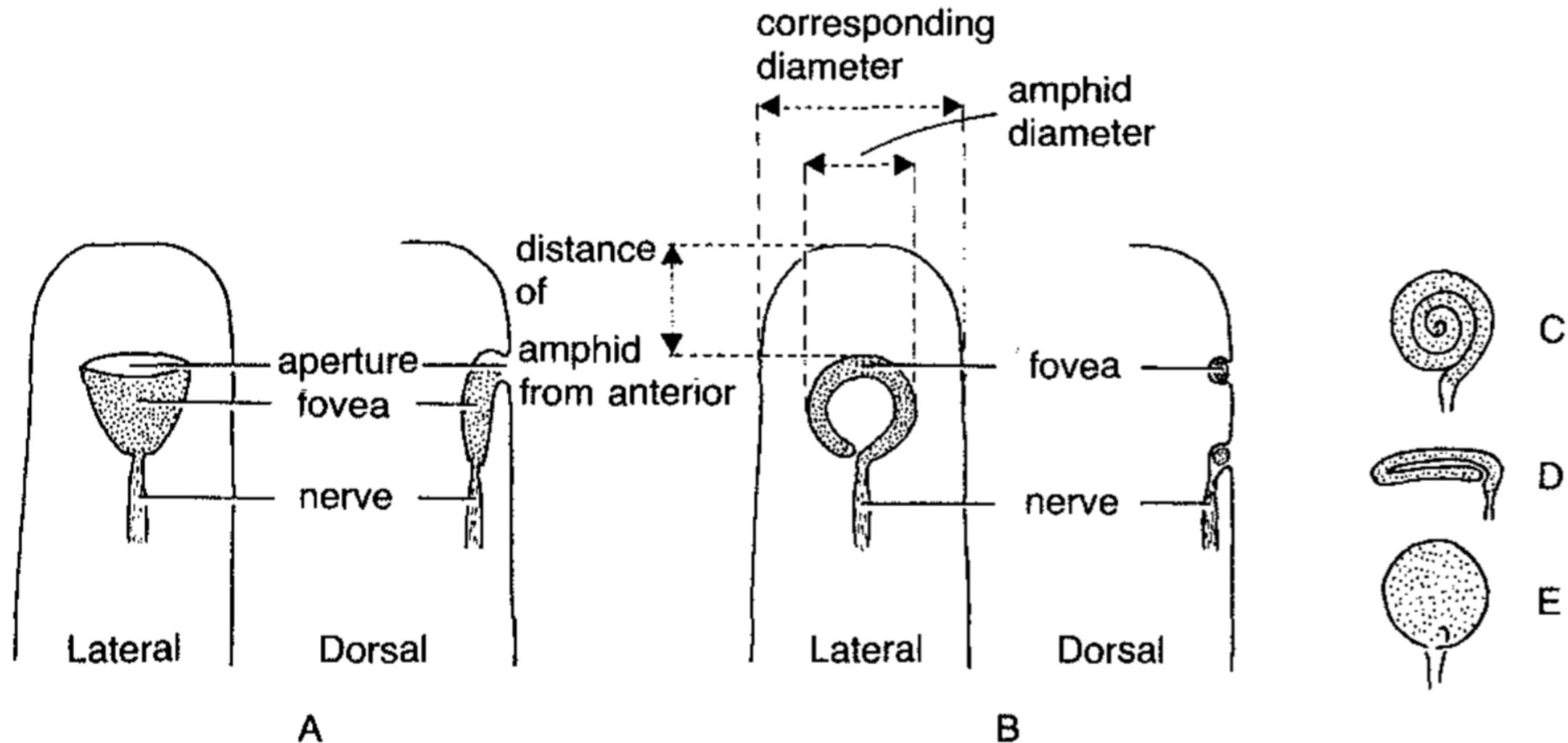


Fig. 3. Amphids. A, Pocket type. B, Spiral type with a single turn, also showing amphid measurements. C, Multi-spiralled type. D, Transverse slit type. E, Circular type.

fovea filled with gelatinous substance (**corpus gelatum**)

Warwick, Platt & Somerfield (1998) Free-Living Marine Nematodes, Part III: Monhysterids

Morphological Character: **Buccal Cavity (mouth)**

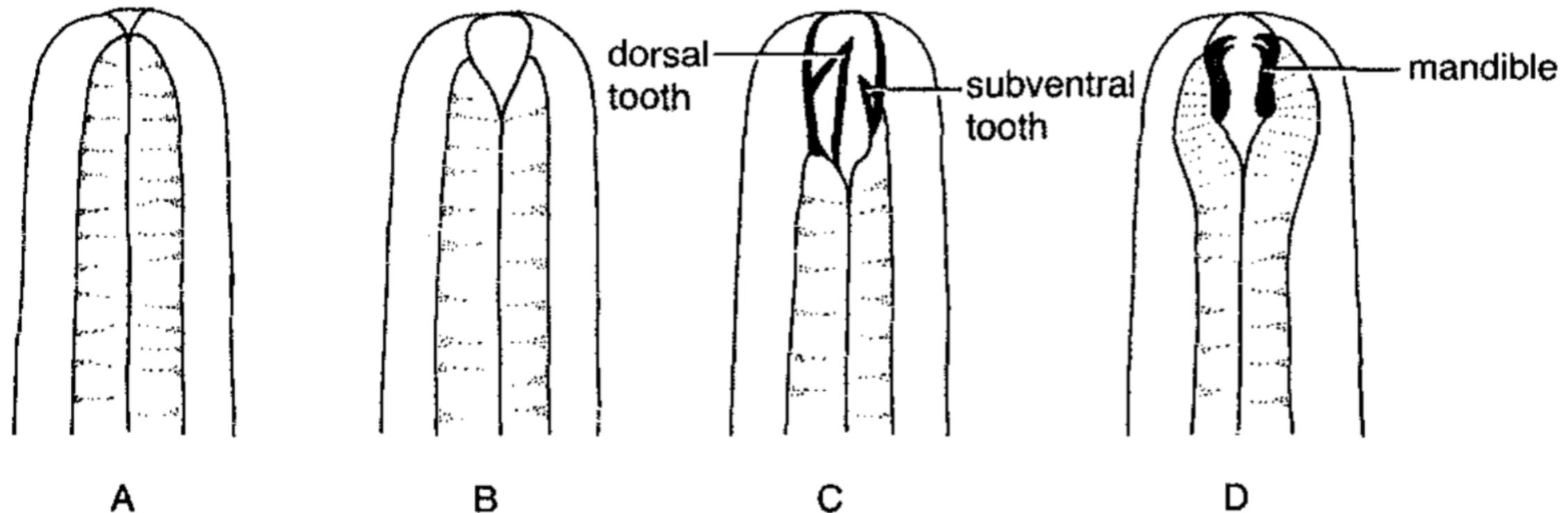
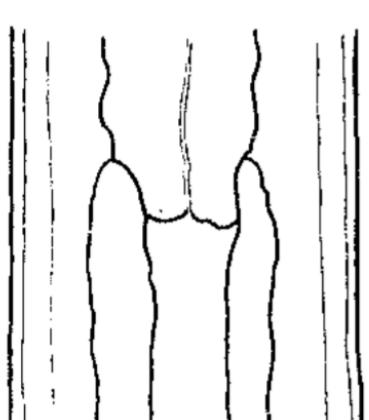


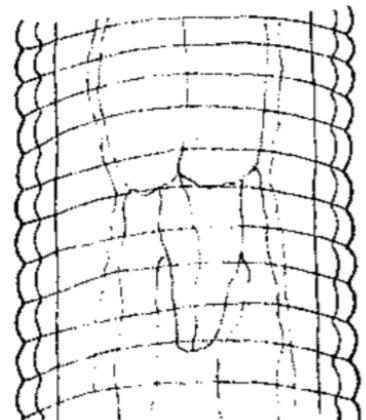
Fig. 4. Buccal cavities. A, Minute form. B, Unarmed form. C, Form with fixed teeth. D, Form with moveable mandibles.

Warwick, Platt & Somerfield (1998) *Free-Living Marine Nematodes, Part III: Monhysterids*

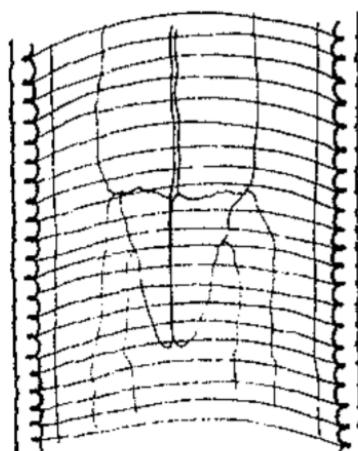
Morphological Character: Cuticle Surface



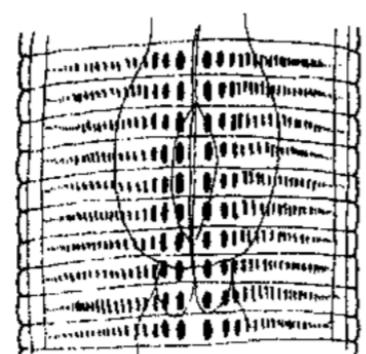
A



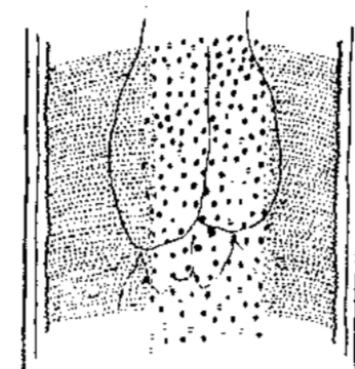
B



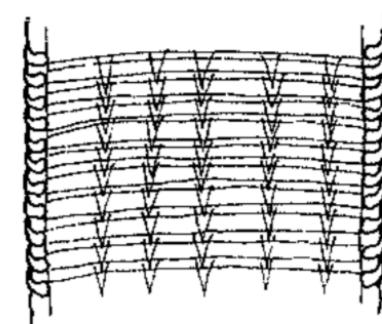
C



D



E



F

- A.** Smooth Cuticle
- B.** Coarsely Striated
- C.** Externally smooth, striations appear deeper in body
- D.** Transverse rows of dots
- E.** Fine dots, irregular laterally
- F.** Longitudinal rows of structures

Morphological Character: **Sensilla**

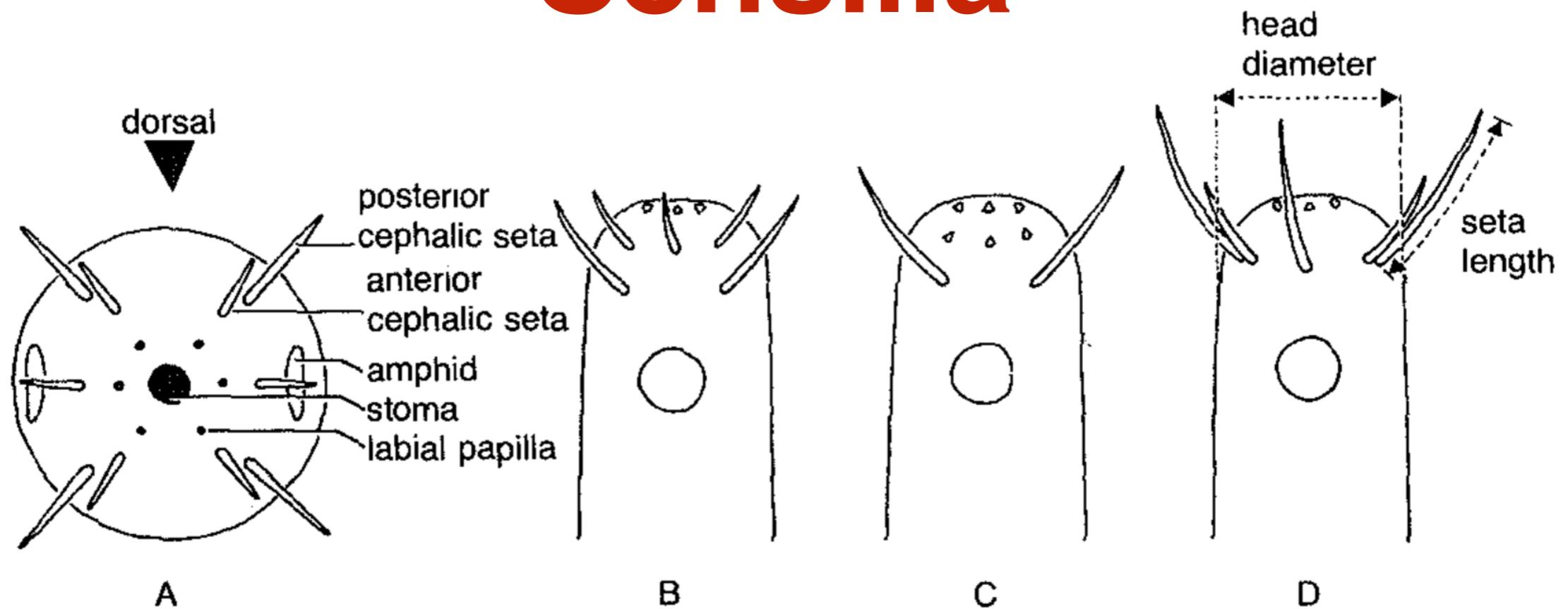


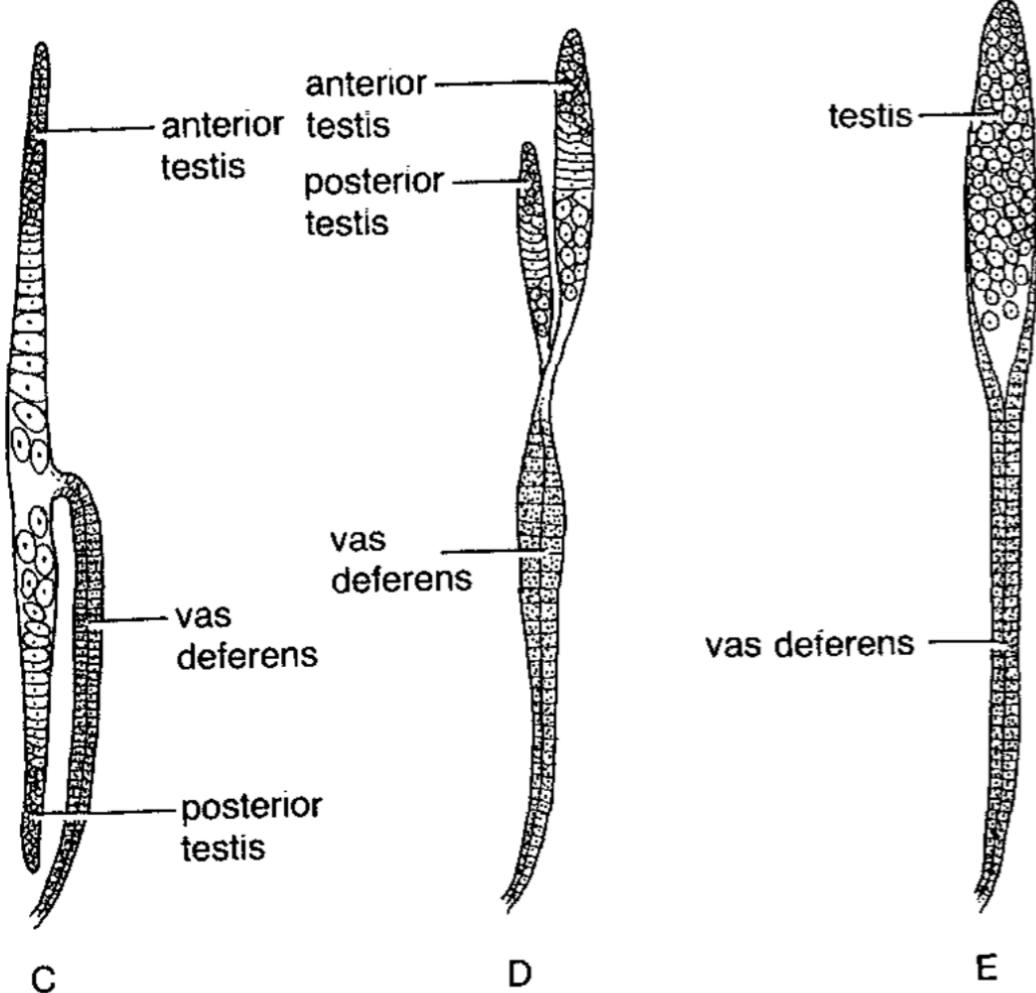
Fig. 2. Head sensilla. A, Apical view showing typical distribution of head sensilla. B, Head sensilla in the 6 + 6 + 4 pattern. C, Head sensilla pattern where only the third circle are setiform. D, Head sensilla in the 6 + 10 pattern, also showing setal measurement (B-D, lateral views).

Papillae (singular: **papilla**) = bump-like
Setae (singular: **seta**) = long and hair-like

Warwick, Platt & Somerfield (1998) *Free-Living Marine Nematodes, Part III: Monhysterids*

Morphological Character: Reproductive Structures (Males)

Layout of Testes

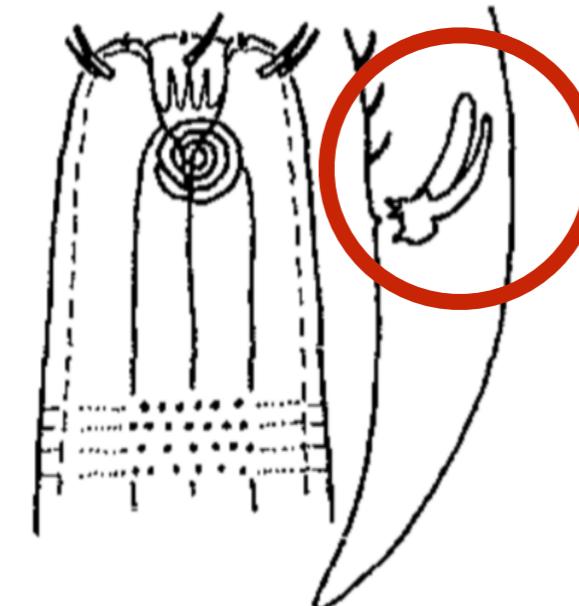


**Two
Opposed**

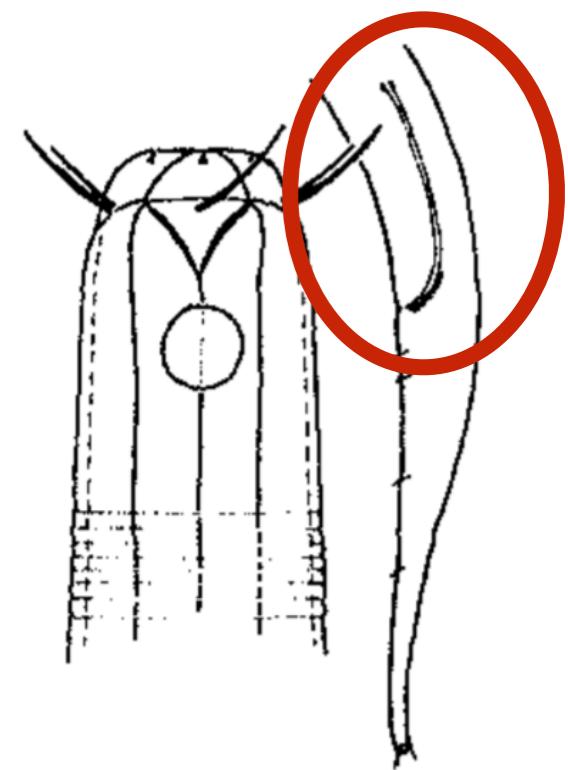
**Two
Tandem**

Single

Spicules and Gubernaculum



F. Praeacanthonchus

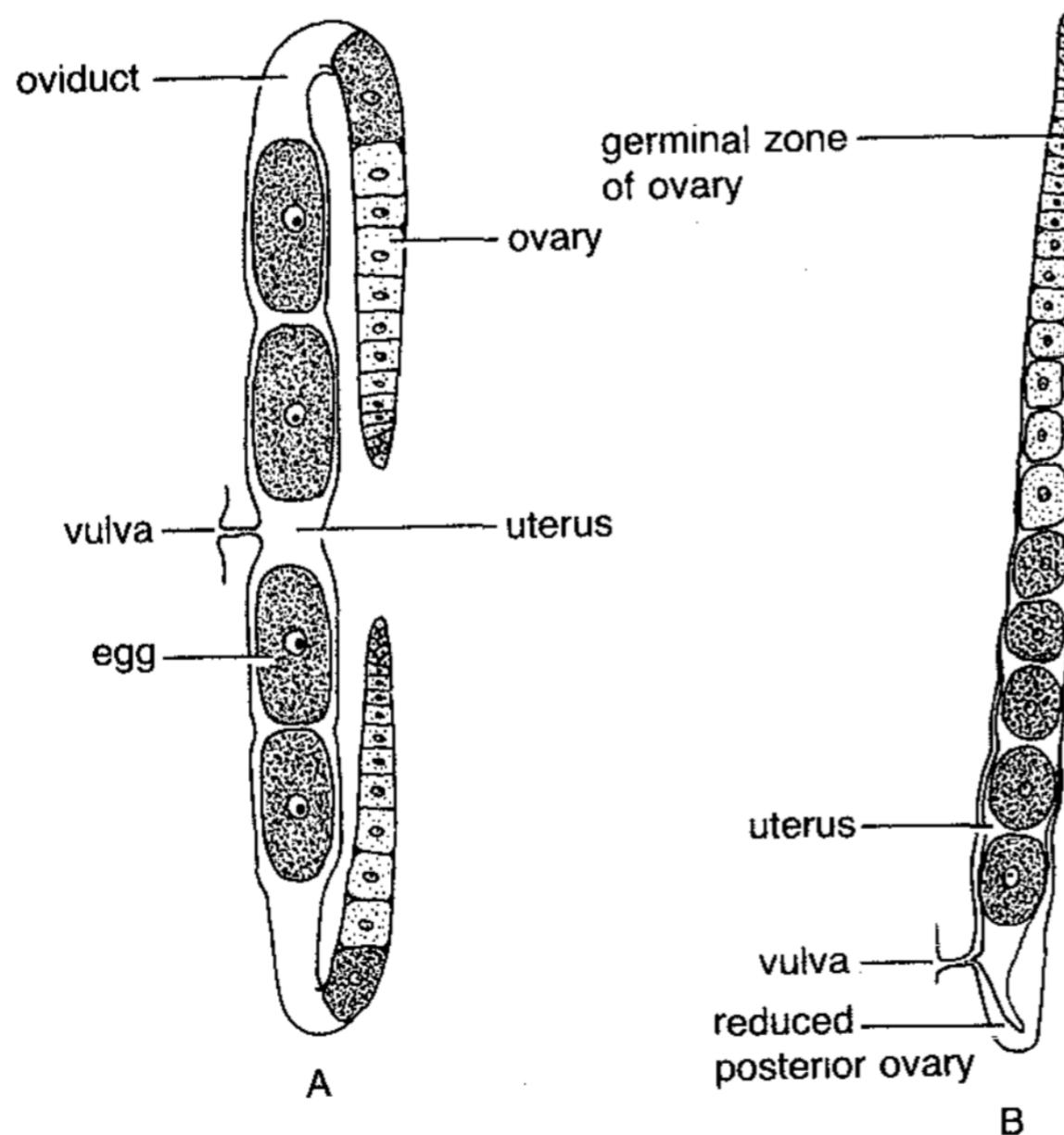


J. Paramonohystera

Morphological Character: **Reproductive Structures (Females)**

Layout of Ovaries

**Opposed
and
Reflexed**

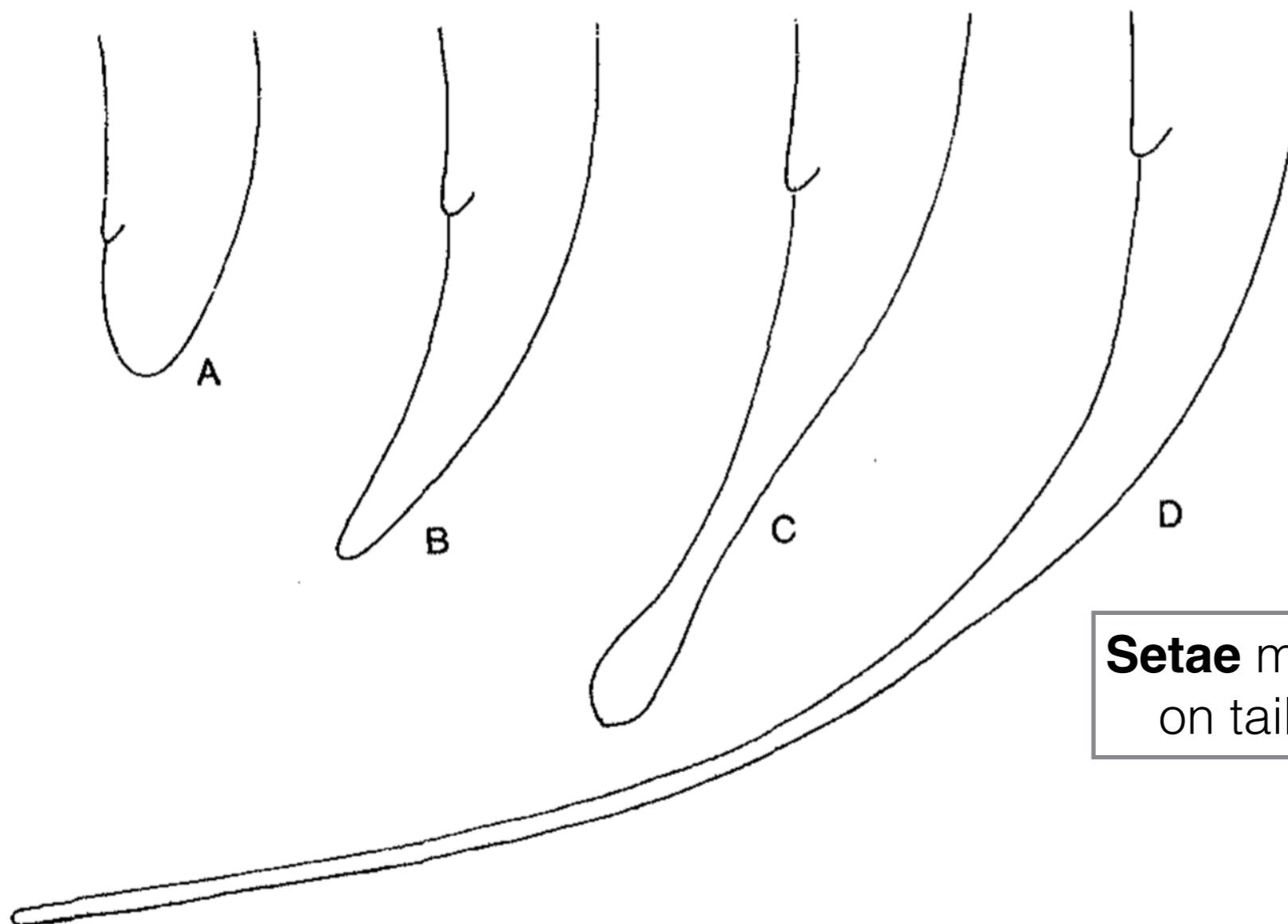


**Singular
and
Outstretched
(towards anterior)**

Warwick, Platt & Somerfield (1998)
Free-Living Marine Nematodes,
Part III: Monhysterids

Morphological Character:

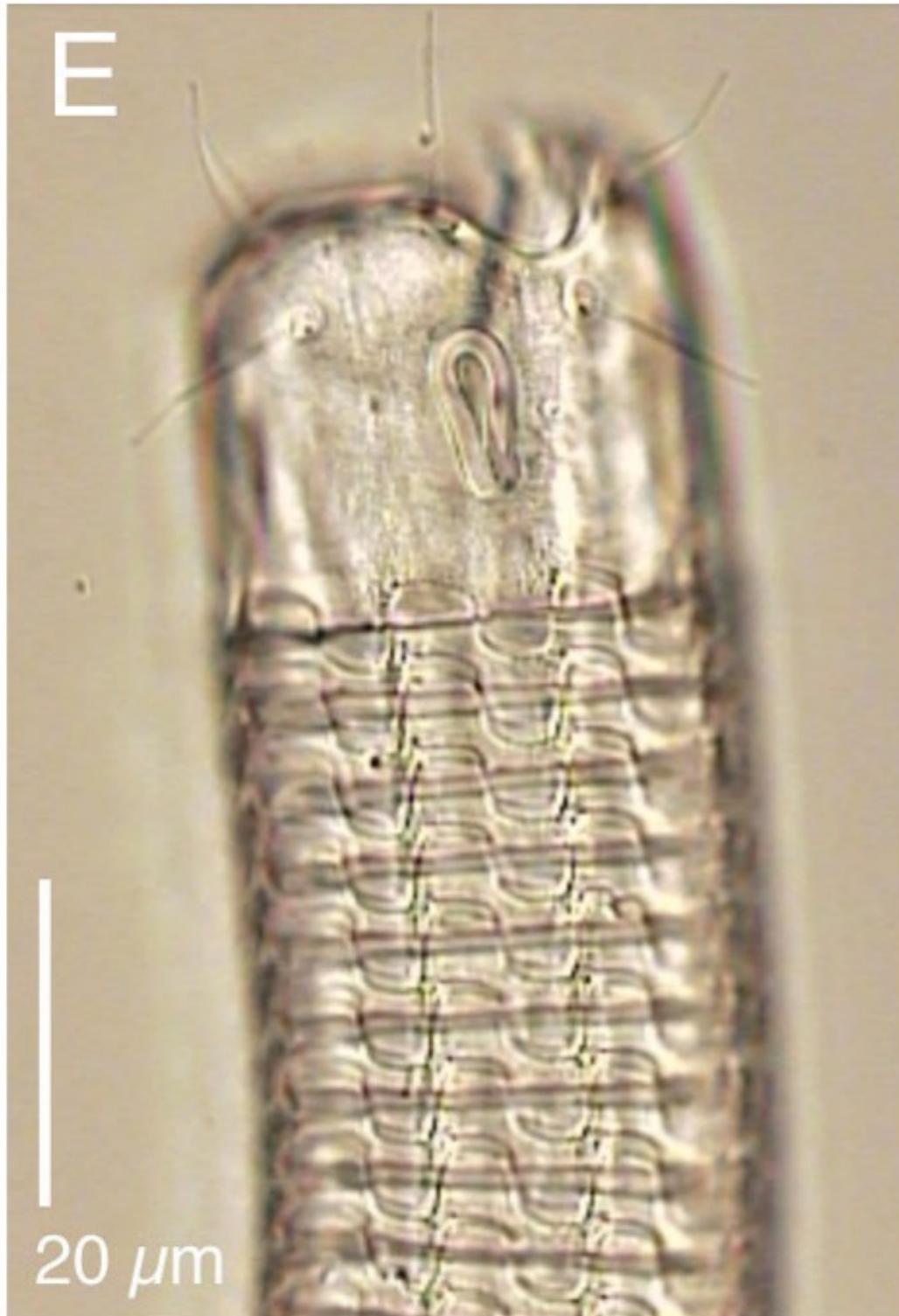
Tail Shape



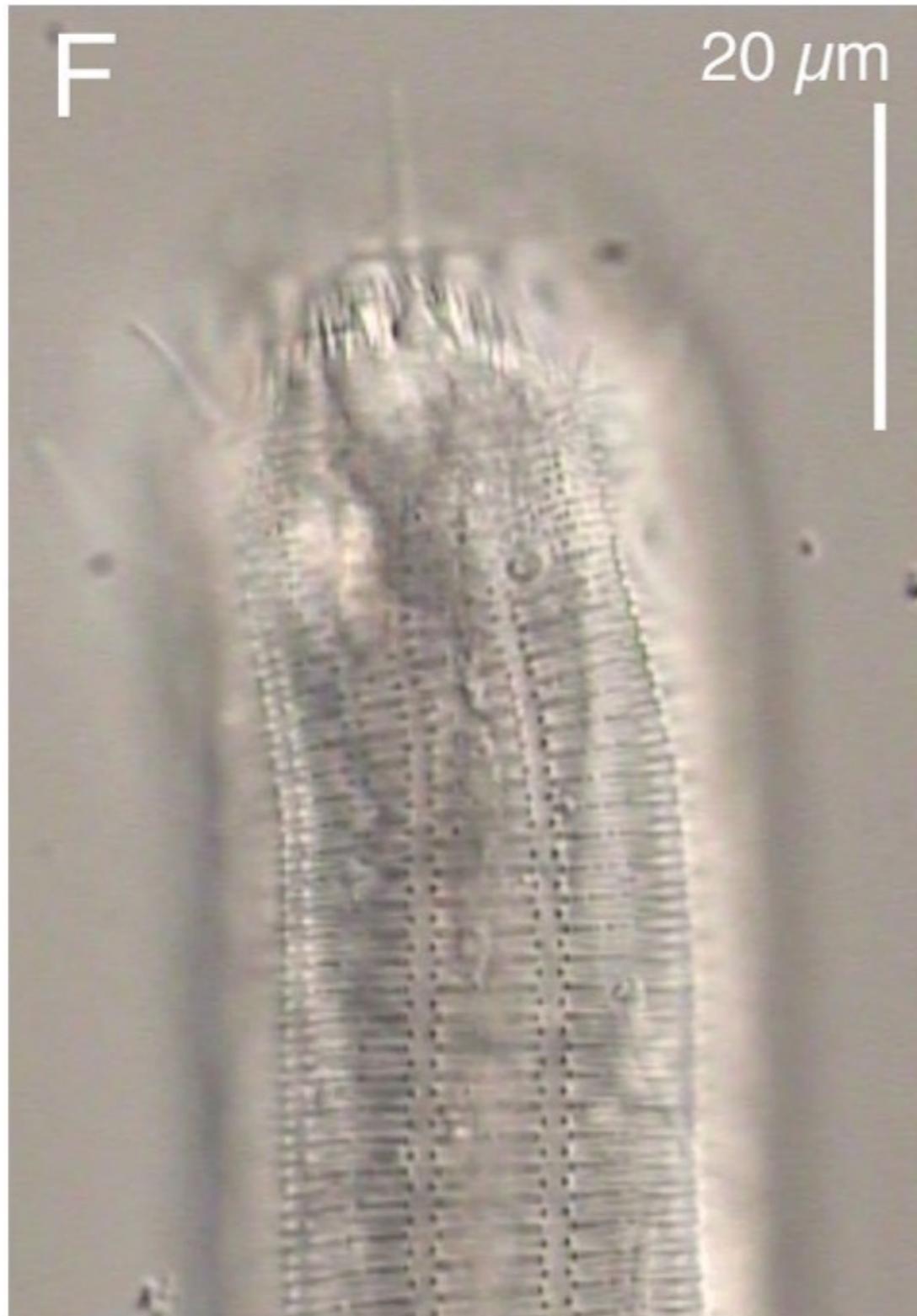
Setae may also be present
on tail and along body

Fig. 7. Tail shapes. A, Short and round. B, Conical. C, Conico-cylindrical with swollen tip (clavate). D, Elongated, filiform.

Warwick, Platt & Somerfield (1998) Free-Living
Marine Nematodes, Part III: Monhysterids



What
morphological
characters do
you see?



What
morphological
characters do
you see?



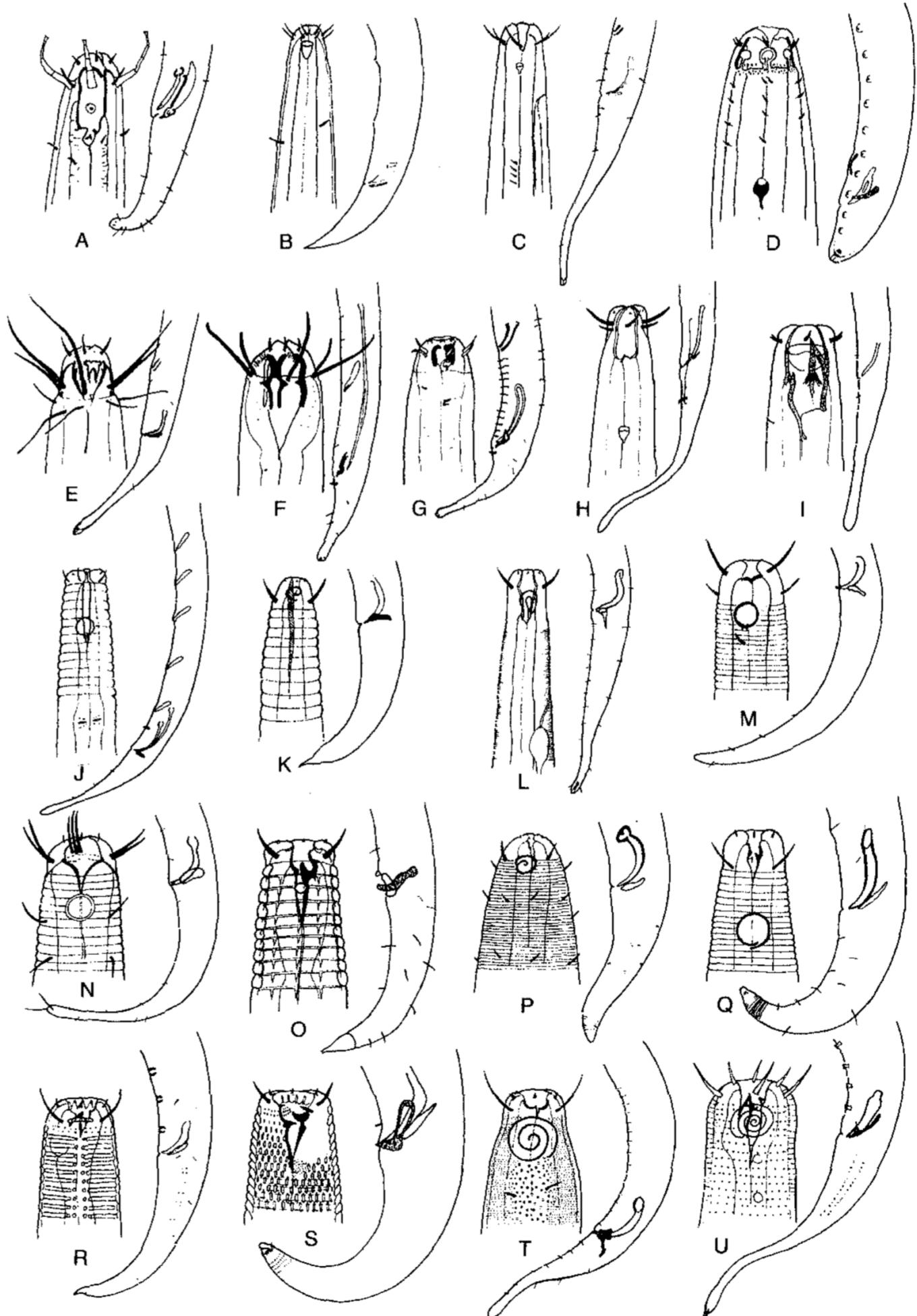
What
morphological
characters do
you see?

De Ley 2006, Wormbook

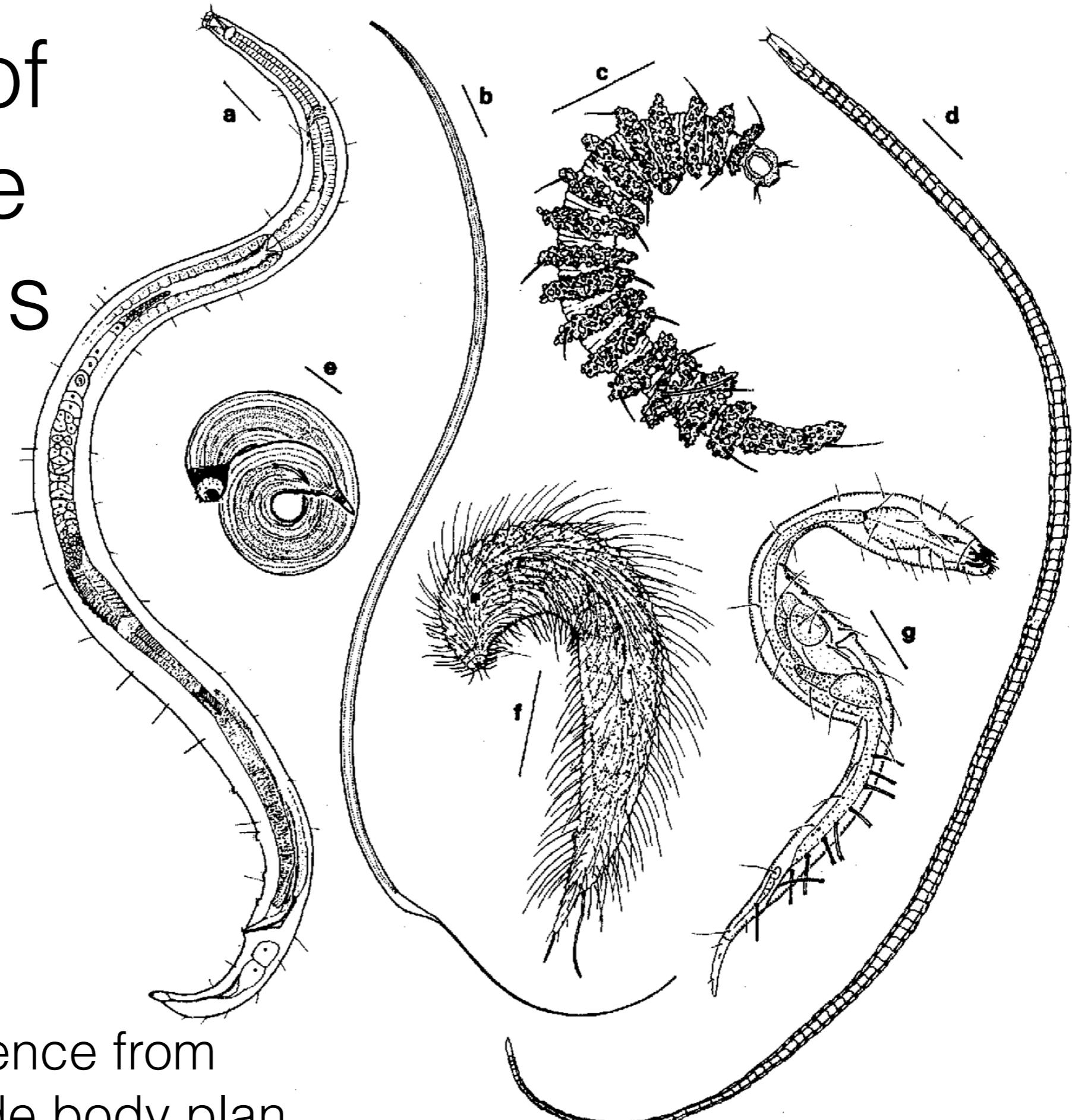
Diversity of nematode morphology

**Common Genera from
British marine sediments**

Warwick, Platt & Somerfield (1998) Free-Living
Marine Nematodes, Part III: Monhysterids



Diversity of nematode body plans



Lots of divergence from
“typical” nematode body plan

DNA Sequencing

- Objective type of data - no expert bias
- Evolutionary history of species
- No visual information about morphology
- Dependent on database matches
- Best used in conjunction with morphology (e.g. from single worms)

```
>Nem47
ATAGCTCATTACAACAGTCGTAGTTATTAGAAAGTATCTTCTGGATAAC
TGTGGTAATTCTAGAGCTAACATGTTCAAGCCCTGACTAACGAAAGG
GTGCATTATTAGAACAAAGCCAATCAGACTTCGGTCTGTCTCAGGTTGA
CTCTGAATAACTTGCTAATCGCACAGTCTTGCAGTGGCGATGTATCTT
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GCATGGAATAATGGAATAGGGCCTGGCTTATTTGTTGGTTAGGAG
CACGAGGCAATGATTAAGAGAAAACAGACGGGGCATTGTATTGCGGCGT
```

A screenshot of a terminal window on a Mac OS X system. The window title bar shows the name of the terminal session. The terminal itself displays a DNA sequence followed by several lines of MacQIIME configuration output. The configuration output includes the last login time, the MacQIIME version (1.8.0-20140103), and instructions for sourcing environment variables. A message at the bottom indicates that the terminal is different due to MacQIIME's modifications to the PATH.

```
hollybik — bash — 104x33
Last login: Mon Mar 30 08:55:00 on ttys000
Hollys-MacBook-Retina:~ hollybik$ macqiime

MacQIIME version:
MacQIIME 1.8.0-20140103

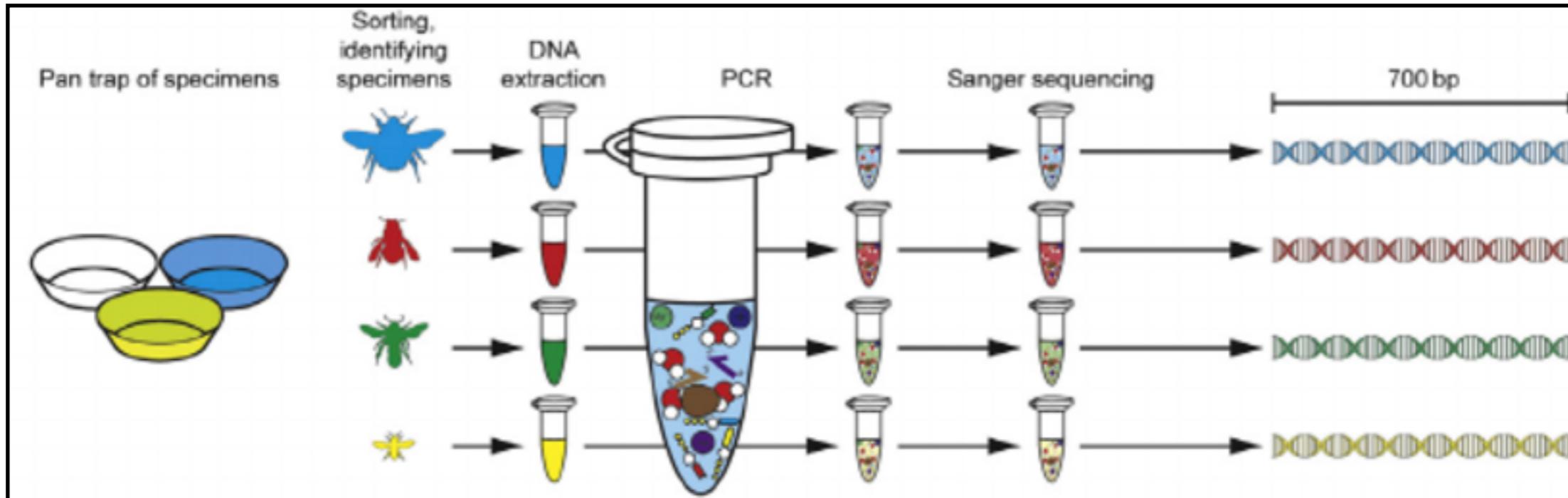
Sourcing MacQIIME environment variables...

This is the same as a normal terminal shell, except your default
python is DIFFERENT (/macqiime/bin/python) and there are other new
QIIME-related things in your PATH.

Type "exit" (return) to go back to your normal shell

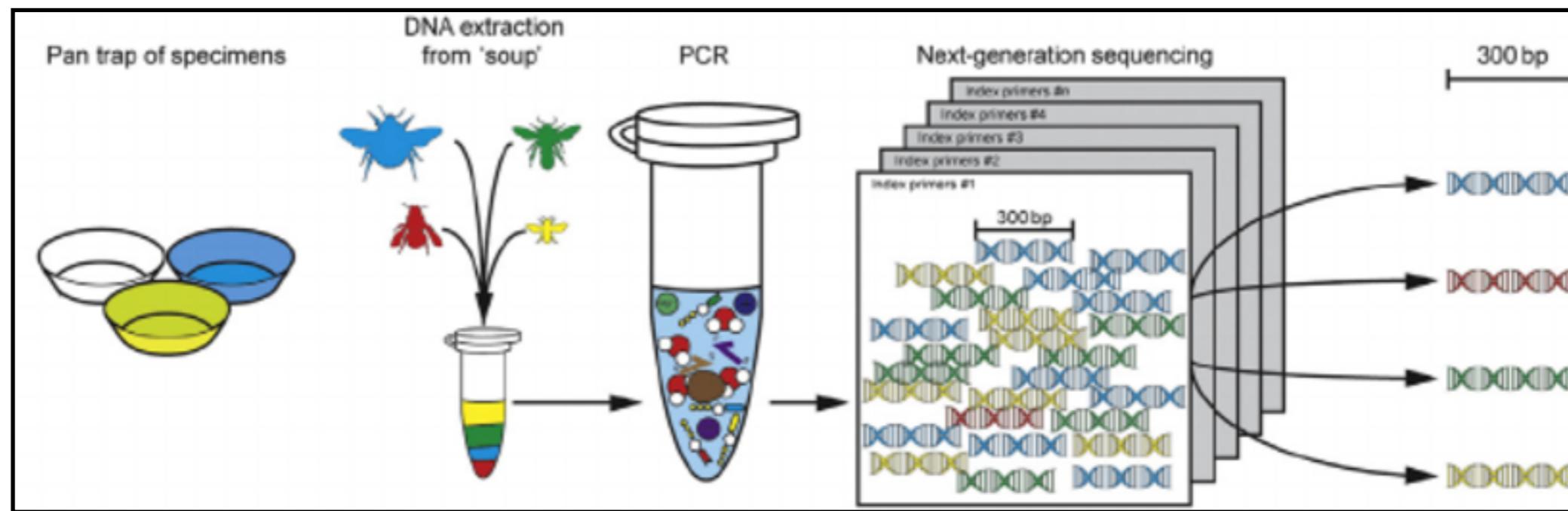
MacQIIME Hollys-MacBook-Retina:~ $
```

DNA Barcoding from Single Nematode Specimens



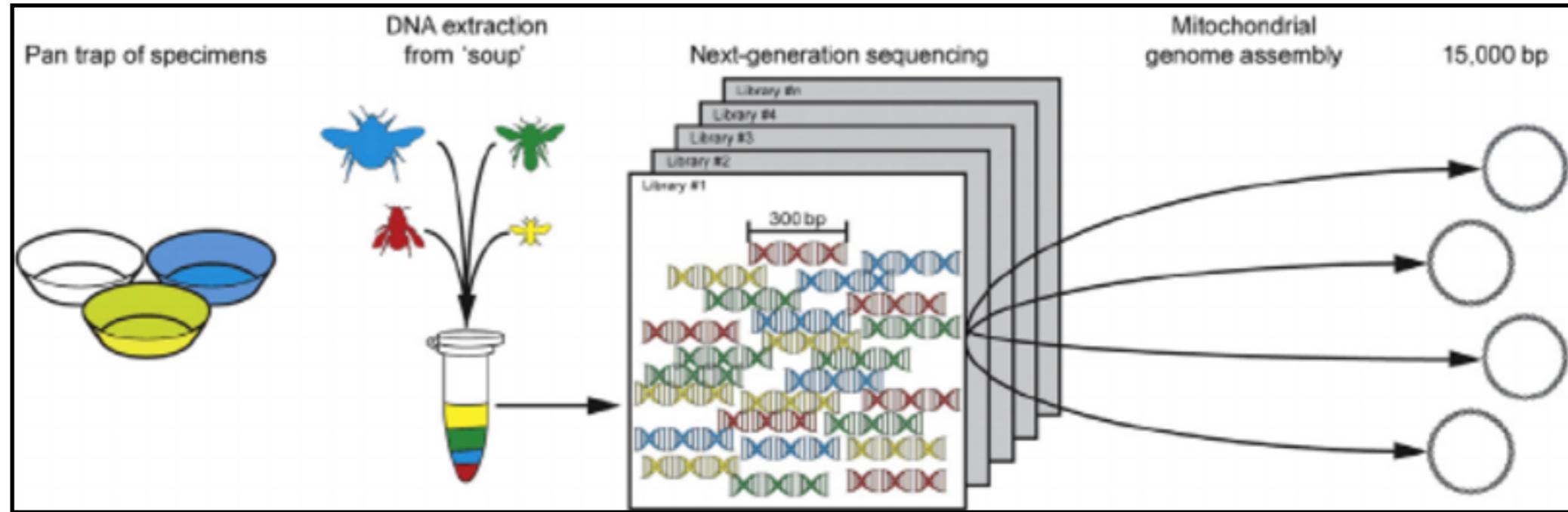
- Experts pick one worm into one tube (usually after taxonomic identification)
- A “barcoding gene” is copied from each nematode using the Polymerase Chain Reaction (PCR)
- Barcoding gene used for nematodes is a 400bp -1600bp fragment of the **18S ribosomal rRNA gene** (nuclear gene encoding the ribosome)

DNA Metabarcoding from a mixture of nematodes



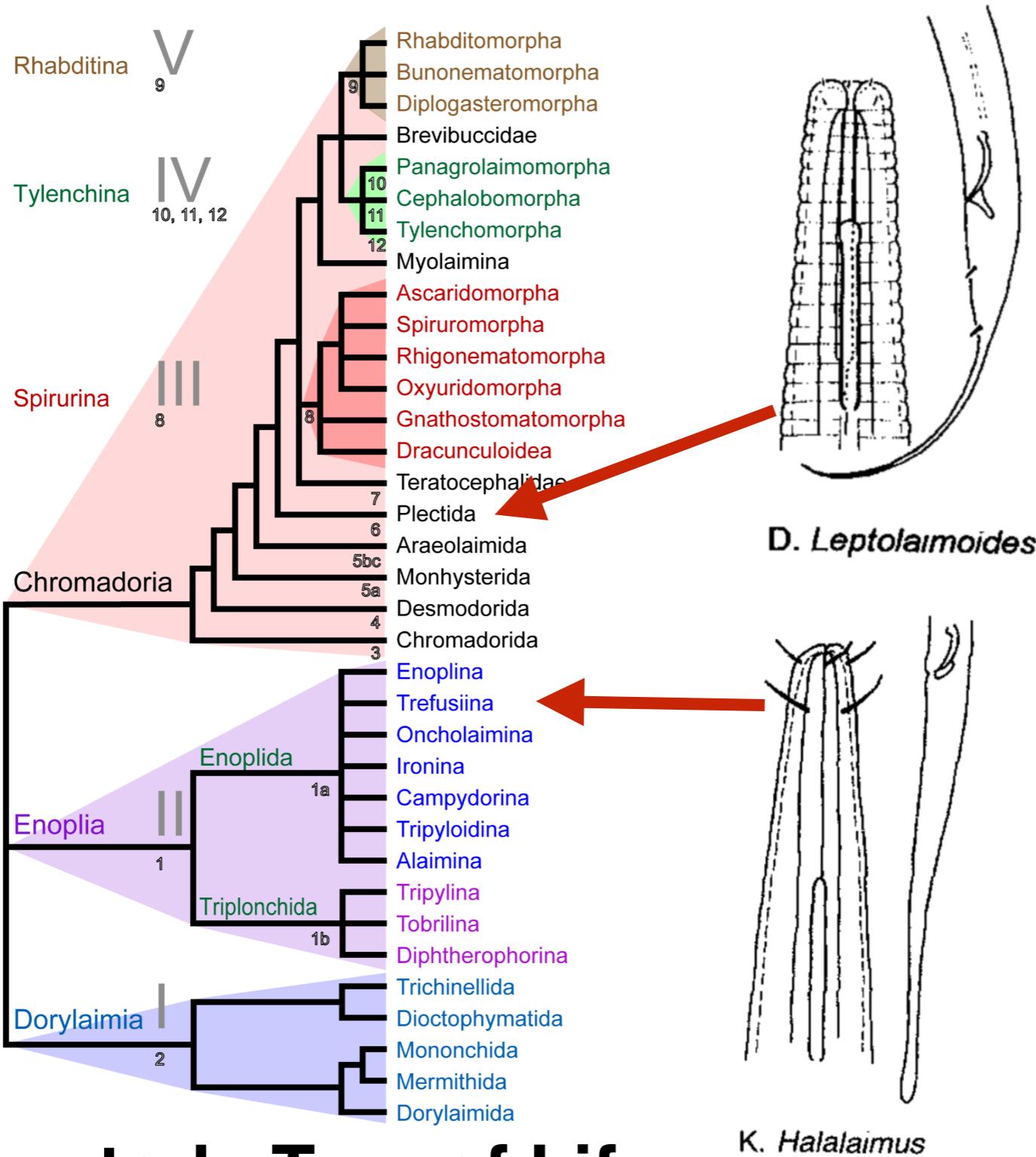
- Nematodes are not separated into individual tubes - DNA is extracted from a large mixture of species poured into one tube
- DNA barcoding from all specimens happens at the same time (parallel PCR amplification of **18S rRNA barcoding genes**) - uses short barcodes ~400bp
- DNA barcodes are separated out after sequencing, using **bioinformatics** (computational data analysis and computer scripts that cluster and match sequences to a database)

Metagenomics from a mixture of nematodes



- Nematodes are not separated into individual tubes - DNA is extracted from a large mixture of species poured into one tube
- Metagenomics involves NO PCR amplification; random genomic fragments are sequenced directly
- Requires intensive **bioinformatics** to pull out useful information - e.g. assembling whole mitochondrial genomes (can also mine for 18S rRNA genes)

Convergent Evolution



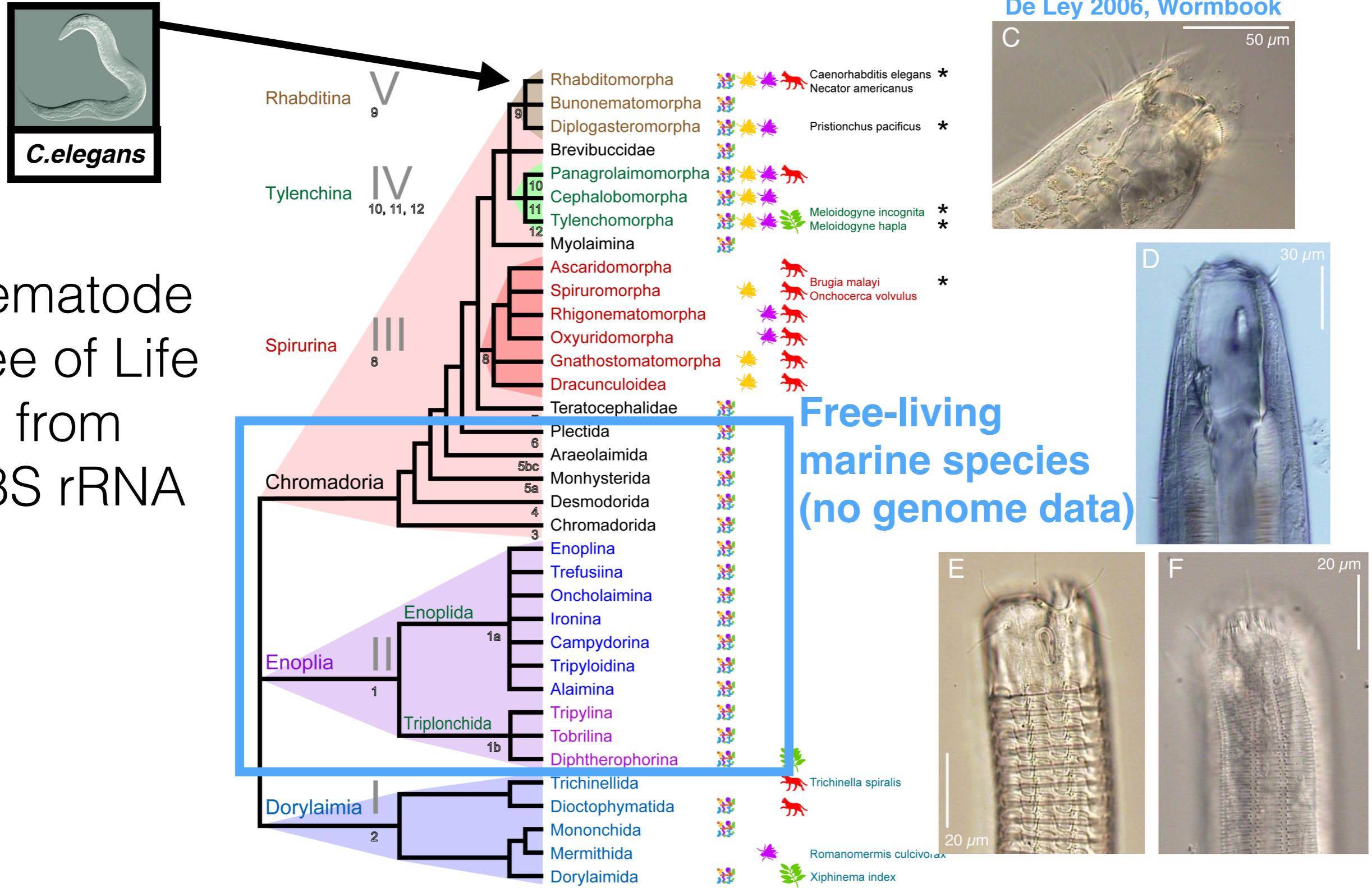
Same body structures have evolved multiple times, independent of each other

Can only be detected by using DNA sequencing

Nematode Tree of Life

DNA sequences overturned previous morphological classifications of nematodes

Nematode
Tree of Life
from
18S rRNA

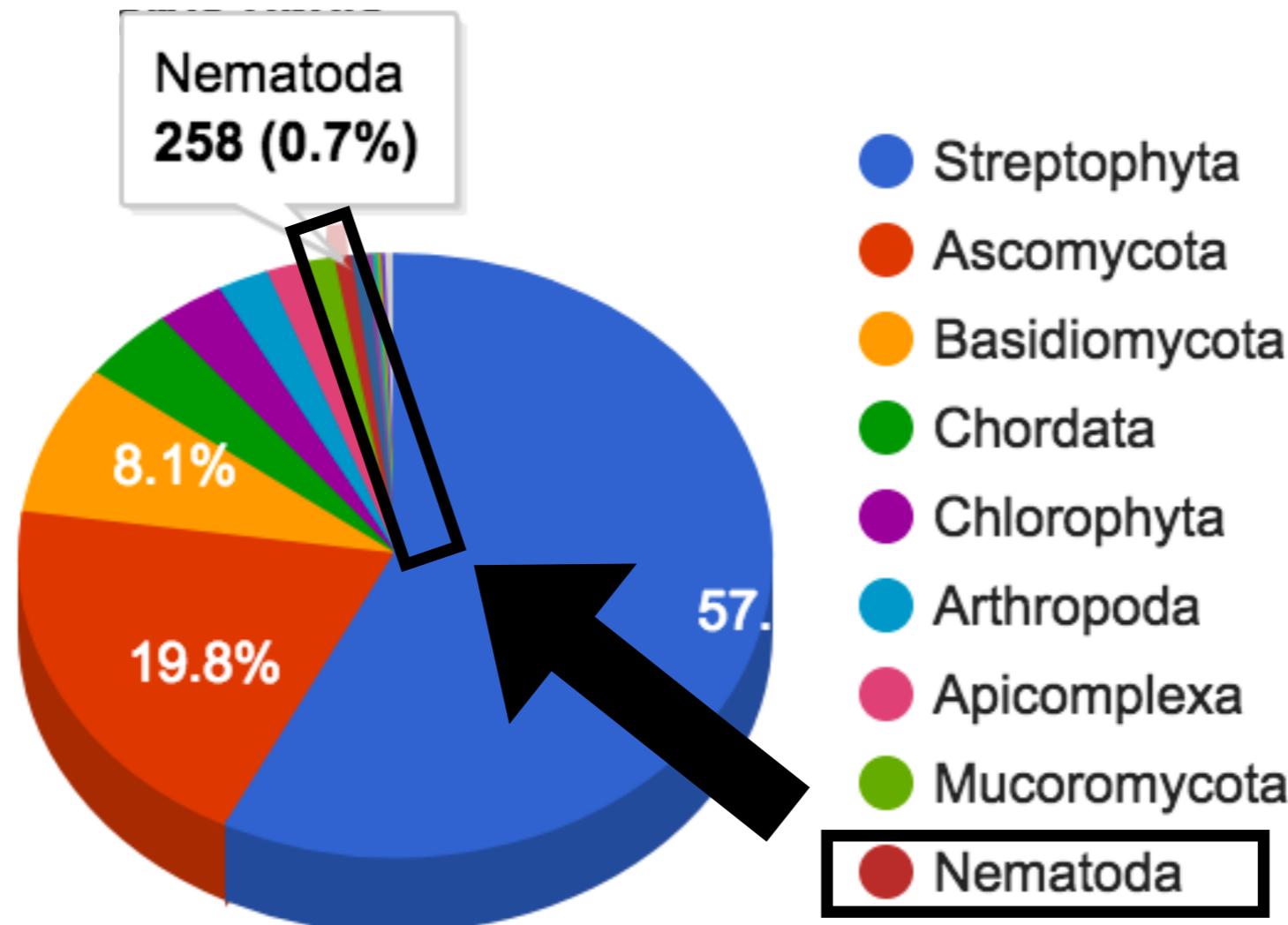


Published Nematode Genomes are *Terrestrial* or *Parasitic*

<i>Acanthocheilonema</i>	<i>Gongylonema</i>	<i>Rhabditophanes</i>
<i>Ancylostoma</i>	<i>Haemonchus</i>	<i>Romanomermis</i>
<i>Angiostrongylus</i>	<i>Heligmosomoides</i>	<i>Rotylenchulus</i>
<i>Anisakis</i>	<i>Heterohabditis</i>	<i>Soboliphyme</i>
<i>Ascaris</i>	<i>Howardula</i>	<i>Steinernema</i>
<i>Brugia</i>	<i>Litomosoides</i>	<i>Strongyloides</i>
<i>Bursaphelenchus</i>	<i>Loa</i>	<i>Strongylus</i>
<i>Caenorhabditis</i>	<i>Meloidogyne</i>	<i>Syphacia</i>
<i>Cycliostephanus</i>	<i>Nippostrongylus</i>	<i>Teladorsagia</i>
<i>Dictyocaulus</i>	<i>Onchocerca</i>	<i>Thelazia</i>
<i>Dirofilaria</i>	<i>Oscheius</i>	<i>Toxocara</i>
<i>Dracunculus</i>	<i>Panagrellus</i>	<i>Trichinella</i>
<i>Elaeophora</i>	<i>Parascaris</i>	<i>Trichuris</i>
<i>Enterobius</i>	<i>Parastonyloides</i>	<i>Wuchereria</i>
<i>Globodera</i>	<i>Pristionchus</i>	

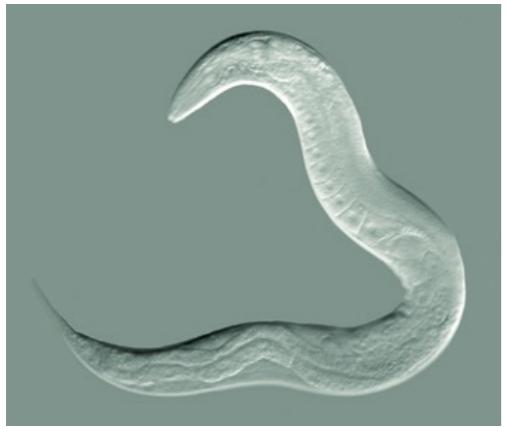
Scientific databases contain few genomes from microbial metazoan phyla such as nematodes

Eukaryotic Phyla



258 nematode genomes completed or in progress

89.3% of genome projects are vertebrates, plants, and fungi



Nematodes

Eukaryotes

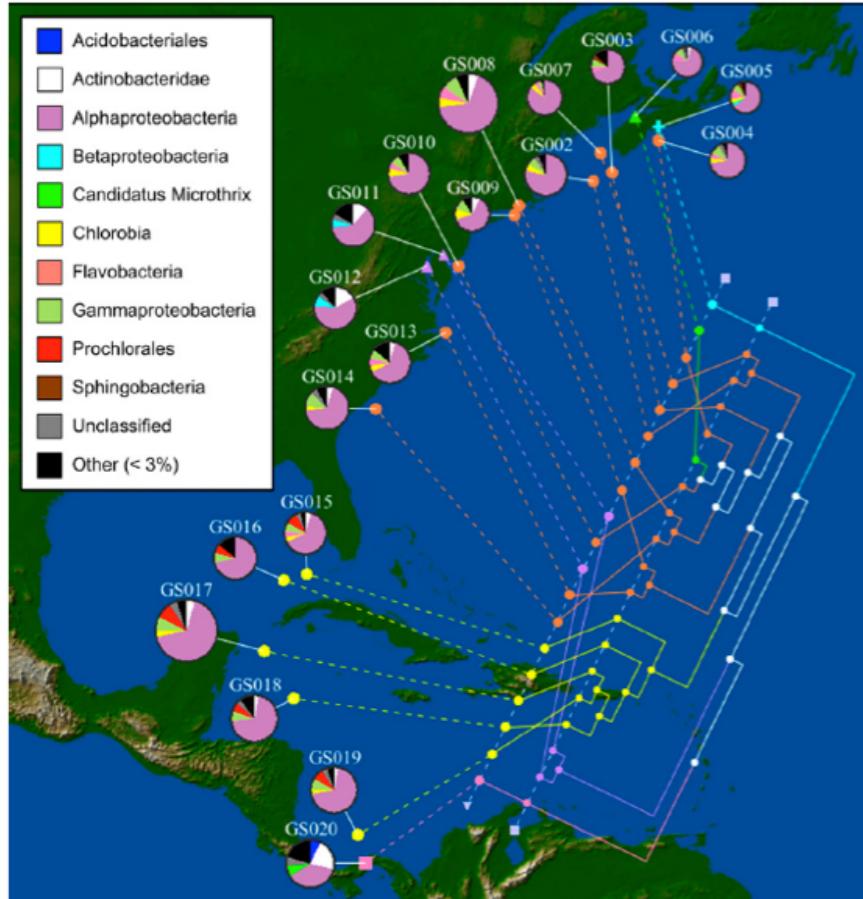
Tree of Life
(from genomes)
itol.embl.de/

Most DNA sequencing & bioinformatics happens here (bacteria and archaea)

Sample Collection



Biological Patterns



DNA Metabarcoding Workflow

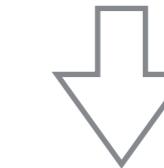
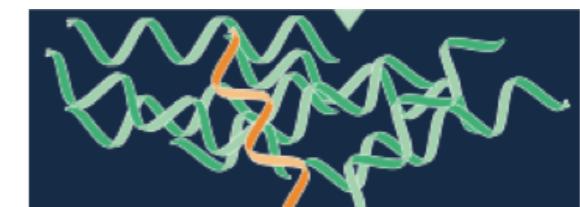
bottleneck

DNA Sequencing



isolate
organisms

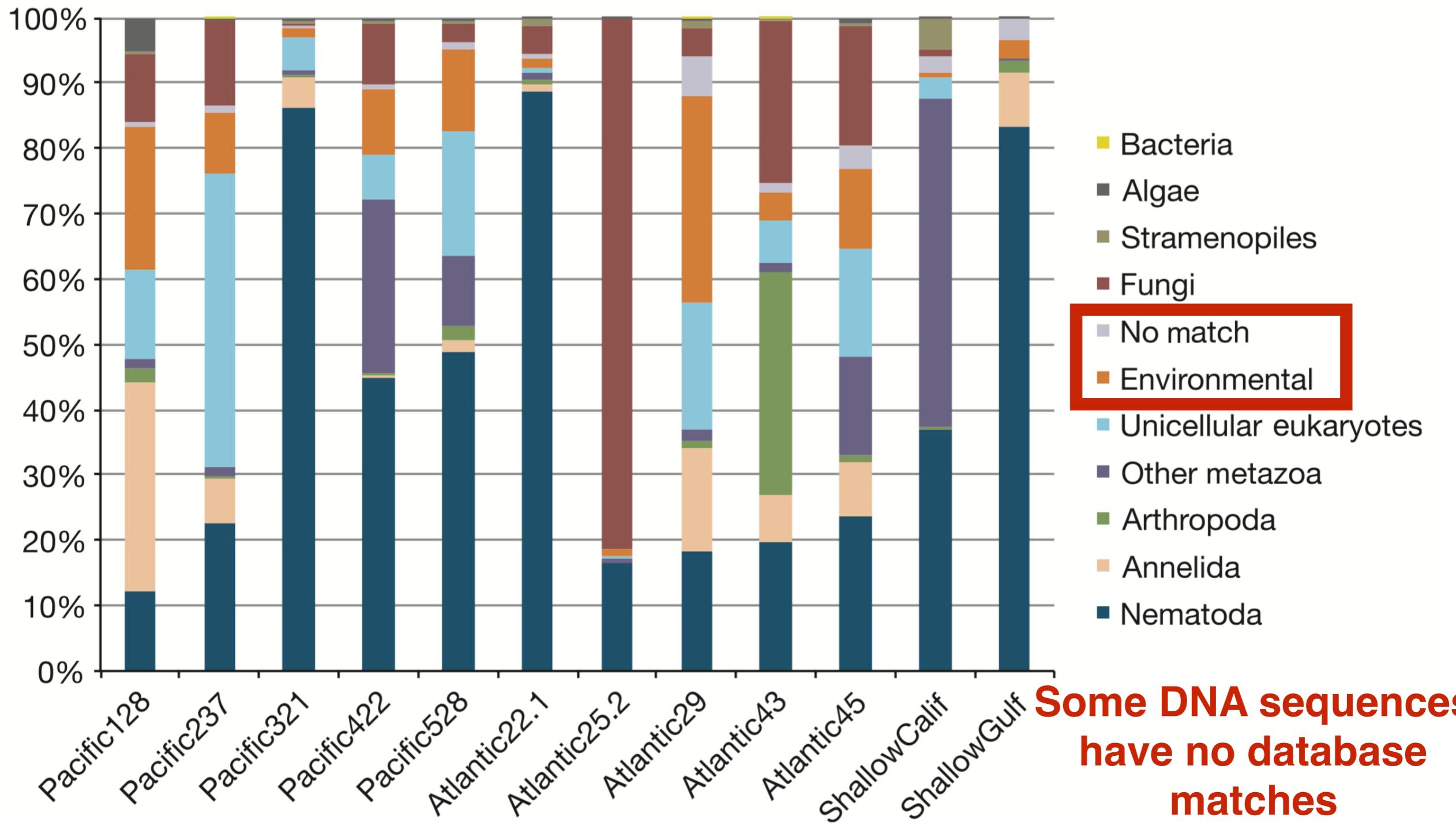
DNA Extraction



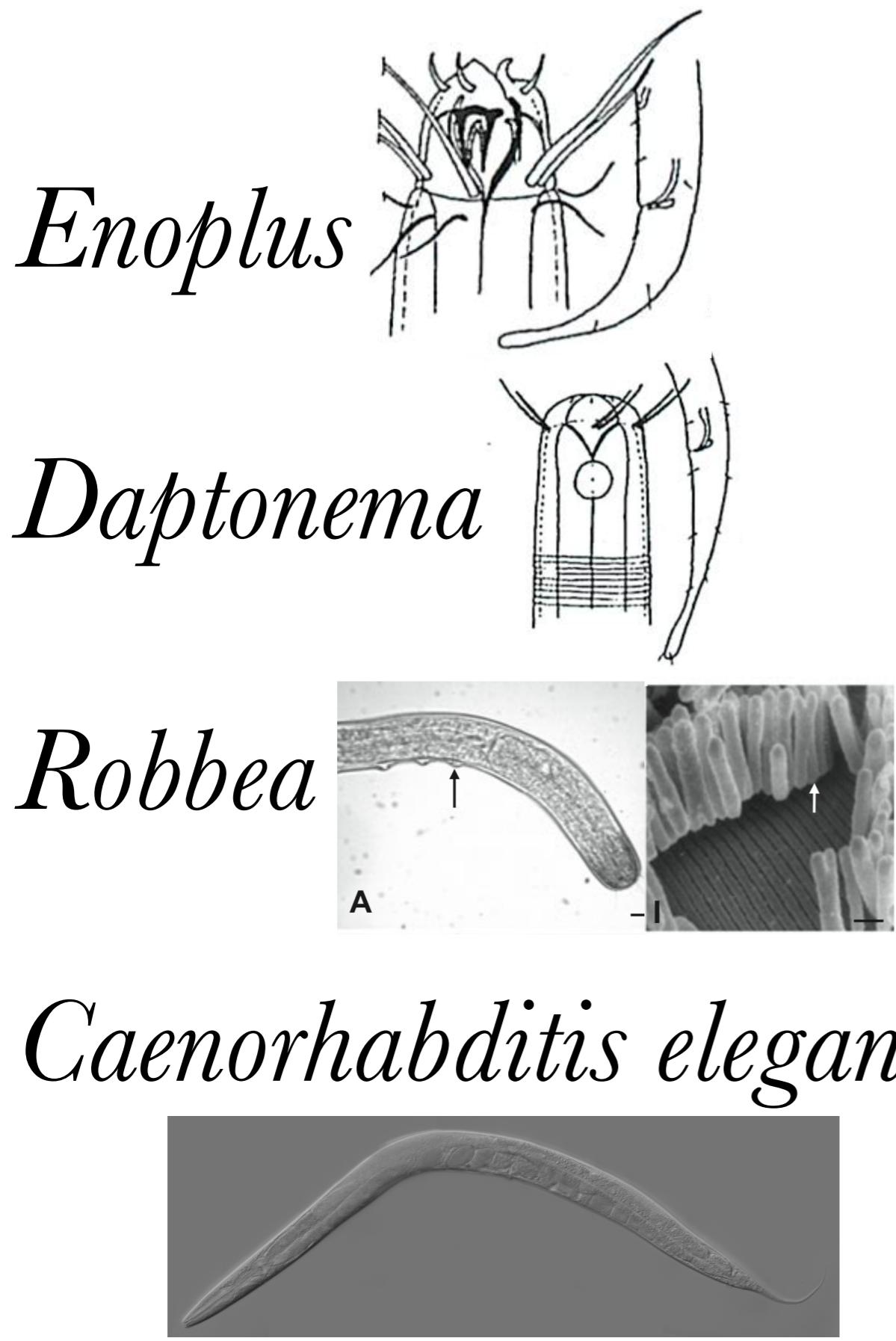
Amplify 18S rRNA



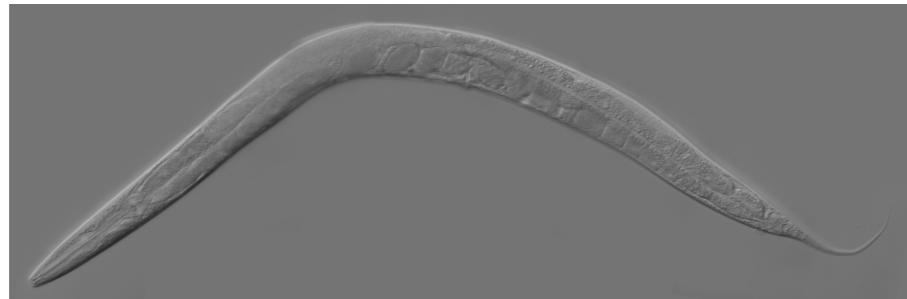
Environmental sequencing recovers a broad range of taxa (not just nematodes)



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Caenorhabditis elegans



Actinomyces spp.

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Clostridium spp.

?????

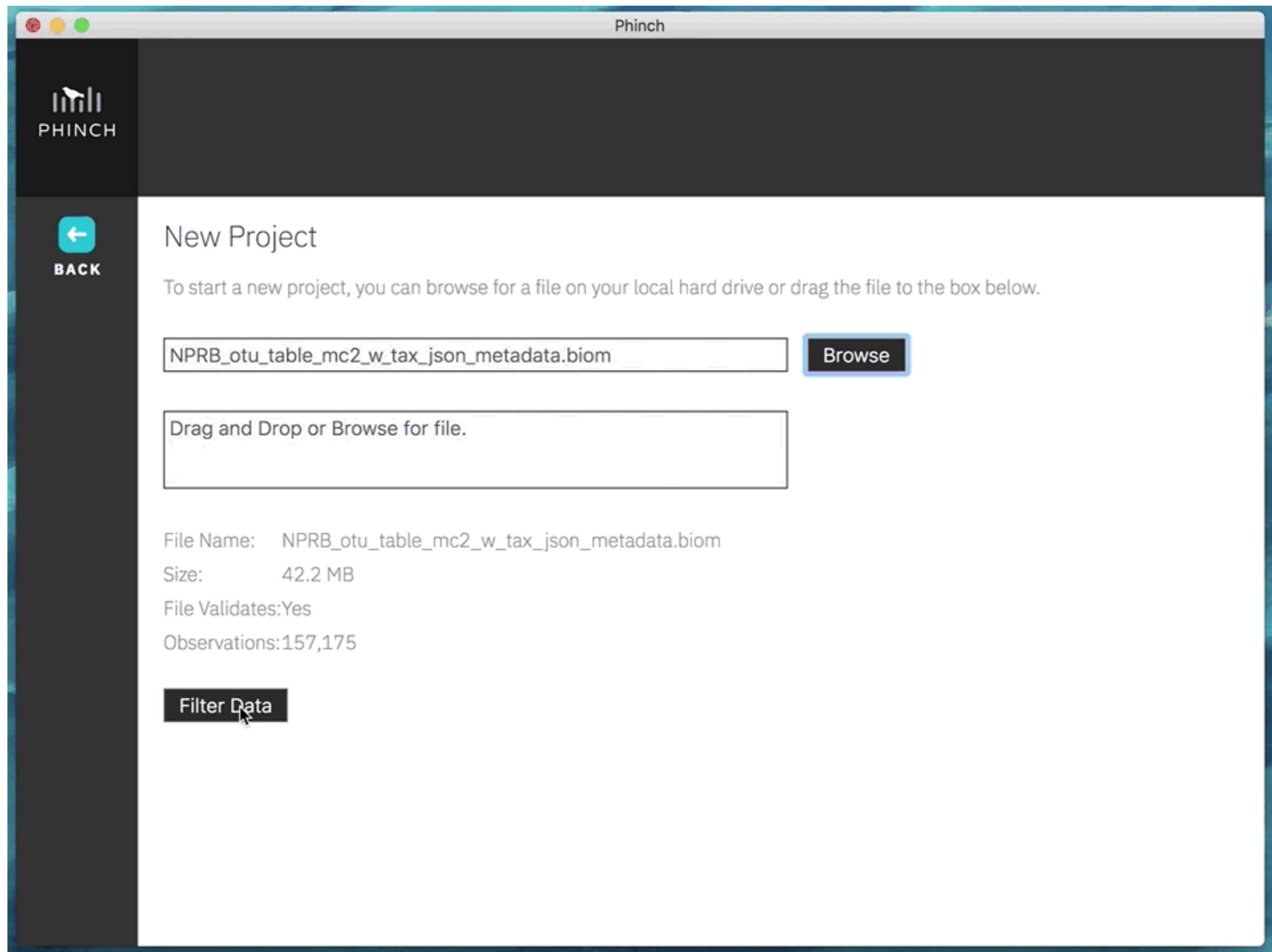
Listeria spp.

?????

Synechococcus spp

?????

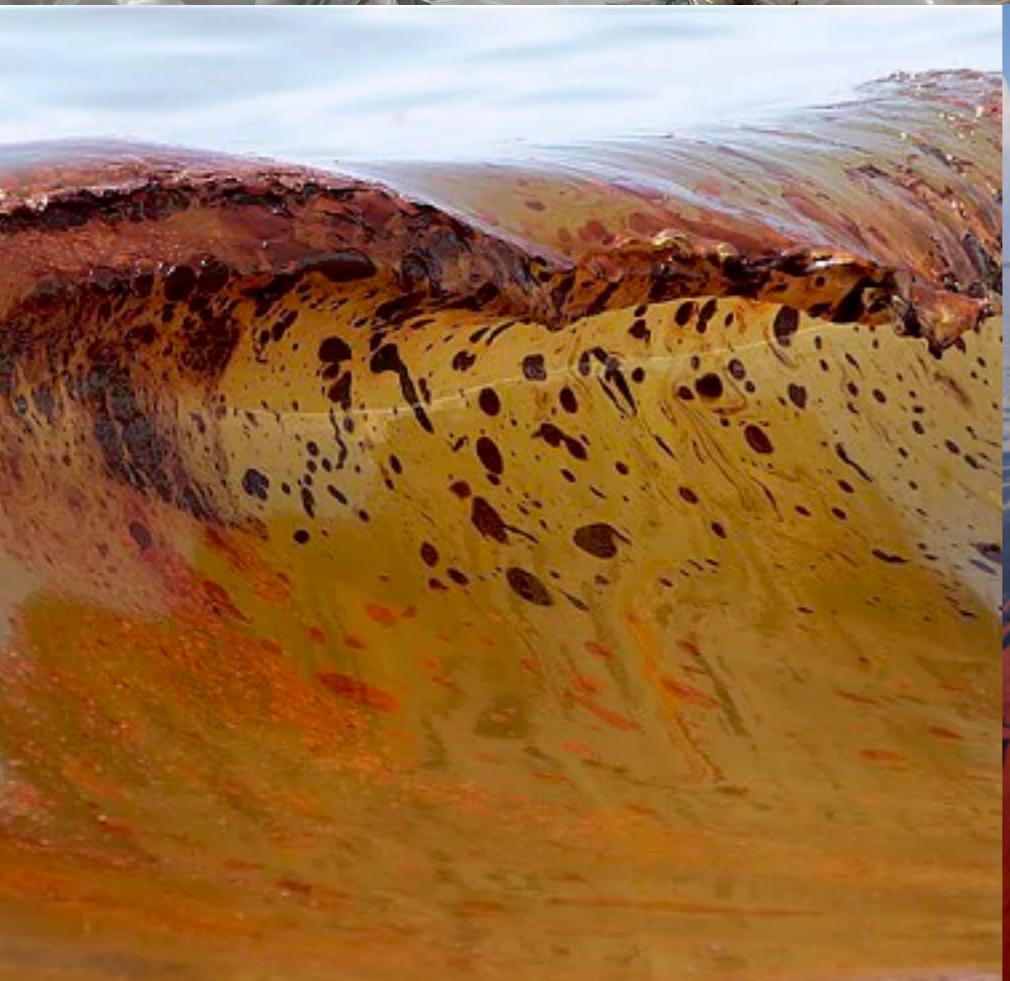
Phinch 2.0 <http://phinch.org>



Applications of DNA metabarcoding:

Deepwater Horizon Oil Spill

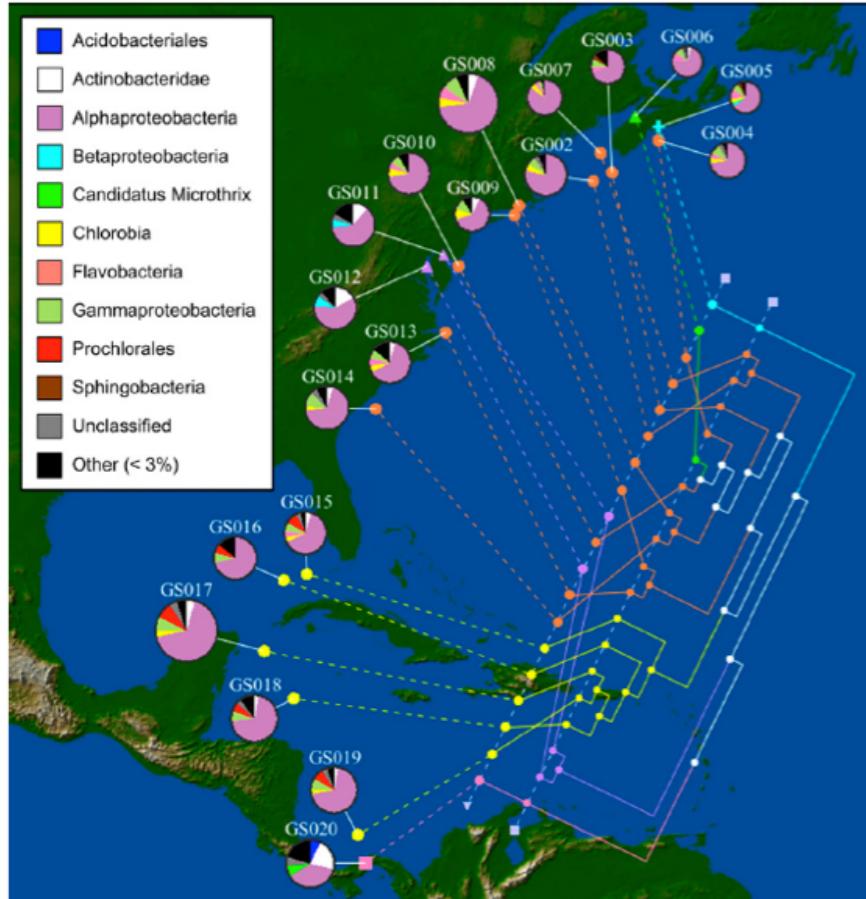




Sample Collection



Biological Patterns



DNA Metabarcoding Workflow

isolate
organisms

DNA Extraction



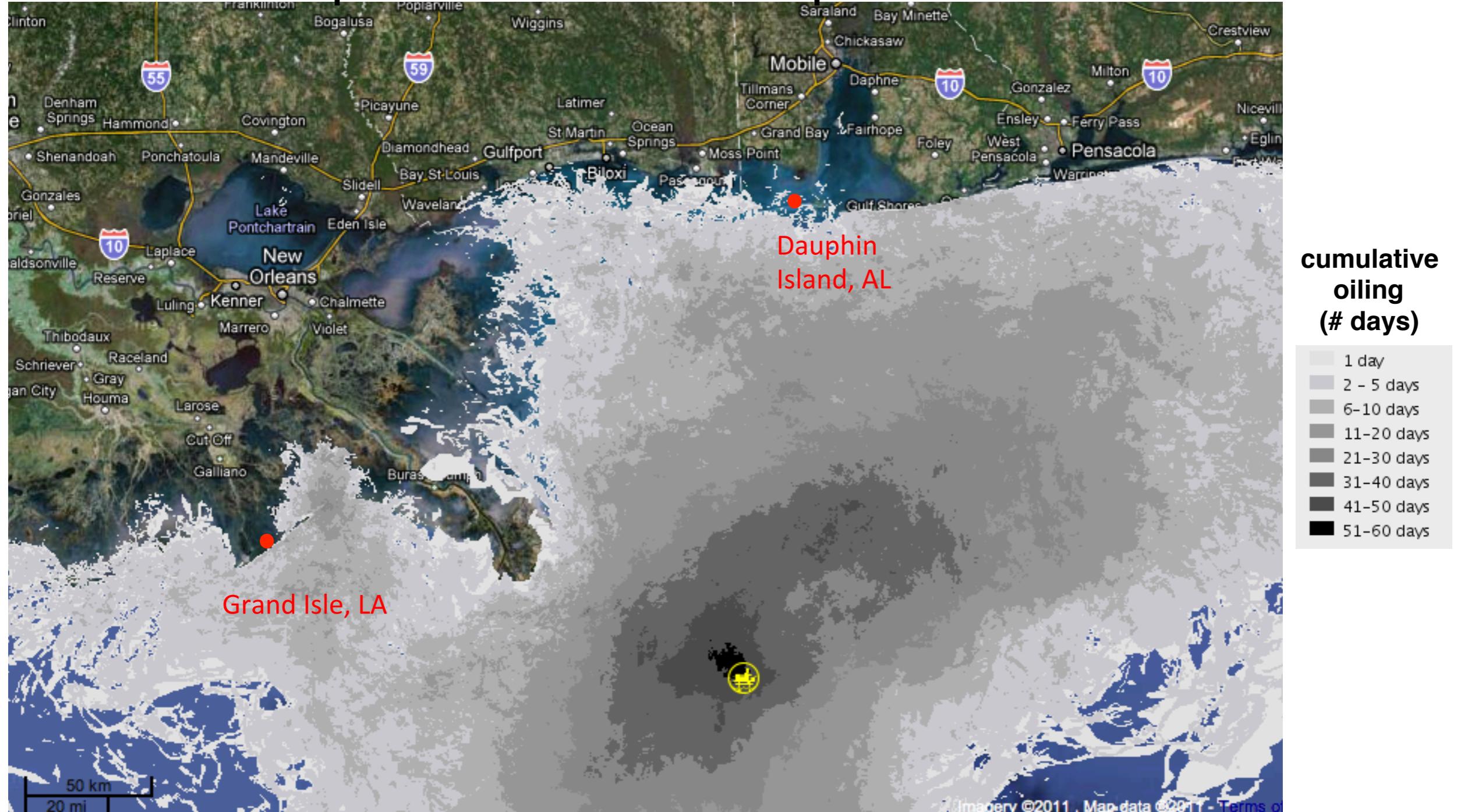
DNA Sequencing



Amplify 18S rRNA

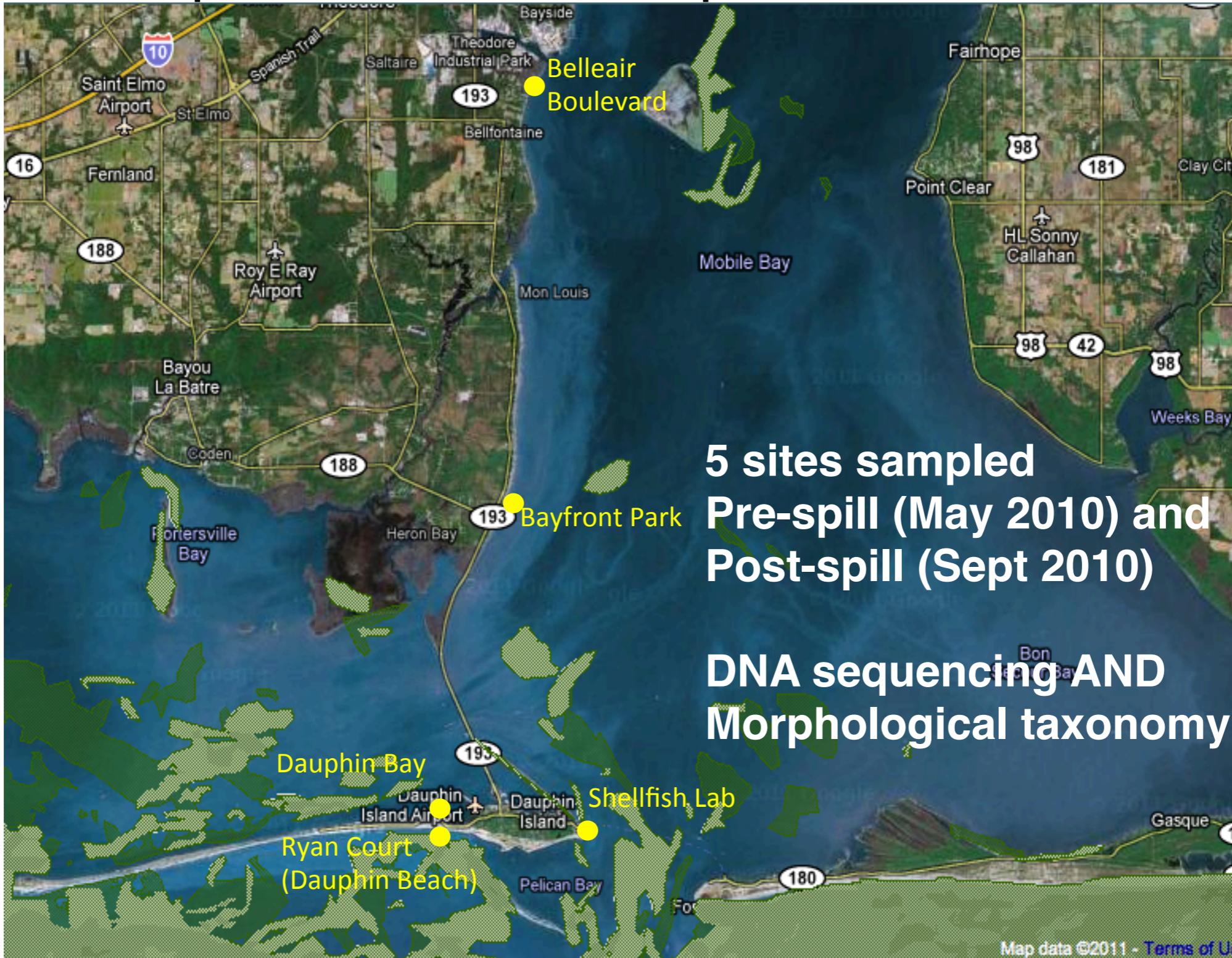


Using DNA Metabarcoding to study the impacts of Deepwater Horizon



Bik et al. (2012) Dramatic Shifts in Benthic Microbial Eukaryote Communities following the Deepwater Horizon Oil Spill. *PLOS ONE*, 7(6): e38550

Using DNA Metabarcoding to study the impacts of Deepwater Horizon

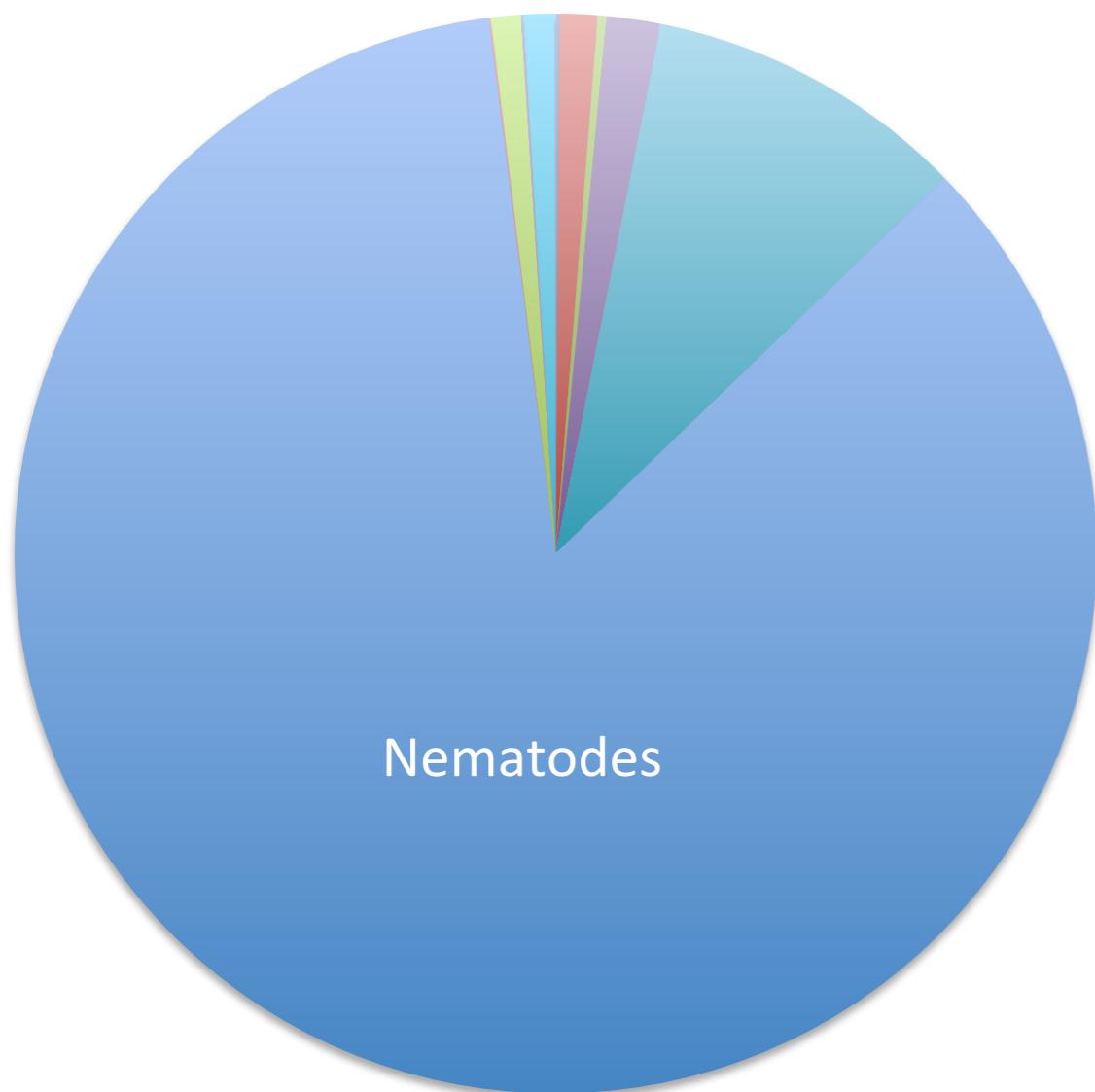


Using DNA Metabarcoding to study the impacts of Deepwater Horizon

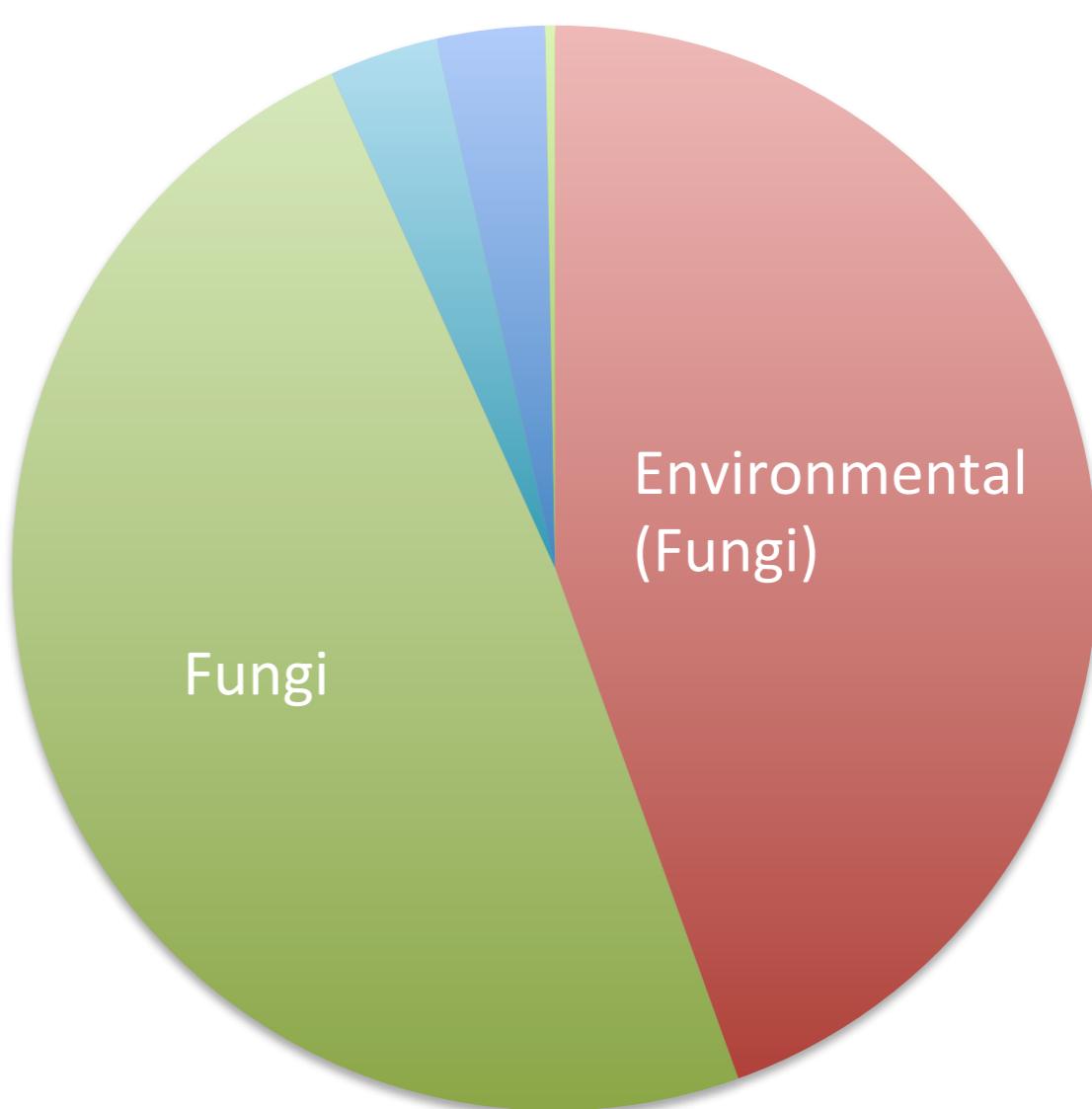


DNA Metabarcoding shows impacts of Deepwater Horizon

Shellfish Lab, Dauphin Island
Pre-spill (May 2010)



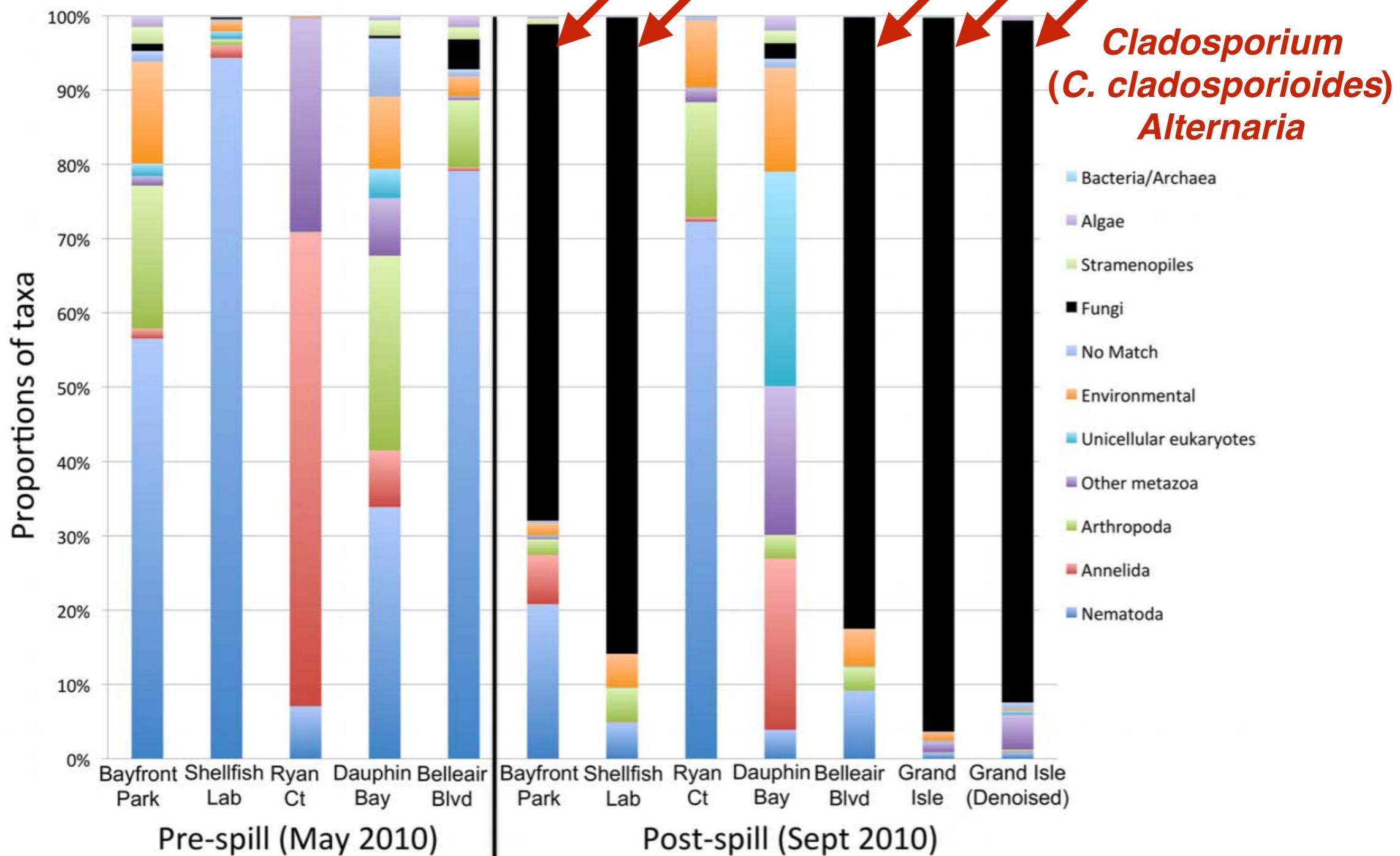
Shellfish Lab, Dauphin Island
Post-spill (Sept 2010)



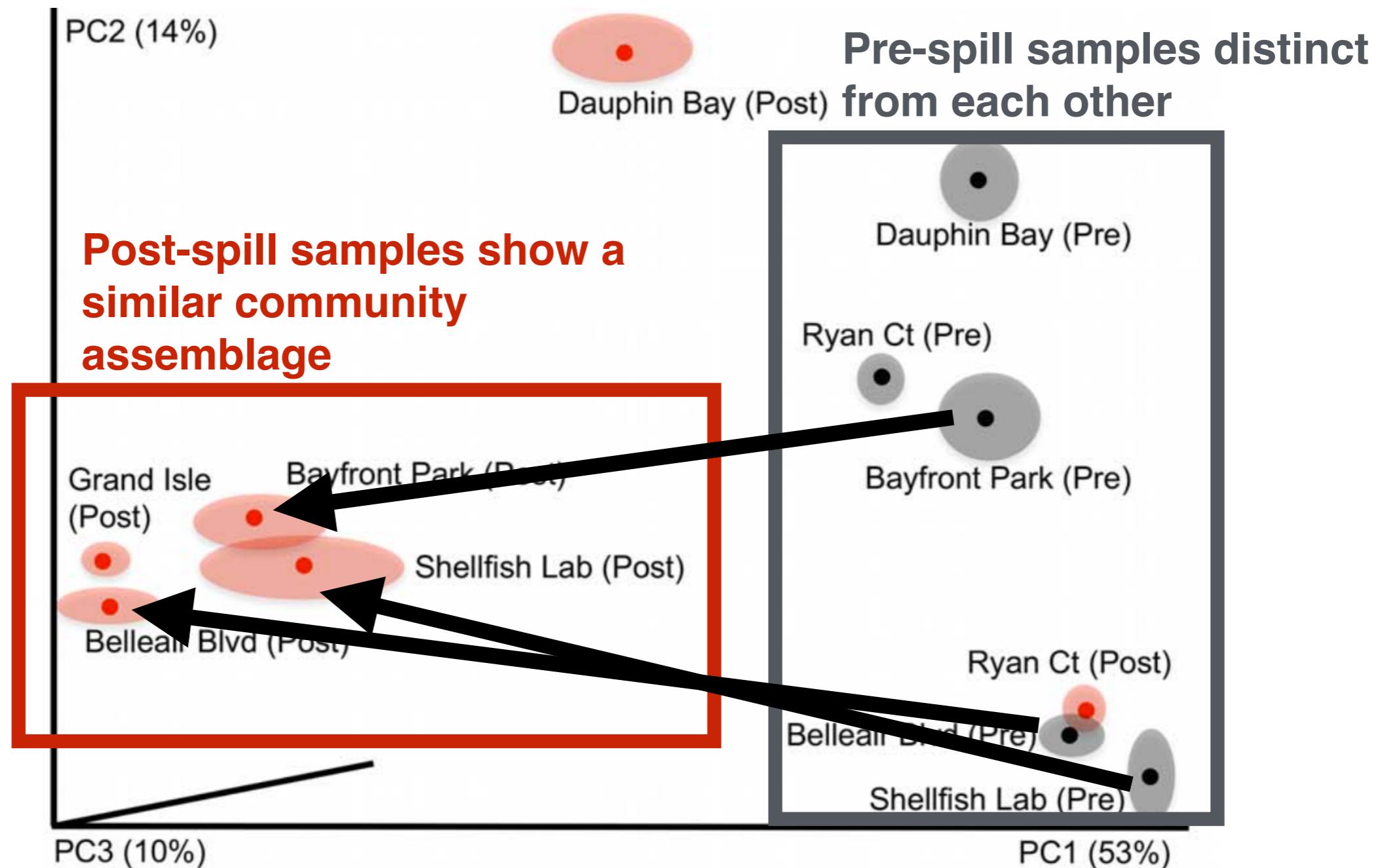
Typical meiofauna assemblage → **Low diversity community**

DNA Metabarcoding shows impacts of Deepwater Horizon

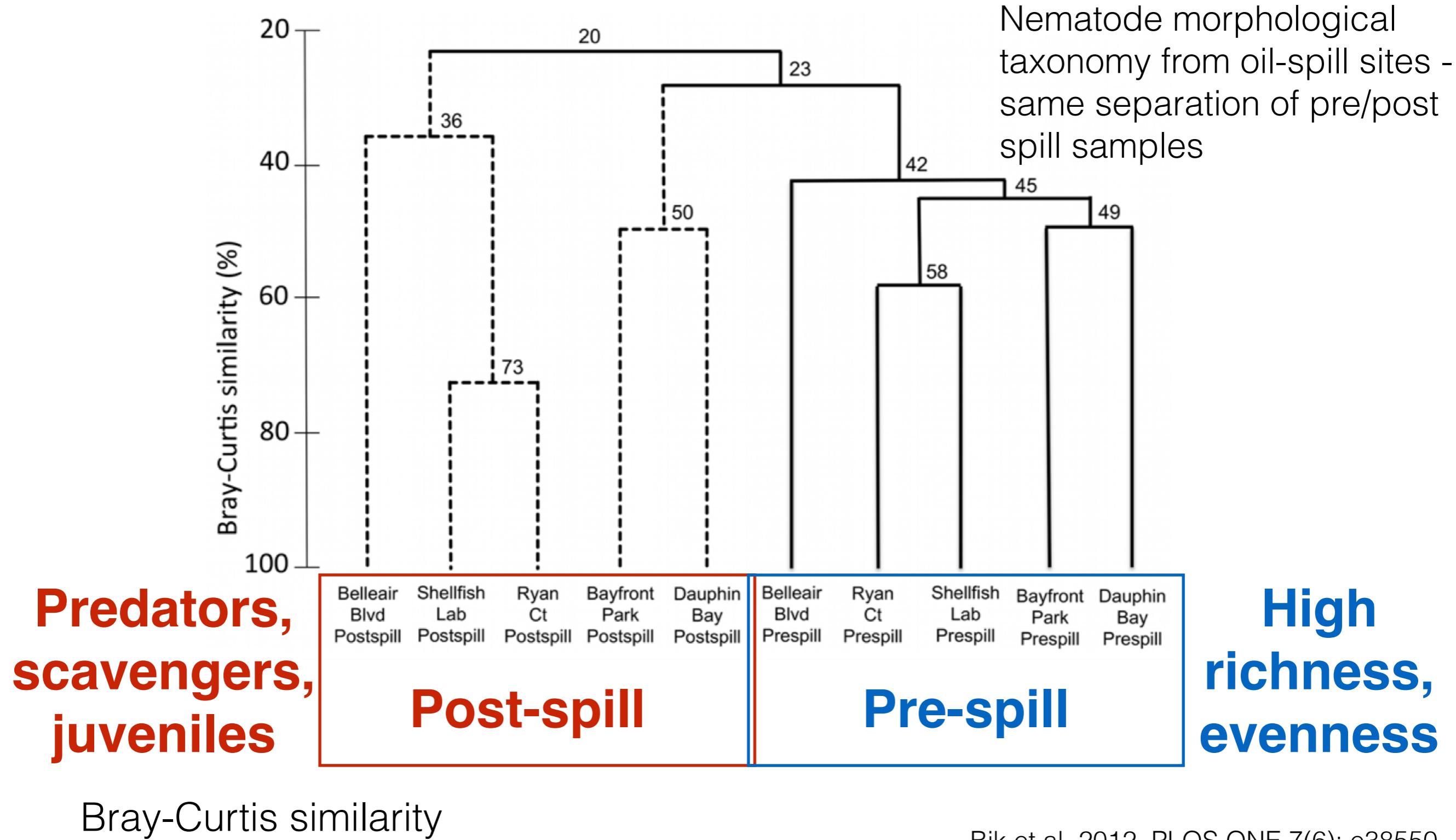
Consistent dominance of fungi



DNA Metabarcoding shows impacts of Deepwater Horizon

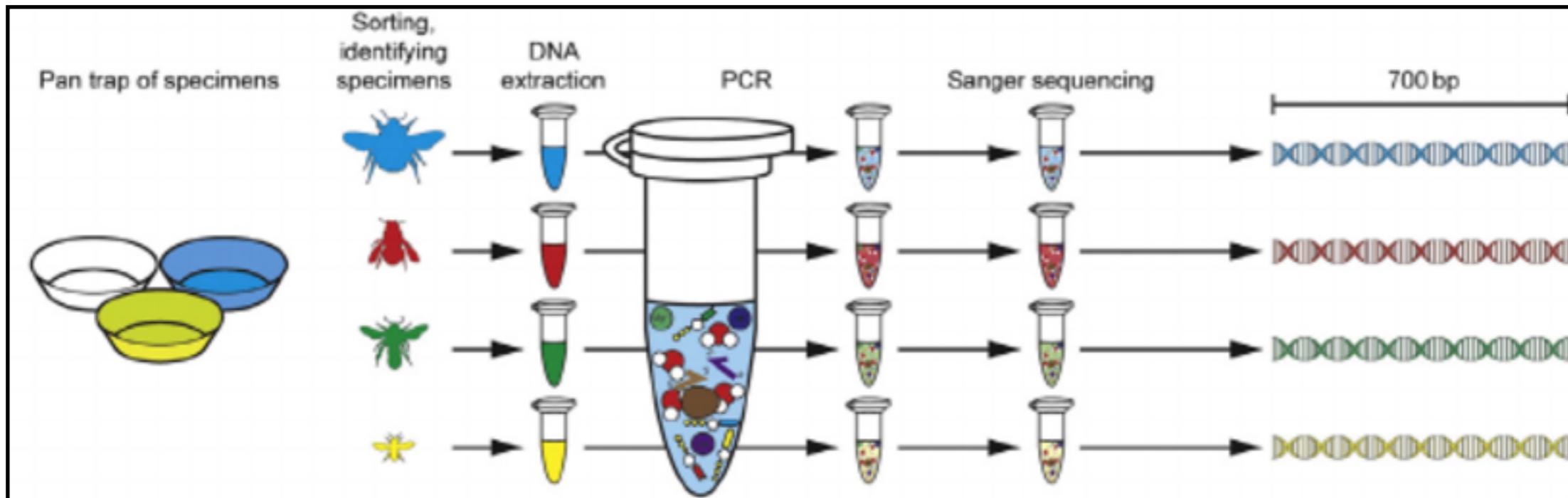


DNA Metabarcoding agrees with morphological taxonomy data



Dynamic Genome Class:

DNA Barcoding Single Nematodes



- You will be given a tube containing one worm (an unidentified nematode isolated from soil or marine sediments)
- You will PCR amplify the **full-length 18S ribosomal rRNA gene** (~1600bp amplified in 2 fragments) from each worm using the Polymerase Chain Reaction (PCR)
- You will compare 18S rRNA sequences to online databases (using BLAST) to try to identify the nematode Genus and its corresponding morphology