



# Introduction to the taxonomy of marine free-living nematodes

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Meioscool, Palma de Mallorca, June 2023



# Presentation outline

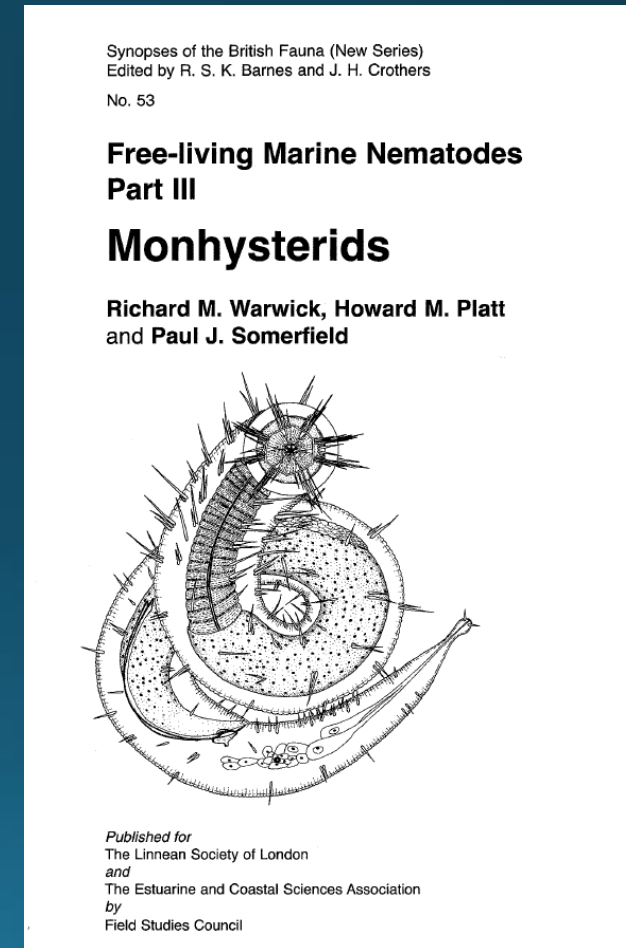
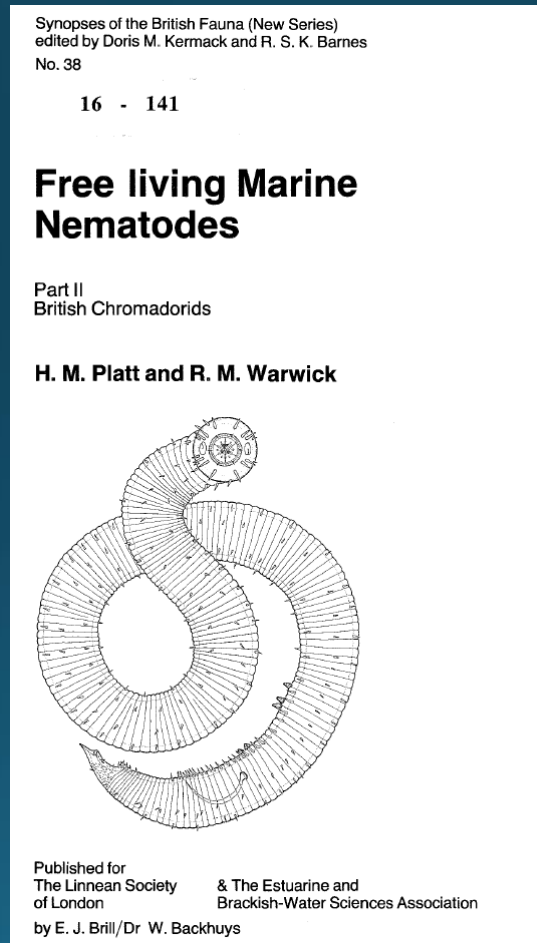
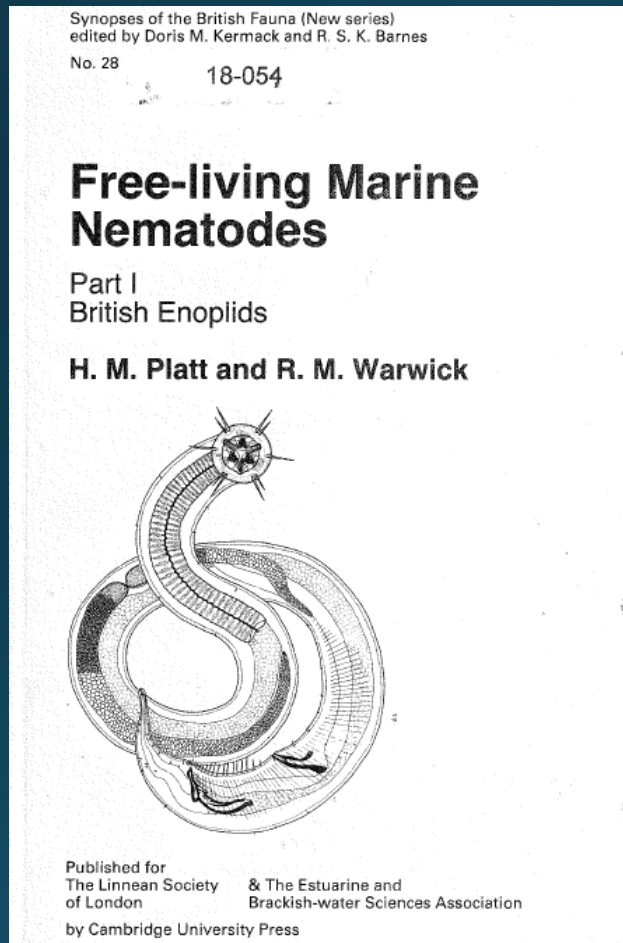
## Marine nematode taxonomy and identification

- Main identification resources
- Broad classification and brief overview of free-living marine nematode orders
- Examples of taxonomically useful morphological traits and their variation
- Recommendations

## Microscopy techniques

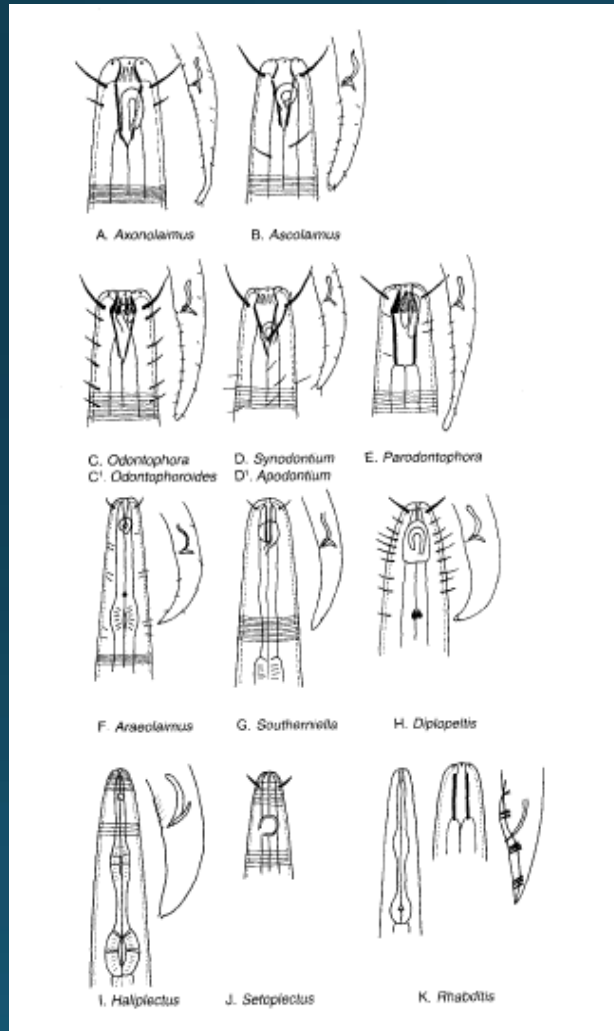
- Light microscopy
- Scanning electron microscopy

# Taxonomy/identification resources:



Out of print – pdfs available to registered users on [nemys.ugent.be](http://nemys.ugent.be)

# Taxonomy/identification resources:

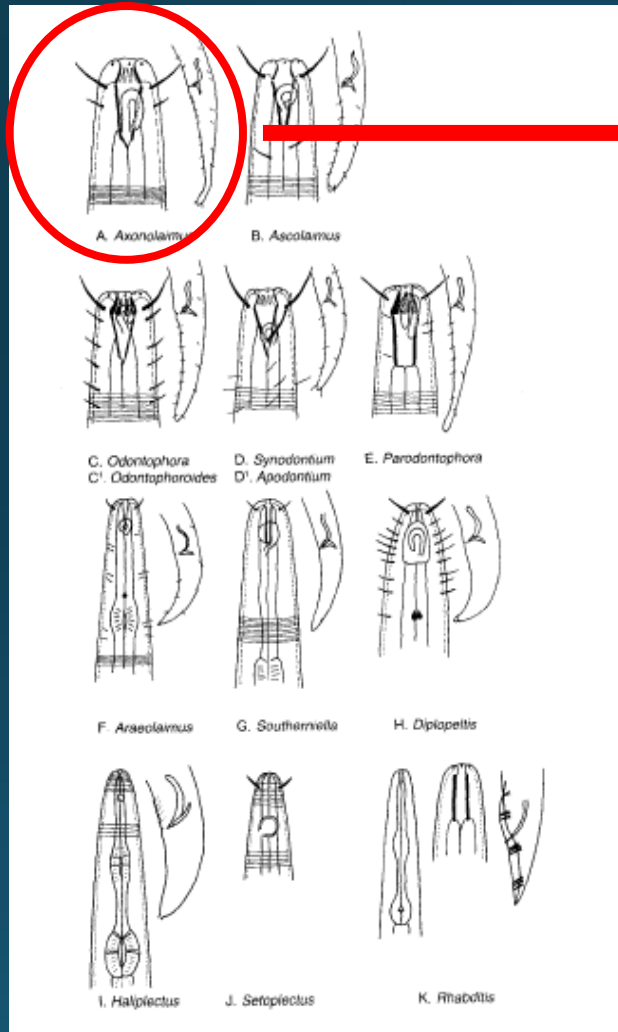


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Fig. 23.

- A, *Axonolaimus*<sup>\*</sup>, buccal cavity double-conical; amphid an elongated loop; female didelphic; L = 1-2.  
 B, *Ascolaimus*<sup>\*</sup>, amphid a rounded loop; tail slightly swollen at the posterior end; L = 2-6.  
 C, *Odontophora*<sup>\*</sup>, buccal cavity with a ring of six teeth in the anterior part (odontia); L = 1-4.  
 C', *Odontophoroides*, similar to *Odontophora* except: lateral subcephalic seta present; female monodelphic; L = 1-1.5.  
 D, *Synodontium*, similar to *Axonolaimus* except: lateral subcephalic seta present; female monodelphic; L = 2-5.  
 D', *Apodontium*, similar to *Synodontium* except: amphids indistinct; female didelphic; L = 2-3.  
 E, *Parodontophora*, walls of buccal cavity parallel; L = 1-2.  
 F, *Araeolaimus*<sup>\*</sup>, buccal cavity narrow, conical; amphid a rounded loop; oesophagus with a bulbous swelling midway; ocellus present, but may be lost on fixation; L = 1-3.  
 G, *Southerniella*, buccal cavity cylindrical; amphid circular; set-off anterior part of oesophagus (corpus); L = 0.5-2.  
 H, *Diplopetis*<sup>\*</sup>, buccal cavity absent; amphids on plaques; ocellus present; L = 2-4.  
 I, *Haliplectus*<sup>\*\*</sup>, cephalic setae absent; amphid circular; mid-oesophageal bulb; pre-cloacal supplements (usually); L = 0.5-2; p. 448.  
 J, *Setoplectus*, similar *Haliplectus* except: four cephalic setae present; L = 1.  
 K, *Rhabditis*<sup>\*</sup>, cephalic sensilla papilliform; amphid absent; buccal cavity tubular; copulatory bursa around cloaca; L = 1-3.

# Taxonomy/identification resources:



58

Fig. 23

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# Taxonomy/identification resources:

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## Family AXONOLAIMIDAE Filipjev, 1918

Axonolaimids typically have the following characters: buccal cavity has a double conical (diamond) shape, often with six tooth-like structures (odontia) in the anterior part; cuticle faintly striated; 6-4 cephalic setae in separate circles, but the anterior six normally reduced to papillae; amphids loop or crook-shaped, often longitudinally elongated; usually two testes and two ovaries.

*Key to genera:* Tarjan & Khuong (1988).

## Genus AXONOLAIMUS De Man, 1889

*Keys:* Wieser, 1959; Hopper, 1963

With reference to the family characters (above), *Axonolaimus* species have elongated amphids and lack odontia in the buccal cavity. In addition to the species described below, there are three other nominal species recorded from the British Isles: *A. filiformis* De Man, 1889, *A. typicus* De Man, 1922 and *A. villosus* Skwarra, 1922. None of these records are accompanied by figures, and require substantiation. *A. filiformis* is, in any case, regarded as a doubtful species (De Coninck & Stekhoven, 1933).

*Species:* 26

### *Axonolaimus helgolandicus* Lorenzen, 1971 (Fig. 107)

*Description:* Body length 1.9 mm. Maximum diameter 39  $\mu\text{m}$  ( $n = 48$ ). Cuticle faintly striated. Six cephalic papillae. Four 16  $\mu\text{m}$  (1.2 h.d.) cephalic setae situated level with the anterior part of the amphids. A 13  $\mu\text{m}$  lateral subcephalic seta situated 2  $\mu\text{m}$  posterior to each amphid. Four additional setae level with the base of the amphids. Somatic setae 79  $\mu\text{m}$ , sparse. Amphids 8  $\mu\text{m}$  wide, 14  $\mu\text{m}$  long, prominent, loop-shaped. Buccal cavity 23  $\mu\text{m}$  long. Oesophagus widens posteriorly, but there is no true bulb. Tail 4.7 a.b.d., conical, with one dorsal and two subventral terminal setae.

Spicules 49  $\mu\text{m}$  (1.4 a.b.d.), dorsally reflexed at the proximal ends. Gubernaculum with a pair of 25  $\mu\text{m}$  slender apophyses. Anterior to the cloaca there is a series of 17 ventral supplements, which are spaced further apart anteriorly.

*Distribution:* Strangford Lough, North East Ireland (intertidal sand).

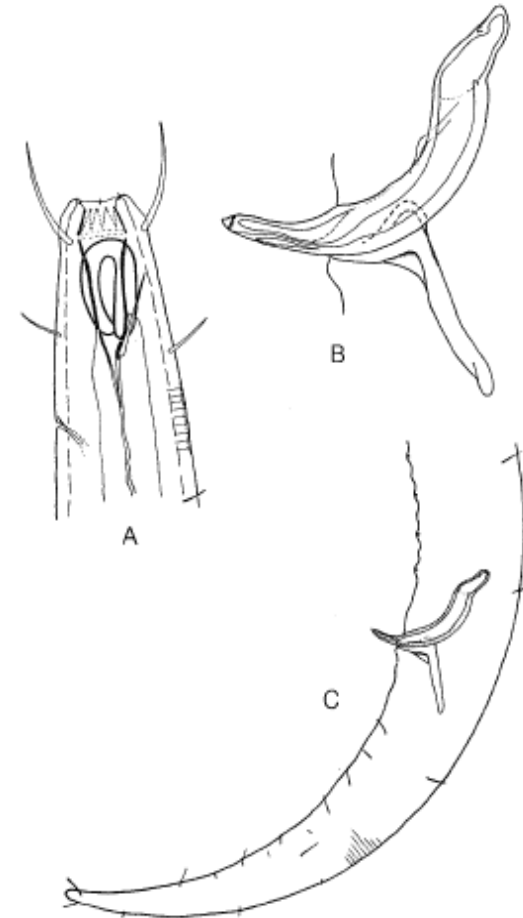


Fig. 107. *Axonolaimus helgolandicus*. A, Male head; B, Spicute and gubernaculum; C, Posterior region of male. Original

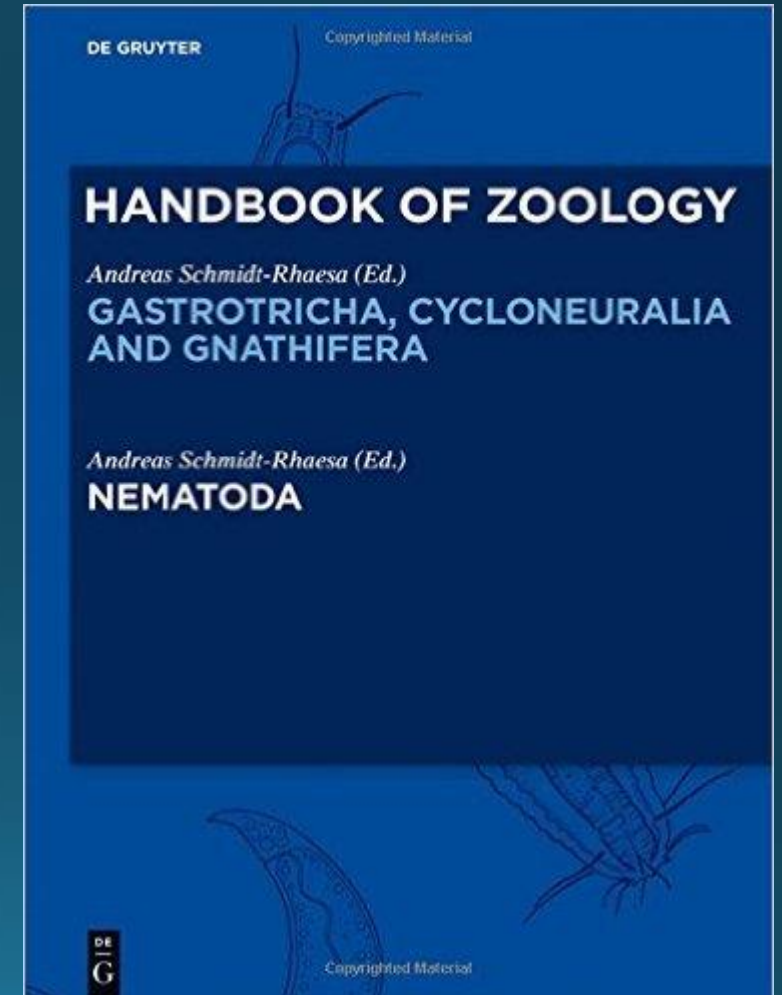
# Taxonomy/identification resources:

New authoritative treatment of nematode taxonomy

One chapter dedicated to each order

Diagnoses of all families & genera

Some keys to families/genera



# Taxonomy/identification resources:

Nemys: Marine nematode database (6564 accepted marine species)

Includes species lists, identification keys, and primary literature sources

Transferred to the World Register of Marine Species (WoRMS) in 2014 –accessible through both Nemys and WoRMS interfaces

15 editors (parasitic taxa now being included)

Still a work in progress



<http://nemys.ugent.be>



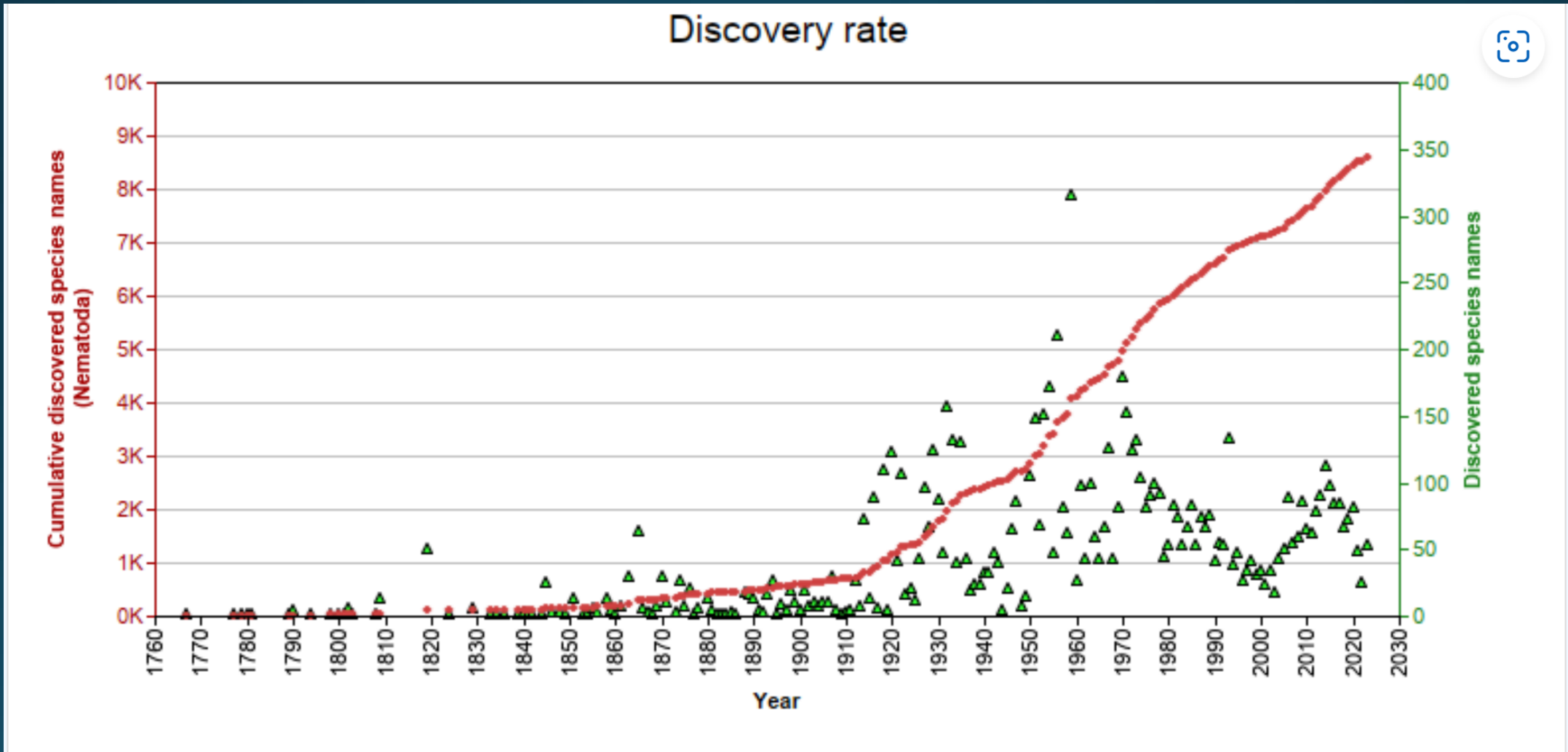


# Taxonomy/identification resources:

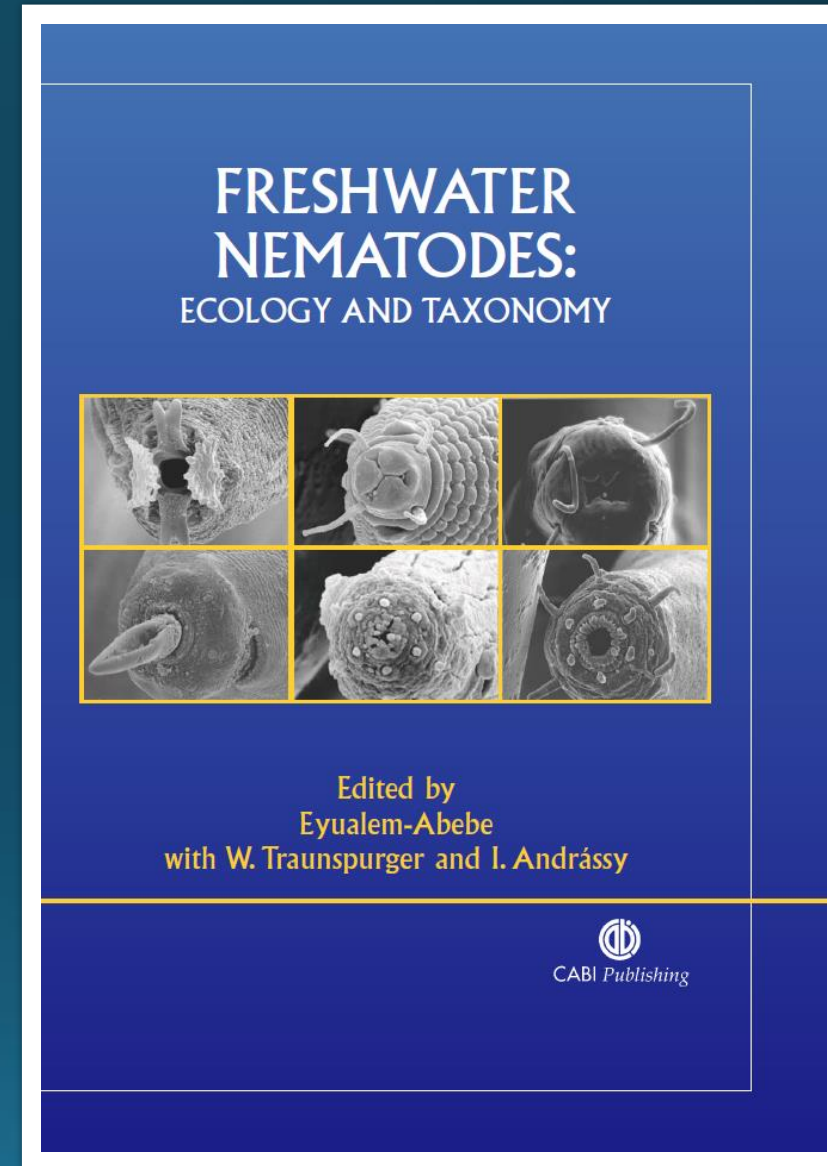
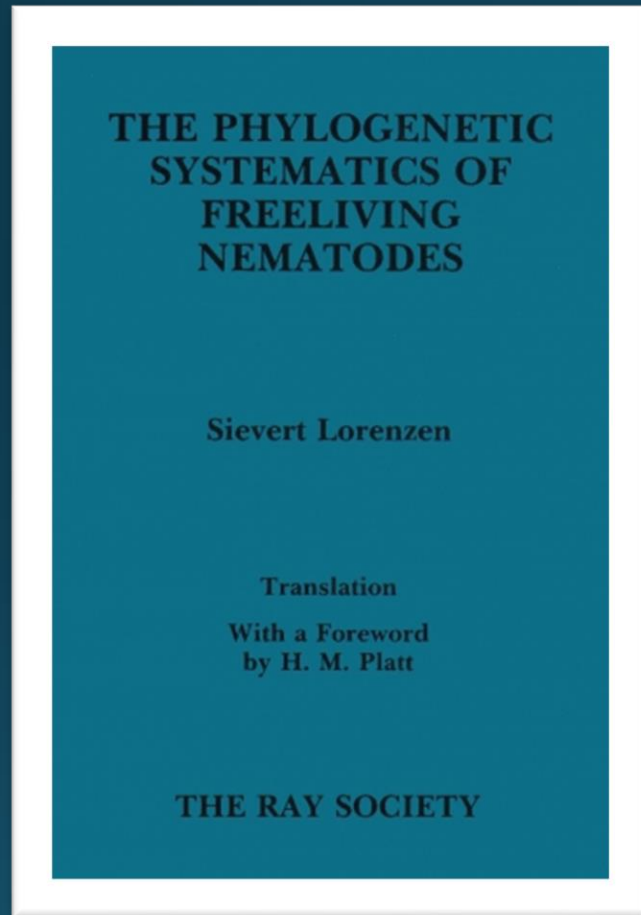
- Contact the relevant WoRMS editors
- Contact details available on Nemys, see also editor workshop report

The screenshot shows the Nemys website interface. At the top, the logo 'Nemys' is displayed in a stylized blue font, followed by the text 'World Database of Nematodes' and 'Linked to the Marine Biology Section, UGent'. A 'login' link is visible in the top right corner. Below the header is a navigation menu with the following items: Start, Browse taxonomy, Search taxonomy, Search literature, Search distributions, Identification keys, Media gallery, Editors, Statistics, Citations, Match taxa, Contact, and Login. The main content area is divided into several sections: 'Introduction' with a paragraph about nematodes and a reference to De Ley & Blaxter (2002, 2004); 'Primary taxonomic literature can be consulted by registered users'; 'The website is integrated into the World Register of Marine Species (WoRMS)'; 'Nemys remains a work in progress!'; and 'News' featuring an announcement for the 'IFNS Three Minute Thesis (3MT®) Competition' dated 2021-08-30. On the right side, there is a 'Quick search' box with a 'Scientific name' input field and a 'Search' button. Below this is a 'Latest taxon additions' section listing several nematode species with their dates of addition, such as 'Rhabditis (Choriorhabditis) marina septentrionalis' added on 2021-09-02. At the bottom right, there is a 'Tweets by @Alex\_Holovachov' section showing a tweet from the 'European Society of Nematologists' (@ESNematologists) dated 2021-09-02.

# Taxonomy/identification resources:



# Other resources:



# Nematode evolution

Nematodes are an ancient group of animals, probably from Precambrian (oldest fossil 395 mya)

Genetic variance is high even among closely related taxa/species

Most genera have a worldwide distribution

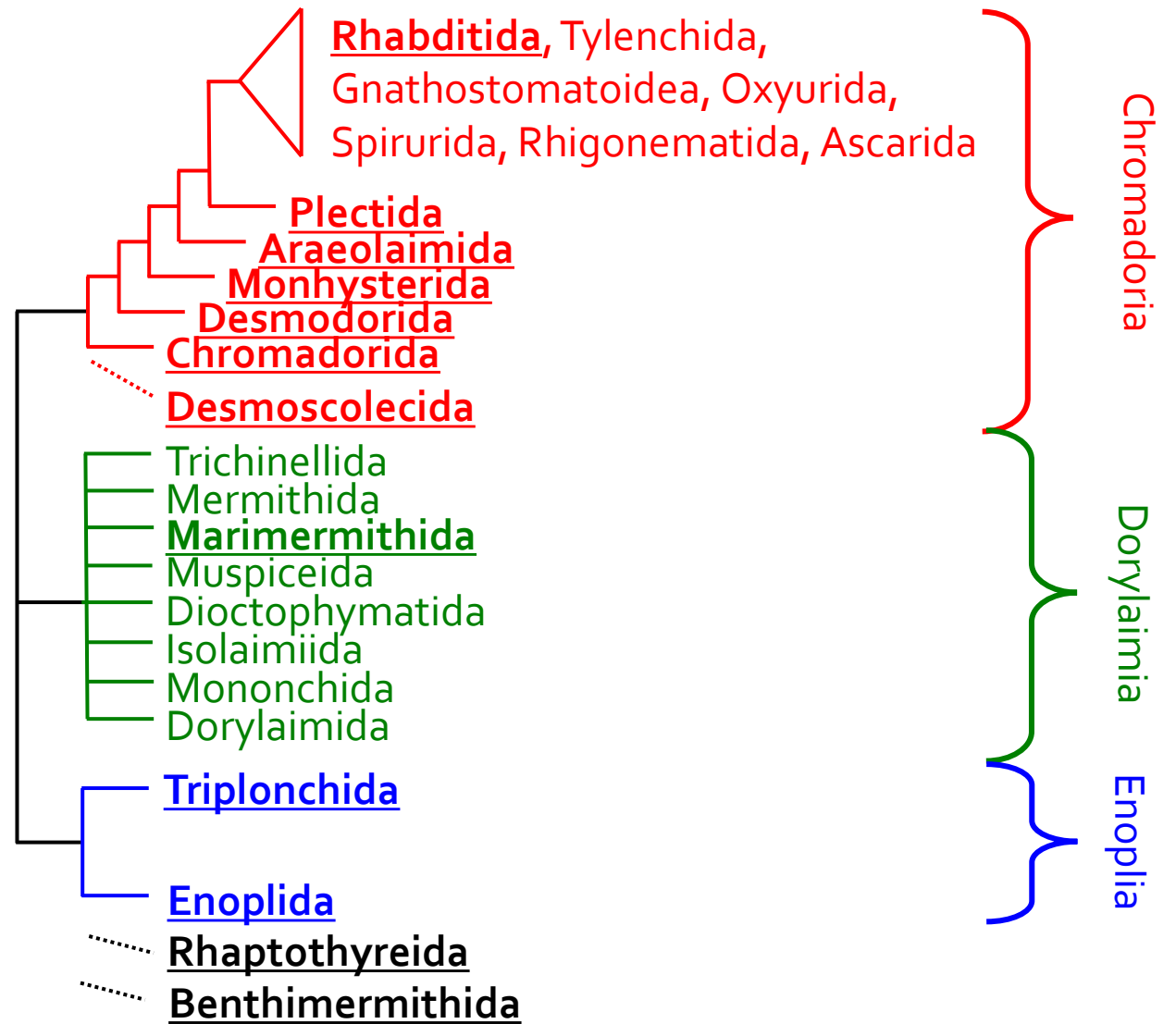
Convergent evolution is common (and confusing for taxonomists!)

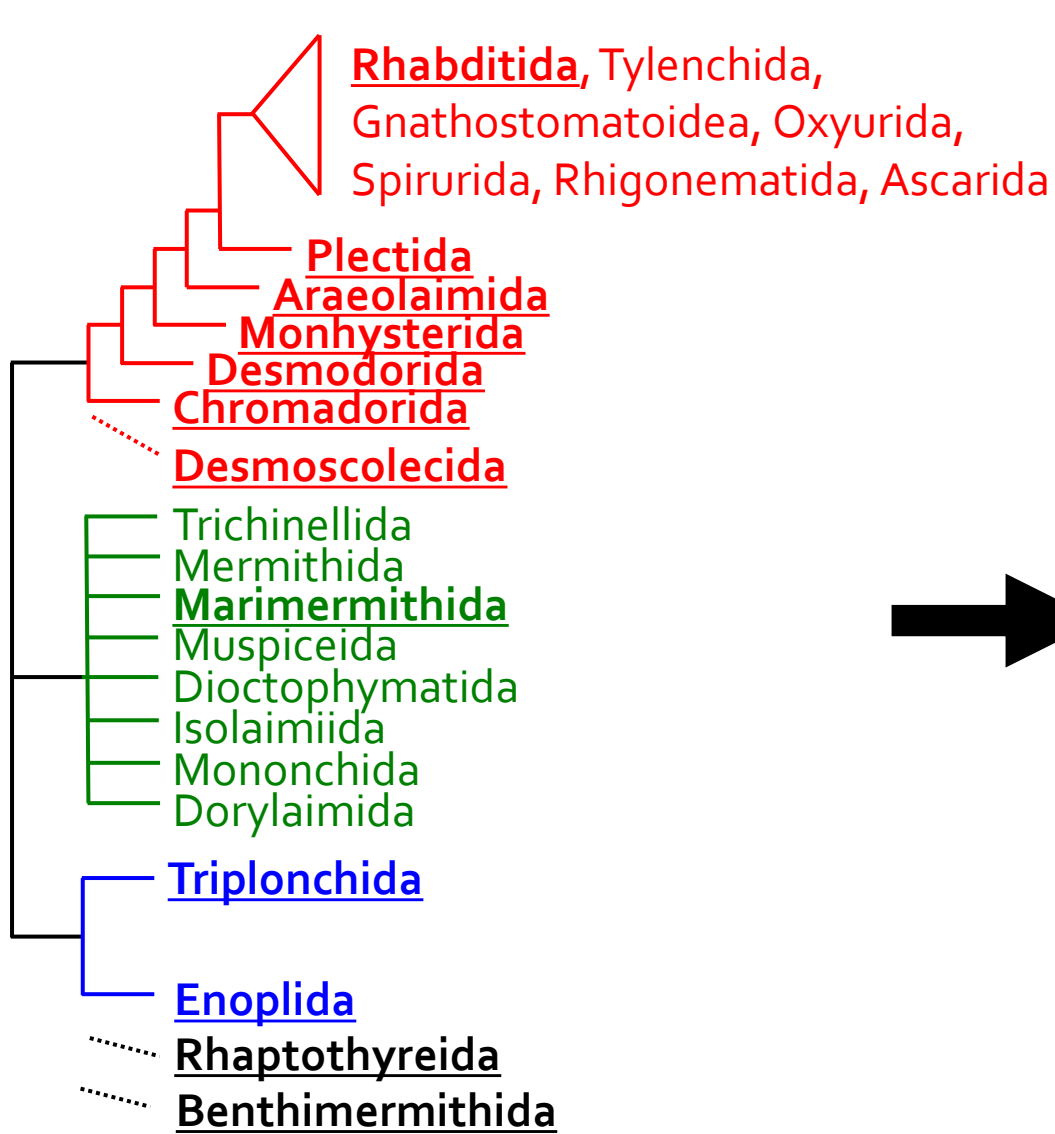
Multiple, often bidirectional habitat transitions between terrestrial, freshwater and marine environments (and between shallow and deep-sea)

Unusually ecological flexibility complicates taxonomy

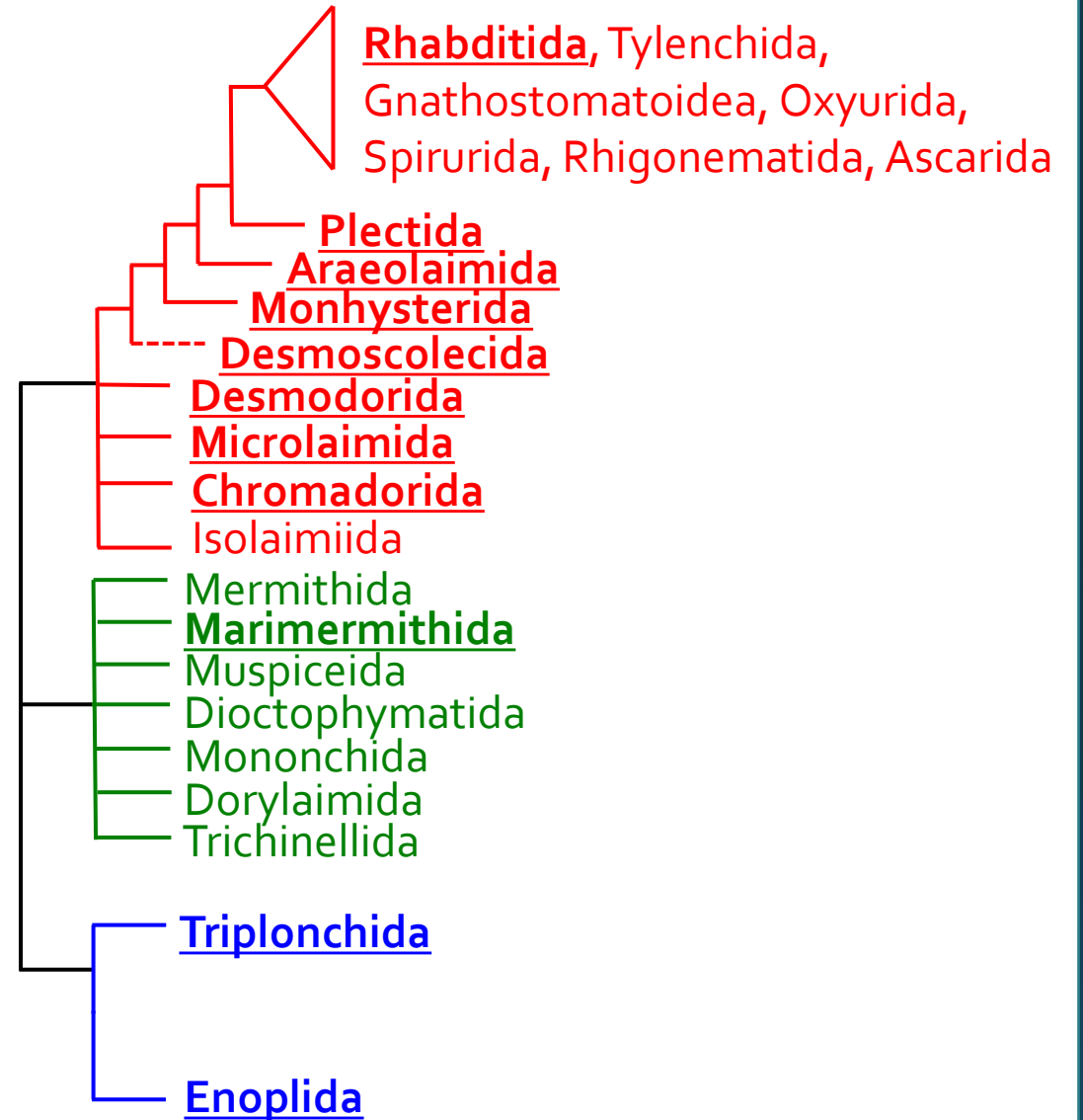
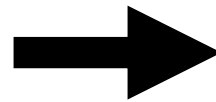
# The nematode tree (2014)

After De Ley & Blaxter (2002, 2004) and Sudhaus (2010, 2011)

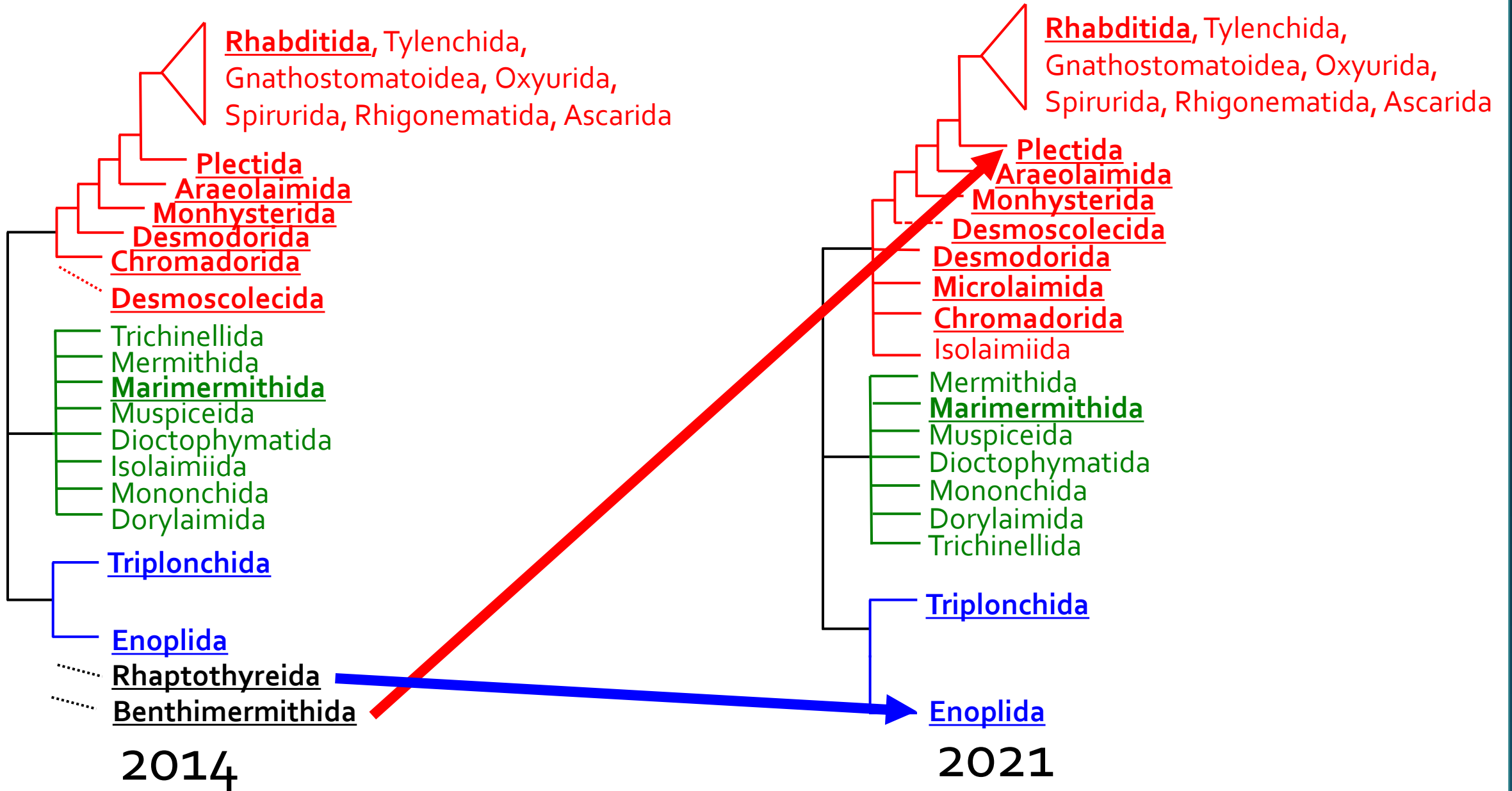


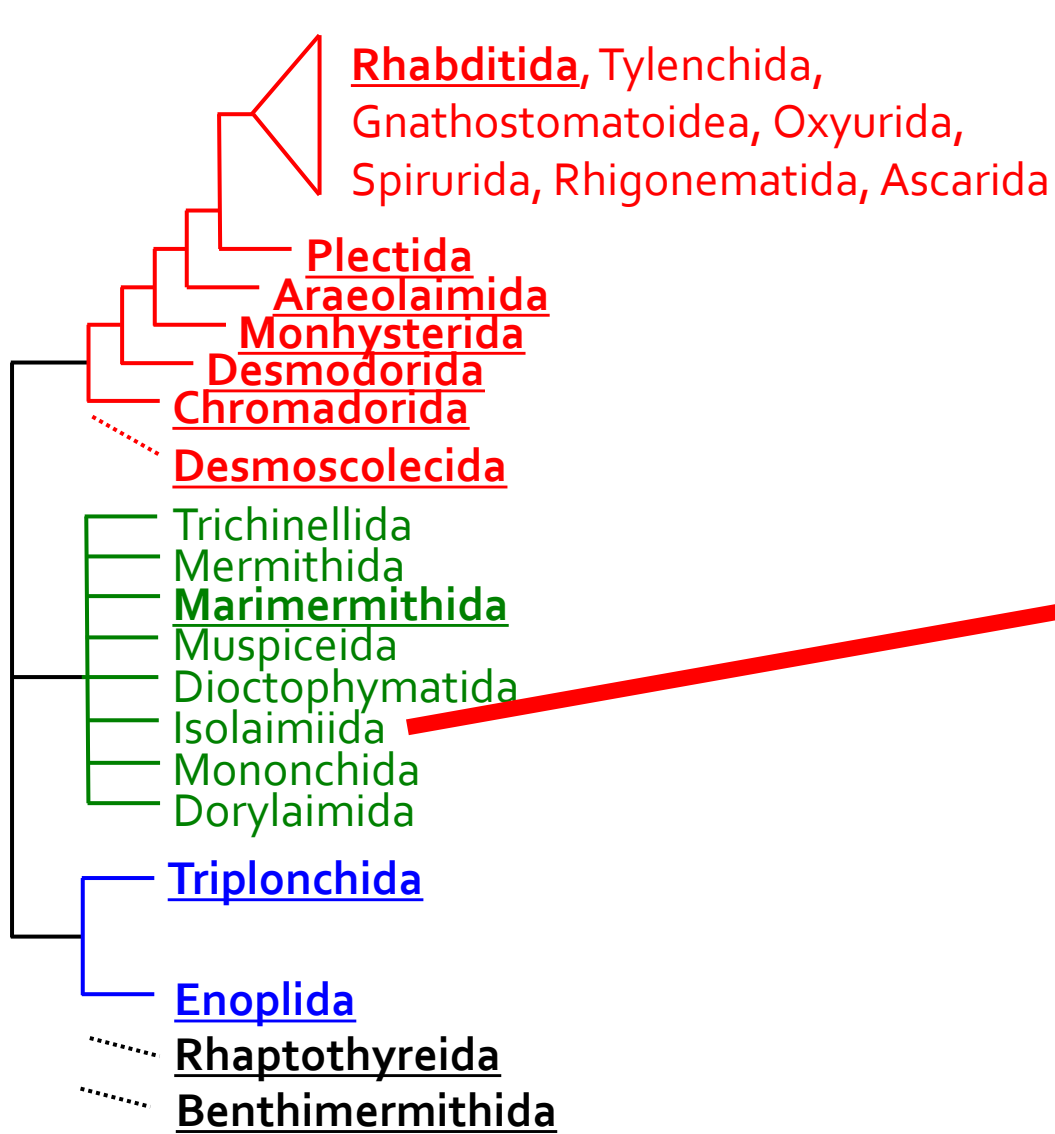


2014

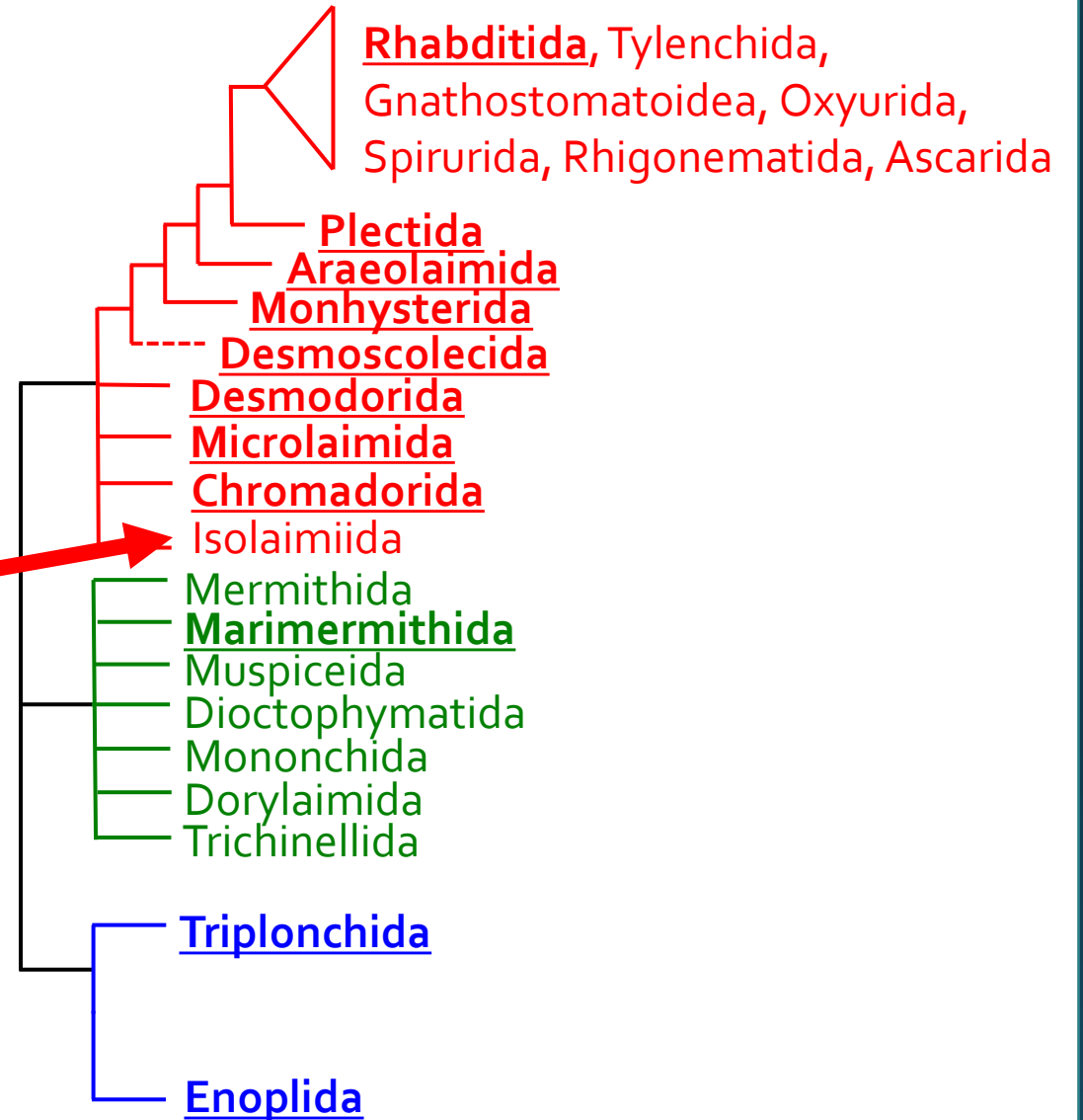


2021



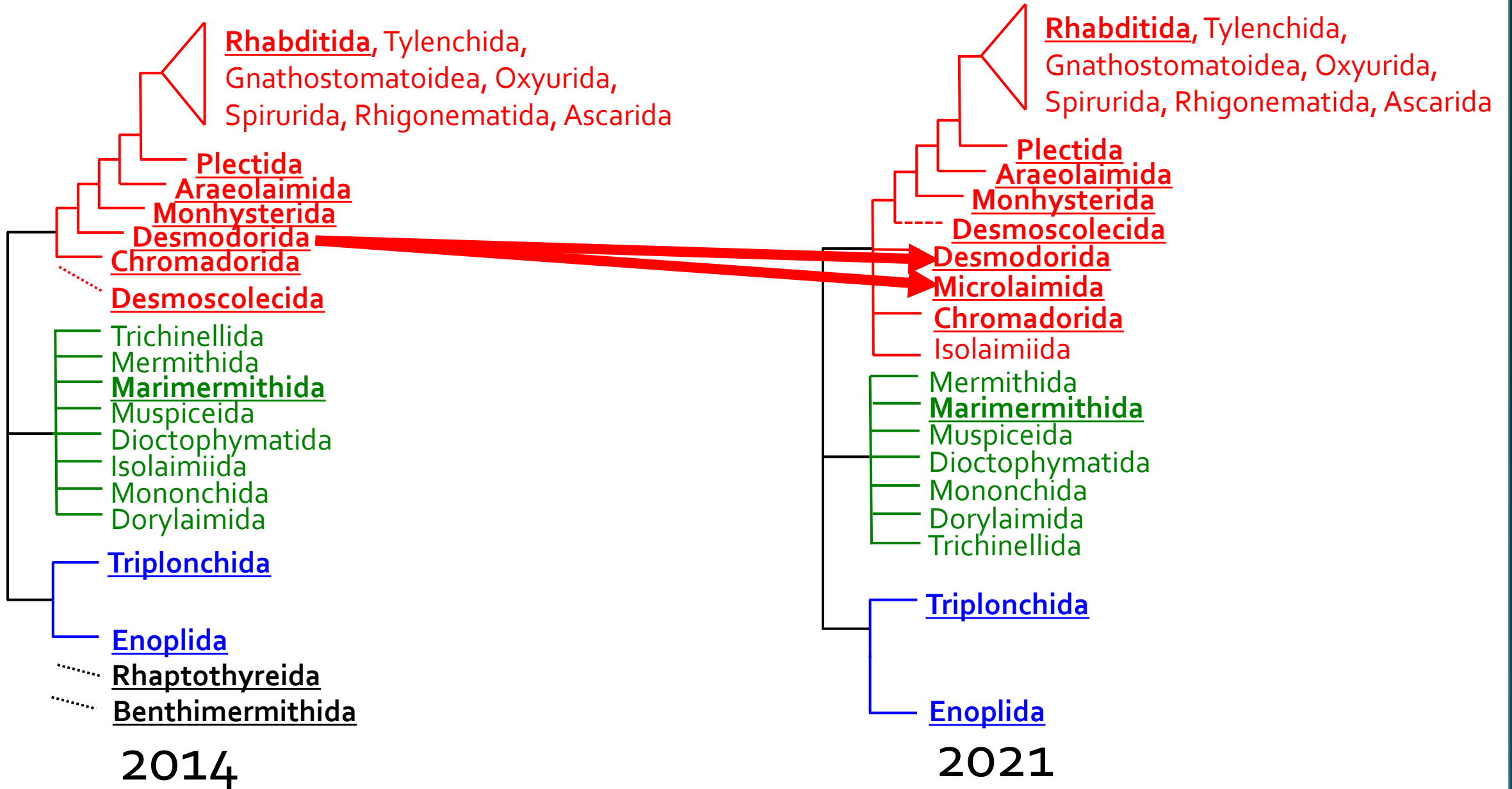


2014



2021

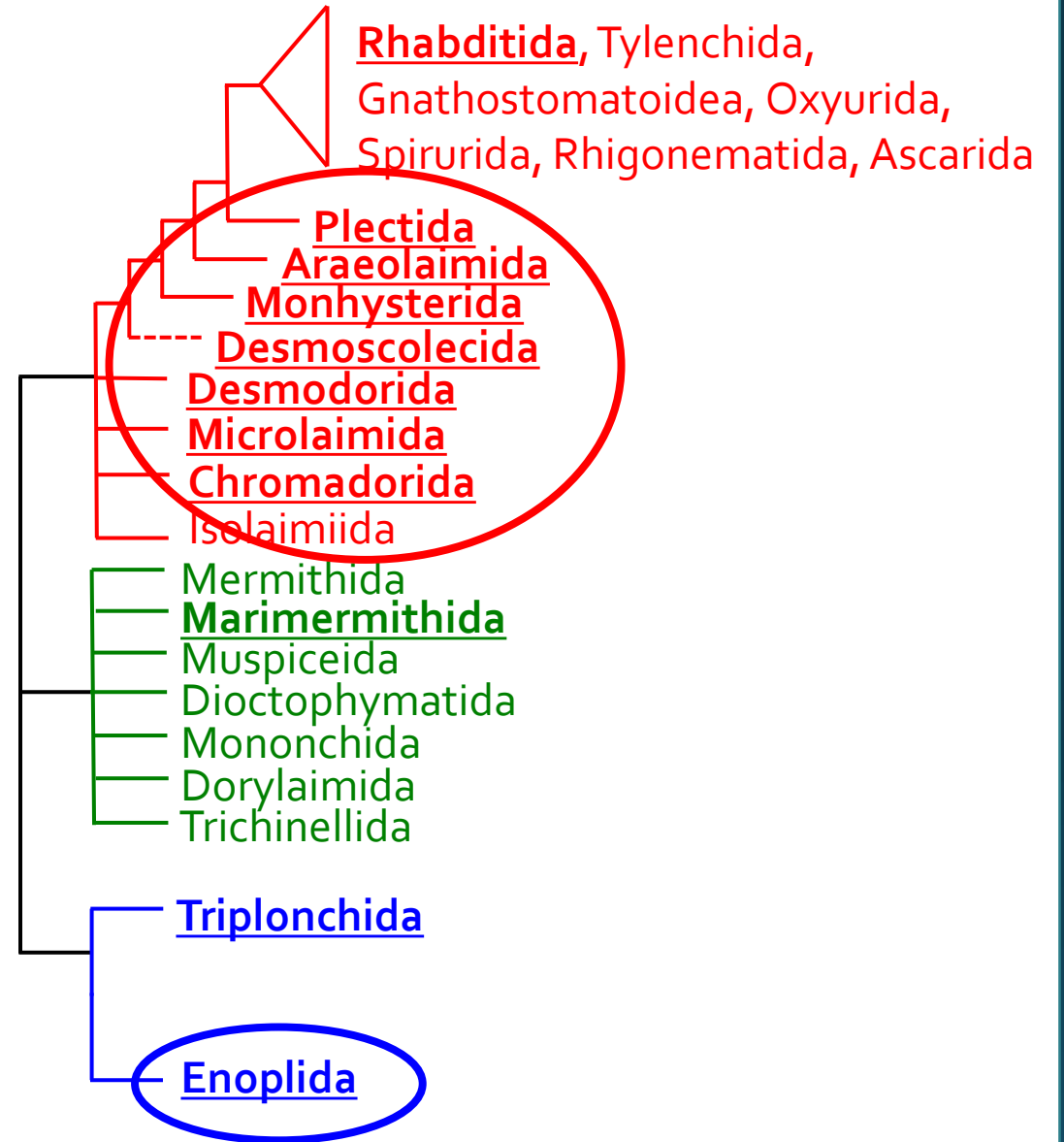




Within **Chromadoria**, 7 orders comprise most free-living marine nematodes (only a few marine representatives in Rhabditida)

Within **Enoplia**, the Enoplida comprises most free-living marine species (a few also in Triplonchida)

No free-living marine nematode species within **Dorylaimia** (only the marine parasitic Marimermithida)



# The main orders free-living marine nematodes:

		Cuticle	Female gonads (shape)	Female gonads (number)	Amphideal fovea	Other traits
Chromadorea	Desmoscolecida	Accretion rings Annulated	Reflexed	2	Circular/Oval	Four cephalic setae on peduncles
	Chromadorida	Punctated	Reflexed	2	Spiral Bowl/Oval Loop	Vestibulum with 12 folds
	Desmodorida	Striated Annulated	Reflexed	2	Spiral/Cryptospiral Circular/Cryptocircular	Vestibulum with 12 folds  Teeth usually present
	Microlaimida	Striated	Outstretched	2	Circular/cryptospiral	Vestibulum with 12 folds  Slightly swollen cephalic region  Teeth usually present but always small
	Monhysterida*	Striated Smooth	Outstretched	1 2	Circular/Oval Unispiral	Precloacal papillae rarely present  Teeth mostly absent
	Araeolaimida*	Striated Punctated	Outstretched	2 1	Loop Spiral Pore Bowl	Four cephalic setae in separate circle  Pharyngeal tubes
	Plectida	Annulated	Reflexed Outstretched	2	Circular/Oval Unispiral Longitudinal groove	Teeth absent (except odontia in Camacolaimidae)
	Enoplea	Enoplida	Smooth Striated	Reflexed	2	Pocket Circular Longitudinal groove

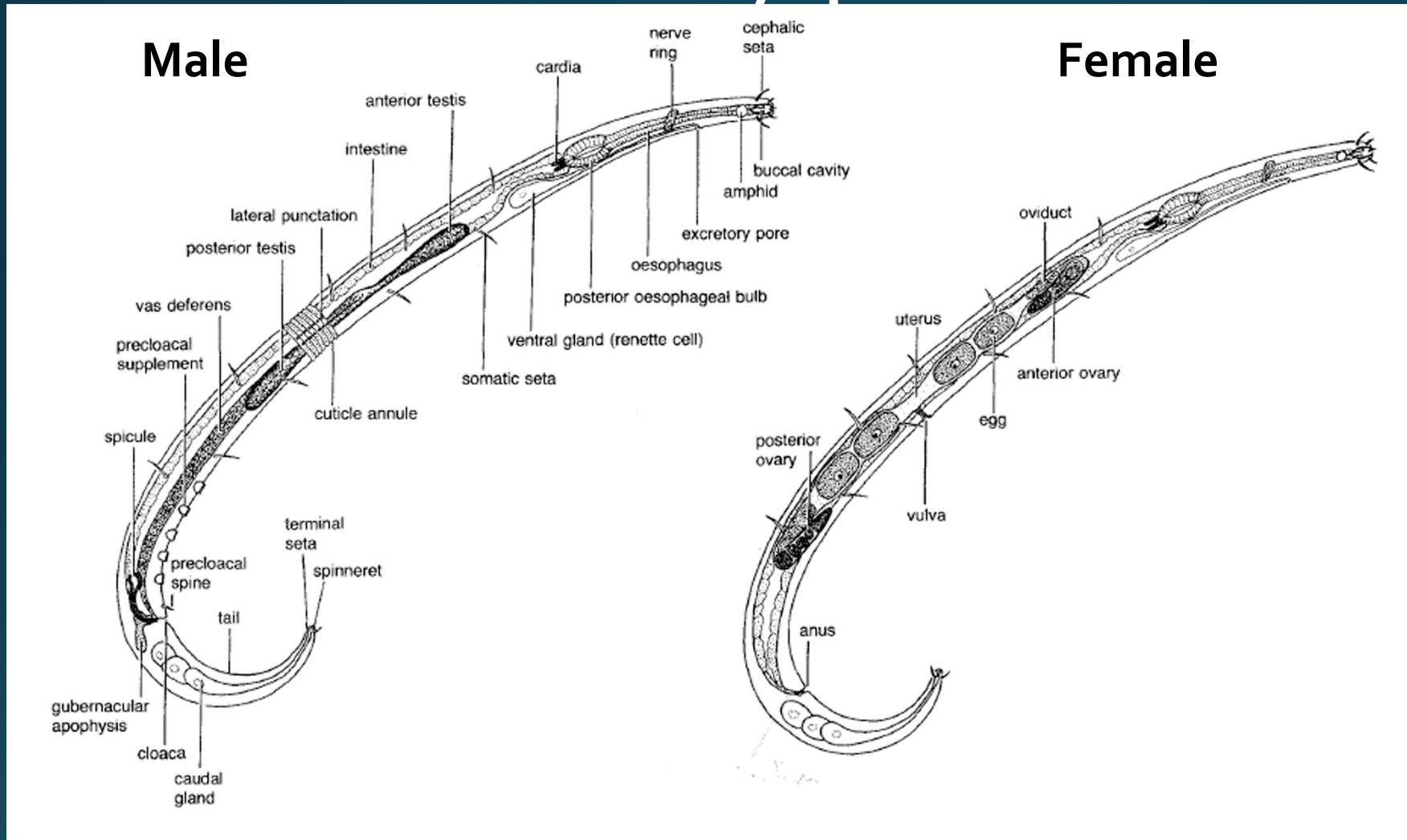
# Nematode identification

It is not straightforward to identify nematode orders, particularly for beginners

The most practical approach remains the identification of genera using pictorial keys

# Key morphological traits

# Nematode body plan



# Key morphological traits: body shape

- The first thing we notice
- May provide some clues, but highly variable



e.g., Desmoscolecida



e.g., Desmodorida



e.g., Comesomatidae



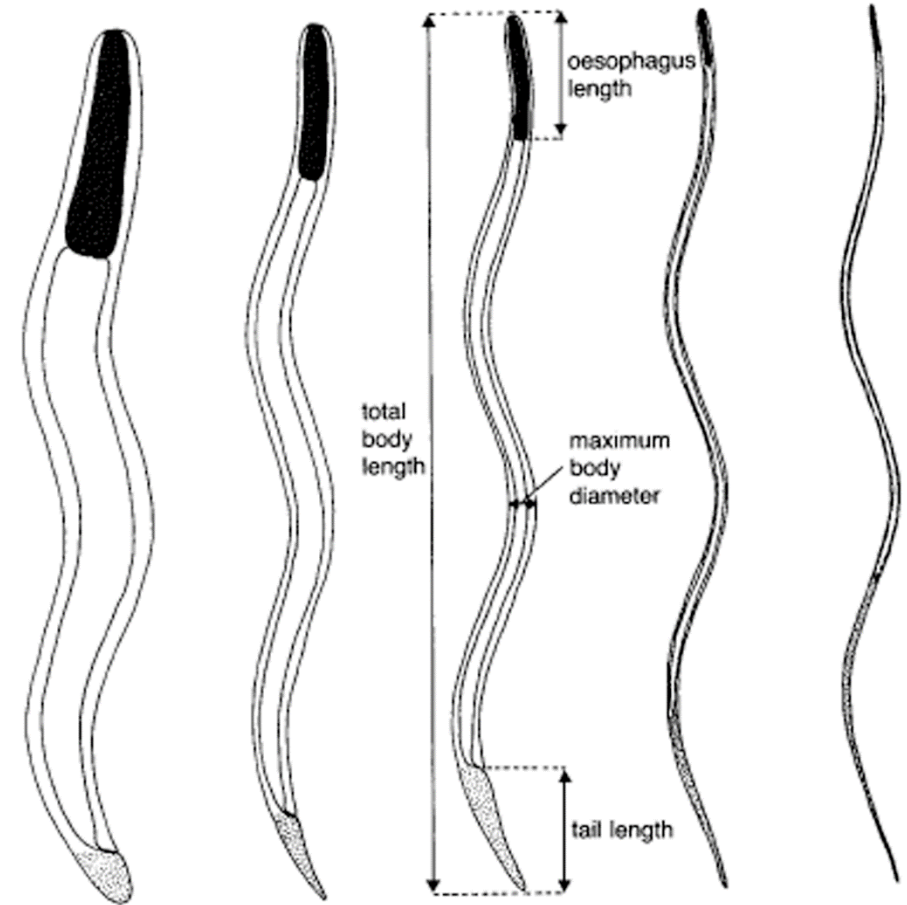
e.g., Stilbonematinae

# de Man ratios

a: body length/max body width

b: body length/pharynx length

c: body length/tail length

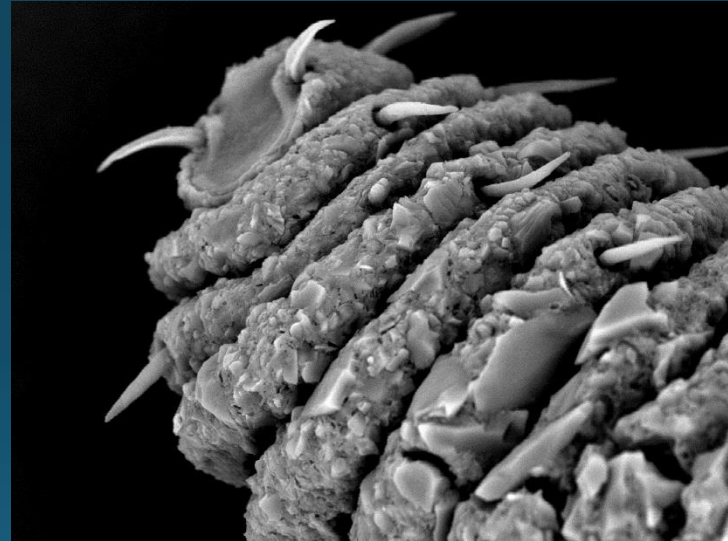


$a = \frac{\text{Body length}}{\text{Body width}} = 10$	20	40	80	120
$b = \frac{\text{Body length}}{\text{Oesophagus length}} = 4$	6	8	16	20
$c = \frac{\text{Body length}}{\text{Tail length}} = 16$	10	7	5	3



# Key morphological traits: cuticle

- Important trait for high level identification
- Desmen (accretion rings on cuticle) are distinctive and only found in Desmoscolecida:

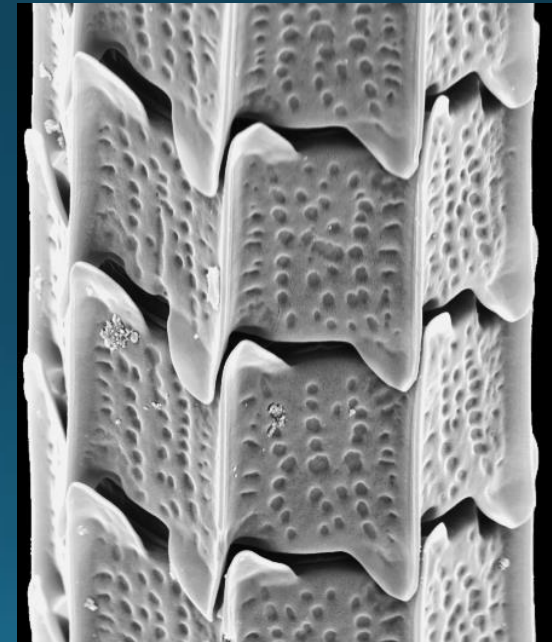
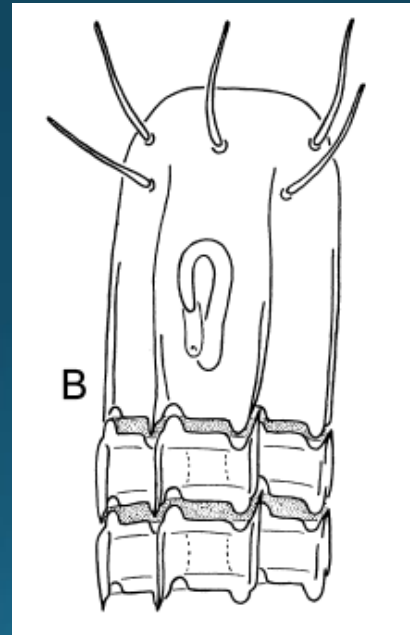


# Key morphological traits: cuticle

- Important trait for high level identification
- Ceramonematina (Plectida): Body annulations divided into plates and with longitudinal ridges/crests



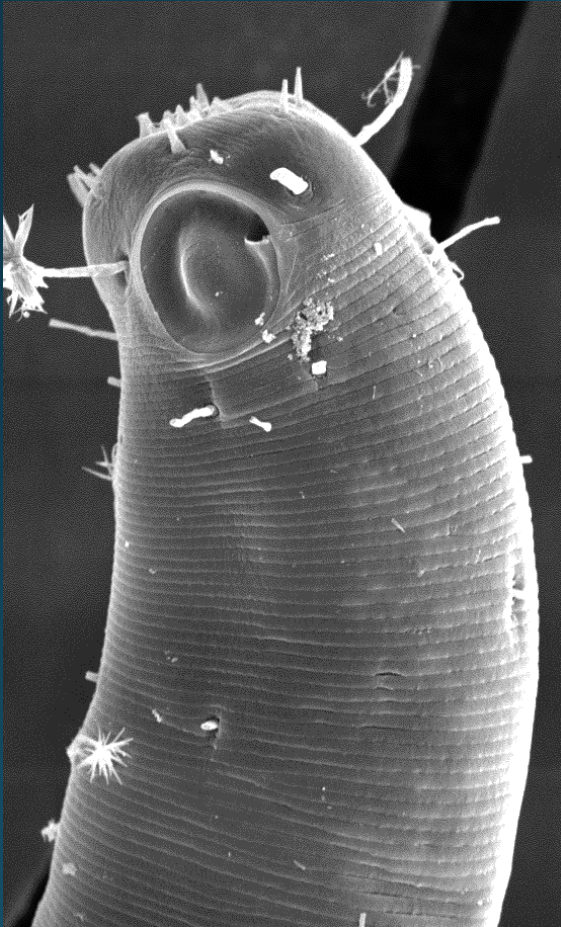
*Metadasynemella*



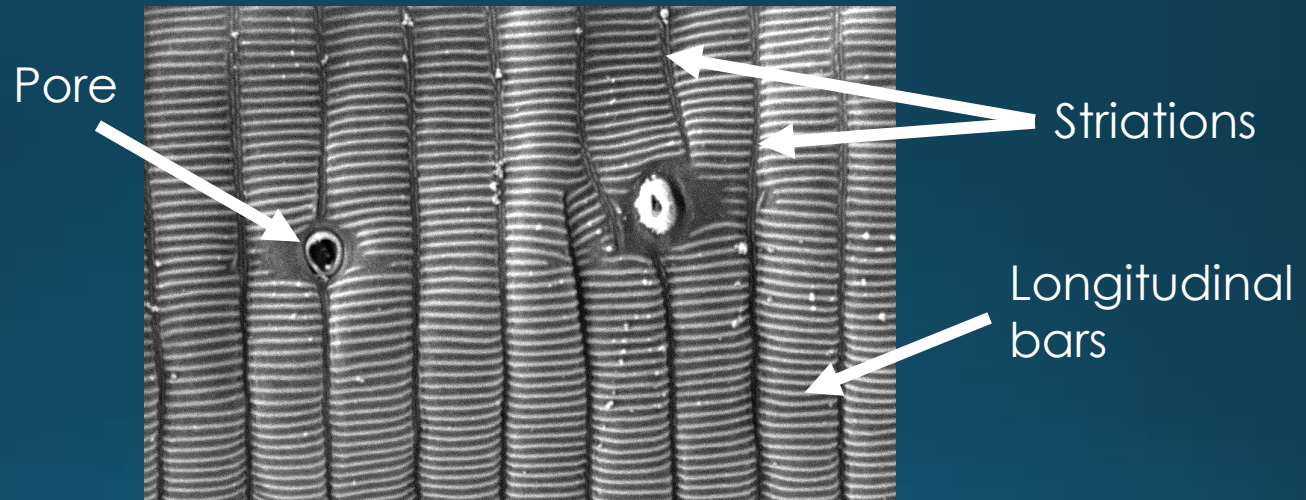
*Ceramonema*  
Holovachov 2008

# Key morphological traits: cuticle

- Striations: do not run all the way through the cuticle



*Metachromadora*: simple striations

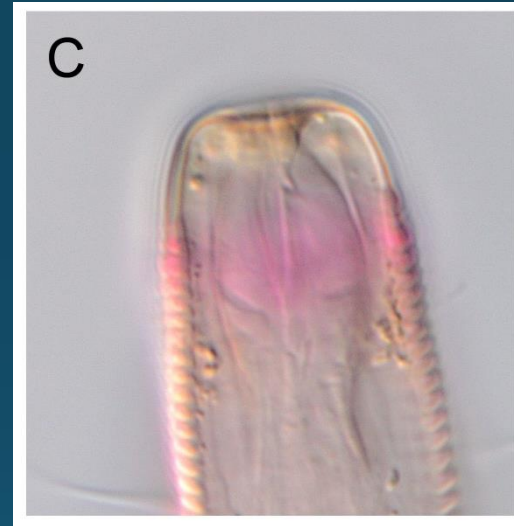
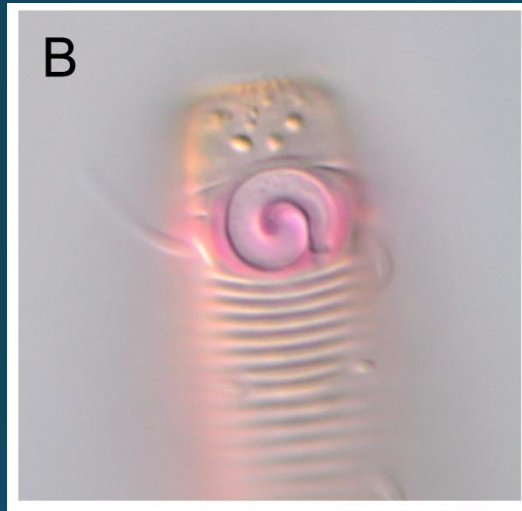


*Microlaimus falciferus*: striations and longitudinal bars

**Striations are found in Monhysterida, Araeolaimida, Desmodorida, Microlaimida, Enoplida. NOT Chromadorida, Plectida**

# Key morphological traits: cuticle

- Annulations: run all the way through the cuticle, more obvious than striations

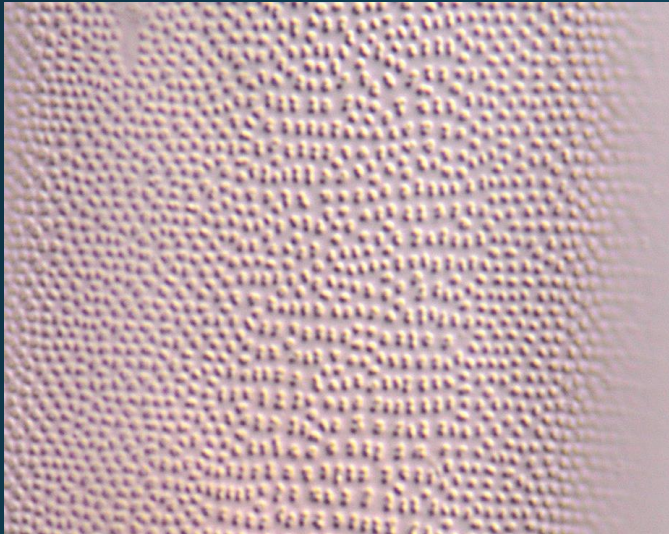


←  
Stygodesmodora confusa  
→

Annulations are found in  
Desmodorida, Plectida,  
Araeolaimida, NOT  
Chromadorida, Enoplida

# Key morphological traits: cuticle

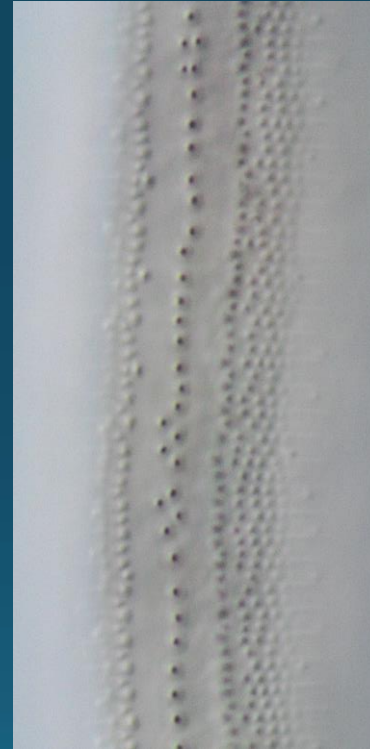
- Punctations



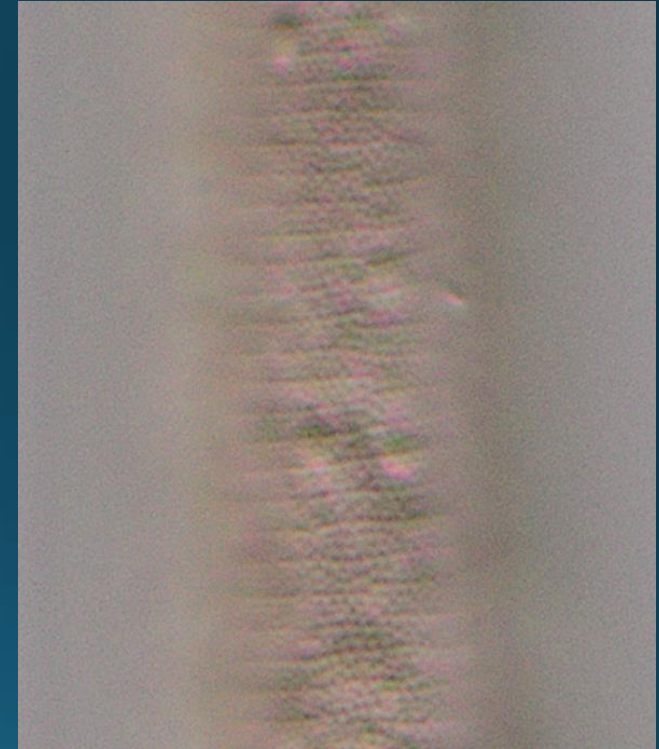
*Sabatieria bathycopia*



*Sabatieria bubulba*



*Bendiella thalassa*

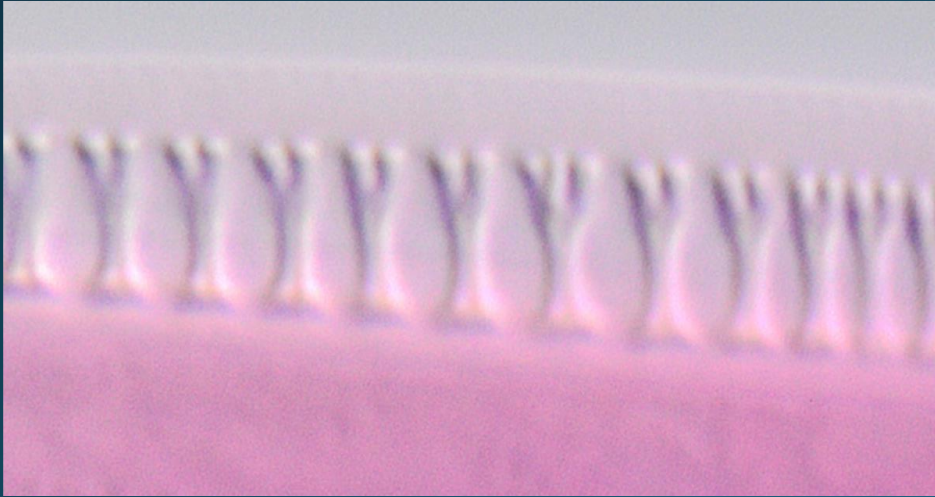


*Sabatieria exculta*

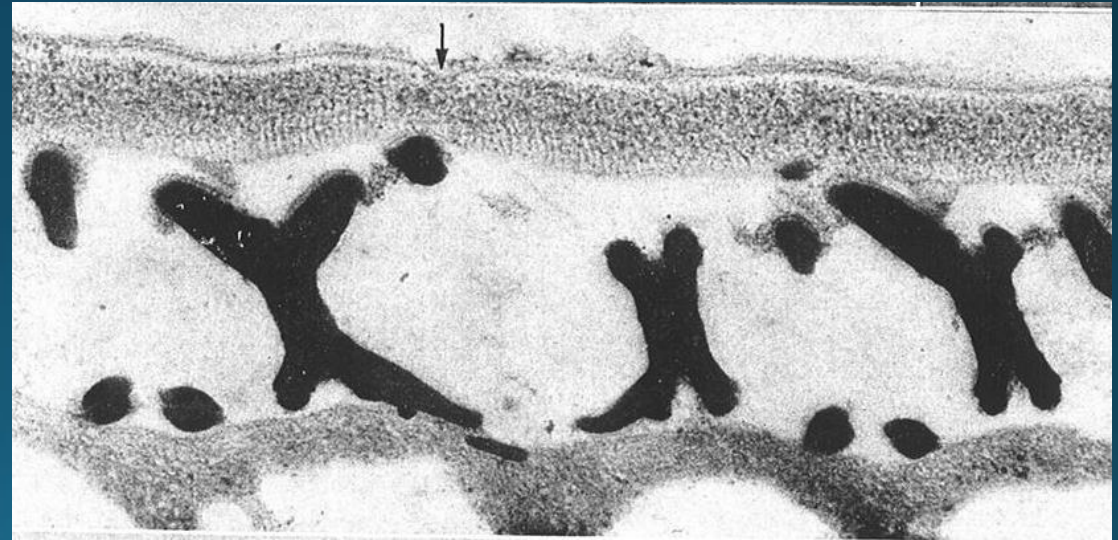
Punctations are found only in Chromadorida & Araeolaimida

# Key morphological traits: cuticle

- Punctations  
→ they are rods in the middle cuticle layer with radiating processes at proximal and distal ends



*Dorylaimopsis nodderi*

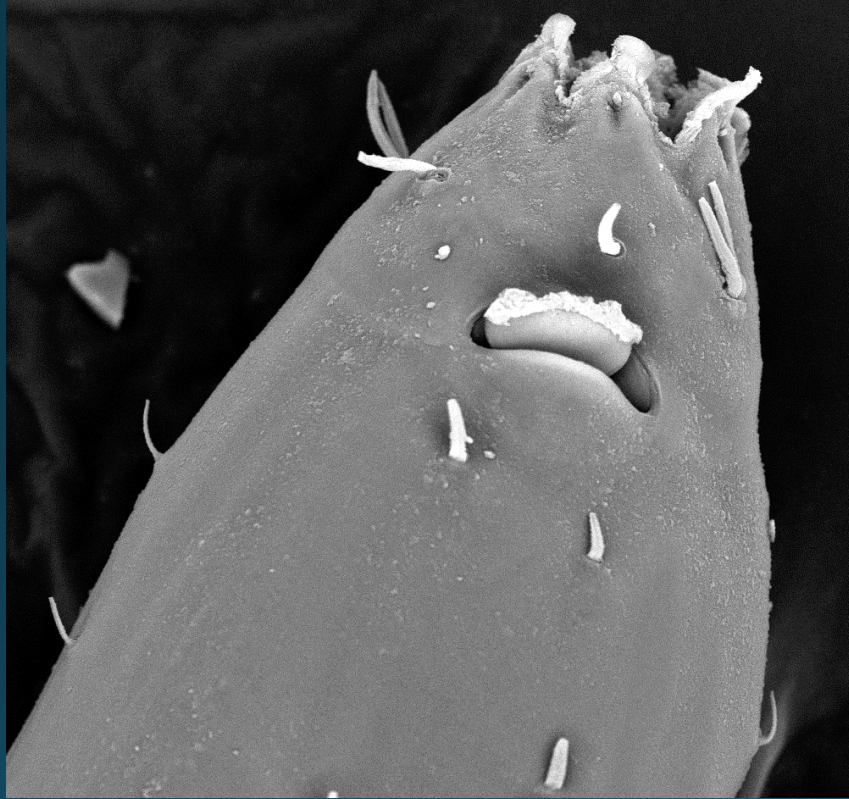


*Acanthonchus duplicatus*, TEM by Wright & Hope (1968)

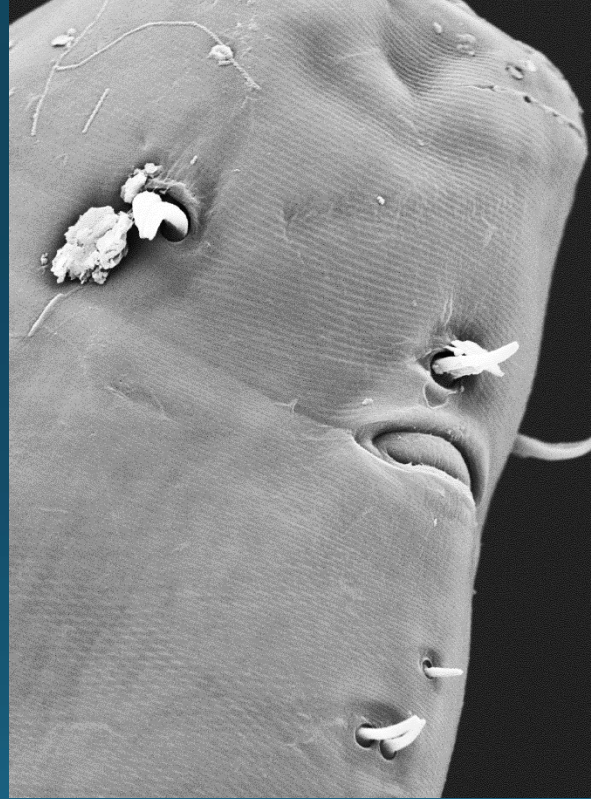
**Punctations are found only in Chromadorida & Araeolaimida**

# Key morphological traits: cuticle

- Smooth



Oncholaimidae

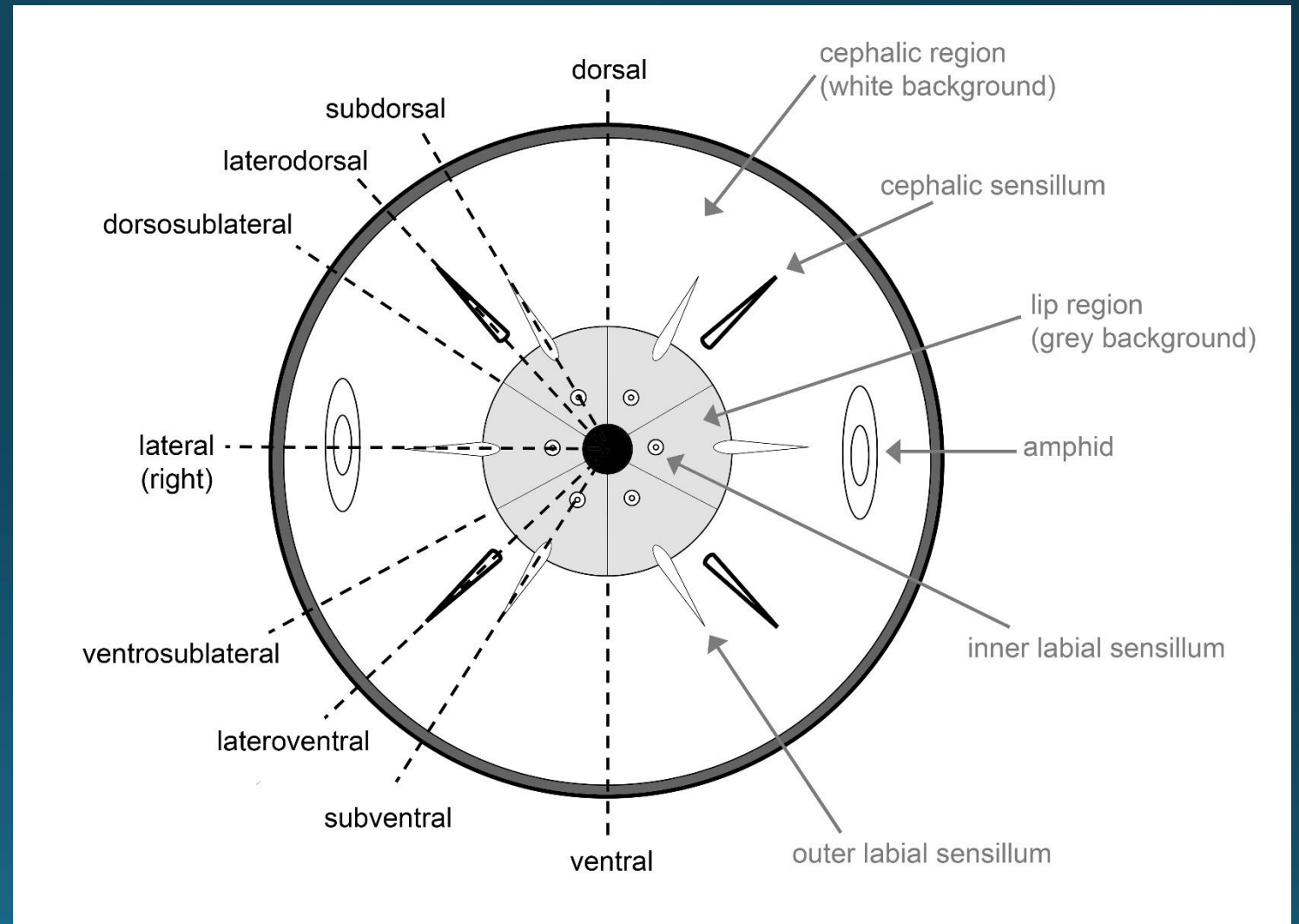


*Deontostoma tridentum*

Smooth cuticle is found in most Enoplida, some Monhysterida, rare in Araeolaimida, Desmodorida, never in Chromadorida or Plectida

# Key morphological traits: cephalic region

- Basic arrangement is three circles of sensilla: 6 inner labial sensilla, 6 outer labial sensilla, and 4 cephalic sensilla
- Sensillae are either papillae or setae; Papillae  $\leq 2 \mu\text{m}$ , Setae  $> 2 \mu\text{m}$
- Triradial (lip region) and bilateral symmetries (cephalic and rest of body region) present
- See Coomans (1979) for terminology (available on Nemys)





# Key morphological traits: cephalic region

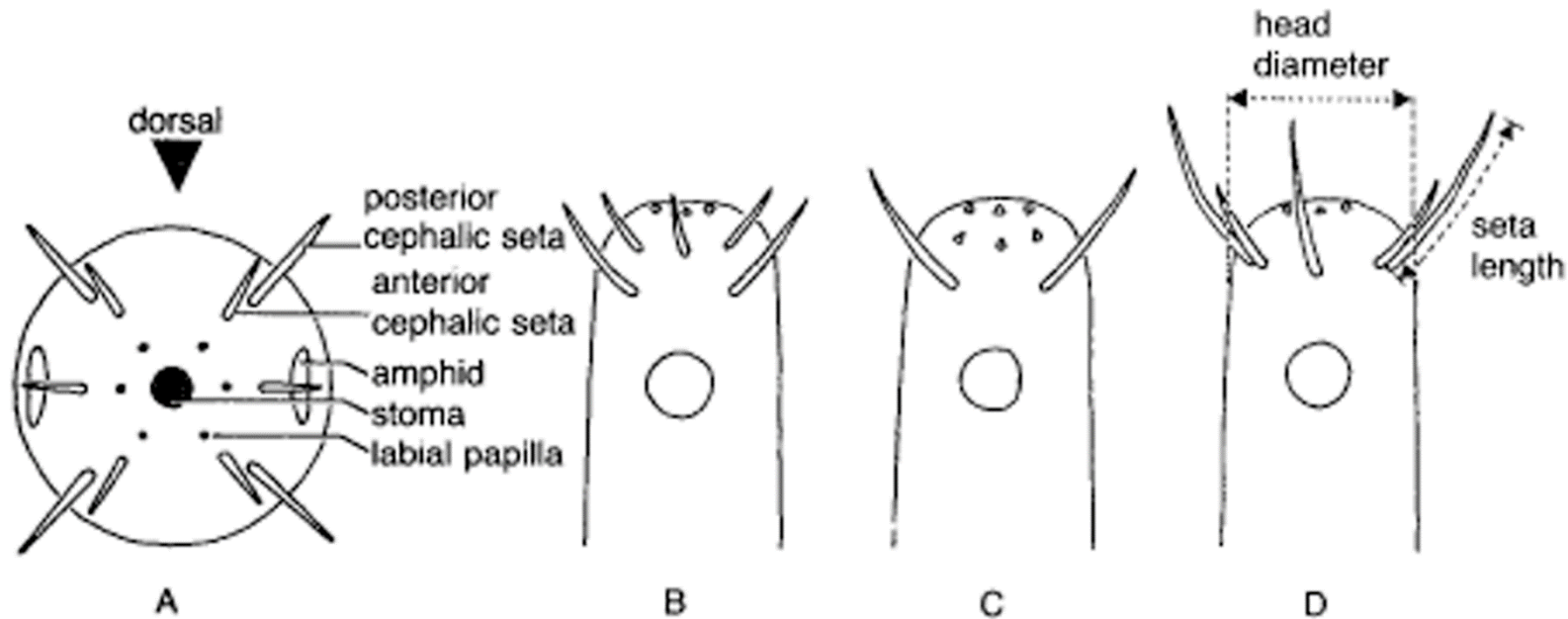
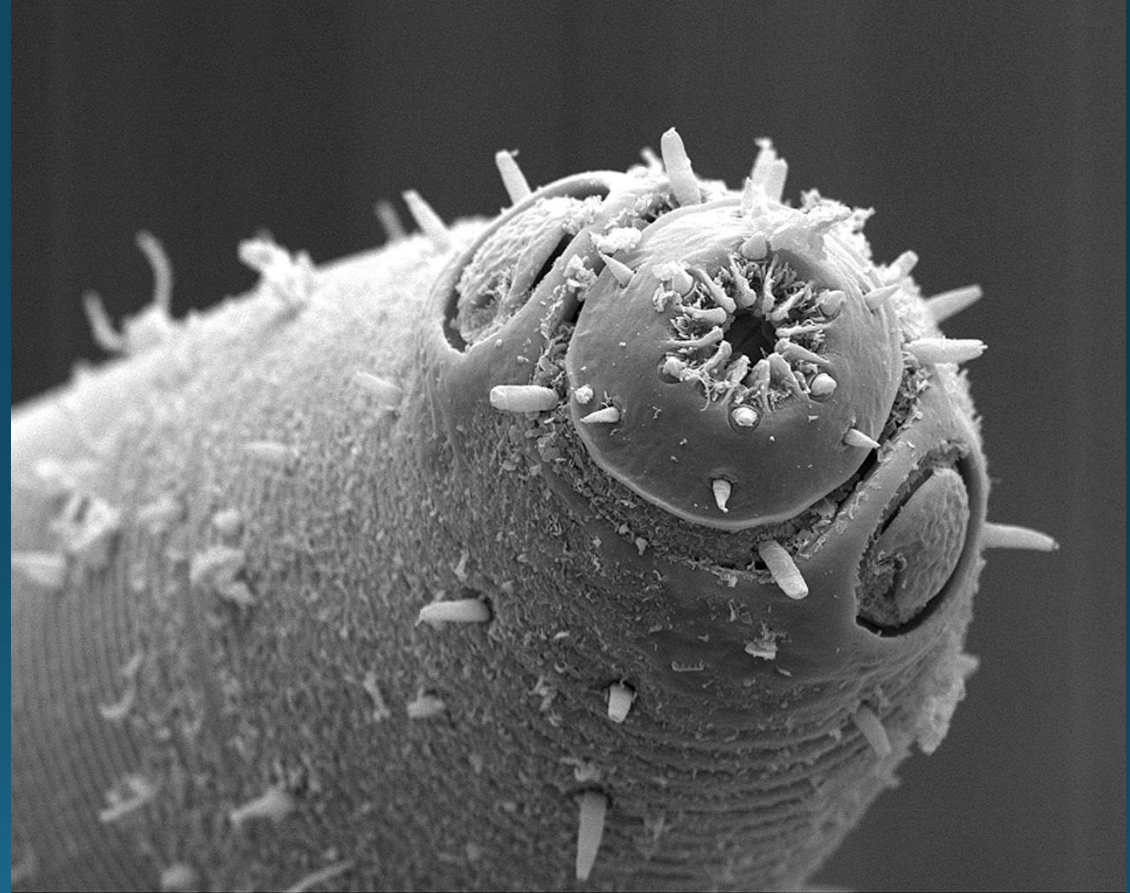


Fig. 2. Head sensilla. A, Apical view showing typical distribution of head sensilla. B, Head sensilla in the 6+6+4 pattern. C, Head sensilla pattern where only the third circle are setiform. D, Head sensilla in the 6+10 pattern, also showing setal measurements (B-D, lateral views).

# Key morphological traits: cephalic region

- Cephalic sensilla

Example of arrangement with *three separate circles*:



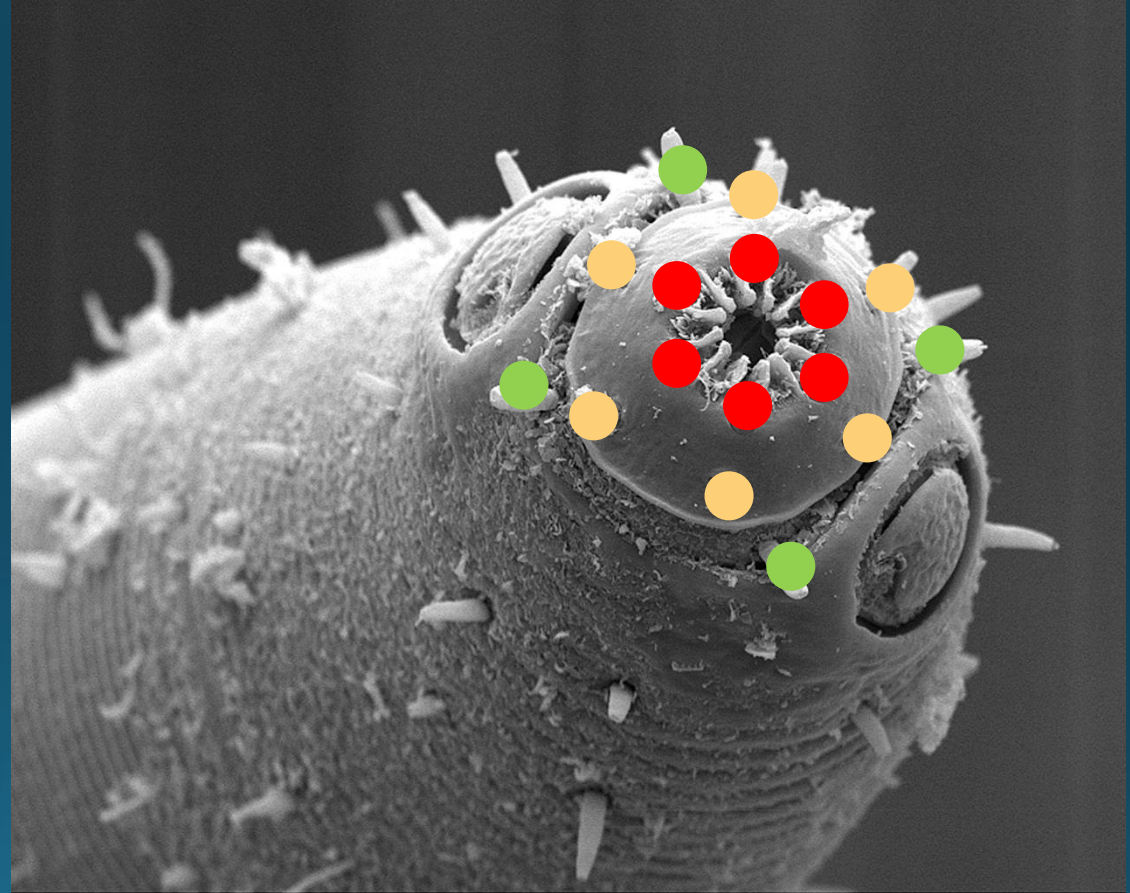
*Pseudodesmodora lacrima*

# Key morphological traits: cephalic region

- Cephalic sensilla

Example of arrangement with *three separate circles*:

6 inner labial papillae  
6 outer labial papillae  
4 cephalic setae



*Pseudodesmodora lacrima*

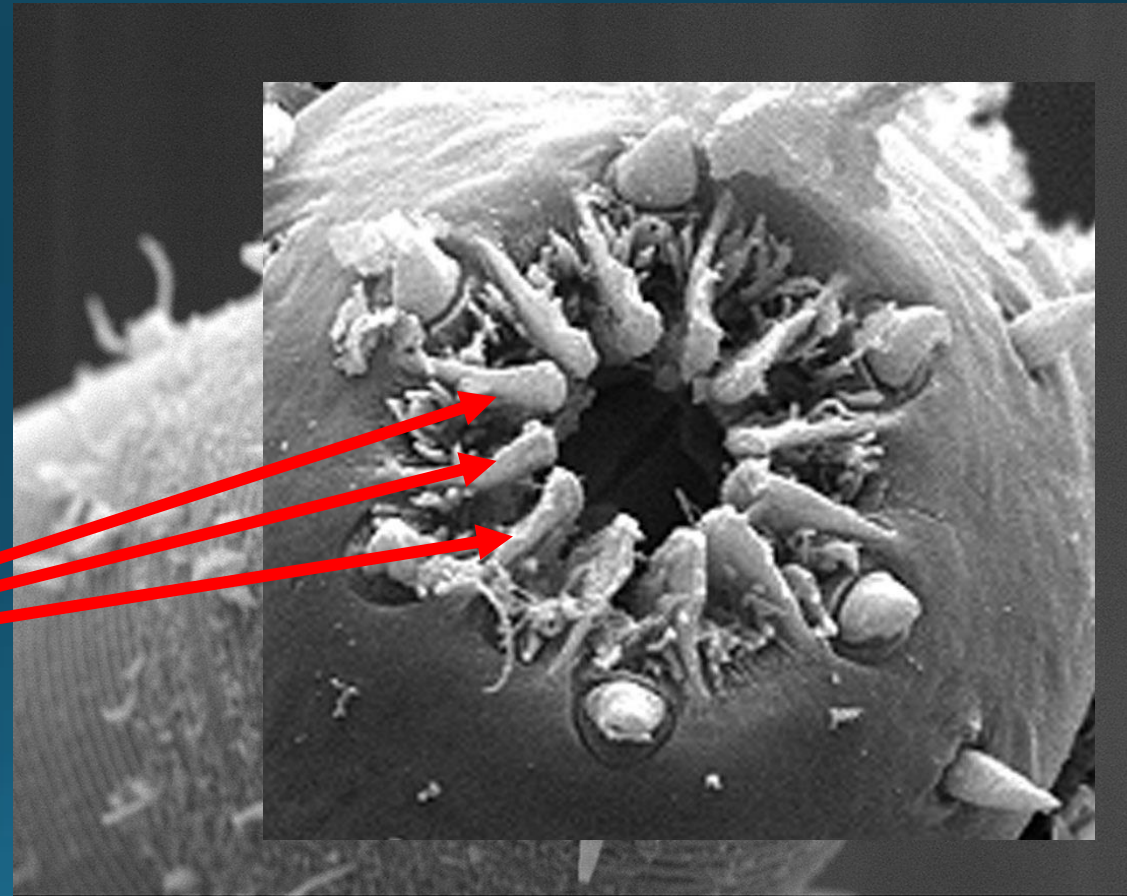
# Key morphological traits: cephalic region

- Cephalic sensilla

Example of arrangement with *three separate circles*:

6 inner labial papillae  
6 outer labial papillae  
4 cephalic setae

Vestibulum folds/rugae  
(Chromadorida,  
Microlaimida and  
Desmodorida)



*Pseudodesmodora lacrima*

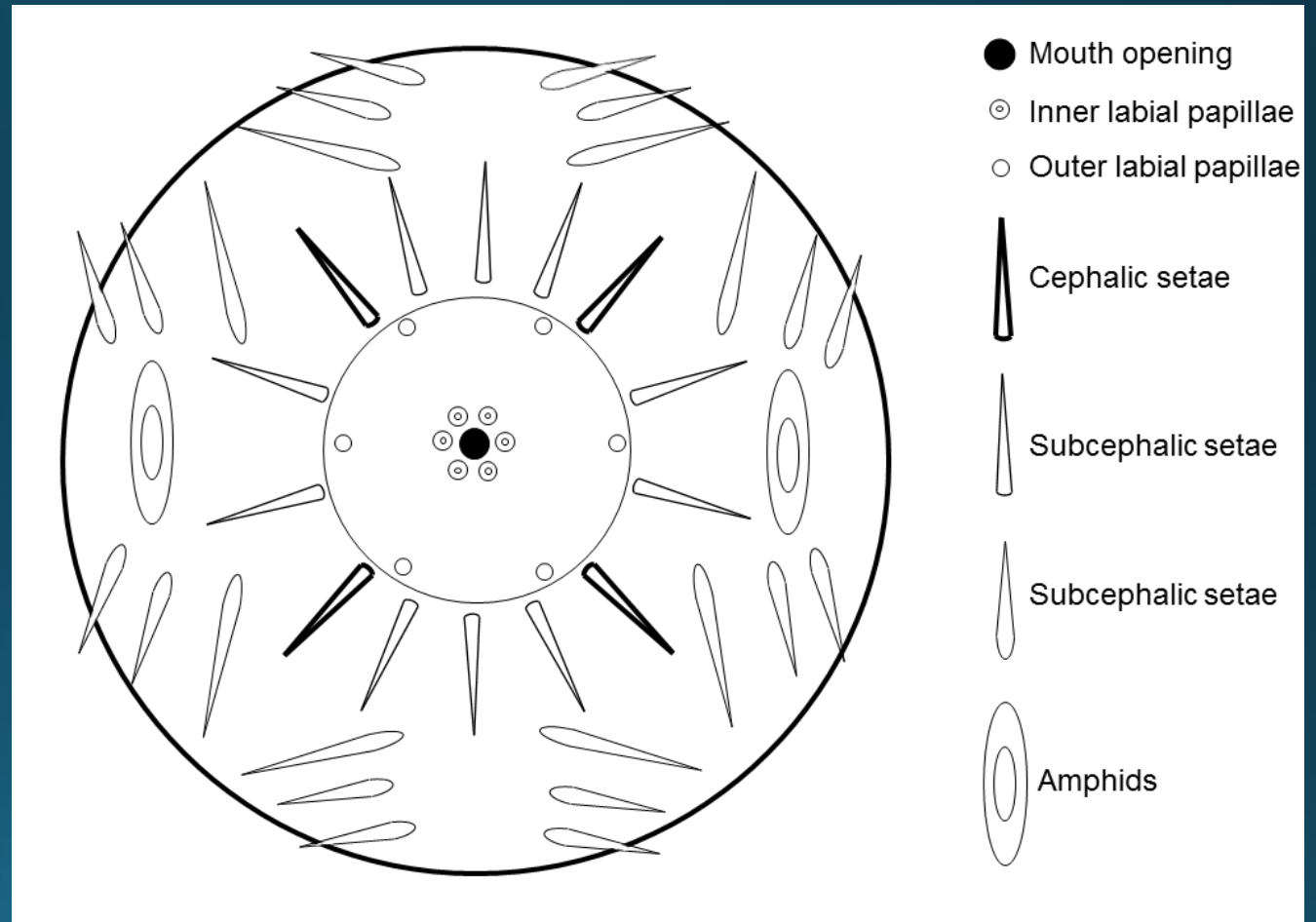
# Key morphological traits: cephalic region

- Cephalic sensillae

Example of arrangement with *three separate circles*:

In this case, additional cephalic setae are situated at same level as the four cephalic setae, and are of same length.

Only way to identify the cephalic setae is by their laterodorsal and lateroventral positions.



*Acanthopharynx dormitata*

# Key morphological traits: cephalic region

- Amphid shape and size
- Amphideal fovea vs amphideal aperture

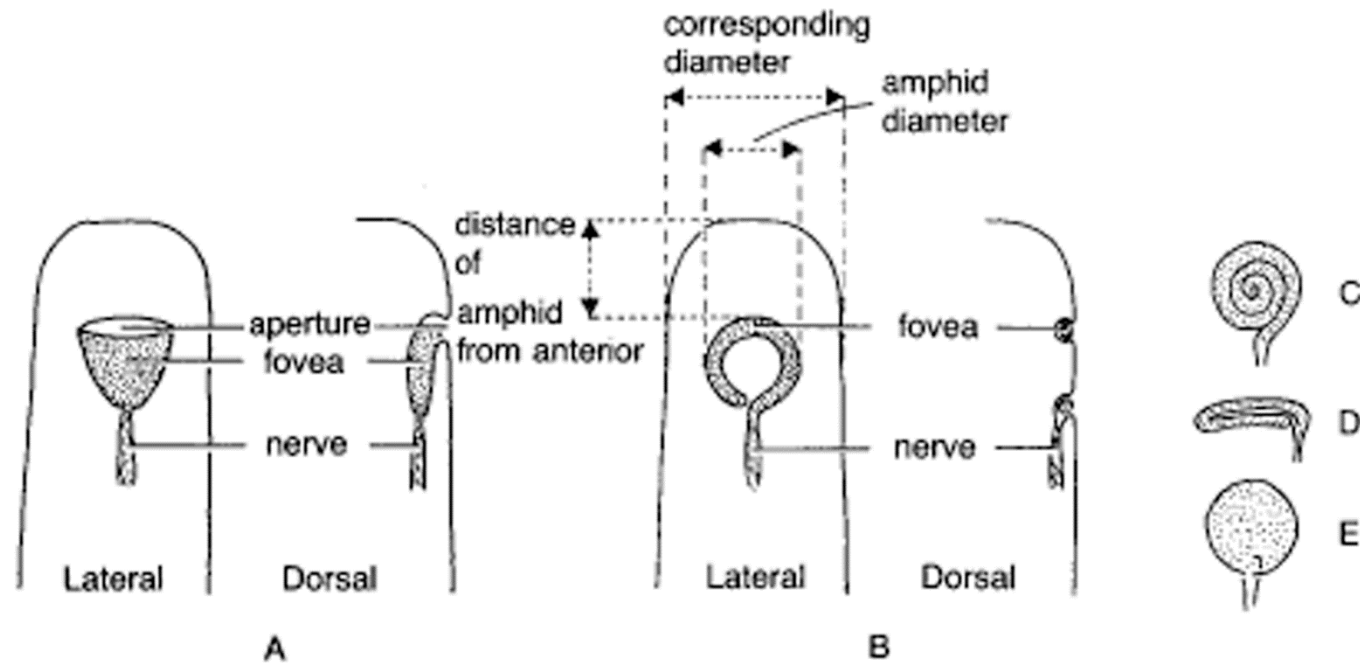
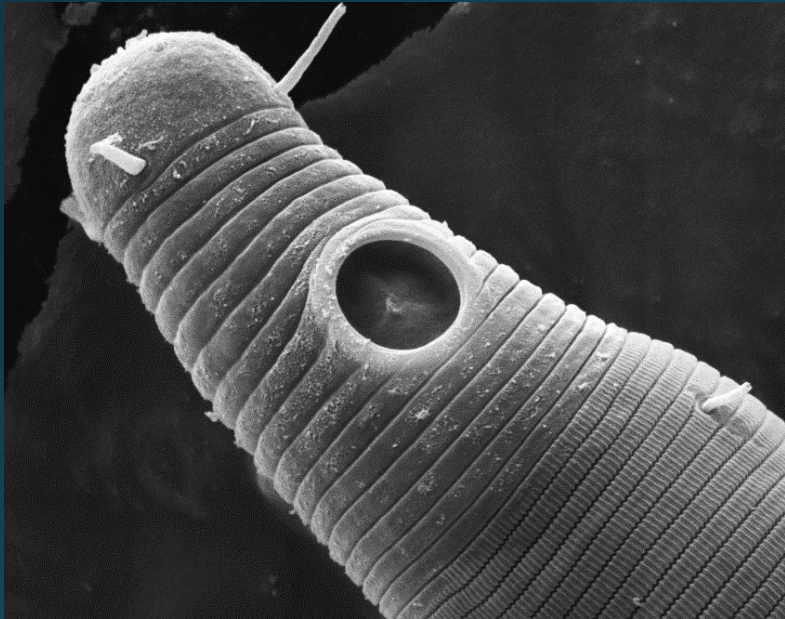


Fig. 3. Amphids. A, Pocket type, B, Spiral type with a single turn, also showing amphid measurements. C, Multi-spiralled type. D, Transverse slit type. E, Circular type.

# Key morphological traits: cephalic region

- Amphid shape and size
- Amphideal fovea vs amphideal aperture



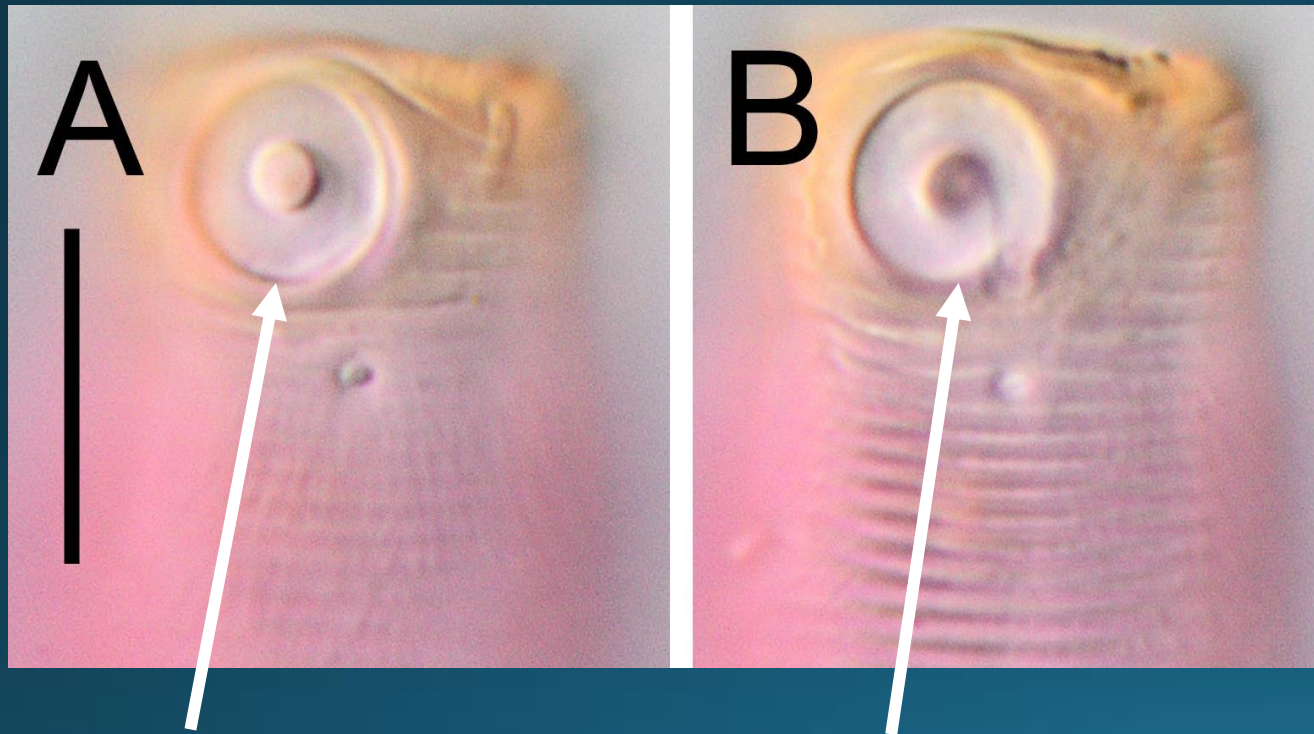
*Aponema subtile*  
Circular amphideal fovea and aperture



*Pseudonchus virginiae*  
Spiral amphideal fovea and circular amphideal aperture

# Key morphological traits: cephalic region

- Amphid shape and size
- Amphideal fovea vs amphideal aperture



*Spirinia verecunda*

Unispiral amphideal aperture and cryptospiral amphideal fovea  
(with broken circle outline)

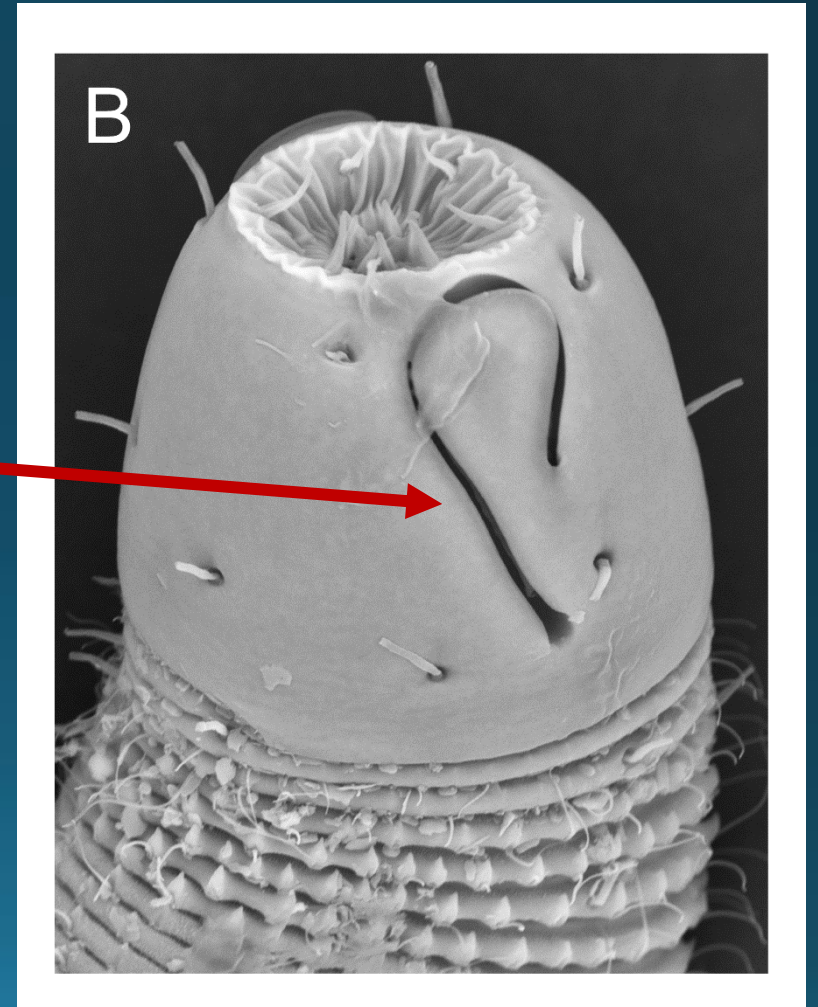
See Leduc & Verschelde 2013  
for further terminology  
(cryptospiral vs cryptocircular  
etc..)



# Key morphological traits: cephalic region

- Amphid shape and size
- Amphideal fovea vs amphideal aperture

Loop-shaped

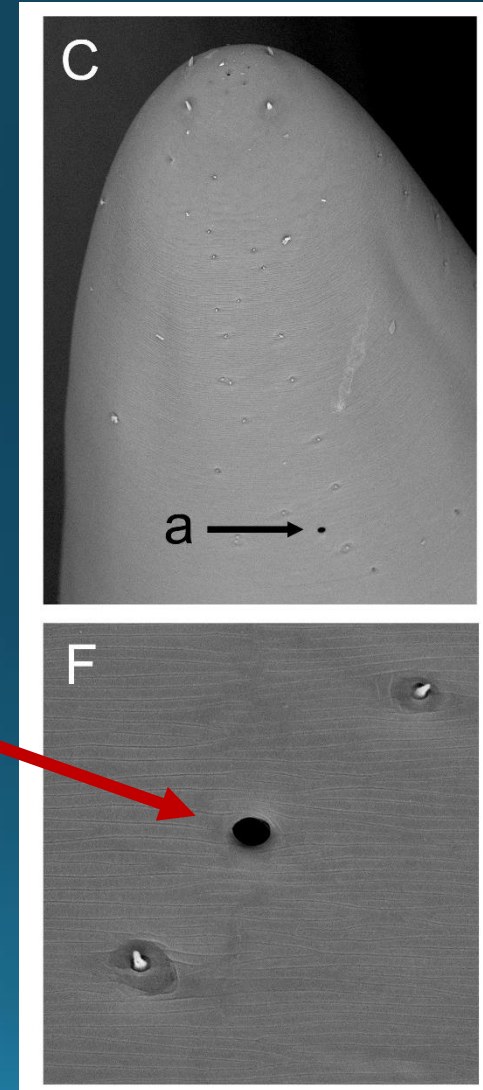


*Desmodorella verscheldei*

# Key morphological traits: cephalic region

- Amphid shape and size
- Amphideal fovea vs amphideal aperture

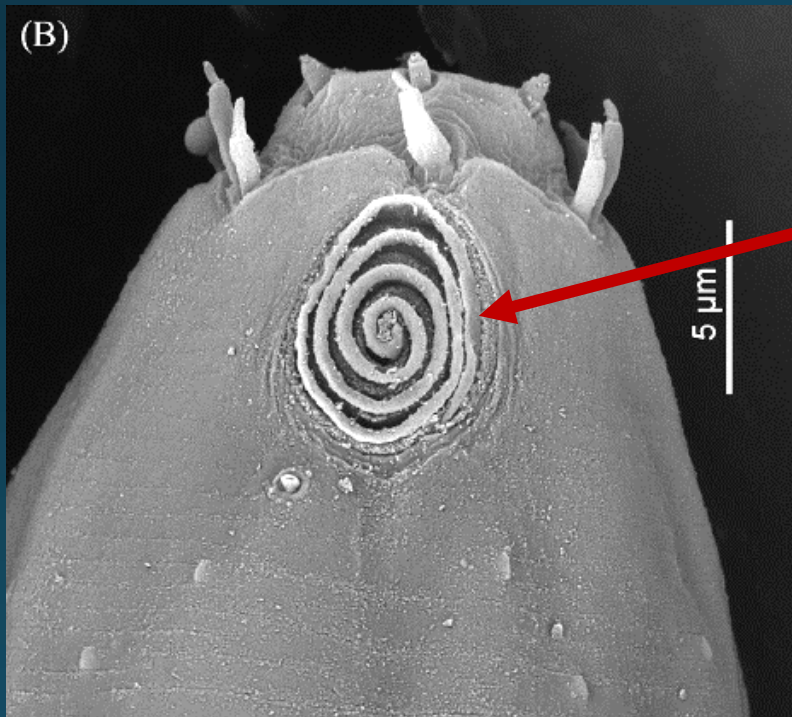
Pore-shaped



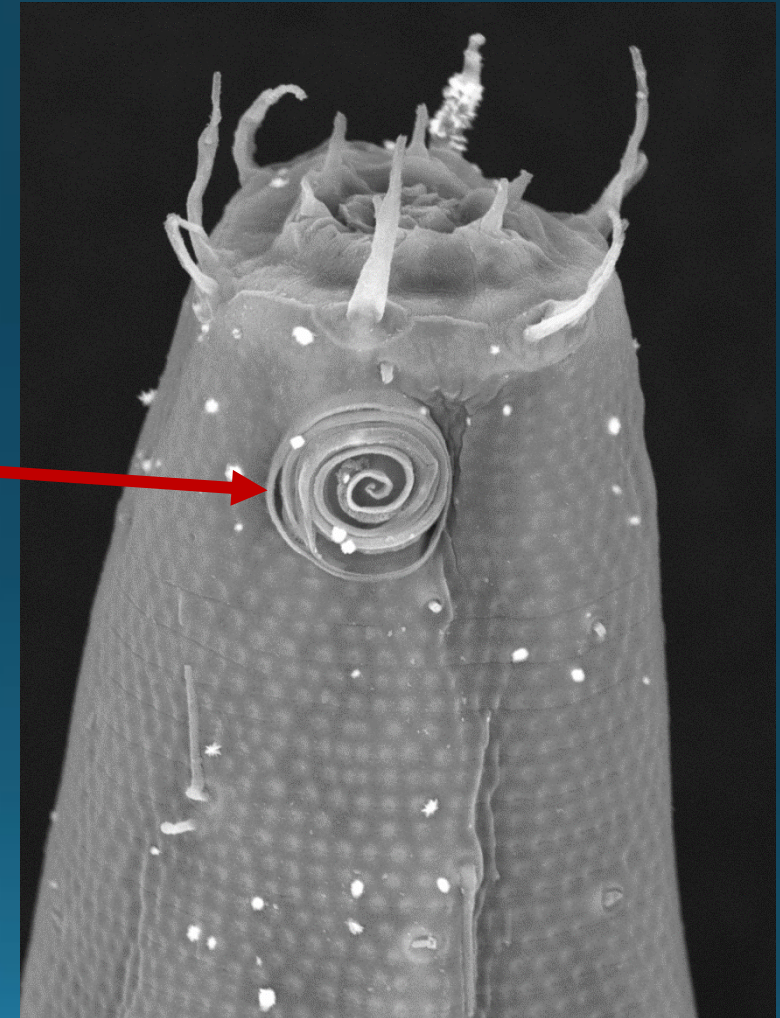
*Trophomera cf marionensis*

# Key morphological traits: cephalic region

- Amphid shape and size
- Amphideal fovea vs amphideal aperture



Spiral

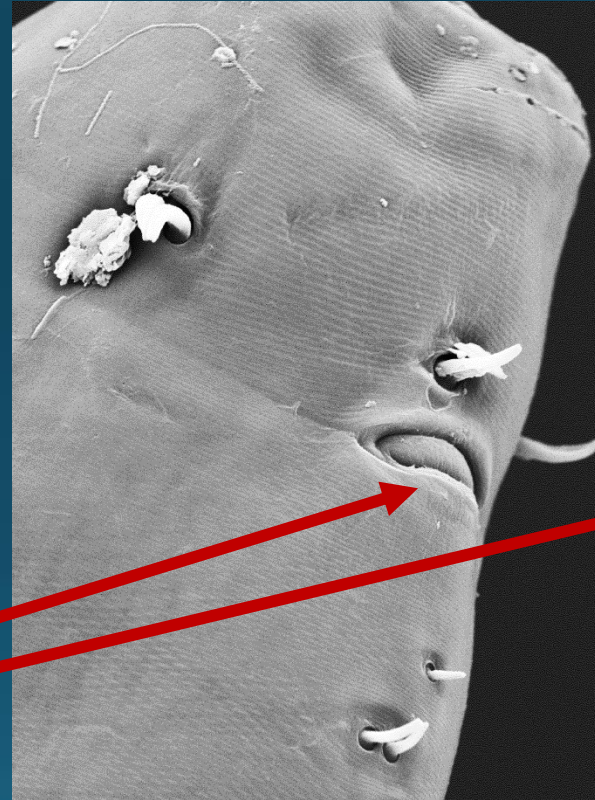
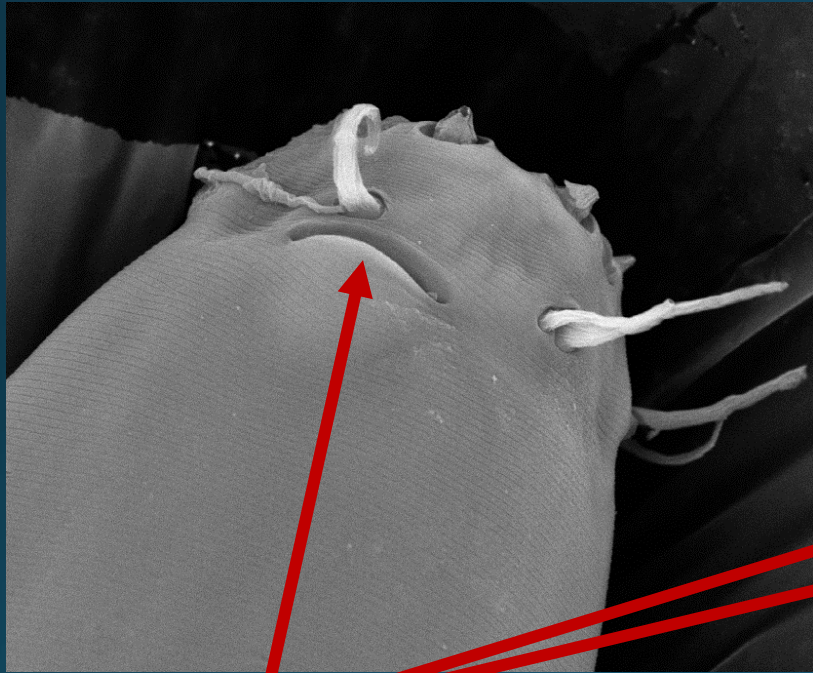


*Paracanthonchus mamubiae* Miljutina & Miljutin 2015

*Paracanthonchus*

# Key morphological traits: cephalic region

- Amphid shape and size
- Pocket-shaped



Pocket-shaped amphids only have a small slit-like aperture. The “pocket” (or fovea) is below the surface.

# Key morphological traits: cephalic region

- Buccal cavity

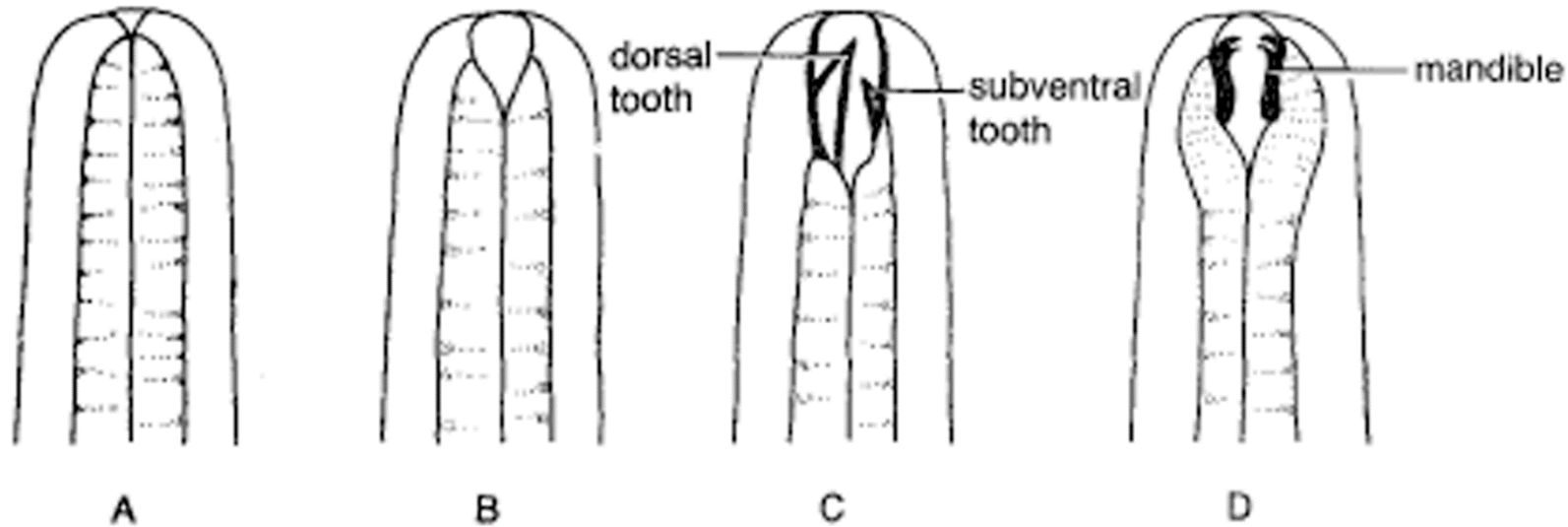


Fig. 4. Buccal cavities. A, Minute form. B, Unarmed form. C, Form with fixed teeth. D, Form with moveable mandibles.

# Key morphological traits: cephalic region

- Buccal cavity: minute



*Anticomidae*



*Metadasynemella*

# Key morphological traits: cephalic region

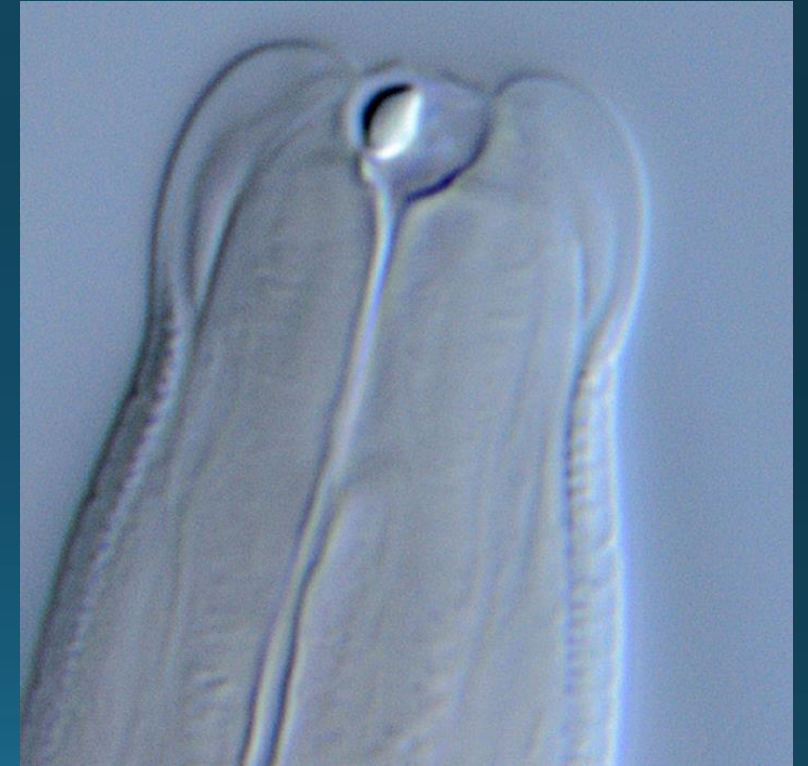
- Buccal cavity: cup-shaped



*Trefusilaimus idrisi*



*Eubostrichus hortulanus*



*Sabatieria bitumen*

# Key morphological traits: cephalic region

- Buccal cavity: large, cuticularised, no teeth

*Elzalia*





# Key morphological traits: cephalic region

- Buccal cavity: medium with teeth



*Onyx*



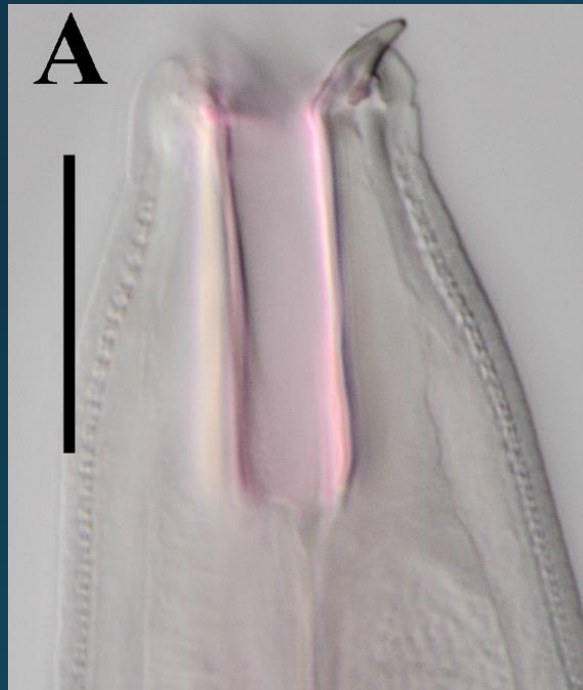
*Acantholaimus*



*Endeolophos*

# Key morphological traits: cephalic region

- Buccal cavity: large with teeth



*Hopperia ancora*



*Parodontophora*



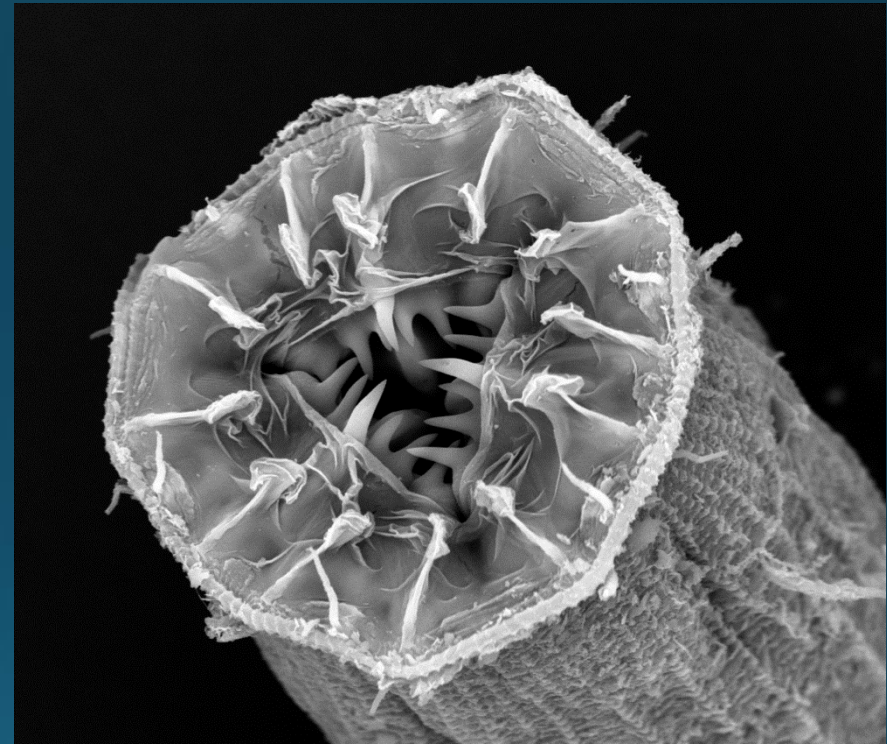
*Viscosia*

# Key morphological traits: cephalic region

- Buccal cavity: mandibles, rhabdions



*Gammanema agglutinans*



*Latronema whataitai*

# Key morphological traits: cephalic region

- Buccal cavity: mandibles, hooks, plates



*Paramesacanthion*



*Sphaerolaimus*

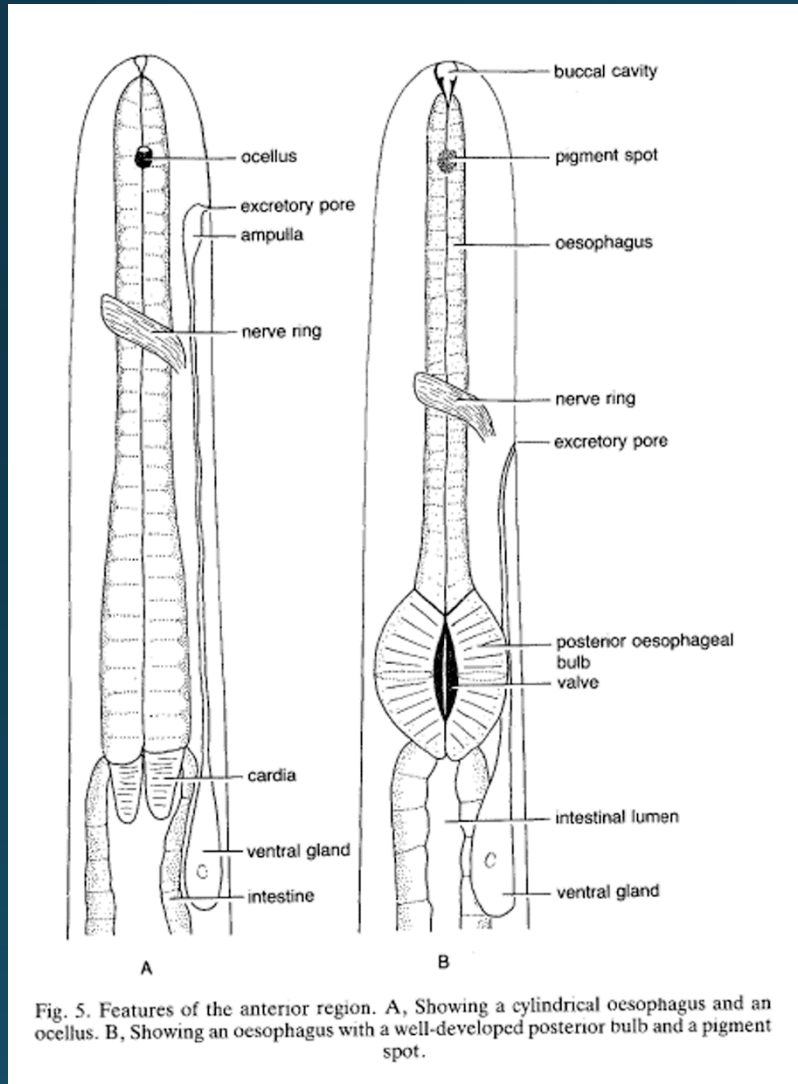
# Key morphological traits: cephalic region

- Buccal cavity: mandibles



*Sphaerolaimus* feeding on *Cervonema*

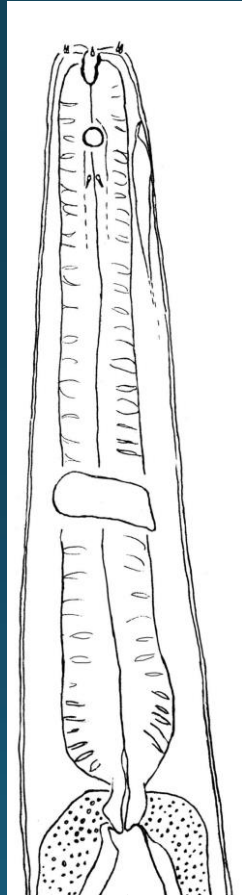
# Key morphological traits: pharynx



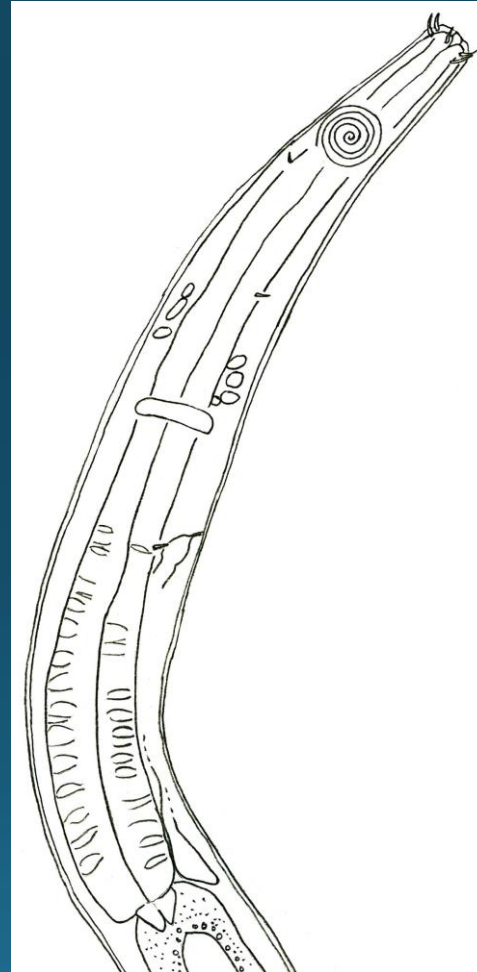
Platt & Warwick 1988

# Key morphological traits: pharynx

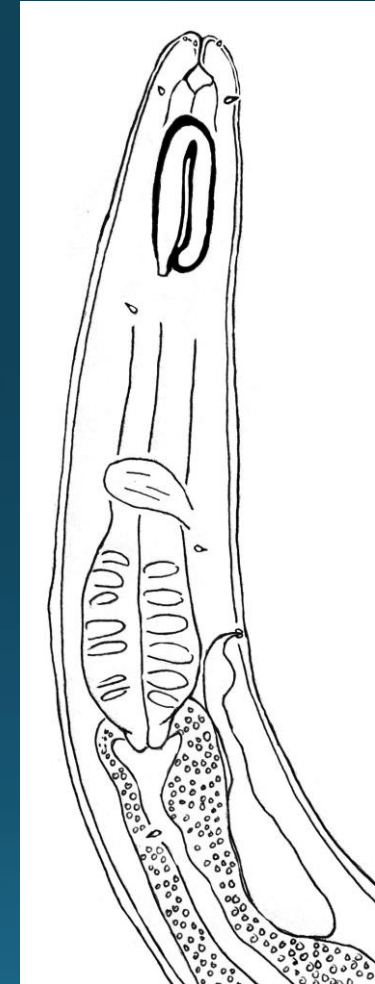
- From undifferentiated to slight posterior bulb...



*Halomonhystera*



*Cervonema heterosperma*



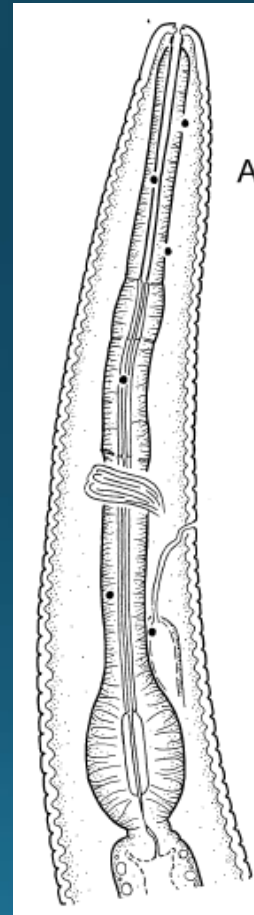
*Mudwigglus patumuka*

# Key morphological traits: pharynx

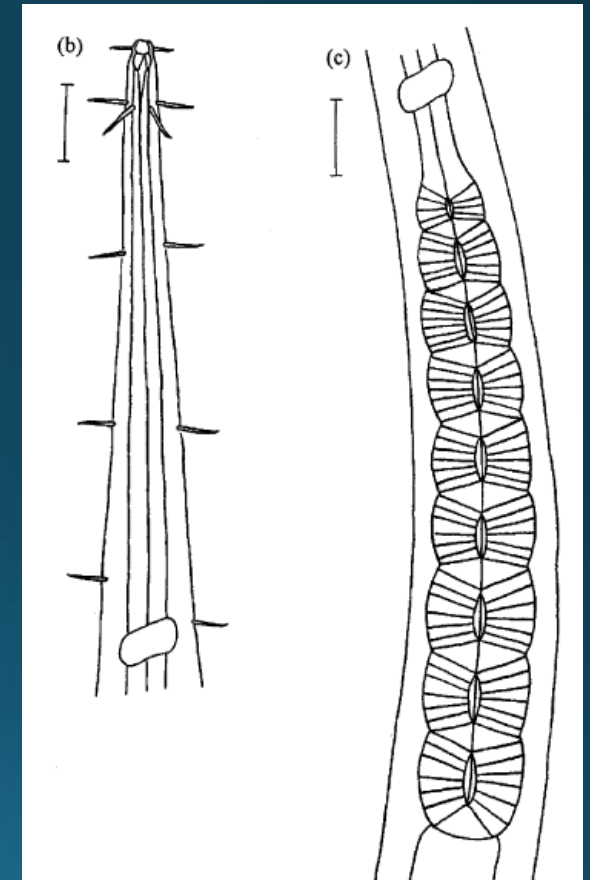
- Differentiation and cuticularisation



*Pseudochromadora*



*Antomicron*  
Holovachov 2012

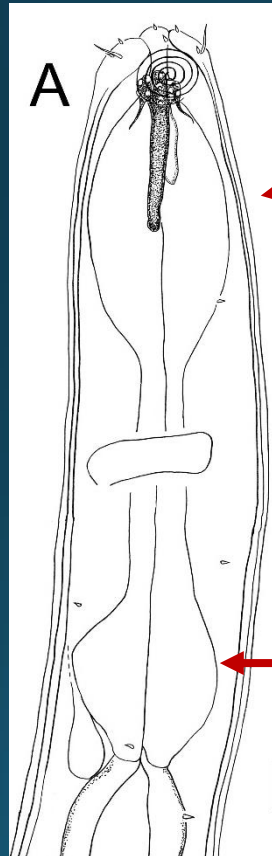


*Belbolla*  
Huang & Zhang 2005

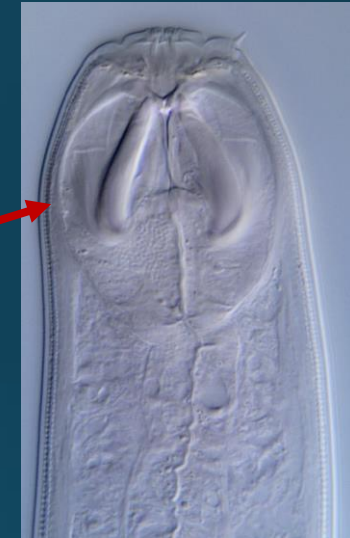


# Key morphological traits: pharynx

- Differentiation and cuticularisation



*Cheironchus haurakiensis*

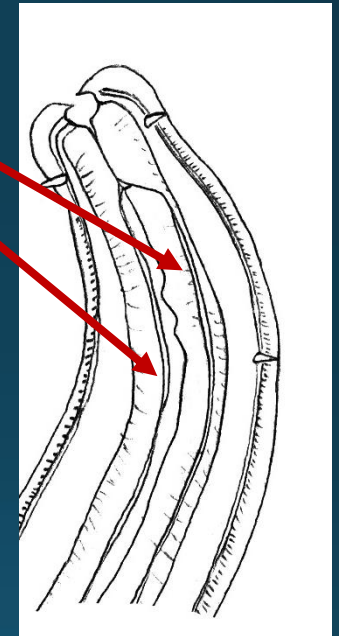
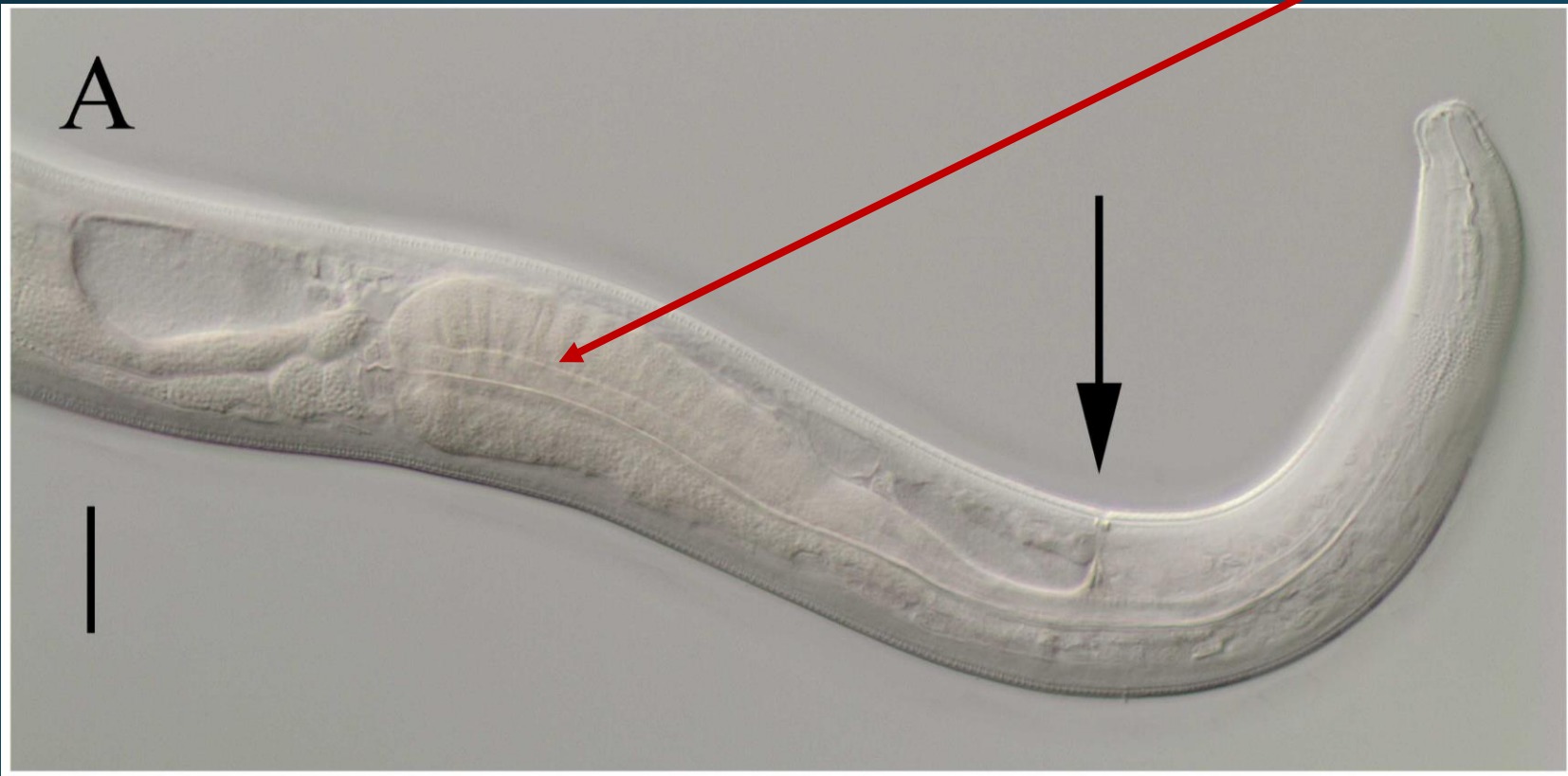


*Pseudocheironchus ingluviusus*

# Key morphological traits: pharynx

- Differentiation and cuticularisation

Pharyngeal tubes



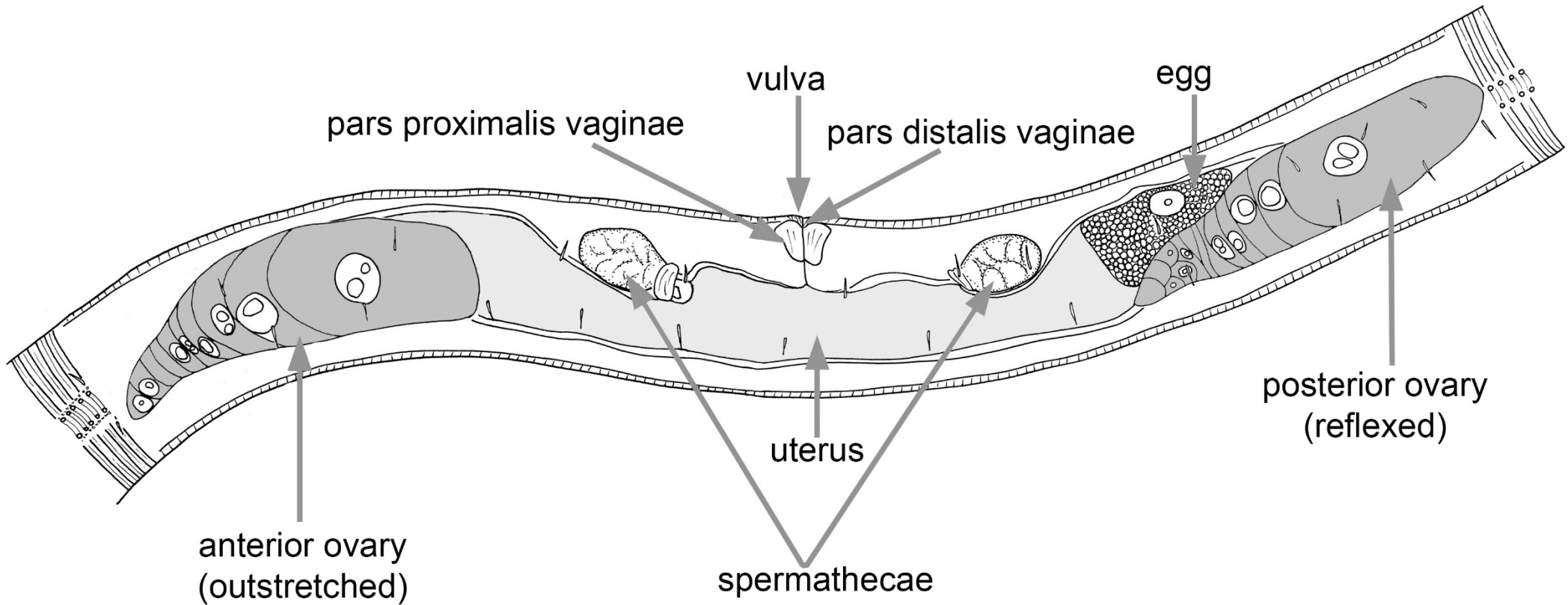
*Sabatieria bubulba*

# Key morphological traits: gonads

Basic features of the gonads:

- Structure: outstretched vs reflexed (“folded”)
- Number: One vs two
- Position: To the right or left of the intestine  
E.g., → differentiation between Xyalidae and Monhysteridae (Monhysterida)

# Female reproductive system



# Key morphological traits: gonads

Head

Tail

Vulva

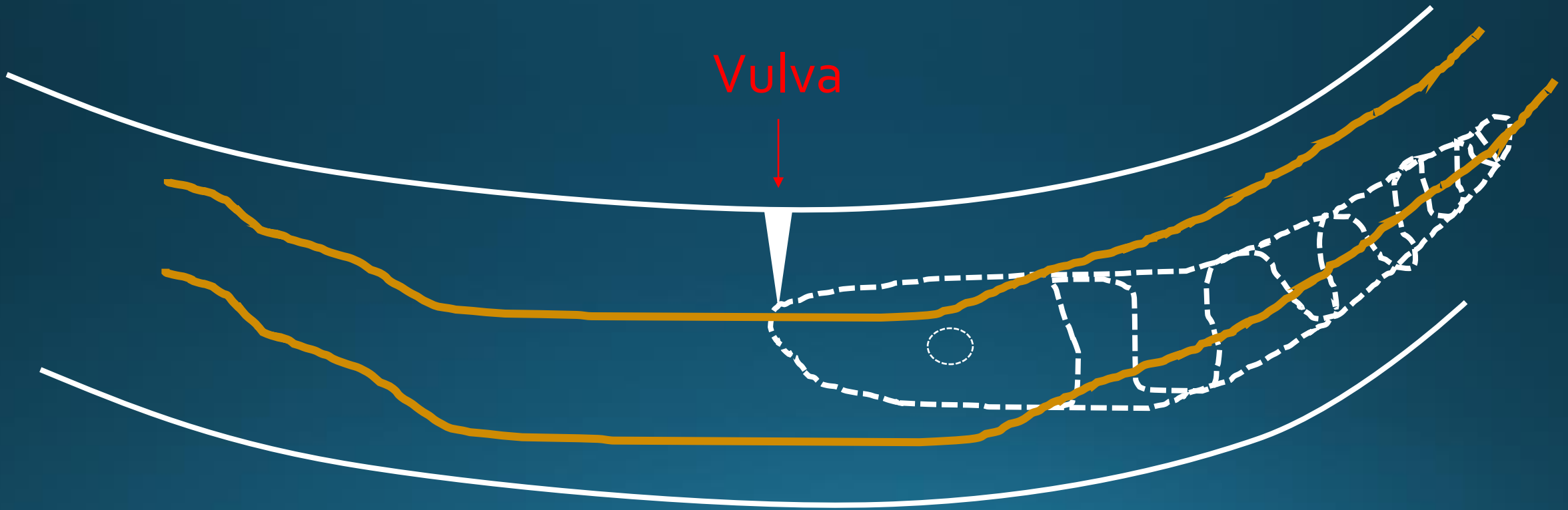


# Key morphological traits: gonads

The intestine is “on top” of the ovary. Is the ovary on the left or right of intestine?

Tail

Head



# Key morphological traits: gonads

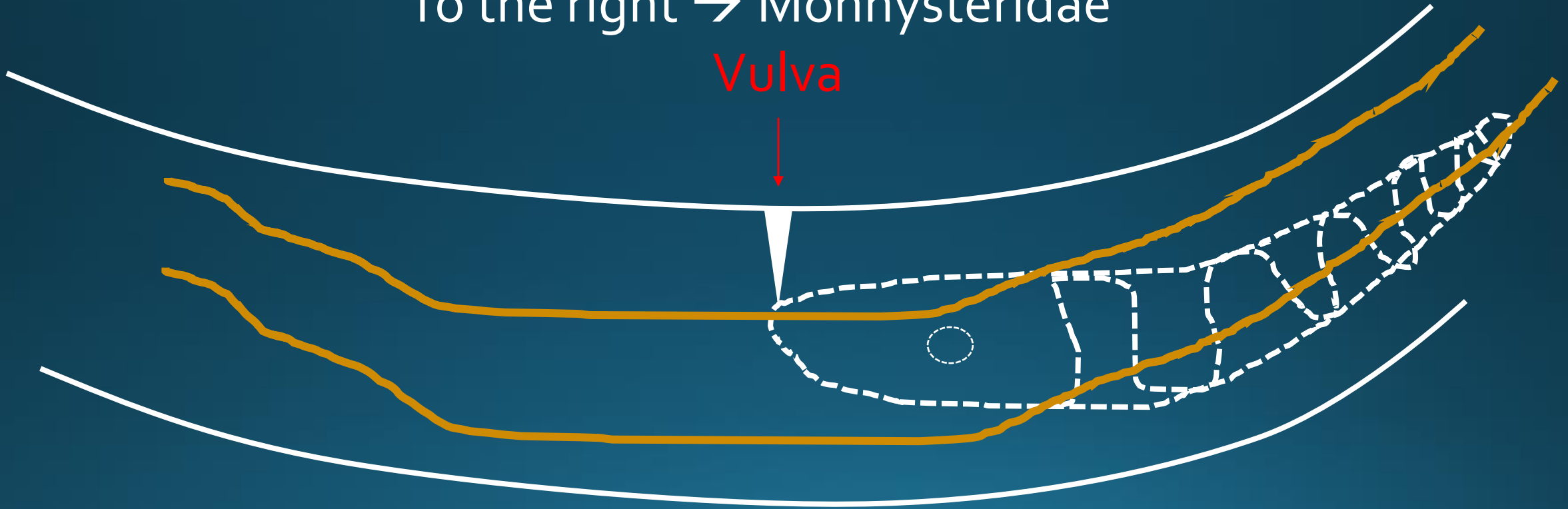
The intestine is “on top” of the ovary. Is the ovary on the left or right of intestine?

Head

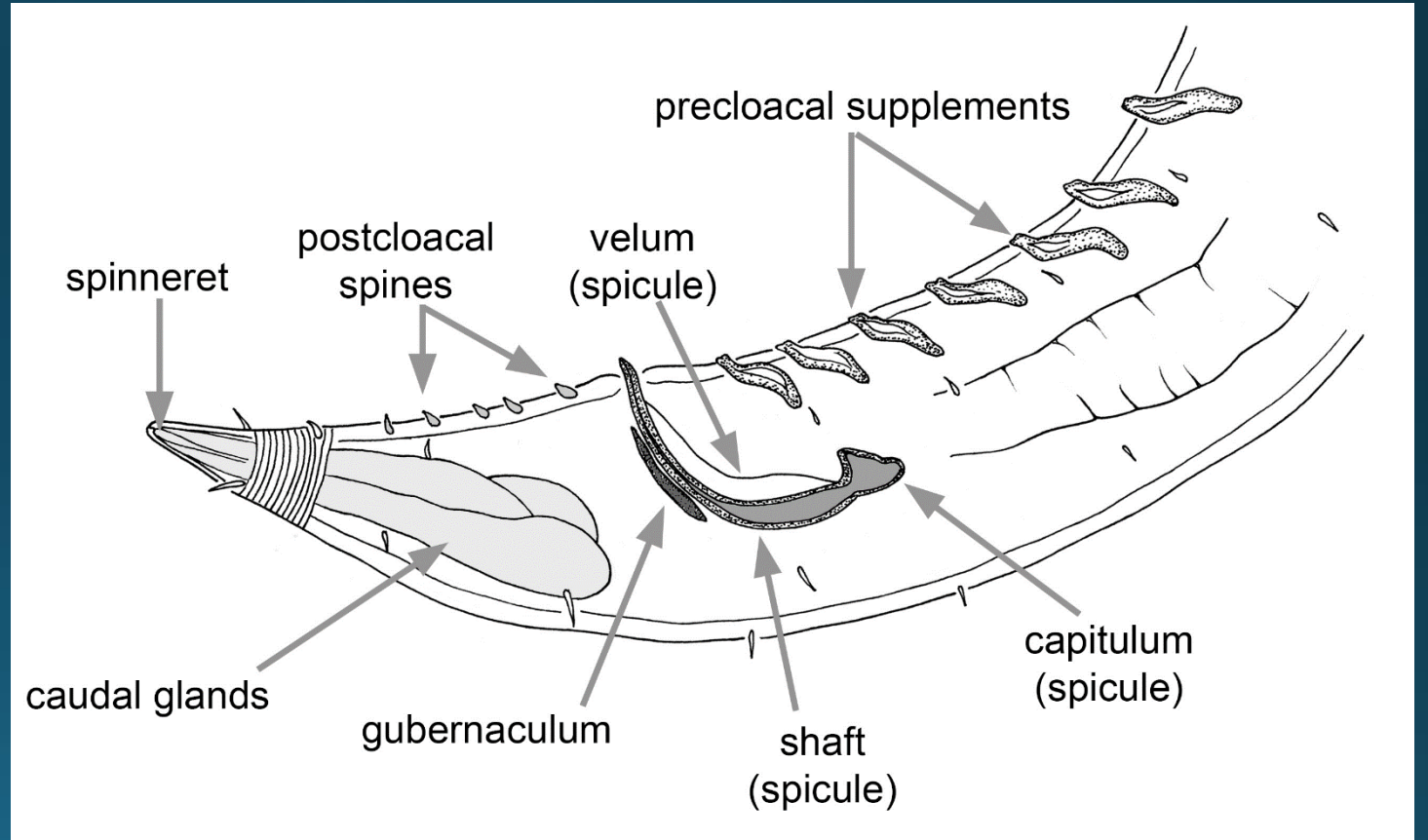
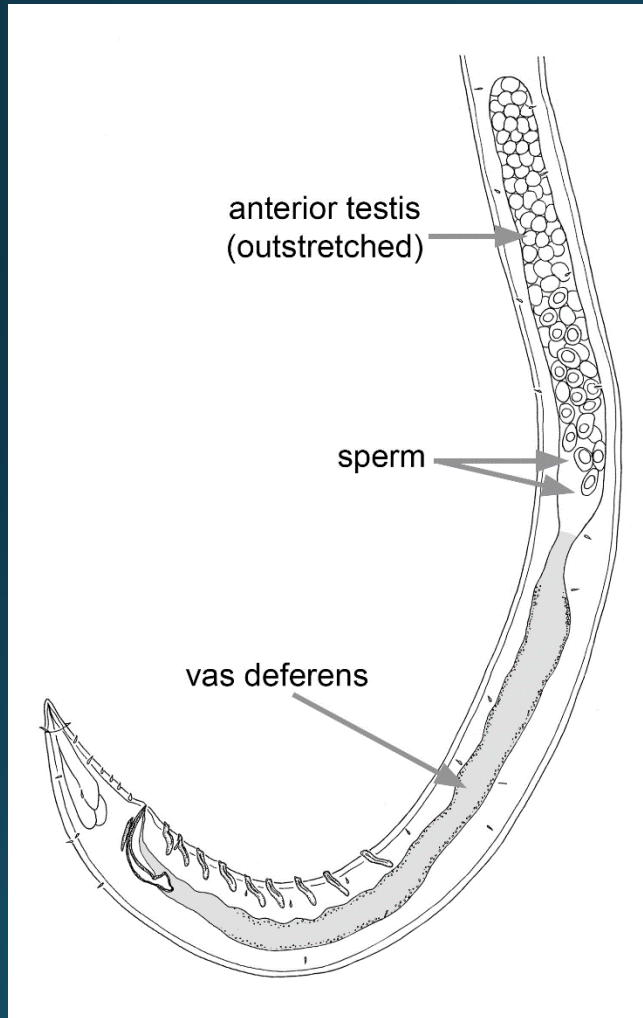
Tail

To the right → Monhysteridae

Vulva



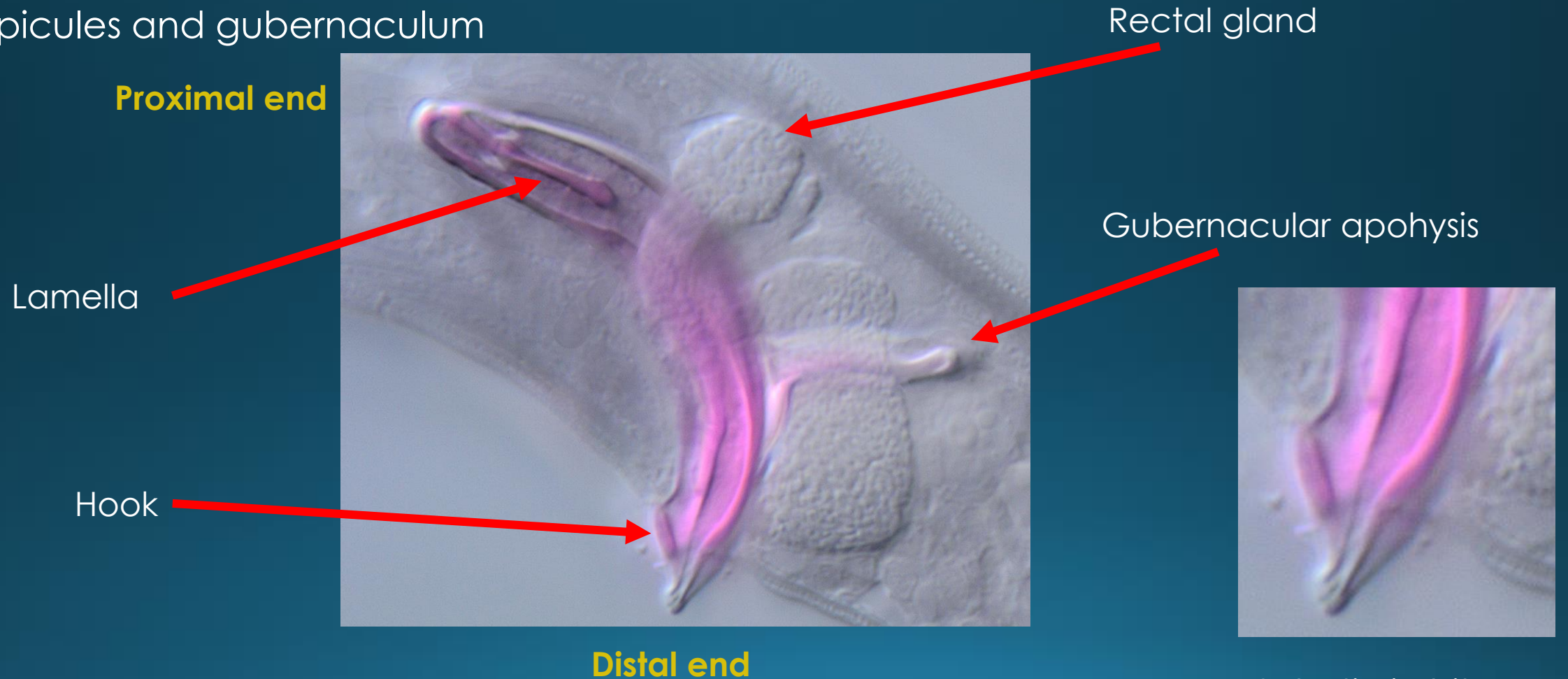
# Male reproductive system





# Key morphological traits: male reproductive structures

- Spicules and gubernaculum

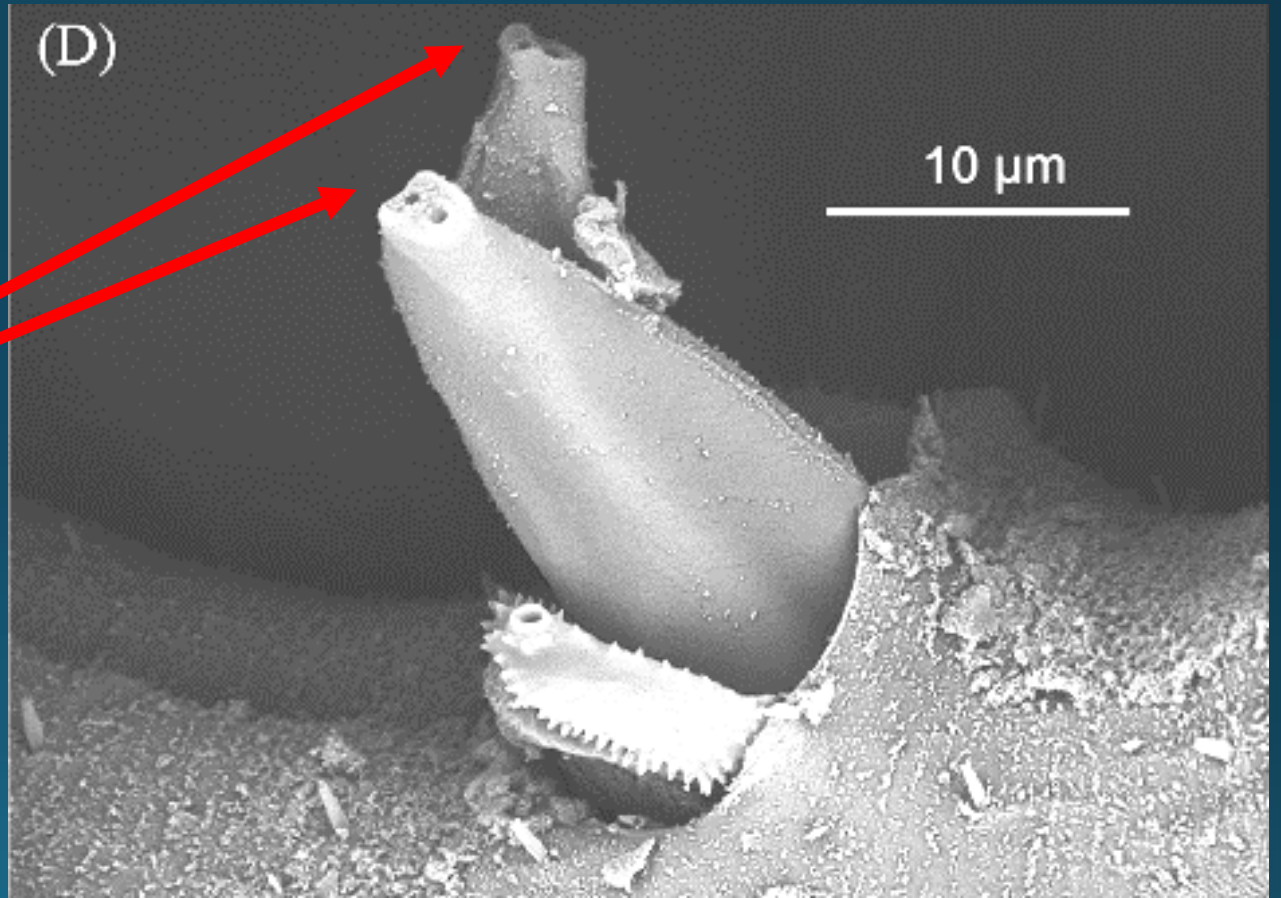


*Sabatieria bitumen*

# Key morphological traits: reproductive structures

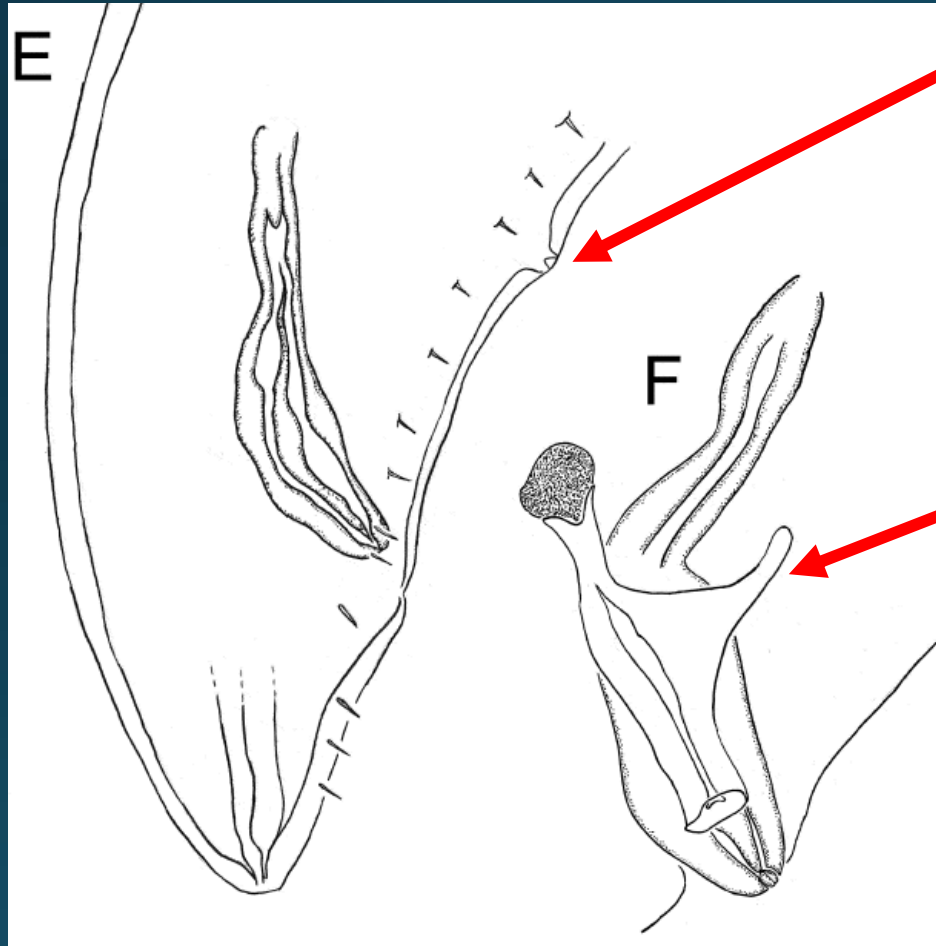
- Spicules transfer sperm and also act as ducts for rectal glands

Apical pores

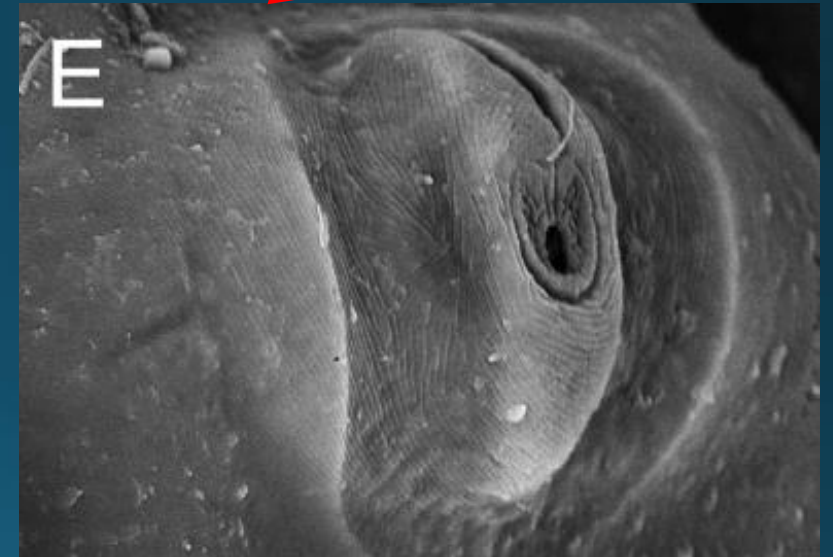


*Paracanthochus mamubiae* Miljutina & Miljutin, 2015

# Key morphological traits: reproductive structures



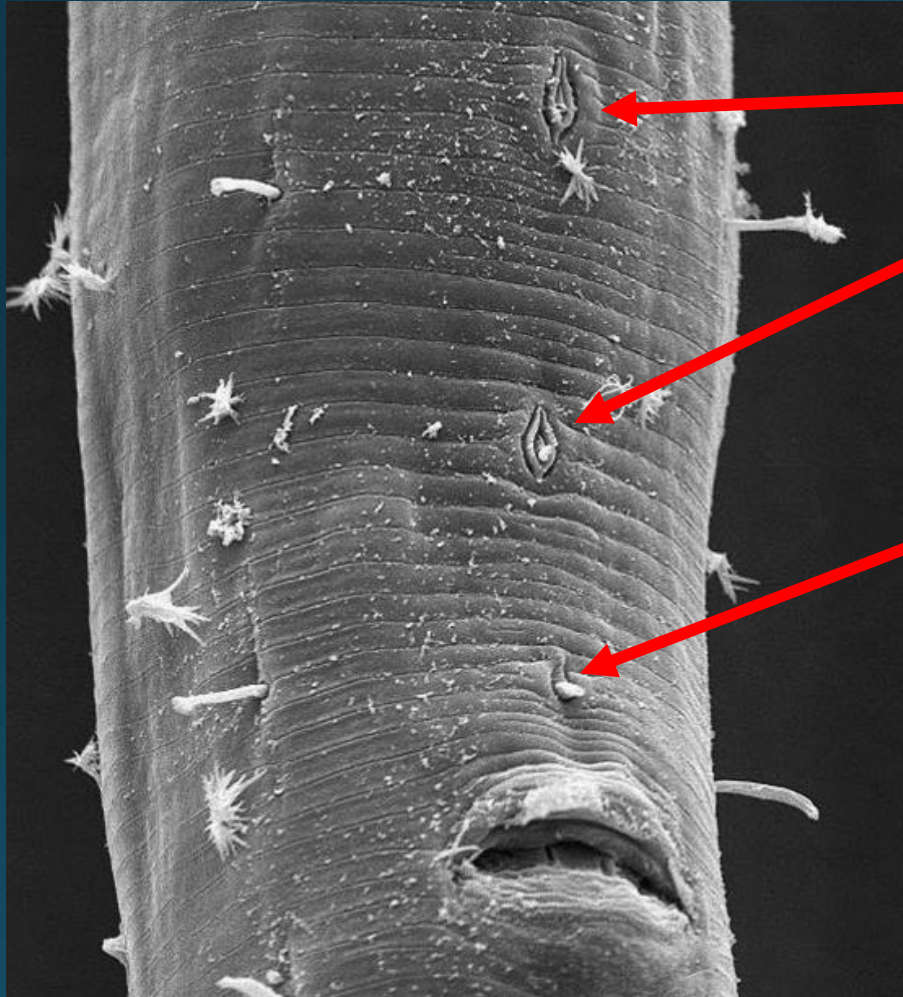
Supplement



Crura

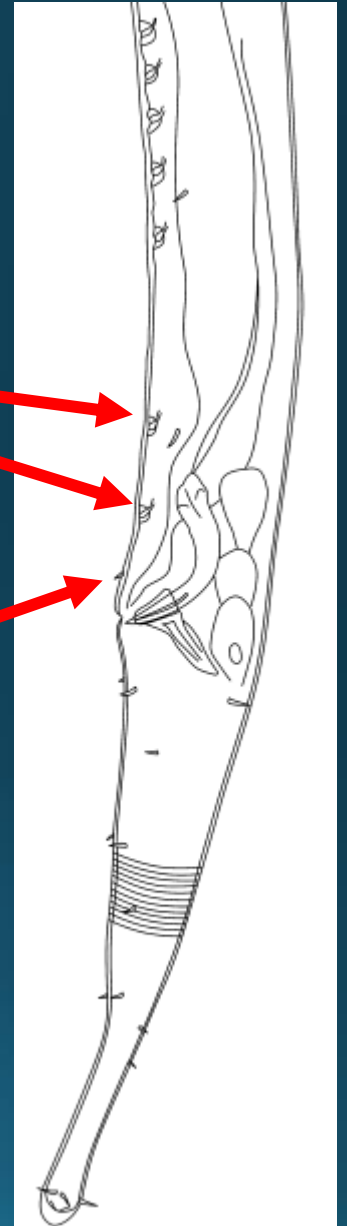
*Deontostoma tridentum*

# Key morphological traits: reproductive structures



Cup-shaped supplement

Pre-cloacal seta



*Sabatieria mortenseni*

# A few final considerations

- What level ID do you really need? Genus, morphospecies, species?
- The more specimens you look at, the better you'll get
- Draw what you see – focus on cuticle, cephalic and pharyngeal body regions, reproductive structures/gonads, tail shape
- Often not possible to make decisive ID based on a single juvenile or female specimen
- Good specimen preparation makes a difference
- Reach out to experts

# Microscopy techniques



# Light microscopy

## ***Specimen preparation:***

Buffered formalin (5%) is best fixative for morphology – leave specimens in for at least 2 weeks

DESS (containing dimethyl sulphoxide, disodium EDTA, and saturated NaCl) is good for both morphology and sequencing

Freezing is also ok for morphology and sequencing. However once thawed, specimens for morphology should be fixed in formalin if they are to be kept for long periods. For sequencing, the quality of sequences decreases over time if specimens kept at -20 C (ok for up to about one year). Better to use -80 C.



*Diplopeltula* sp.

# Light microscopy

## Specimen preparation:

Live samples – remember that nematodes will stay alive for a week or more if kept in the fridge. This includes samples obtained from the continental shelf and even continental slope – they are tough!

When sorting live specimens for sequencing, they can be mounted onto a temporary slide, and immobilized by heating the slides using a hot lamp or hotplate on a low setting. This will allow to take voucher images before preparing specimen for sequencing



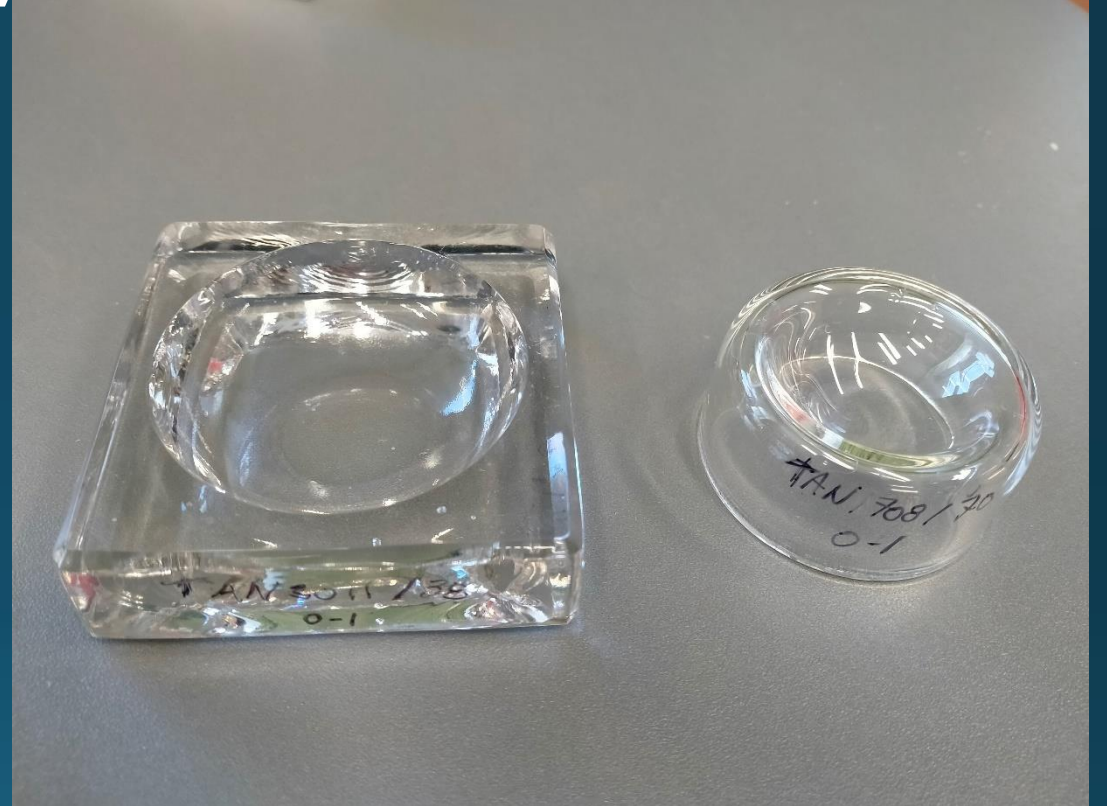
*Hapalomus* sp.



# Light microscopy

## Specimen preparation:

Once fixed, specimen are transferred to a 5% glycerol solution (5% glycerol, 20% ethanol, 75% water), which is left to evaporate to pure glycerol (1-3 days)



# Light microscopy

## Specimen preparation:

Prepare slides with a paraffin wax ring by transferring with a copper pipe



# Light microscopy

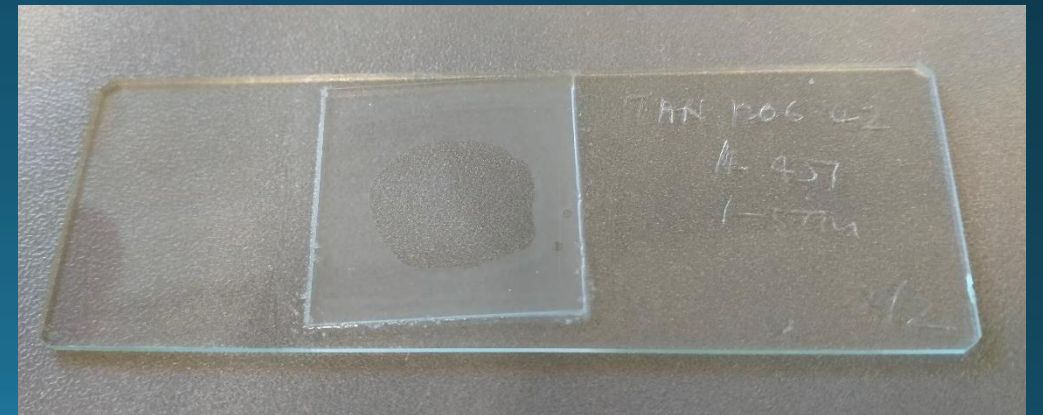
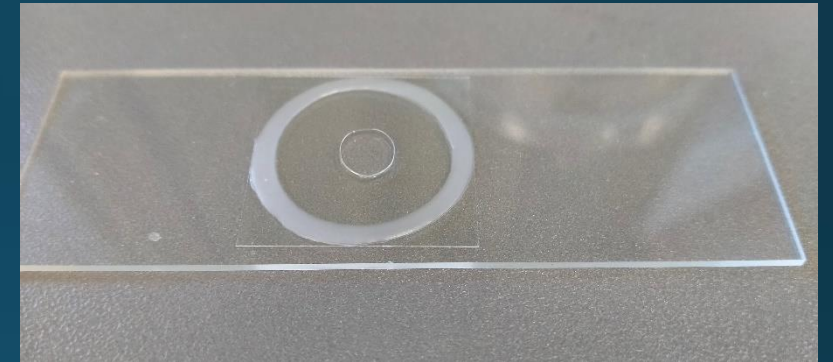
## Specimen preparation:

A fresh drop of glycerol is dropped in middle of a slide with a wax ring

Specimen is laced in glycerol, centered, then coverslip is laid on top

Wax is melted by placing slide on hot plate at ca. 60-70 °C.

Slide is sealed with artist varnish or similar, and labelled



# Light microscopy

## Specimen observation:

Use a compound microscope with:

- Differential Interference Contrast (DIC)

- 4, 10, 20, 40 and 100x objectives

- A camera with image analysis software

A drawing tube is required for detailed drawings



# Scanning electron microscopy

# Why do SEM?

## Nematode identification:

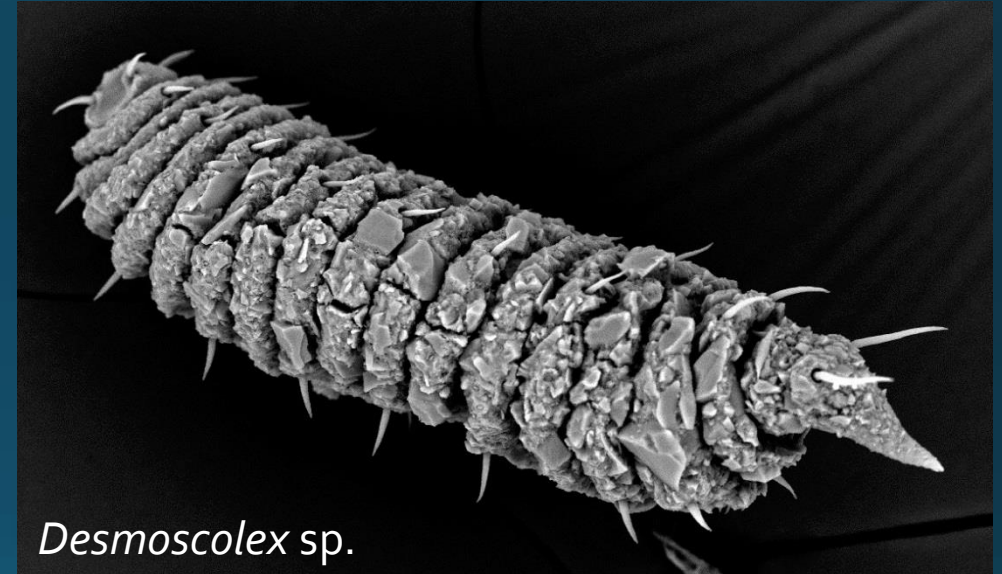
- not needed

## Nematode species descriptions:

- Verify/complement light microscopy observations
- Provide greater number of traits

## Get great pictures:

- Publications, science communication



# Doing SEM is easier than it used to be

- New range of benchtop SEMs relatively cheap and do not require dedicated technician
- The hard part is sample preparation



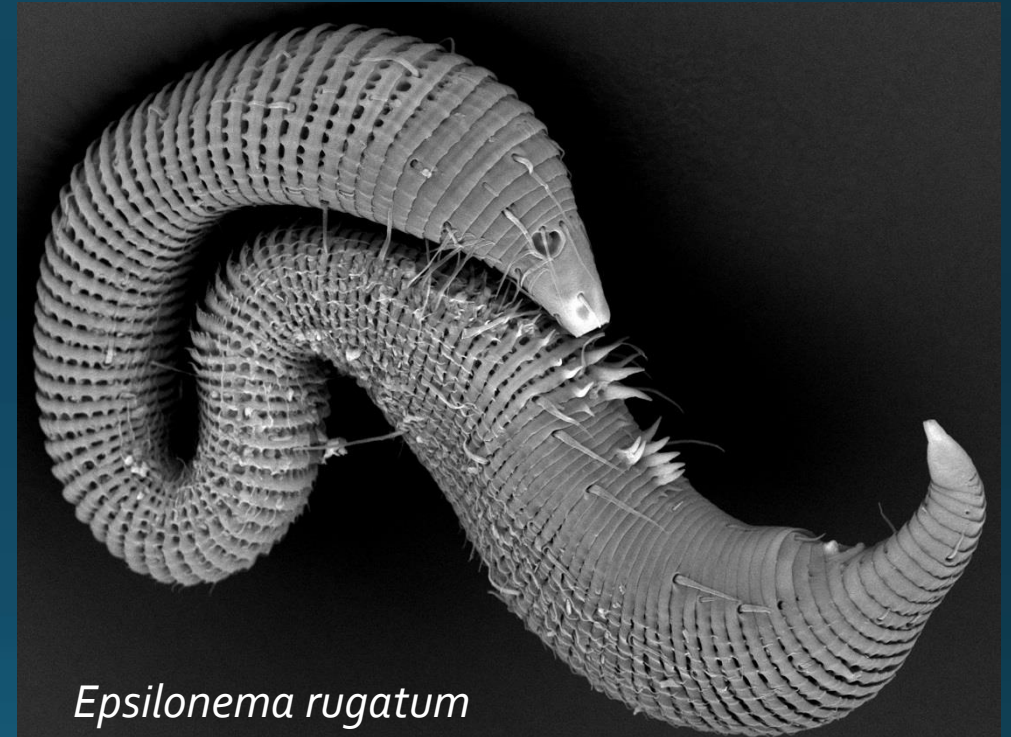
NIWA's Hitachi TM3000 Benchtop SEM

# Sample preparation

## 1<sup>st</sup> step fixation:

- This step required to harden structures through chemical bond formation
- 5-10% buffered formalin, and/or
- 2% glutaraldehyde with sodium cacodylate buffer

→ Important to fix animals for long duration (weeks if possible, longer better)



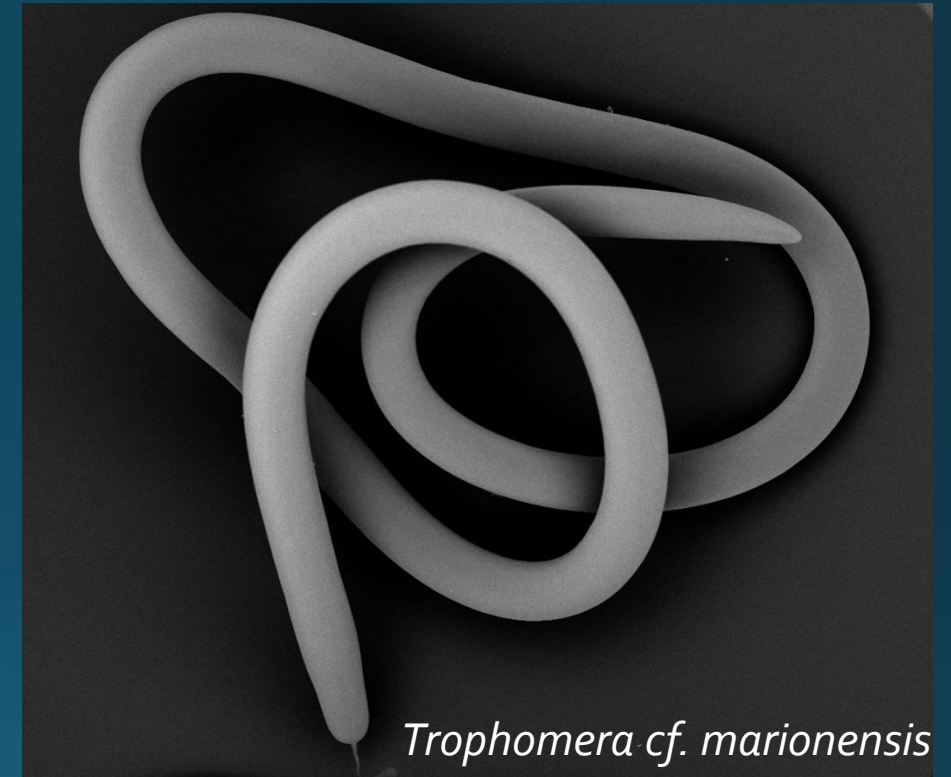
*Epsilonema rugatum*



# Sample preparation

## 2<sup>nd</sup> step fixation:

- 4% Osmium tetroxide for 2-48 hours
  - Extremely toxic, use only under a fume hood and wear gloves and goggles at all times. Only few drops needed.
  - Look for change in colour
  - Acts on lipids, and won't work if specimen has been in contact with alcohol (no change in colour)



# Sample preparation

## Dehydration

- This step required to replace water in specimen by ethanol (or acetone)
- Progressively transfer specimens to 25, 50, 75, 90, and 100% ethanol (~15 min each)

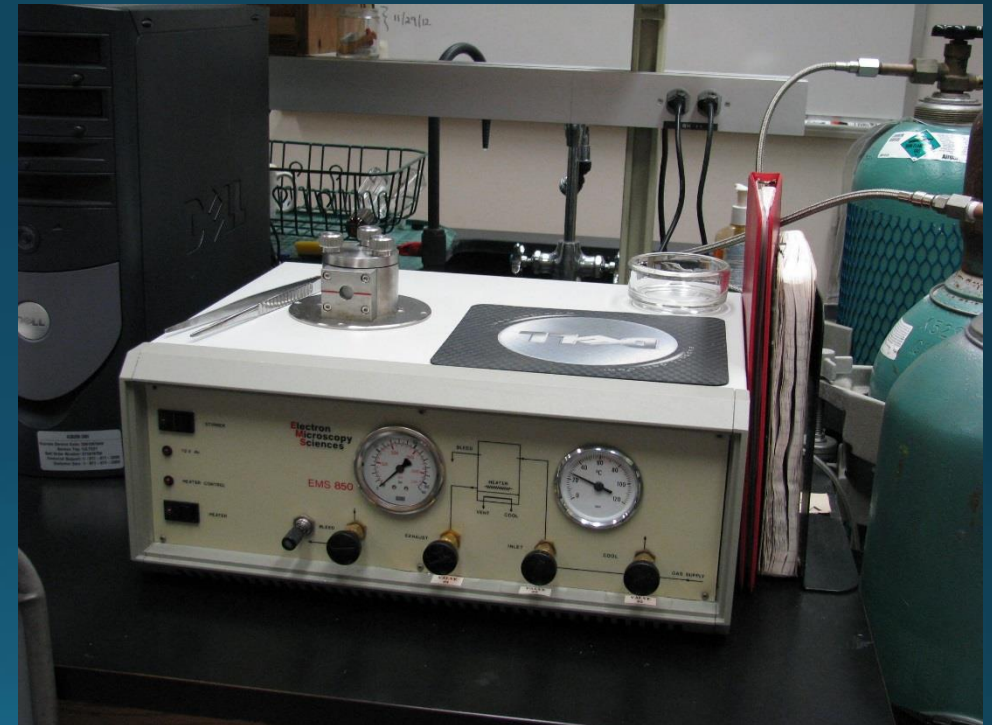


*Pseudochromadora reathae*

# Sample preparation

## Critical point drying

- Specimens need to be dry before entering SEM chamber
- Best method for drying specimens without causing collapse of fine structures or wrinkling of cuticle

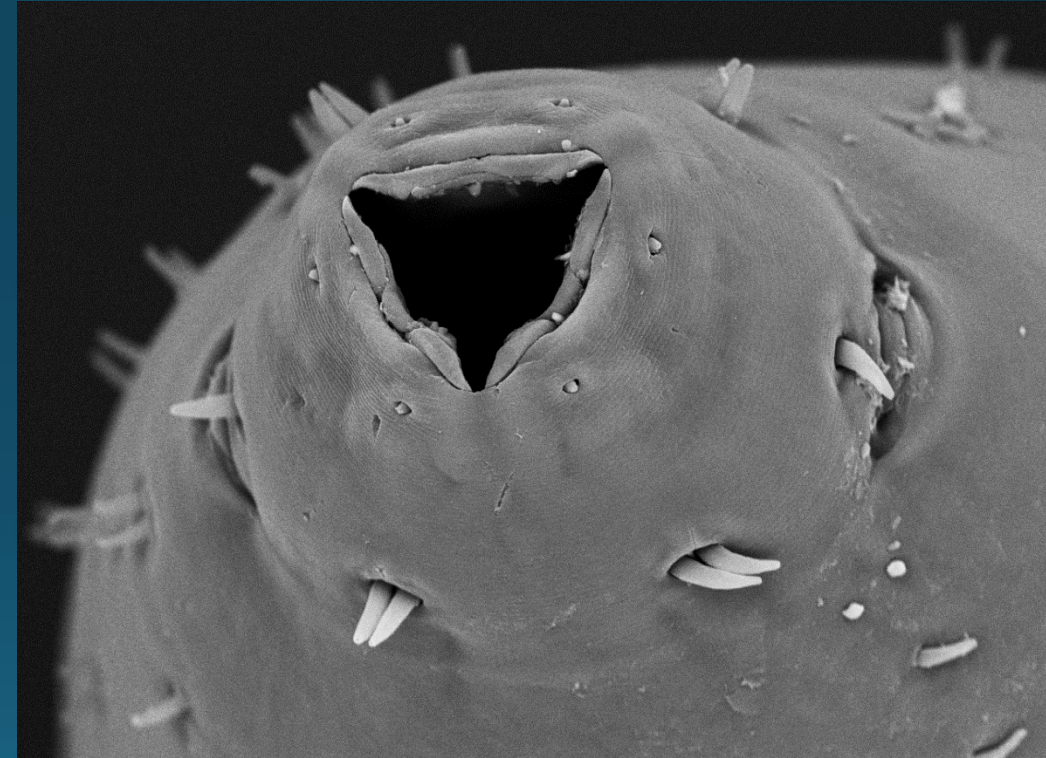


EMS850 CPD

# Sample preparation

## Critical point drying

- Required so that no liquid enters SEM chamber
- Best method for drying specimens without causing collapse of fine structures or wrinkling of cuticle
- Formalin and osmium tetroxide-fixed specimens that have been transferred to ethanol sometimes may look ok after air drying (and coating)

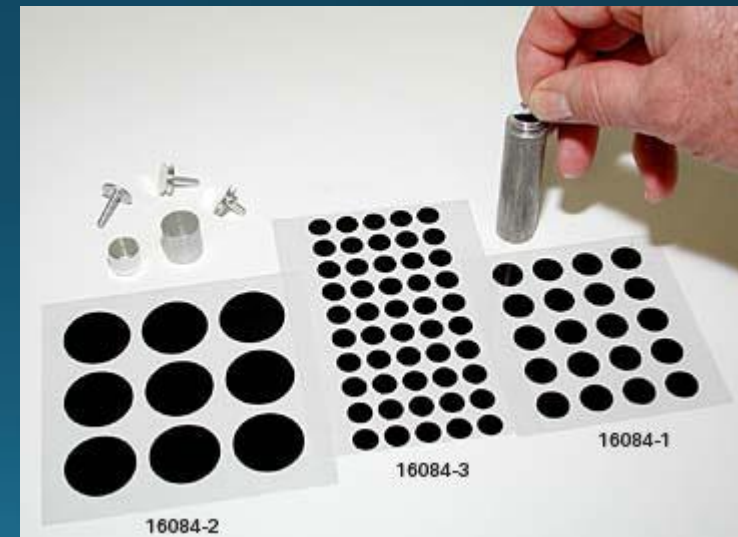
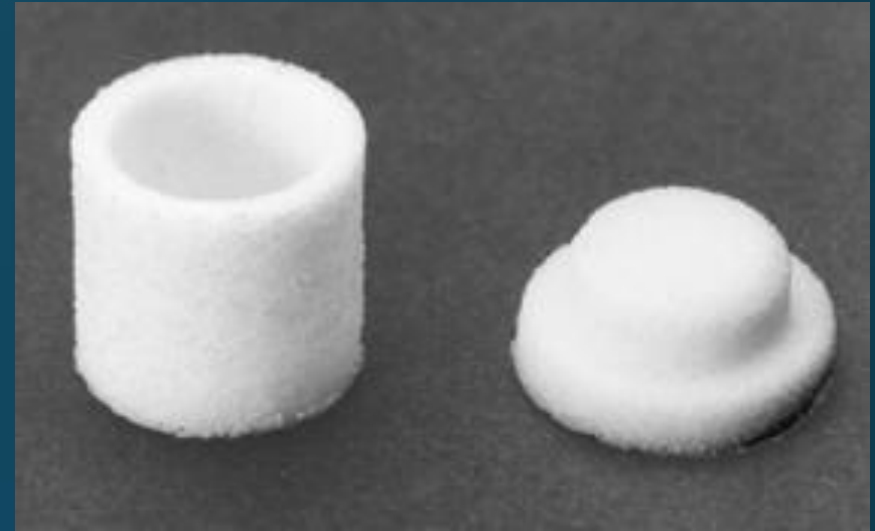


Air-dried *Deontostoma tridentum*

# Sample preparation

## Critical point drying

- Use microporous specimen capsules
- Transfer dry specimens on stub with carbon tape using “eyelash on a stick”
- Make sure to earth your stick and microscope to avoid static problems (works best in high humidity!)
- You will probably lose a few specimens in process



# Sample preparation

## Coating

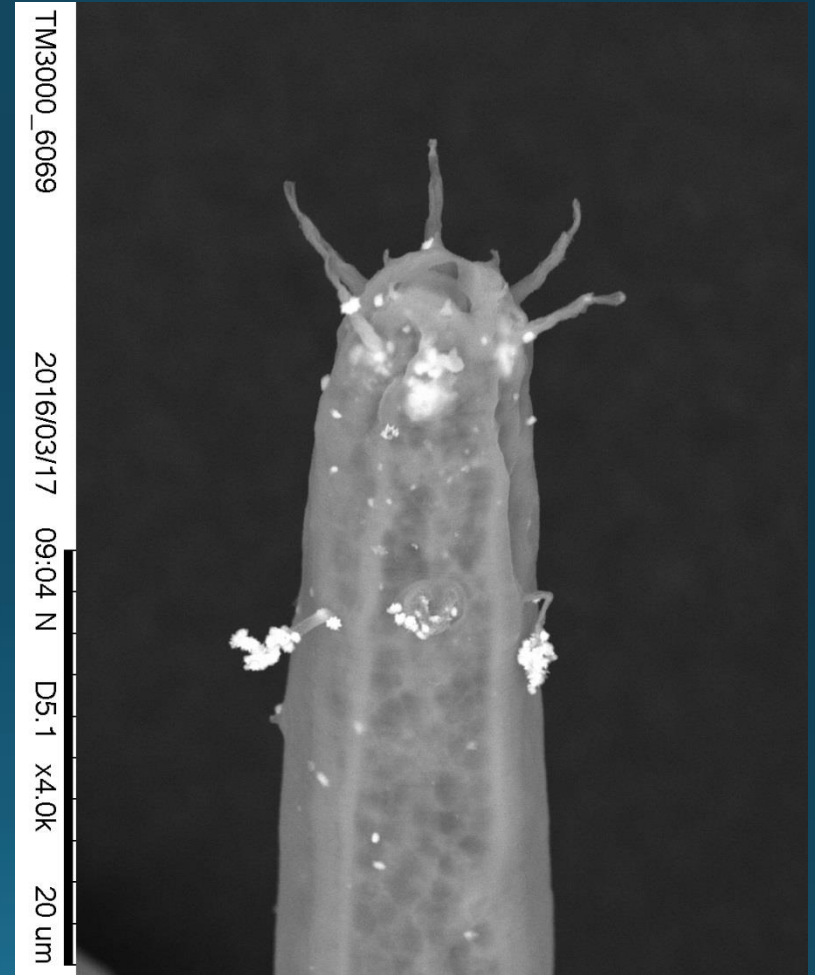
- Required to avoid charging of specimens
- Gold ok, gold/palladium apparently better
- Quick and easy! Just check manufacturers instructions



# Some taxa easier than others

Taxa with thick cuticle tend to look better, e.g., Desmodoridae, Leptosomatidae

Special care (i.e., long fixation) should be taken for taxa with thin cuticle, e.g., Monhysteridae, Linhomoeidae, Trefusiidae

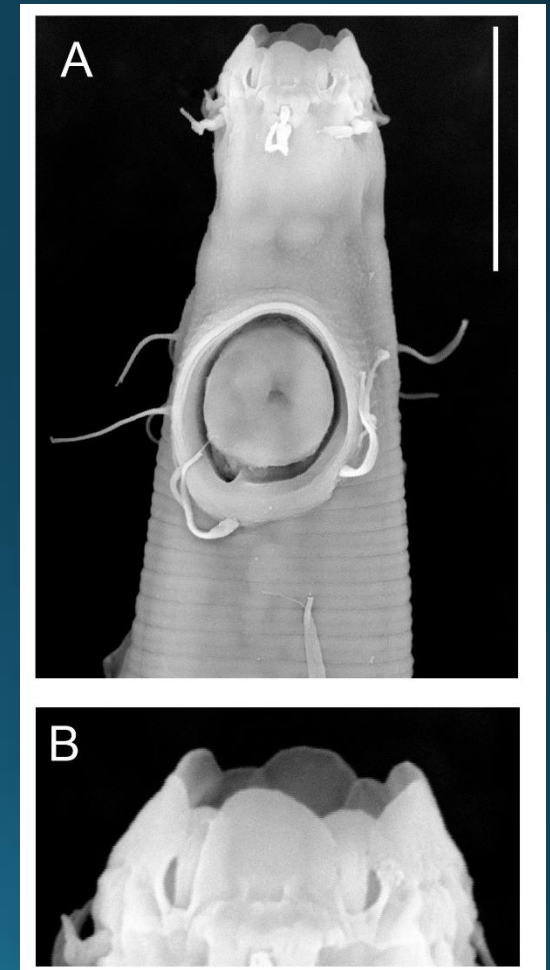
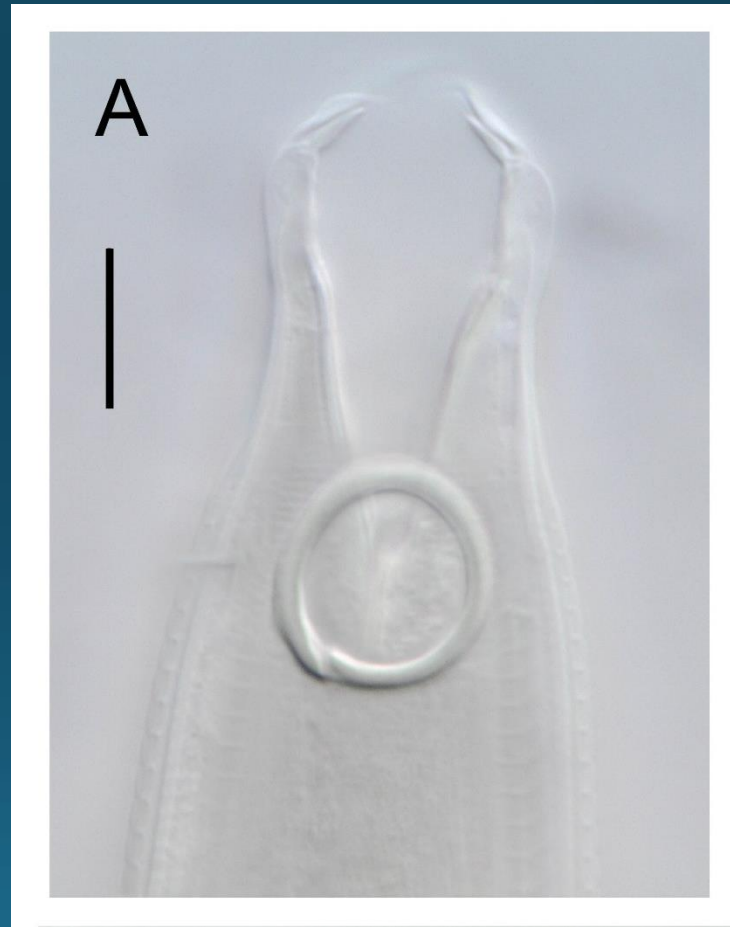


*Trefusia piperata*: too fragile!

# Examples...

*Paramphimonhystrella glossalga*:

- Unusual structure of lips (double set of 6 each)
- Specimens had been in ethanol/glycerol solution, so not in great shape

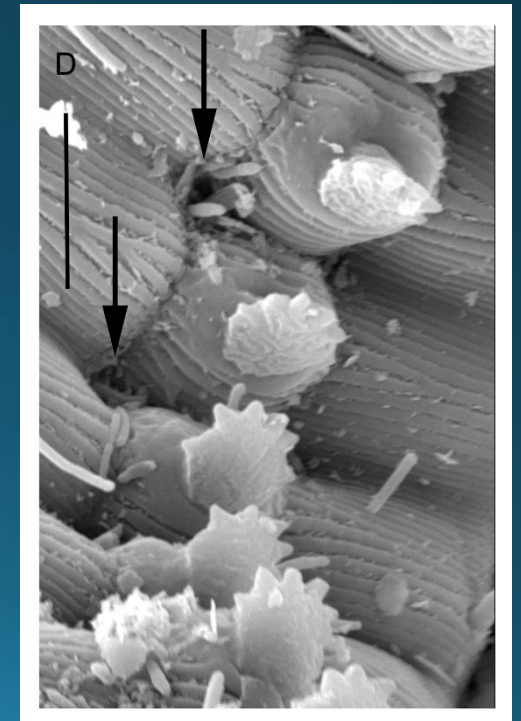
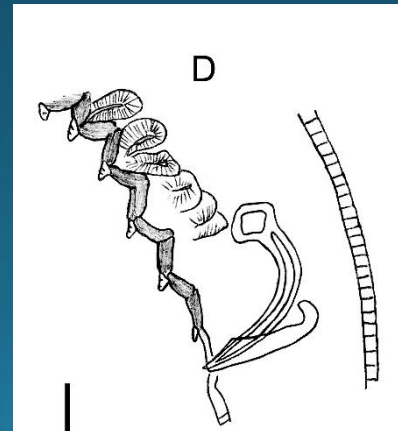
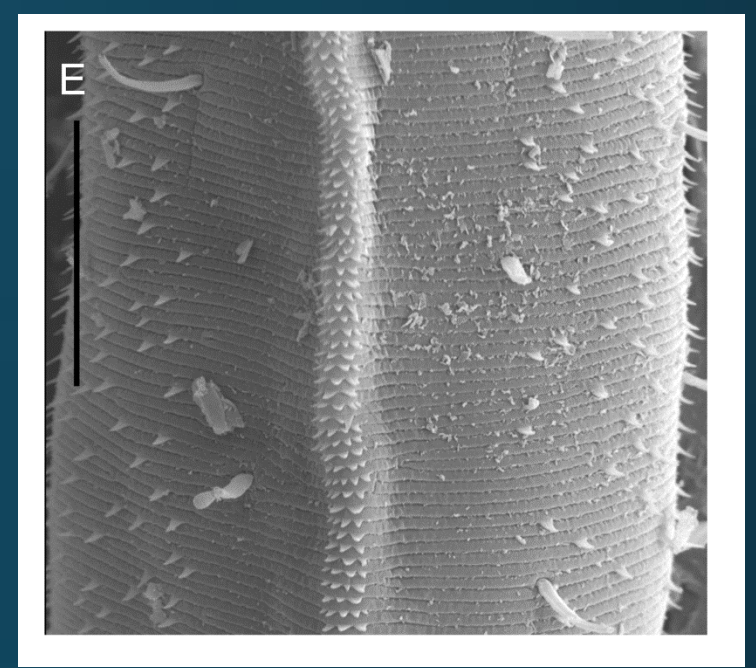
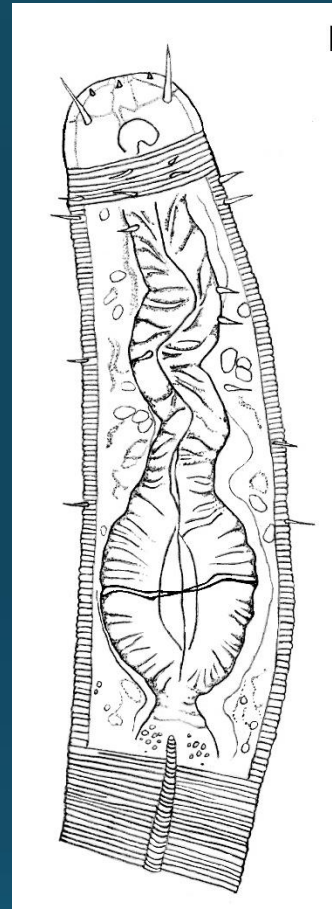




# Examples...

*Pseudochromadora reathae*:

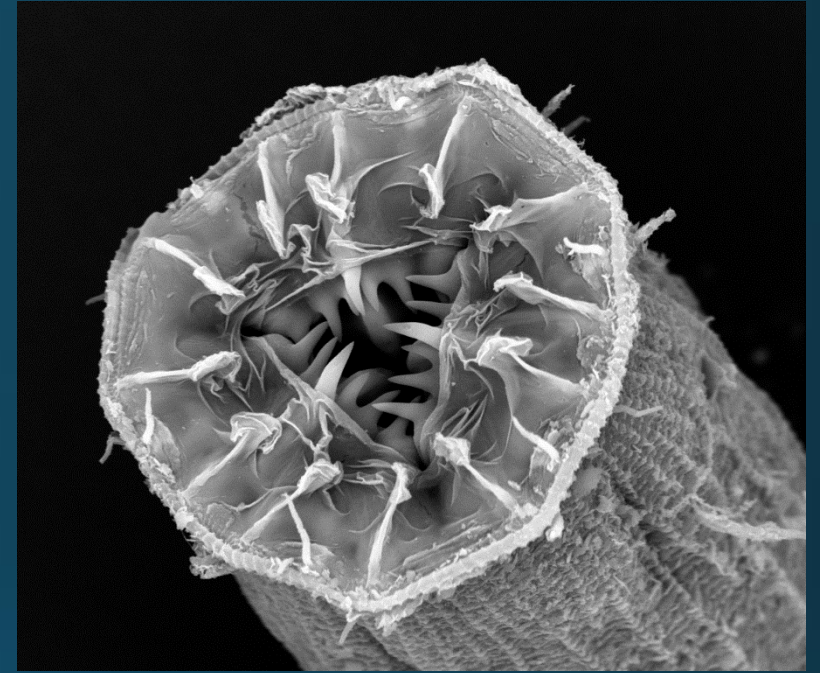
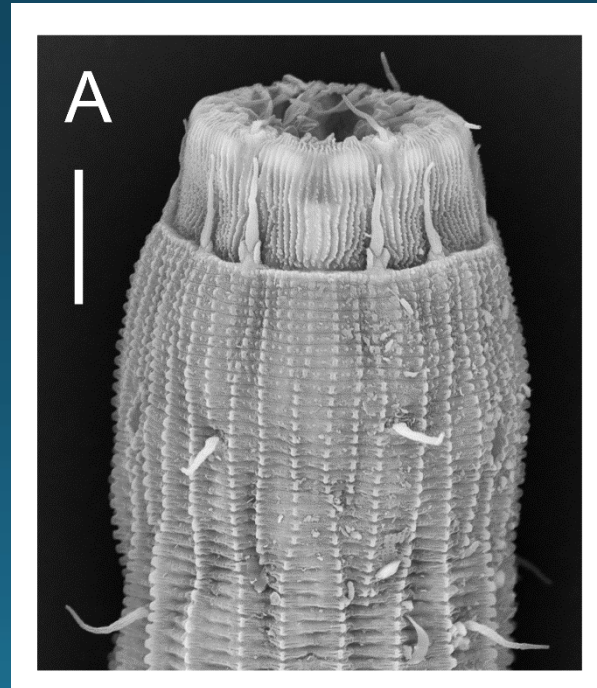
- Fine structure of cuticle and precloacal supplements



# Examples...

*Latronema whataitai*:

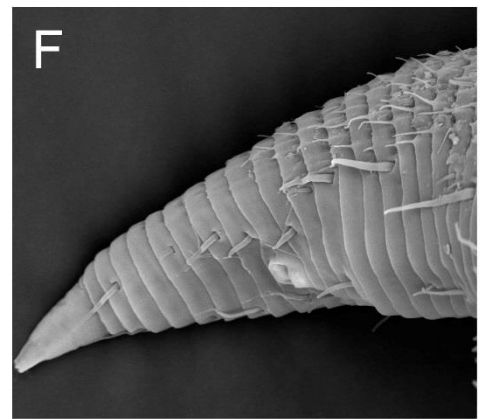
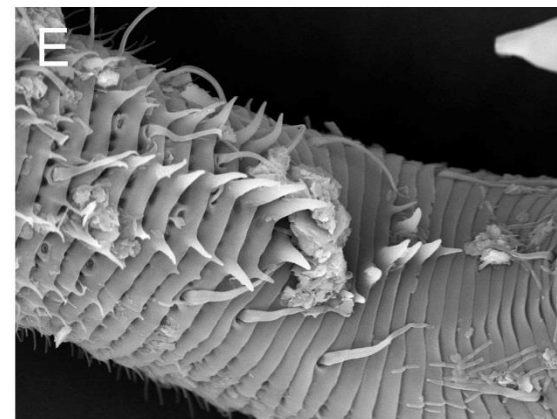
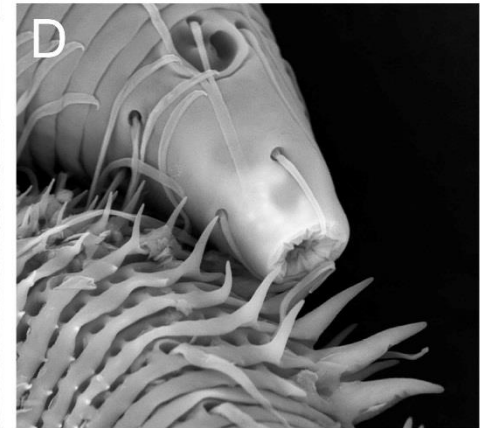
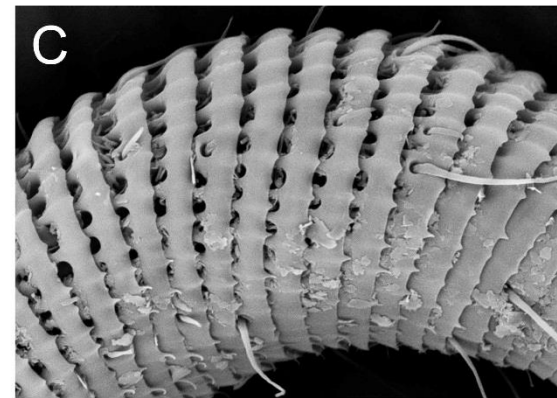
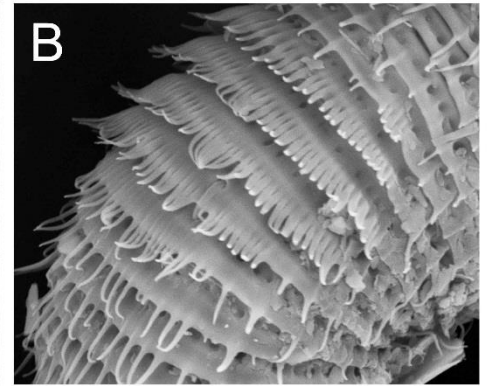
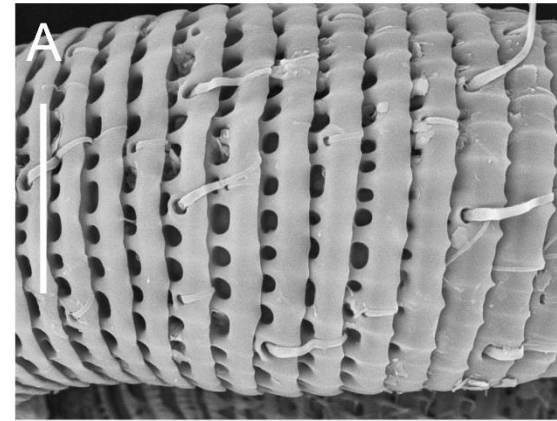
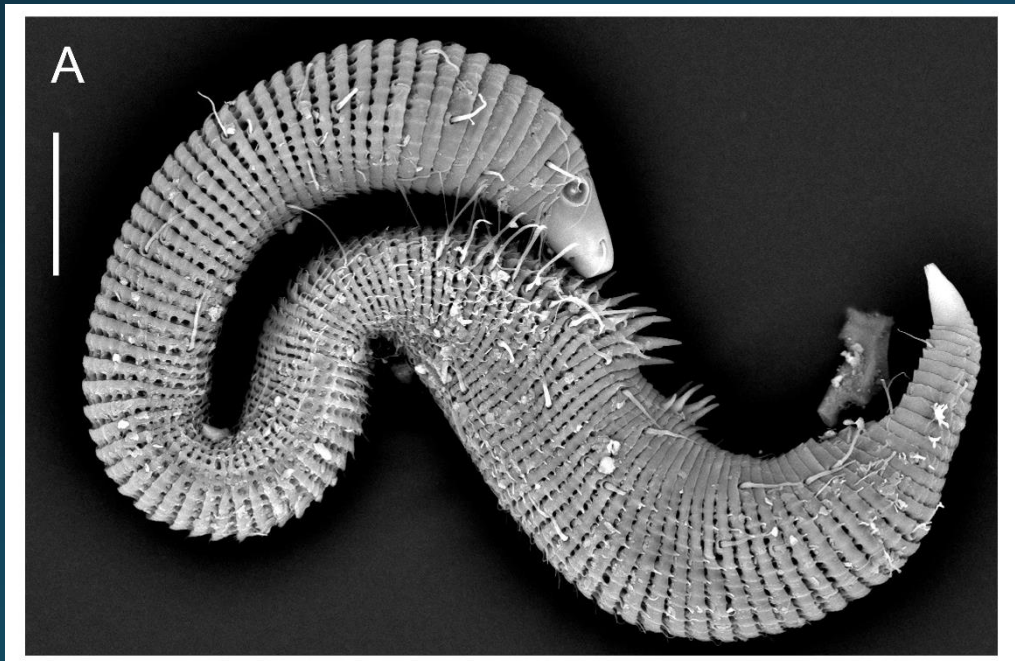
- Number and arrangement of teeth
- Cuticle details



# Examples...

*Epsilonema rugatum*:

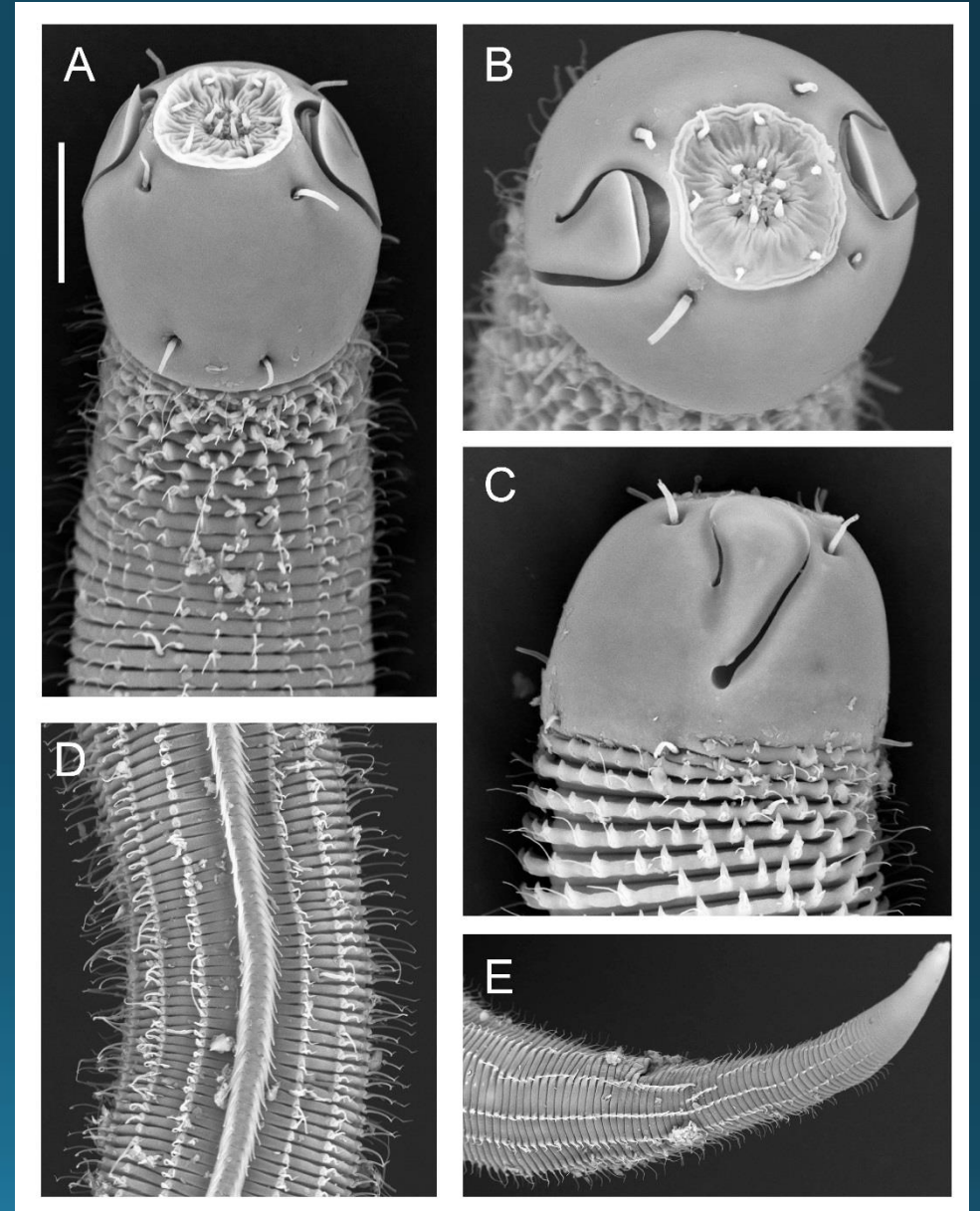
- Details of cuticle



# Examples...

*Desmodorella verscheldei*:

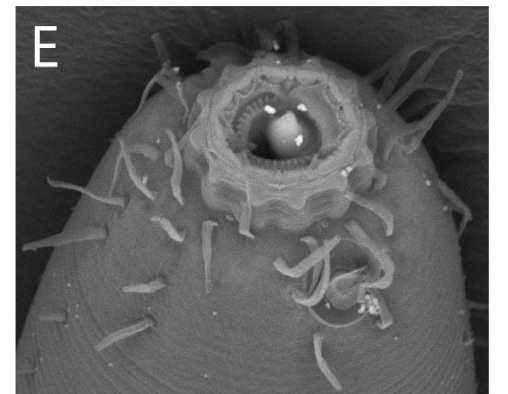
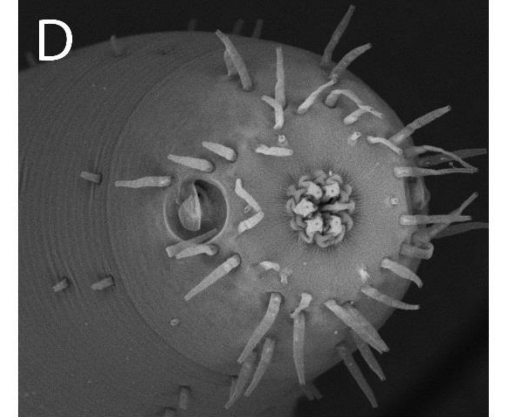
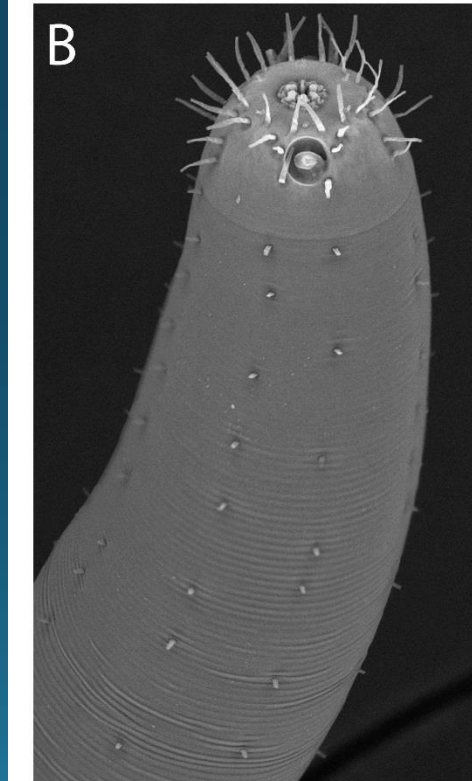
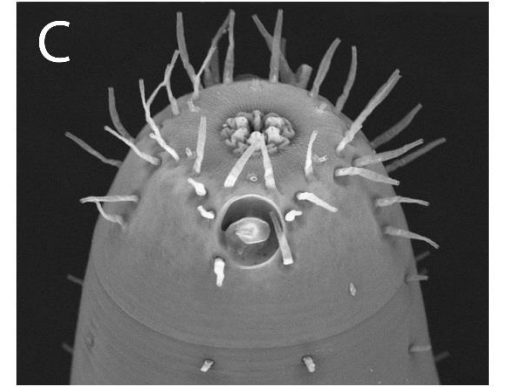
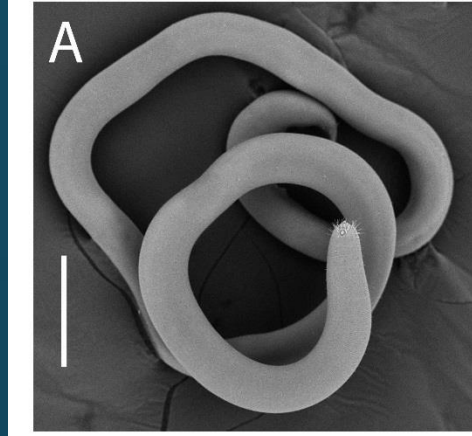
- Details of cephalic structures and cuticle



# Examples...

*Acanthopharynx dormitata*:

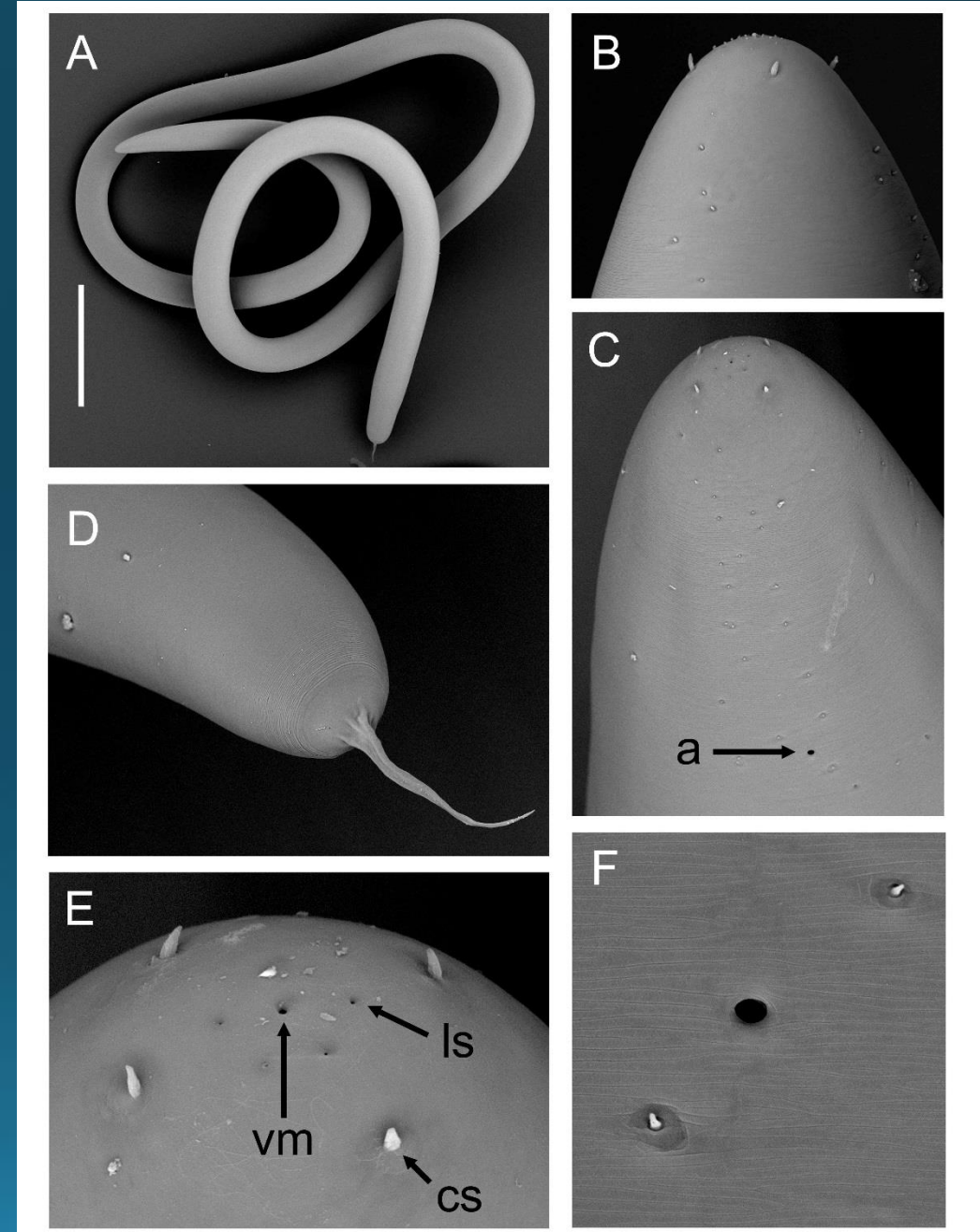
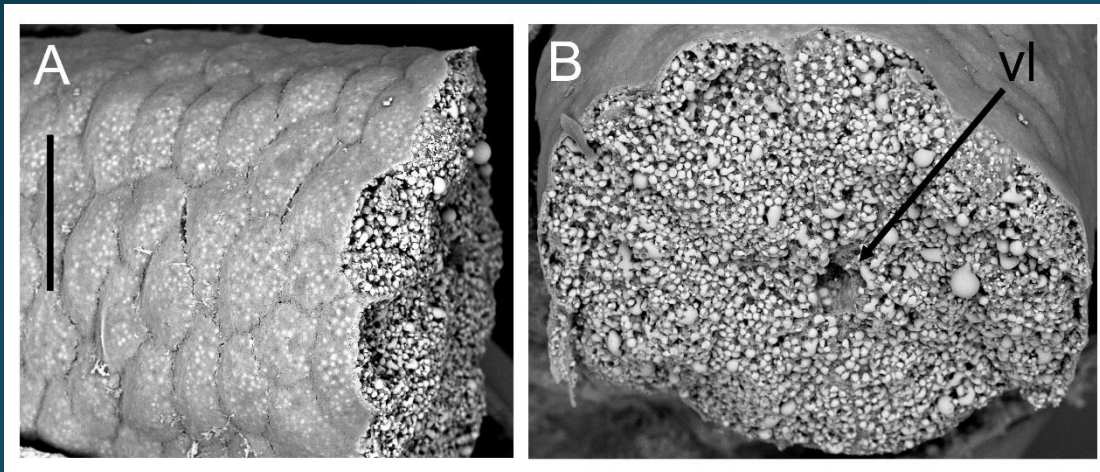
- Cephalic sensilla
- Denticles in mouth cavity



# Examples...

*Trophomera cf. marionensis*:

- Cephalic sensilla
- Small amphid
- Structure of modified intestine



# A few last comments on SEM

- Specimen preparation is key - be patient and try different methods
- Prepare as many specimens as possible, as some specimens usually get lost during processing (it's a bit of a lottery...)
- Often all you need is ONE good specimen
- Don't underestimate the value of good images – good for publications AND engaging public

