Twinning BA/12/IB/AG 01 "Further strengthening of capacities of phytosanitary sector in the fields of plant protection products, plant health and seeds and seedlings, including phytosanitary laboratories and phytosanitary inspections"

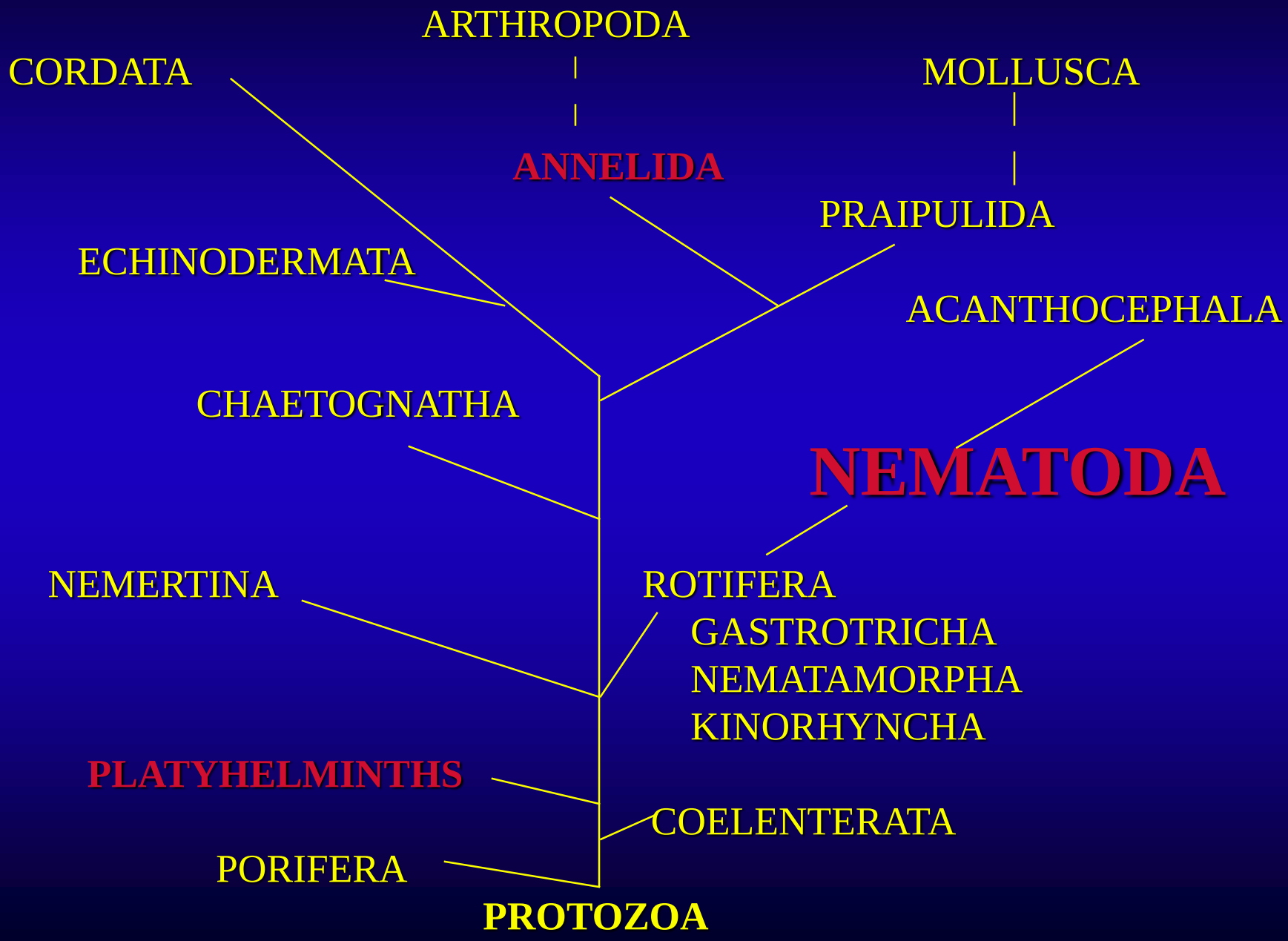
Training course on agricultural nematology

Mostar, March 7-11, 2016

PLANT PARASITIC NEMATODES

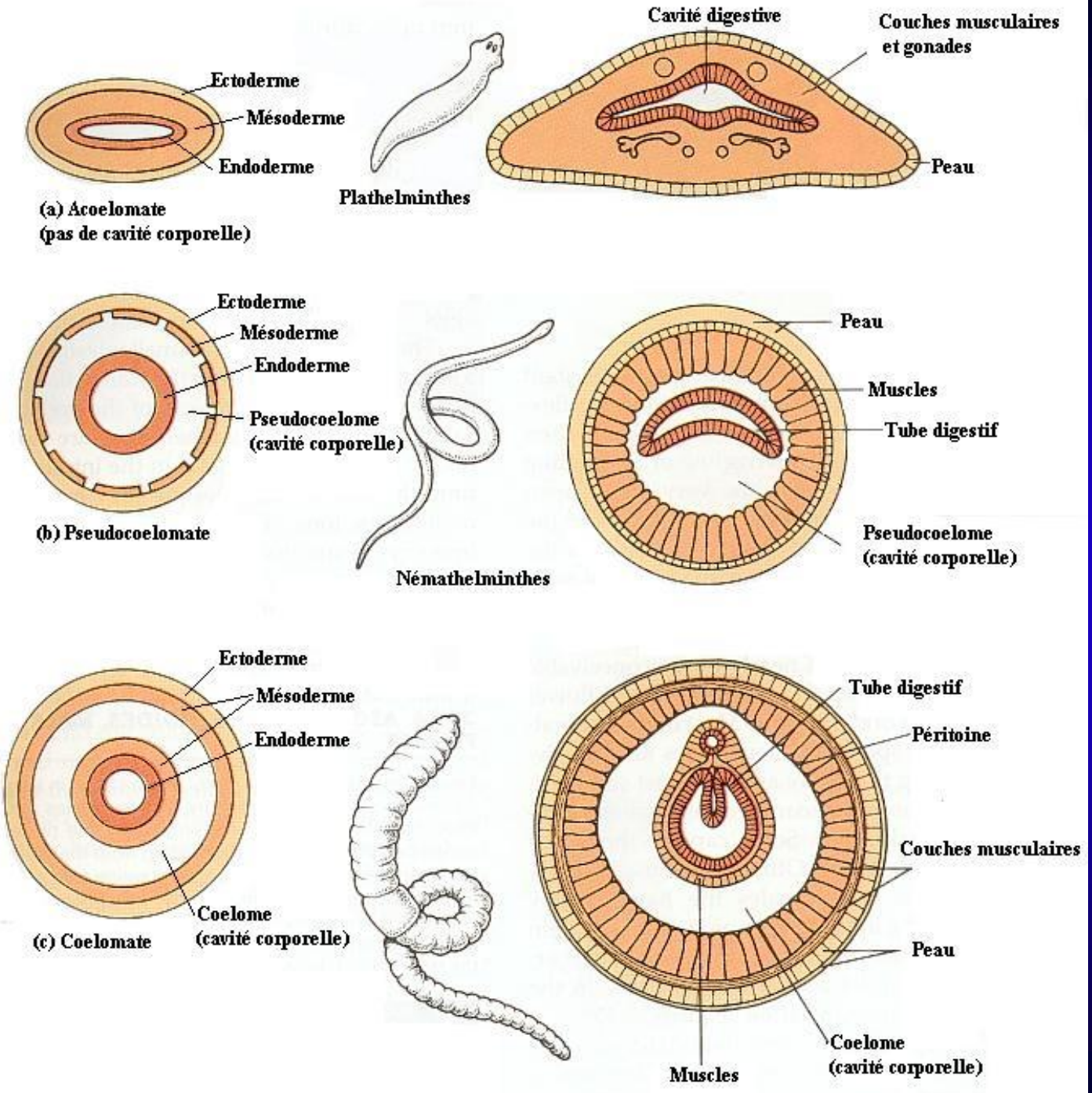
General Introduction

Systematic position of NEMATODA



Pseudocoelomates

Phylum:- Rotifera
Gastrotricha
Kinorhyncha
Loricifera
Priapulida
Nematoda
Nematormorpha
Acanthocephala
Entoprocta



Acoelomate, Pseudocoelomate & Eucoelomate body plan

Pseudocoelomates

(common characteristics)

All have:-

- *a body wall of epidermis*
- *a dermis*
- *muscles surrounding the pseudocoel*
- *complete digestive tract*
- *an epidermis may secrete a non-living cuticle with specialisations e.g. bristles, spines etc.*

Phylum:- NEMATODA

General characteristics:-

- cylindrical shape
- flexible, non-living cuticle
- lack of motile cells
- muscles run longitudinally only (4 bands)
- psuedocoel as hydrostatic organ highly developed
- generally small (<5cm)

General facts and figures:-

1. **7 out of 10** multicellular animals are nematodes
2. They currently number over **40,000** species
3. An acre of good farm soil contains several **100 million to billion** terrestrial nematodes...
4. …. Of which **30%** are likely to be plant feeders
5. Nematodes cause, worldwide, about **10%** loss of agricultural crops
6. They are **small** (average 1mm) and **transparent**
7. All are **aquatic** for part of their life-cycle

General facts and figures:-

8. They exist in all types of habitats
   (i.e. temperatures [below freezing - 50 C], wide range of pH)

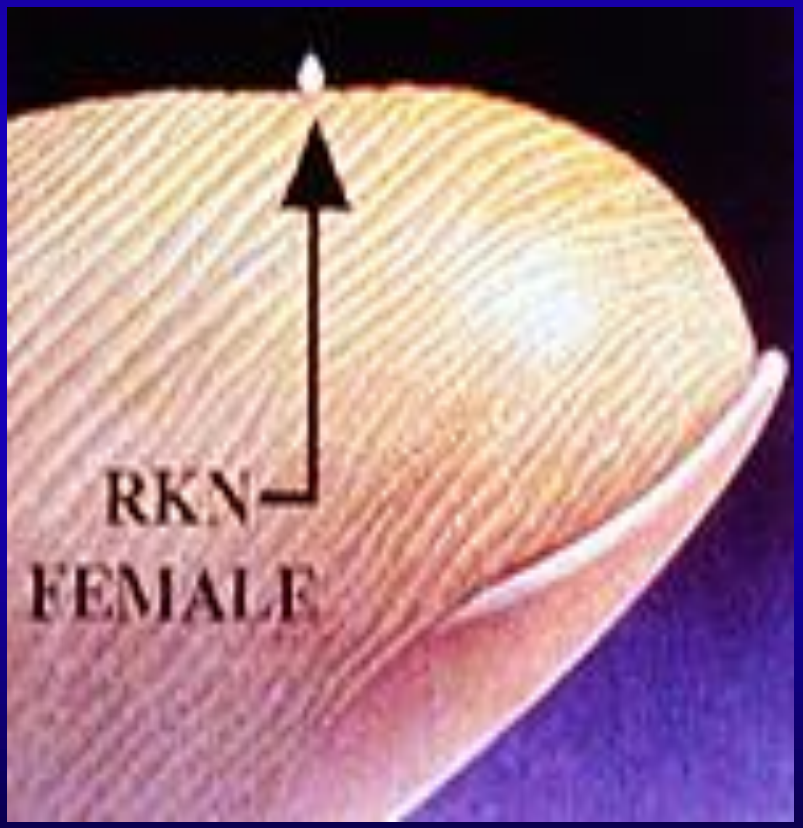
9. No overlap of fresh-water and marine faunas

10. Nematode evolution
   Marine ***Terrestrial*** ***Freshwater***

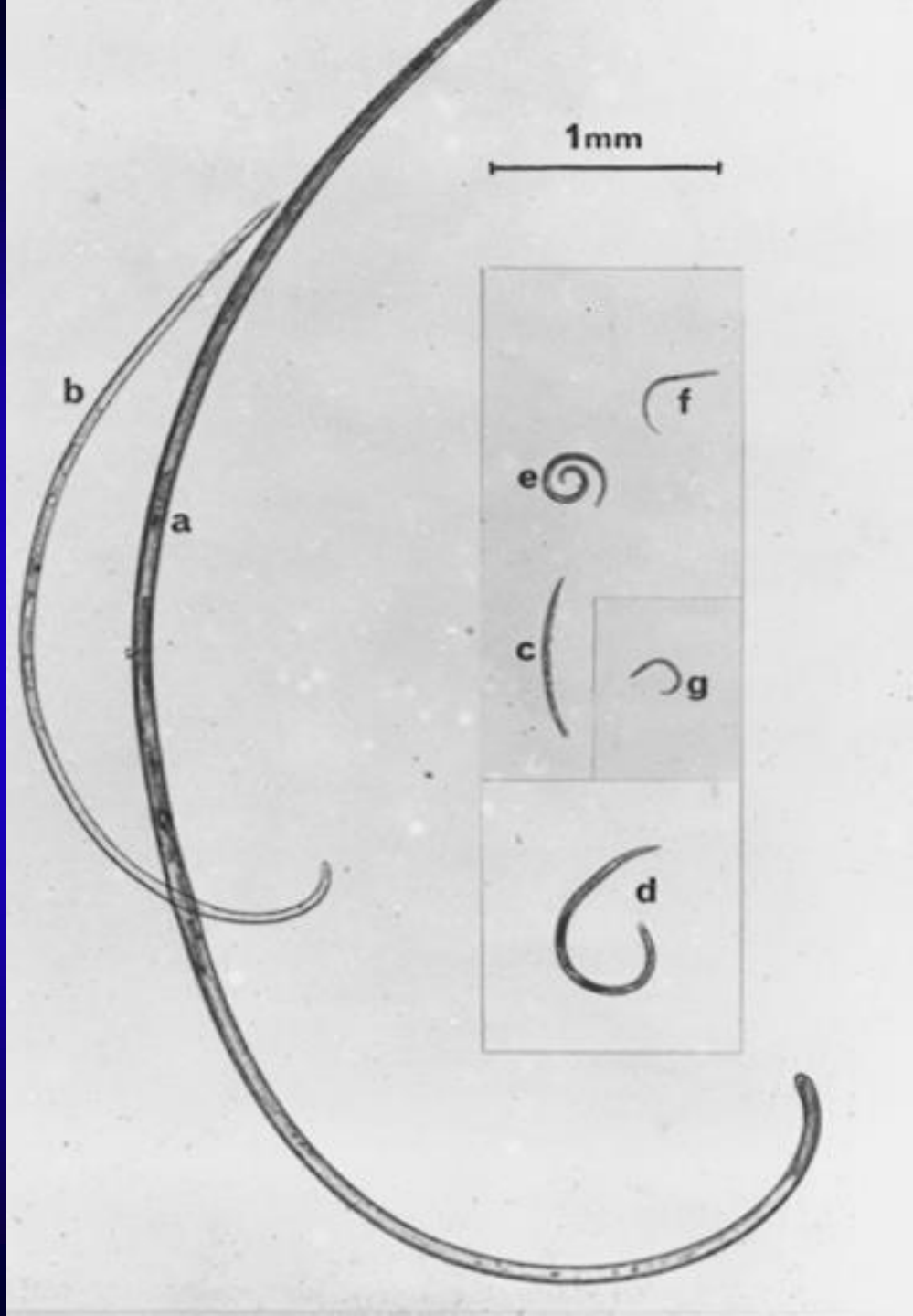
They range in size (from 7.8 m, *Placentonema gigantissima*, whale parasite to 112µ, *Sahaeronema minutissimum*, in citrus plants)

Isolated specimens of *Placentonema gigantissima* (left) and *Ascari lumbricoides* (right) (source unknown)

Most plant nematodes are small (up to 1 mm in length) and transparent (source unknown)



Size range of plant parasitic nematodes



Types (trophic groups) of nematodes

GROUP	Role in soil, crop & animal health
• Animal parasites ..	**Many diseases in animals & man**
• Plant parasites ….	**Primary parasite & secondary as vector, crop yield losses 5-20%**
• Bacterial feeders ..	**Contribute to nutrient cycling in soil**
• Fungal feeders …	**Nutrient cycling, damage in mushroom crops**
• Predators ……	**Feed on plant-parasitic nematodes (role in bio-control)**
• Insect parasites …	**Bio-control of some insects**

Variety of form in nematodes

a. Tetrameres

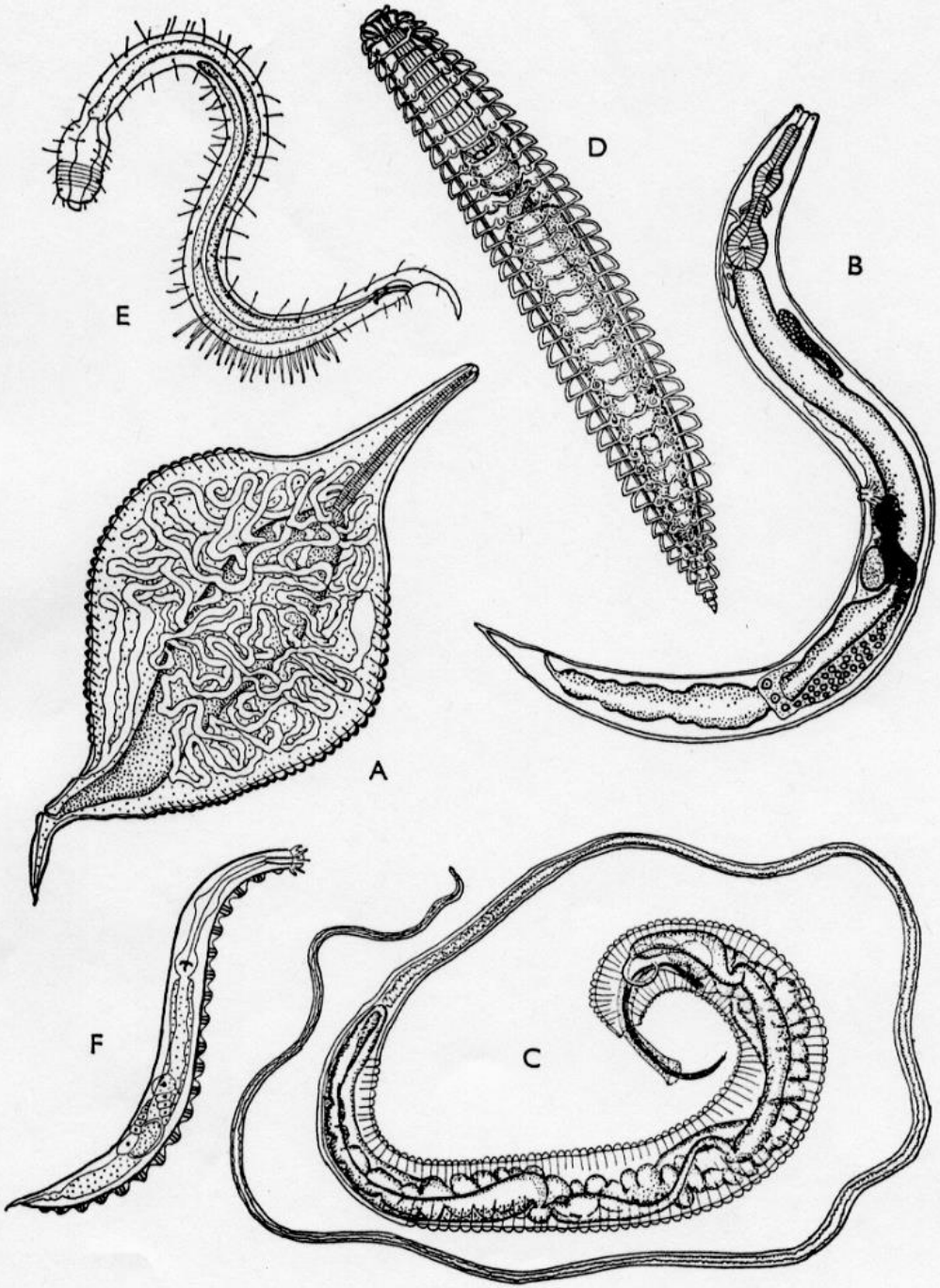
b. Rhabditis

c. Trichuris

d. Criconema

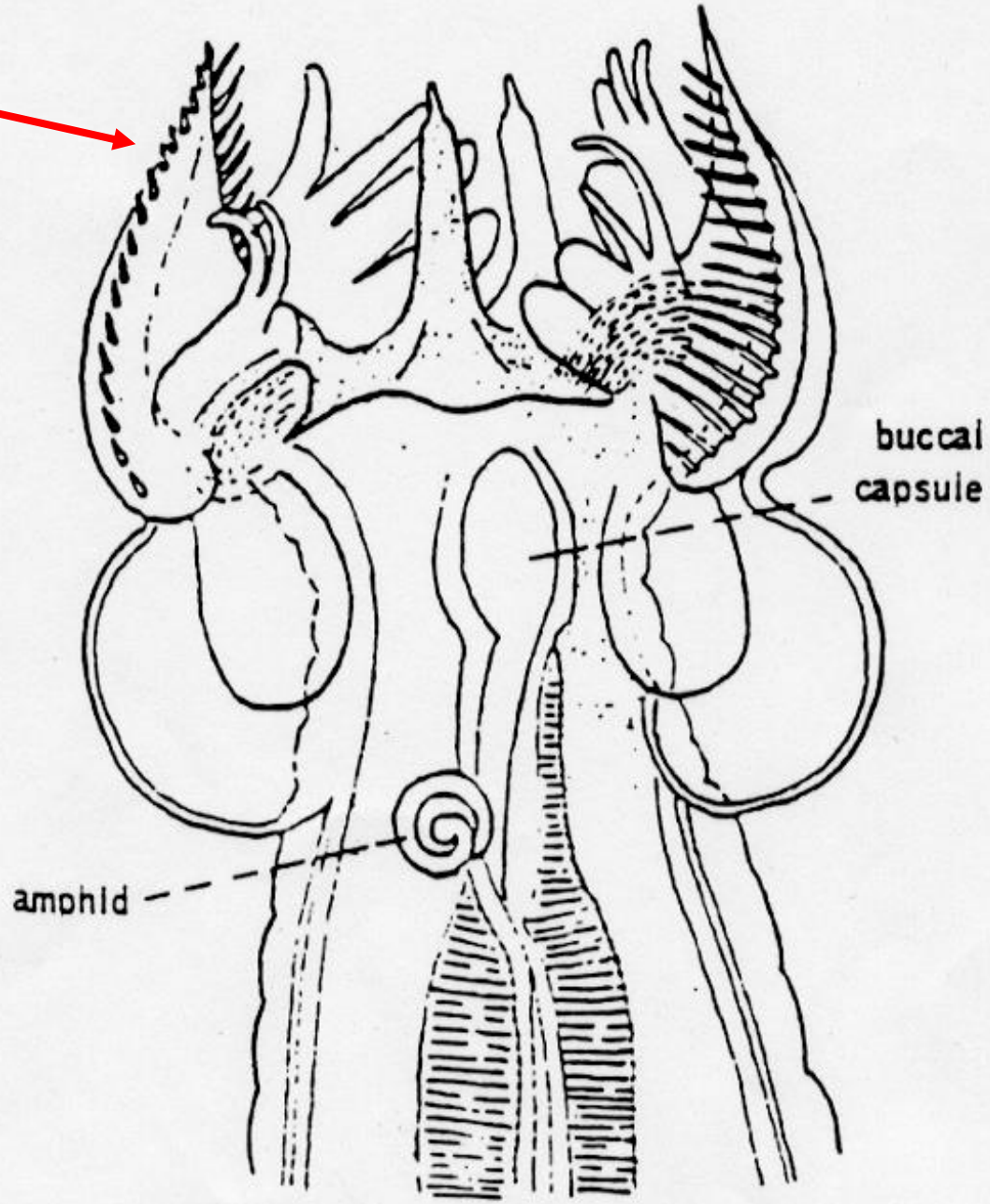
e. Chaetosoma

f. Bunonema



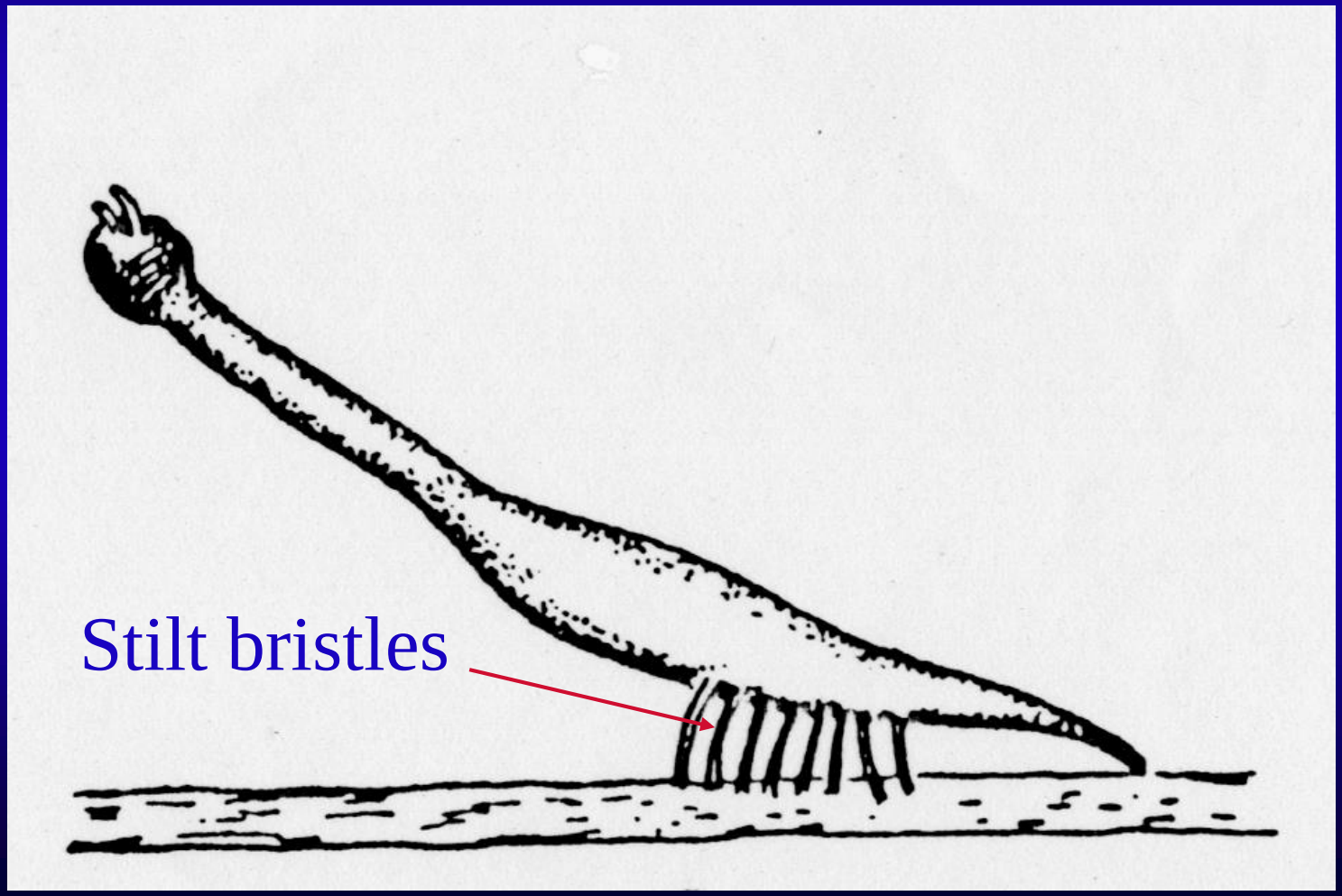
Cuticular projections of head region

[*Wilsonema* with ornate projections on lips]

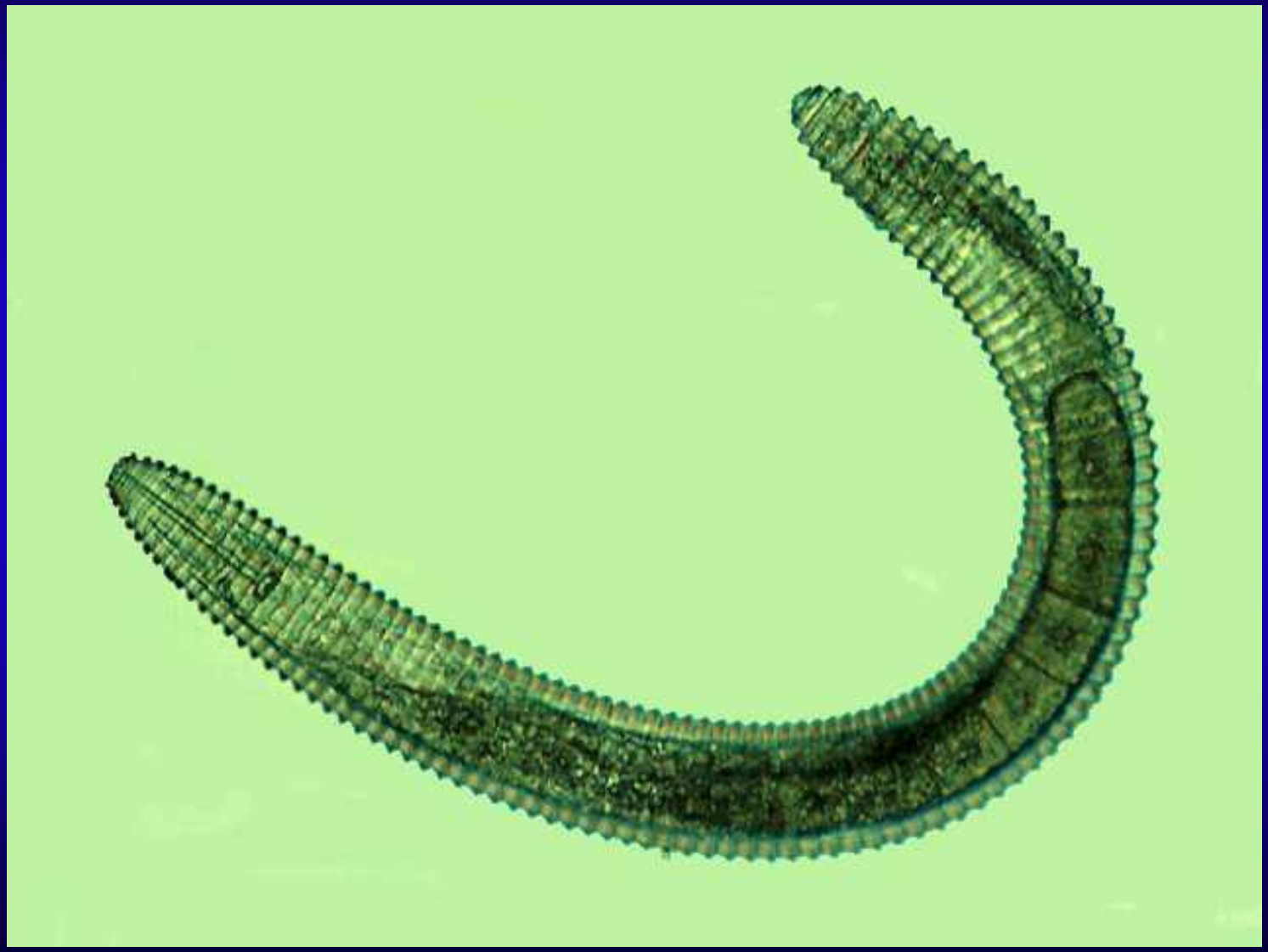


Cuticular projections - stilt bristles

[*Draconematidae*, marine nematode]

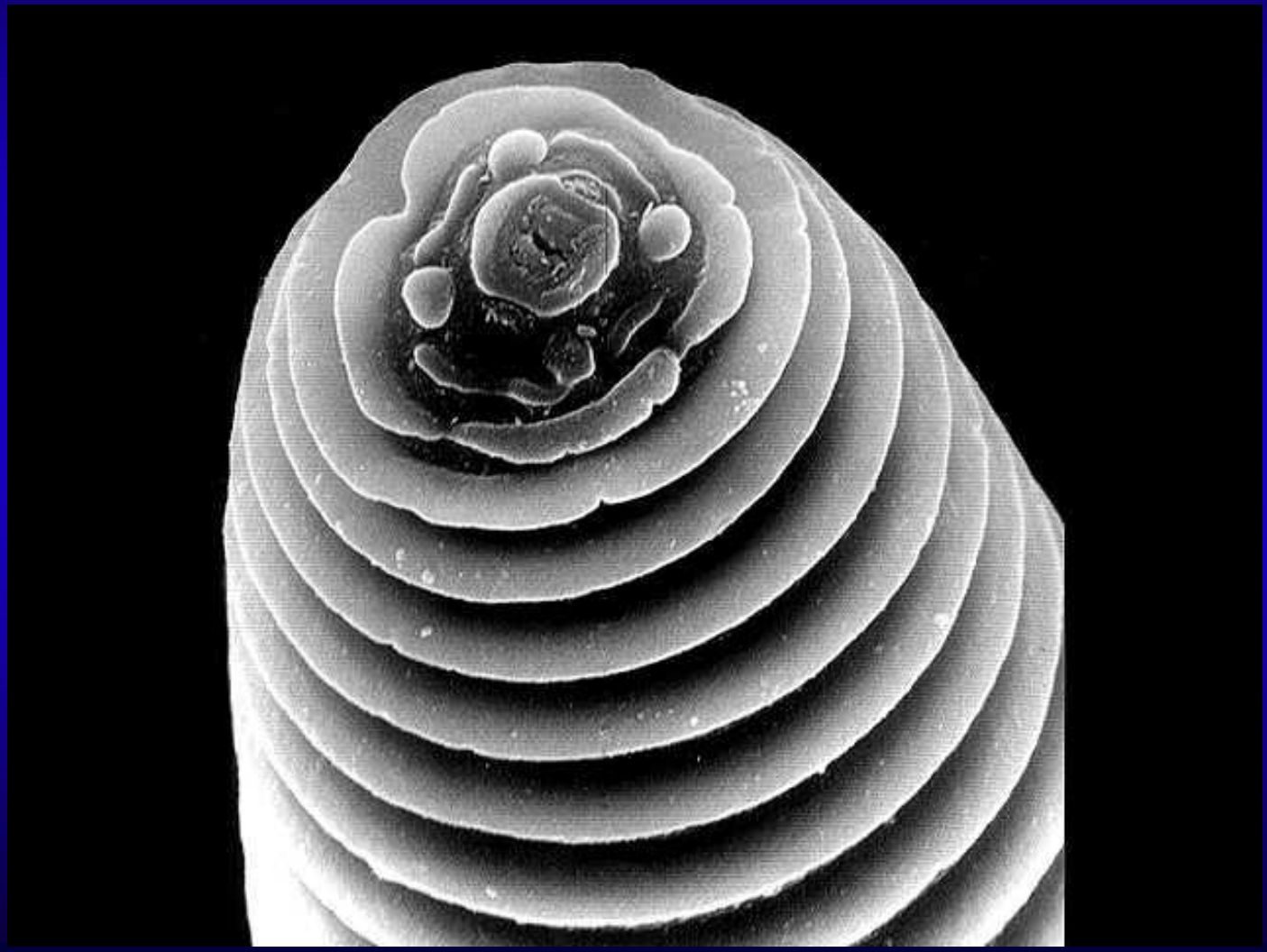


Criconemoides (source unknown)

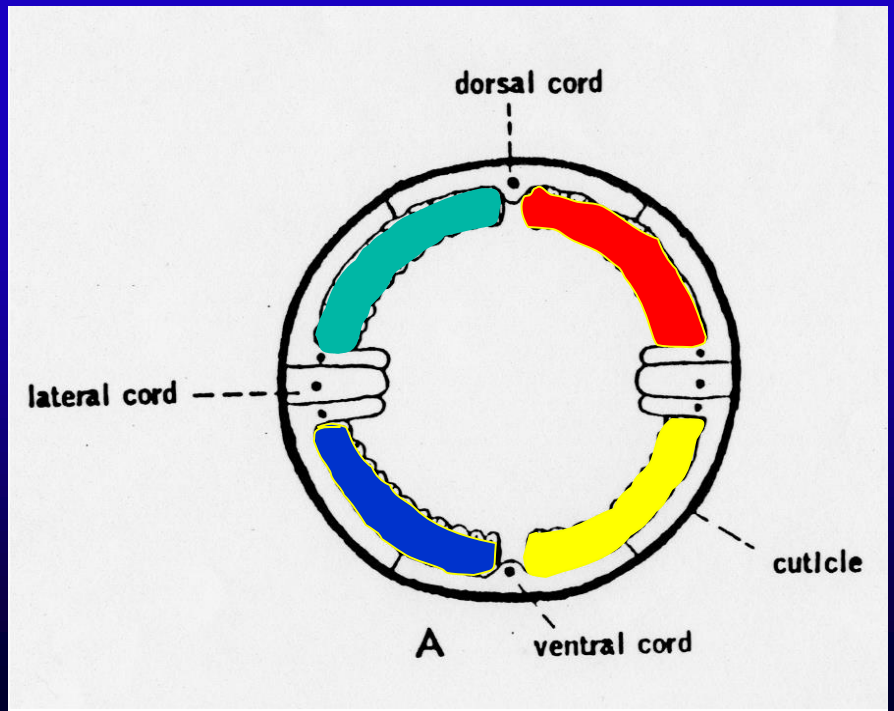


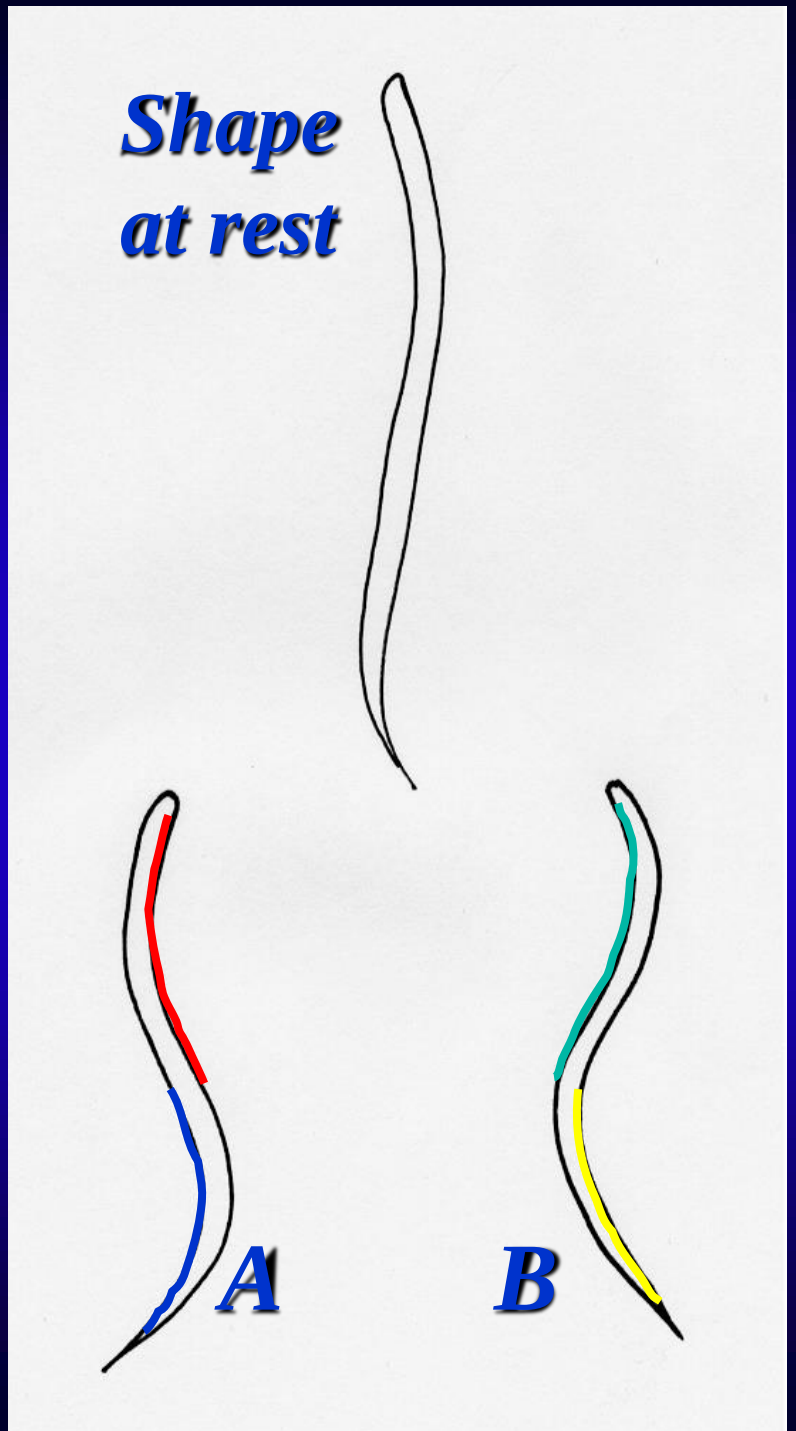
Criconemoides (head region)

(source unknown)



Nematode movement

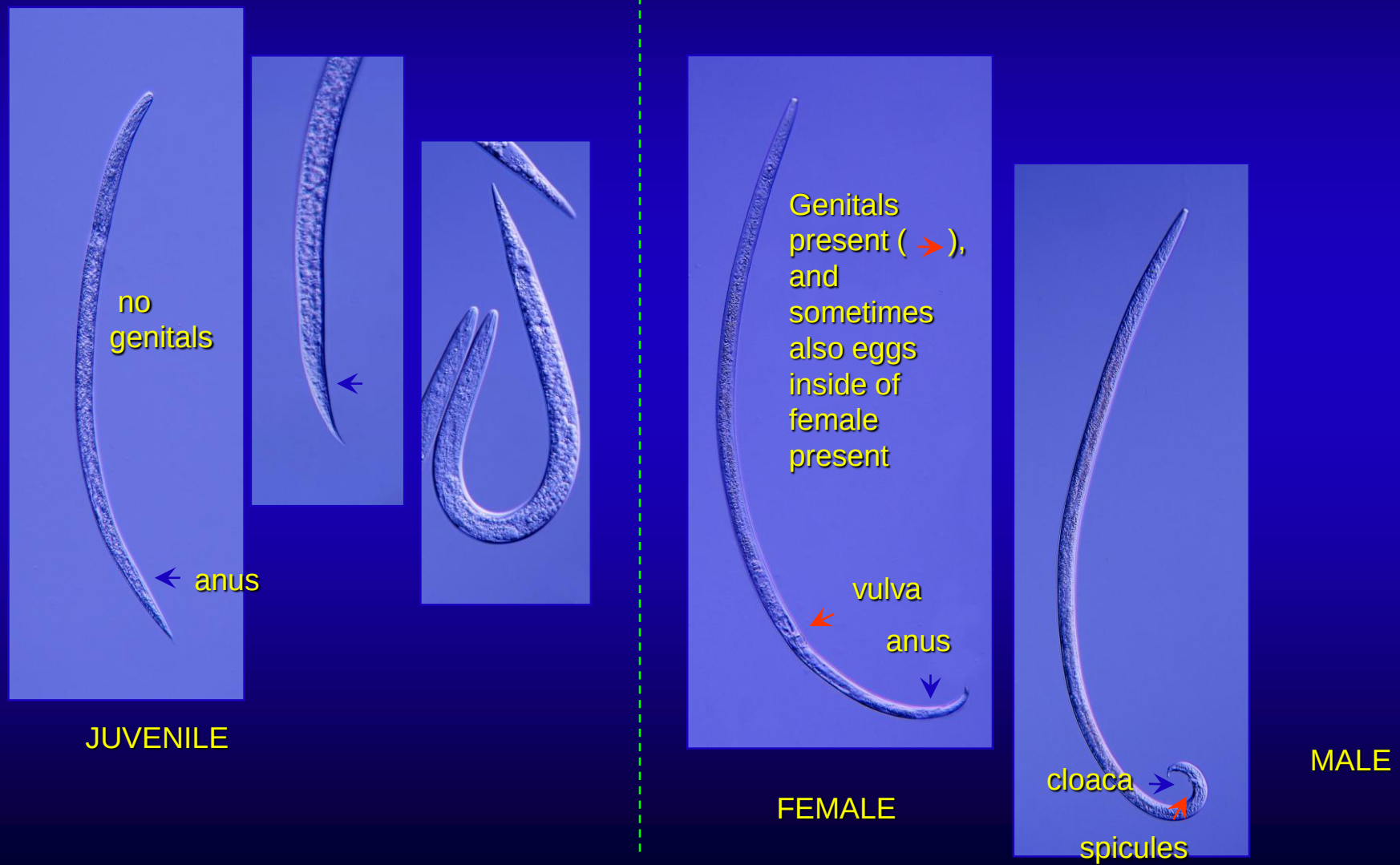


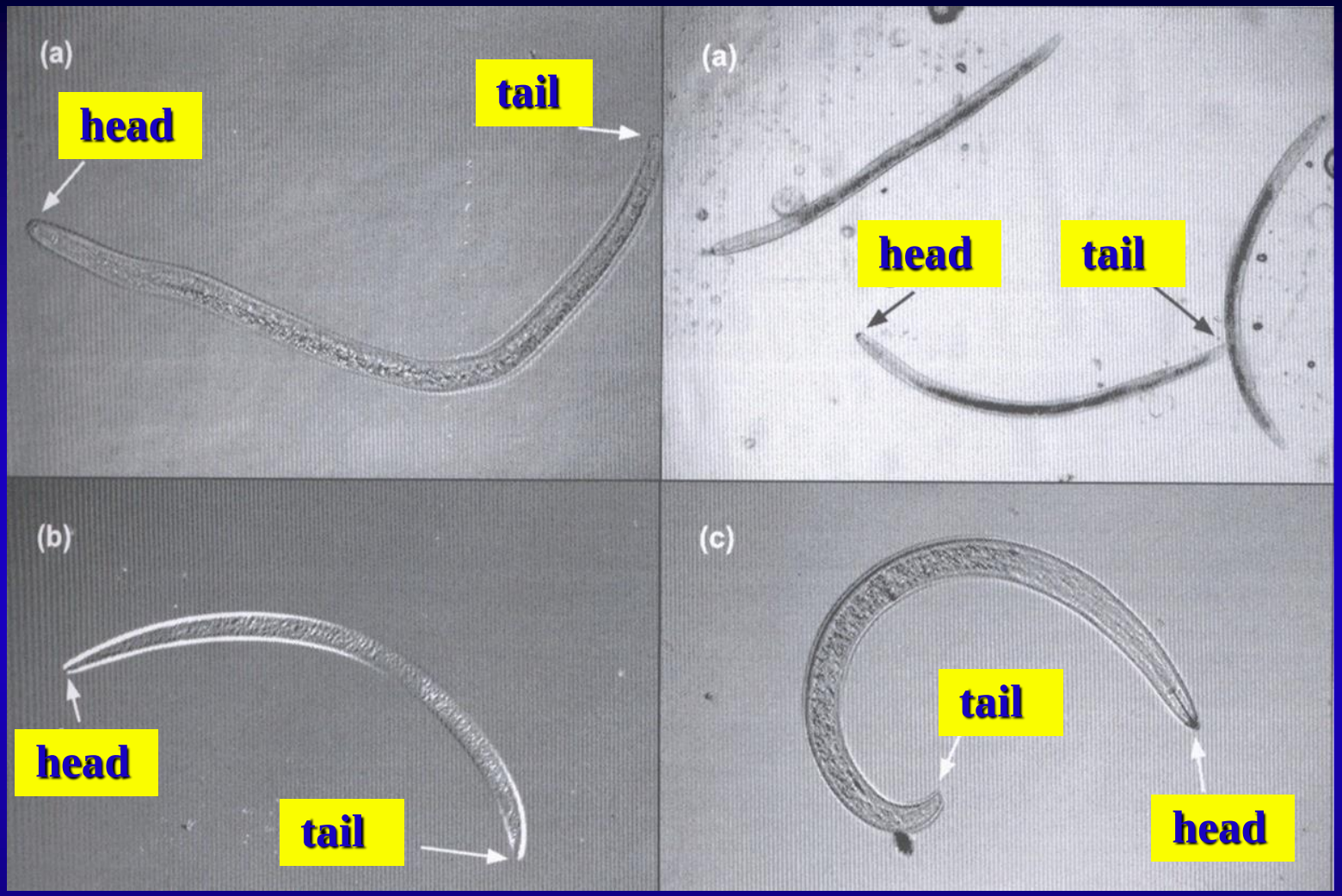


Distinguishung juveniles from adult nematodes

(morphological identification is possoble on adults, only, mainly females

(slide prepared by prof. Marek Tomalak, Institute of Plant Protection – Governmental Research Institute, Poznań, Poland

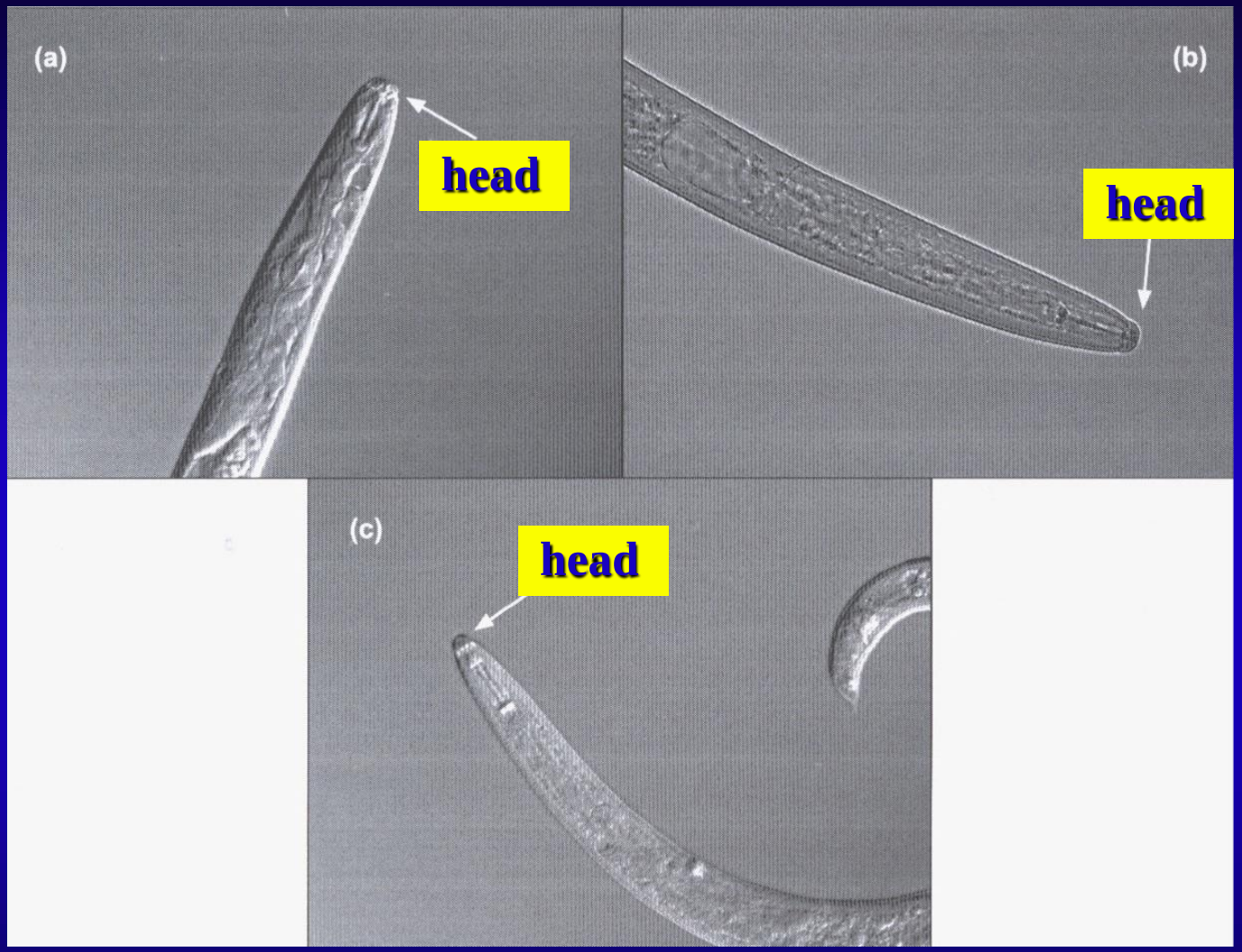




Plant-parasitic nematode juveniles (Tylenchida)

(a) Pratylenchus, (b) *Belonolaimidae*, (c) *Hoplolaimidae*

(phot. Renata Dobosz, Institute of Plant Protection, Poznań, Poland)



Head of plant-parasitic nematode juveniles (Tylenchida)

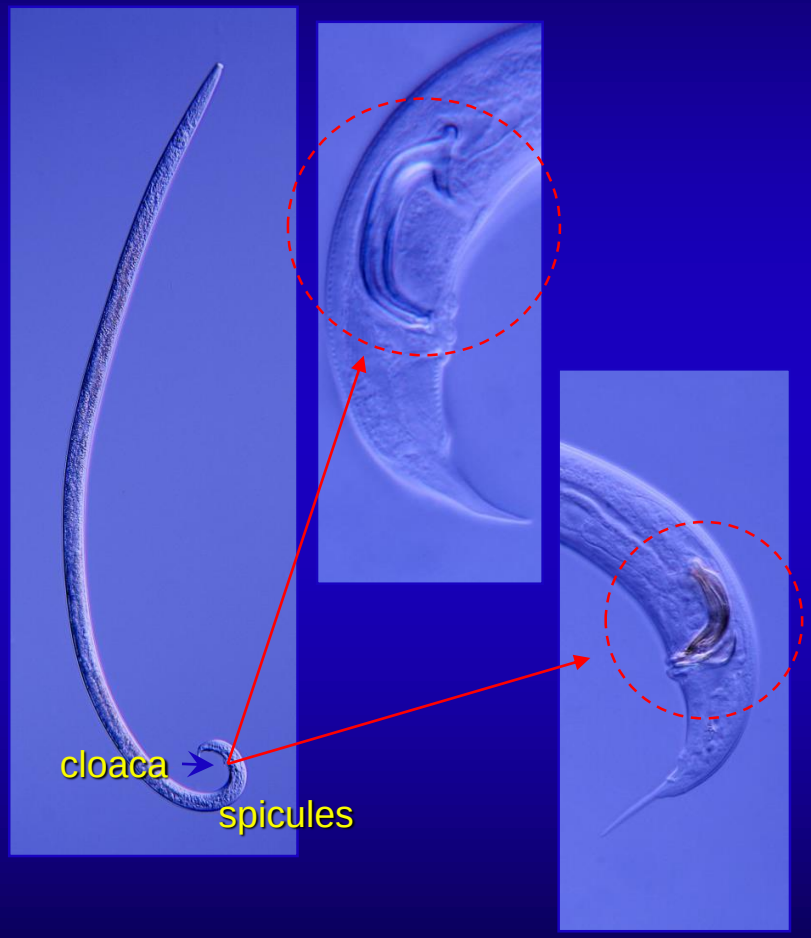
(a) Pratylenchus, (b) *Belonolaimidae*, (c) *Hoplolaimidae*

(phot. Renata Dobosz, Institute of Plant Protection, Poznań, Poland)

Distinguishung females from males

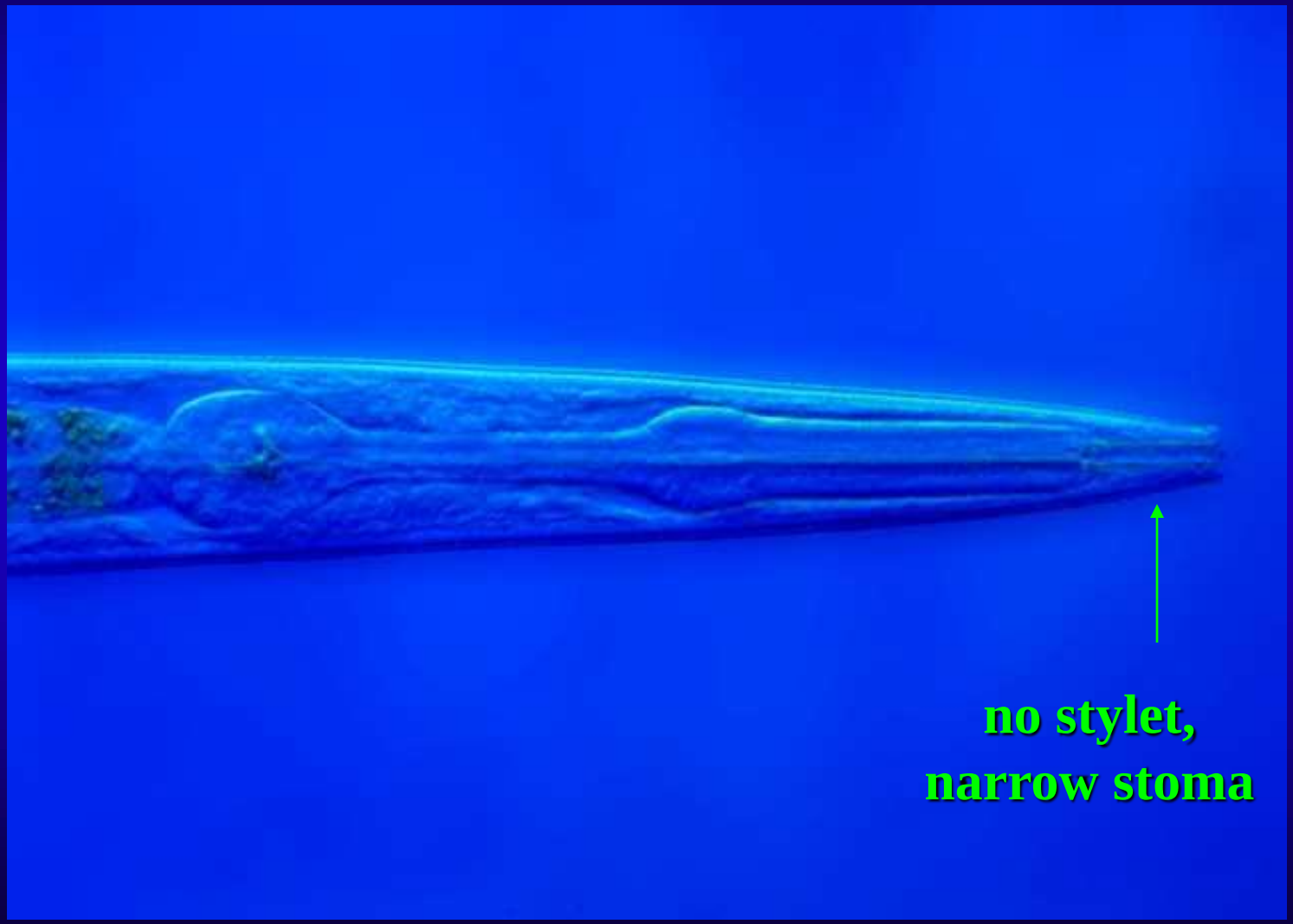
slide prepared by prof. Marek Tomalak, Institute of Plant Protection – Governmental Research Institute, Poznań, Poland

FEMALE– vulva is present



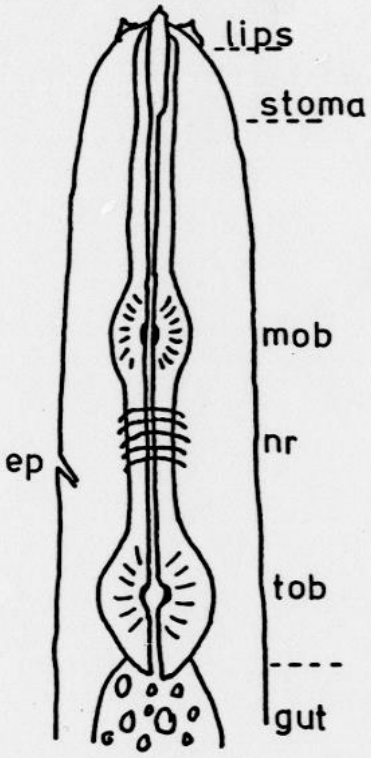
MALE – spicules are present

Bacteria feeder *(Rhabditida)* (source unknown)



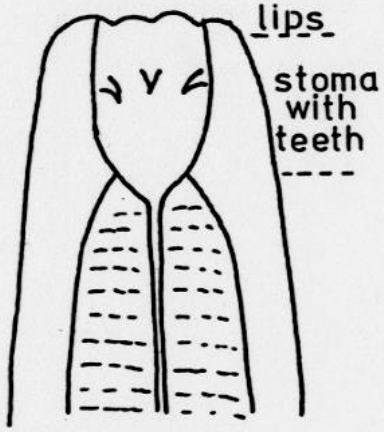
Oesophageal regions

MICROBIVEROUS



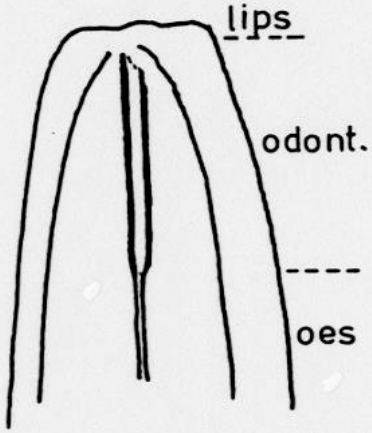
e.g. Rhabditis

PREDACEOUS



e.g. Mononchus

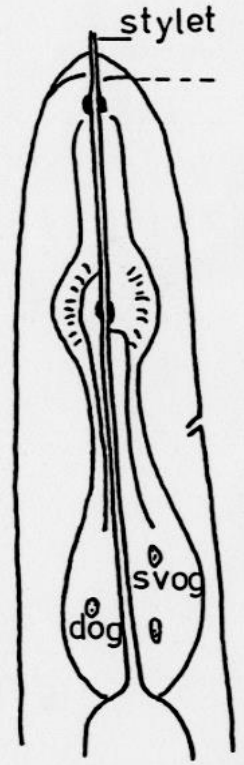
HERBIVOROUS



DORYLAIMIDA

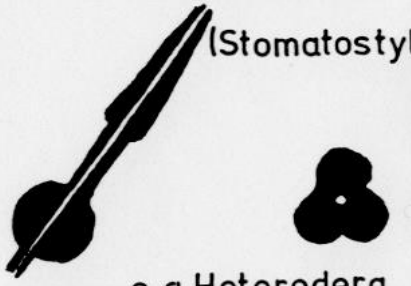
(Odontostylet)

e.g. Longidorus



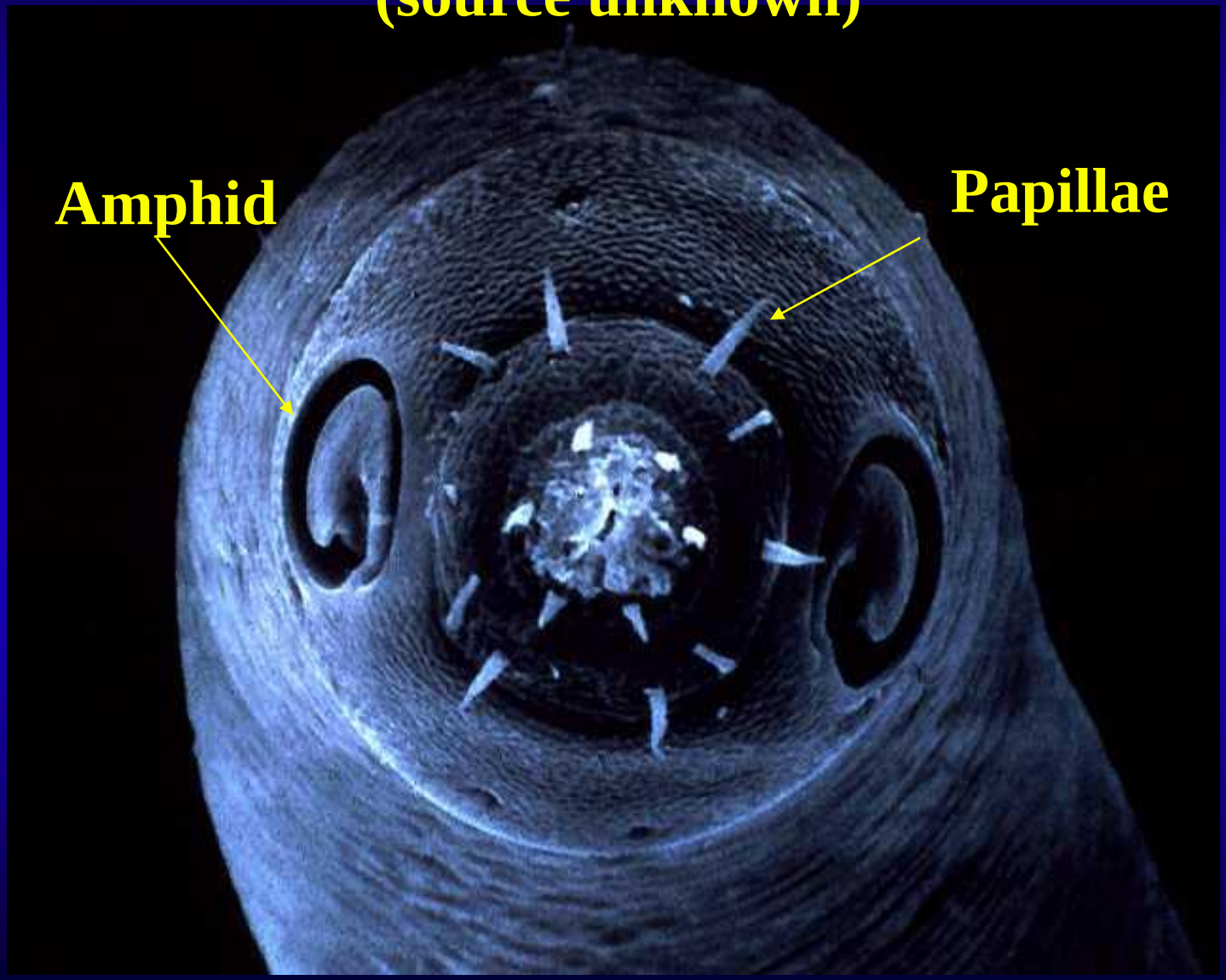
TYLENCHIDA

(Stomatostylet)

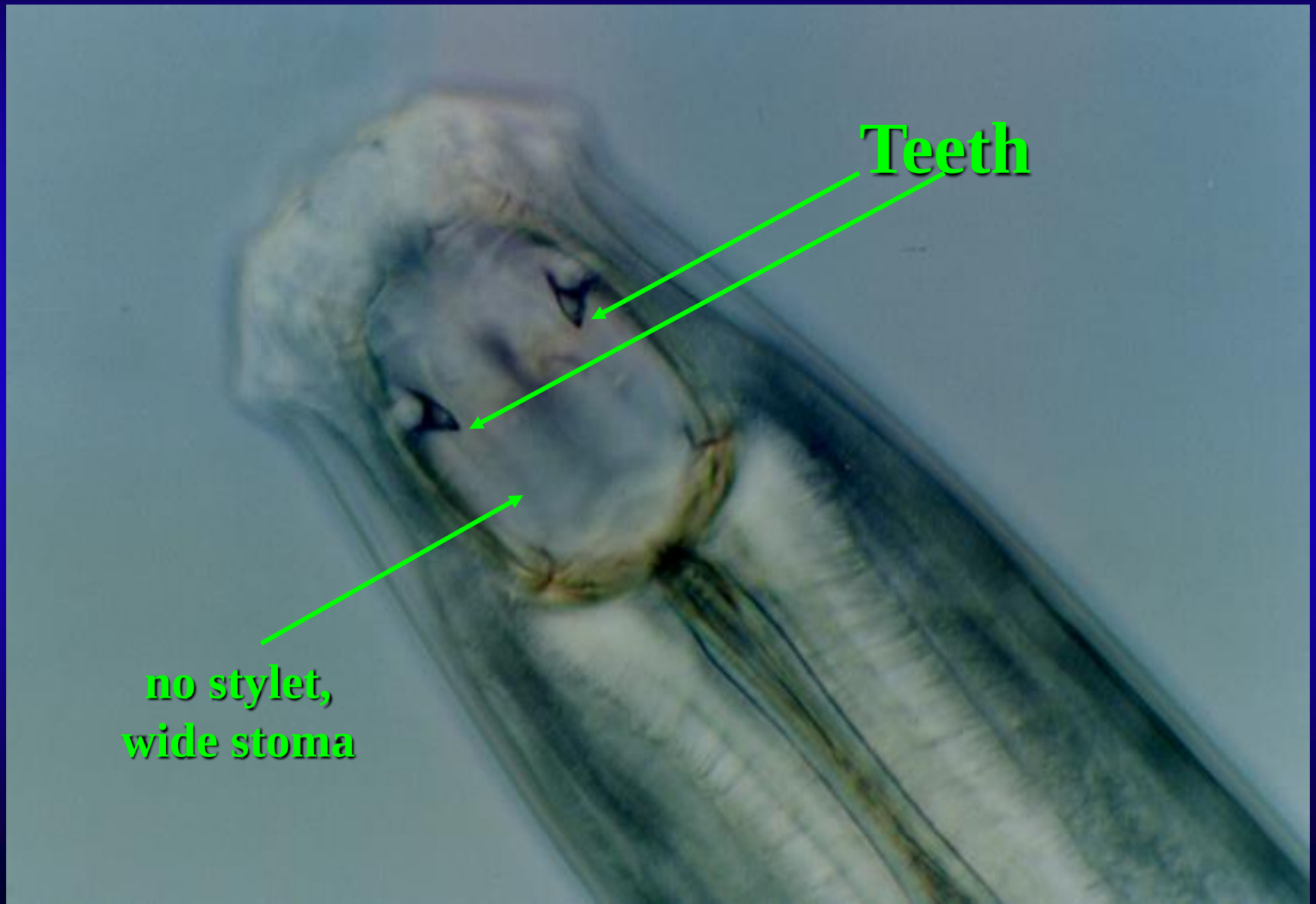


e.g. Heterodera

Amphids & Papillae *(Desmodora pilosa)* **(source unknown)**



Predaceous nematode *(Mononchus)* (source unknown)

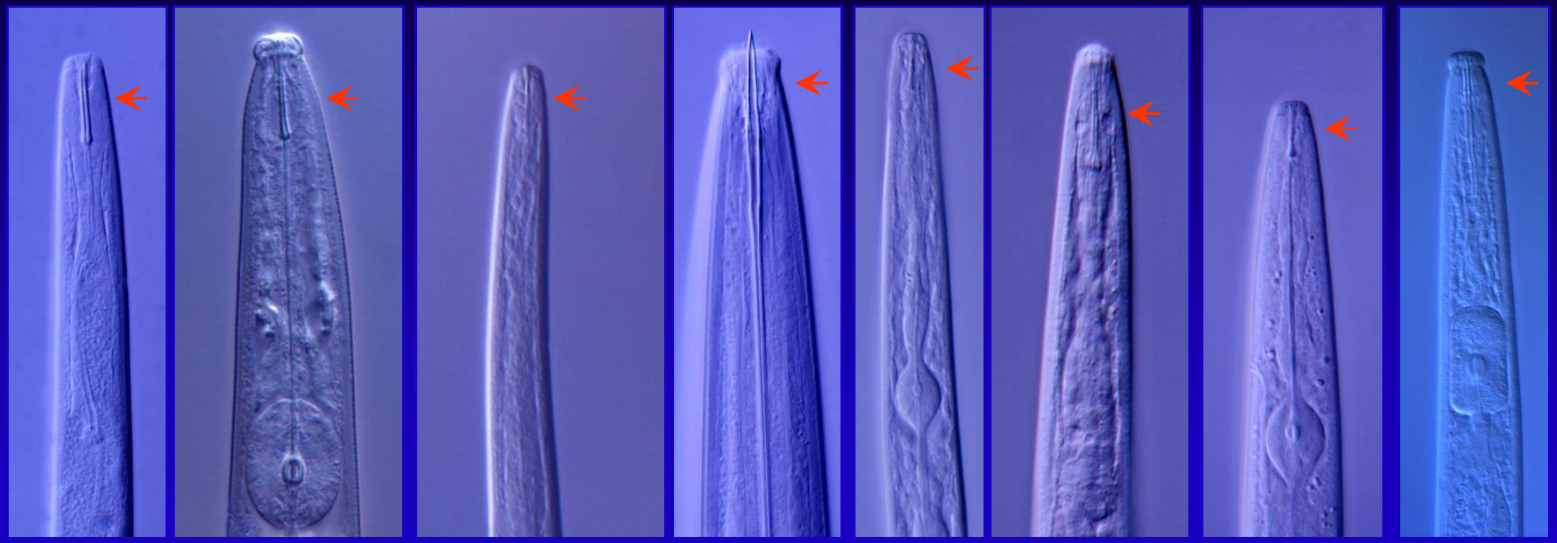


Predaceous nematode *(Mylenchulus)* (source unknown)

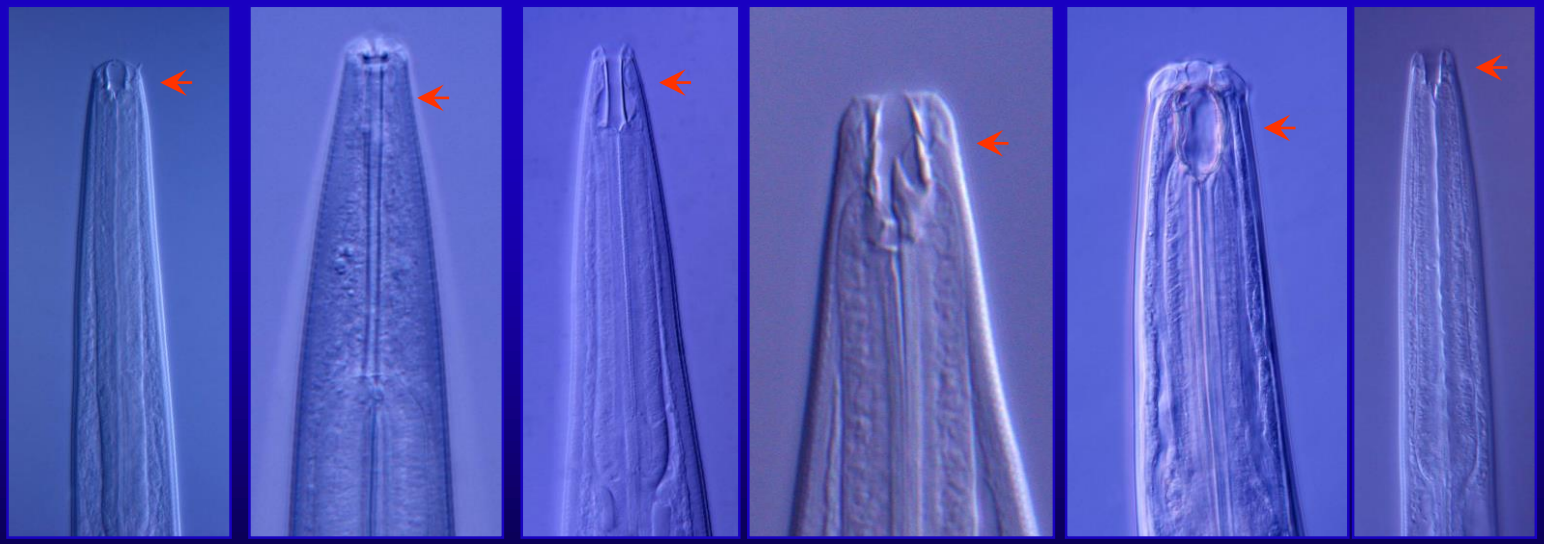
Mononchus feeding on other nematode (source unknown)

Scheme of oesophageal regions

Slide prepared by prof. Marek Tomalak, Institute of Plant Protection – Governmental Research Institute, Poznań, Poland



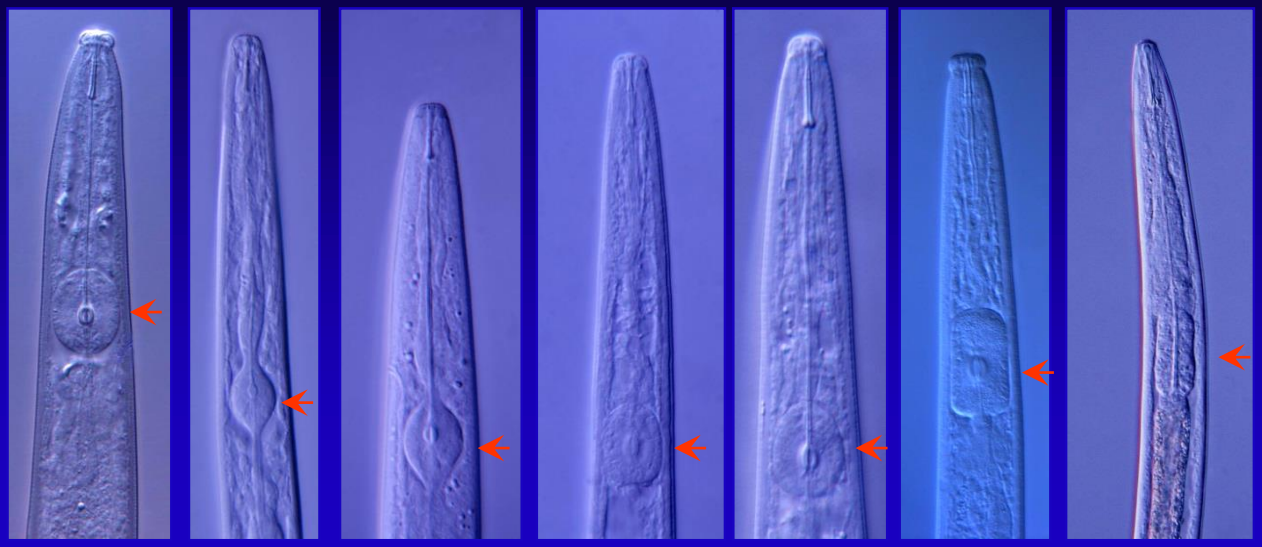
Stylet present



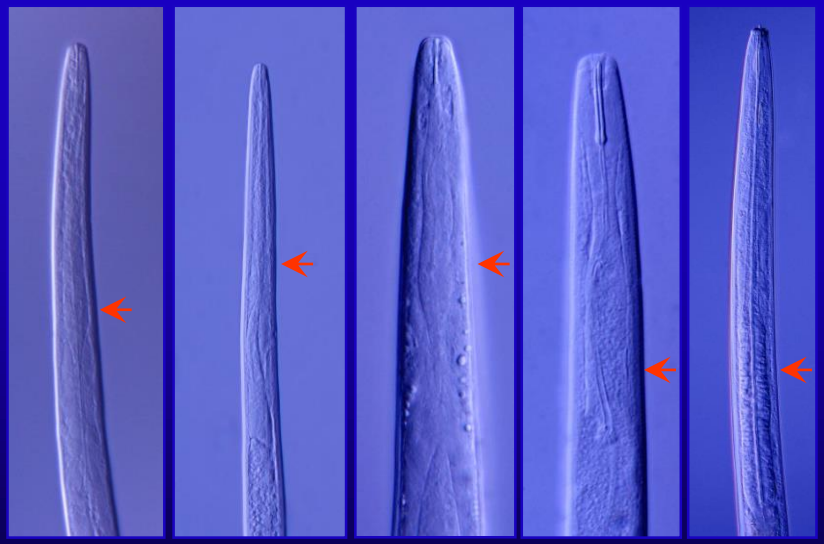
Stylet absent

Scheme of oesophageal region – stylet present

Slide prepared by prof. Marek Tomalak, Institute of Plant Protection – Governmental Research Institute, Poznań, Poland



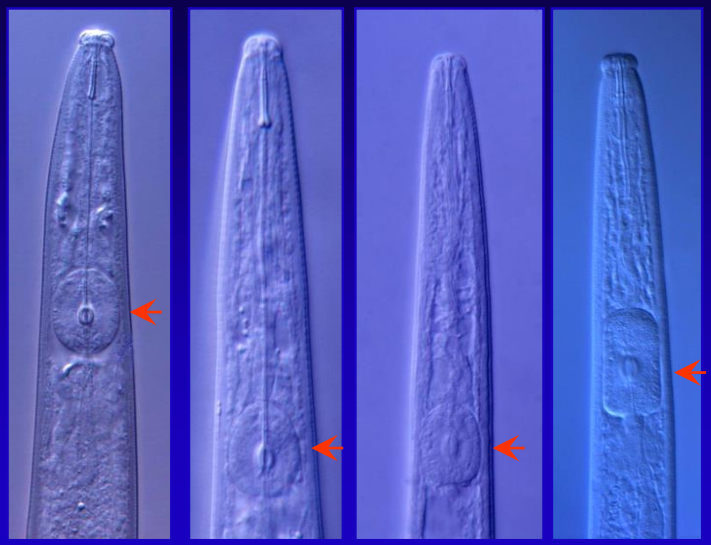
Median bulb present



Median bulb absent

Median bulb present – stylet present

Slide prepared by prof. Marek Tomalak, Institute of Plant Protection – Governmental Research Institute, Poznań, Poland

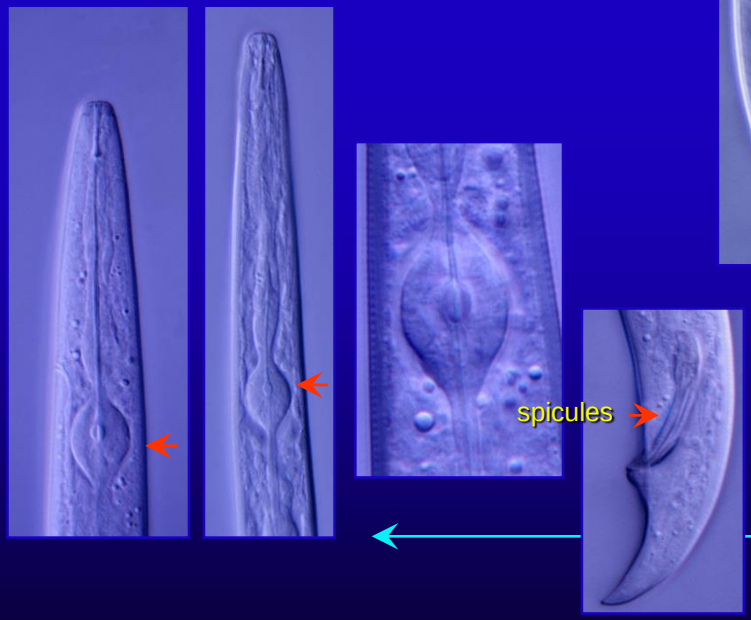


Median bulb prominent, egg-shaped or rectangular with rounded angles, distinctly offset from the rest of pharynx with distinct central valves
Spicules aphelenchidal

Aphelenchida

Median bulb elongated, egg-shaped, without central valves
Spicules dorylaimoidal

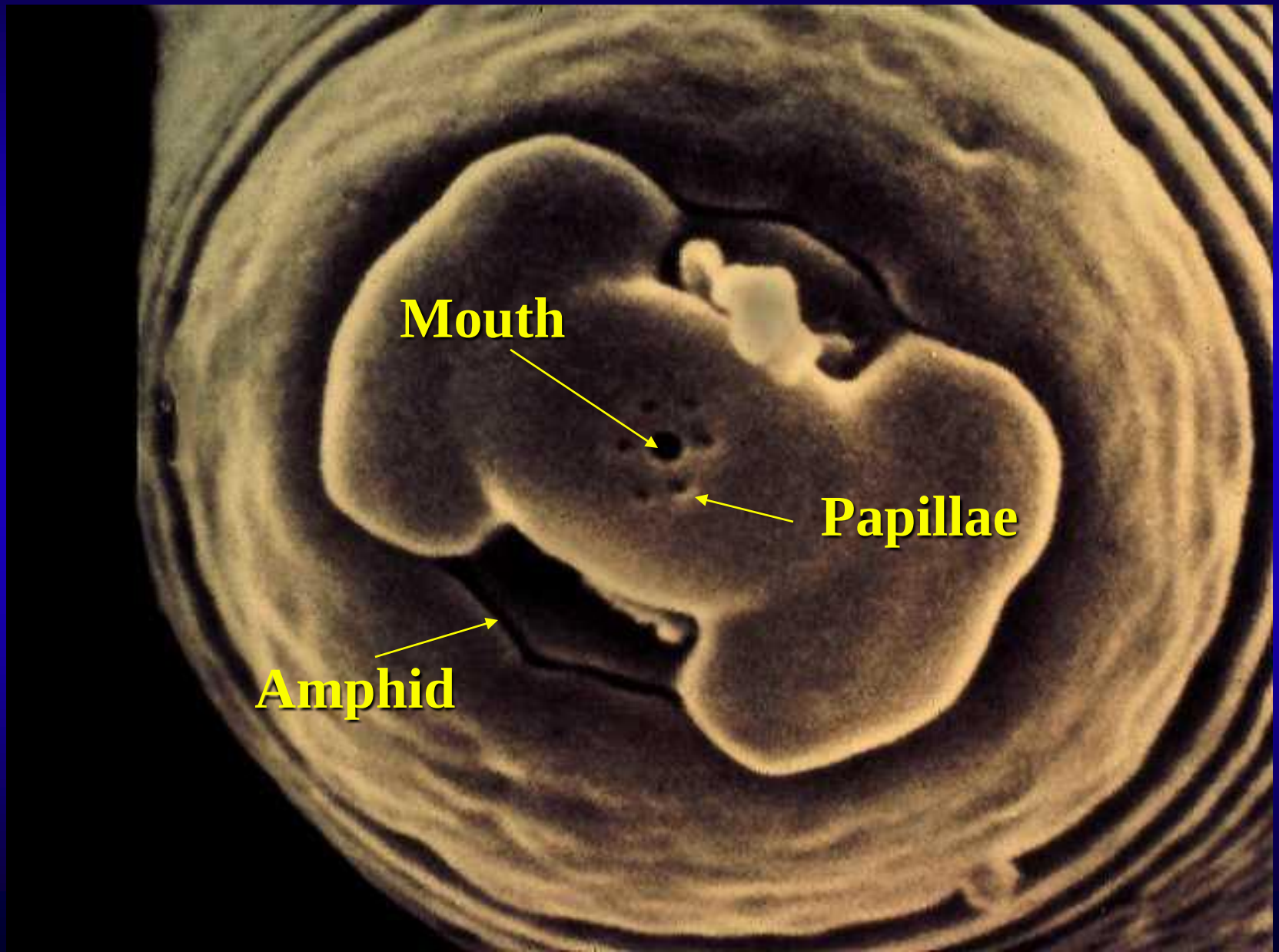
Dorylaimida



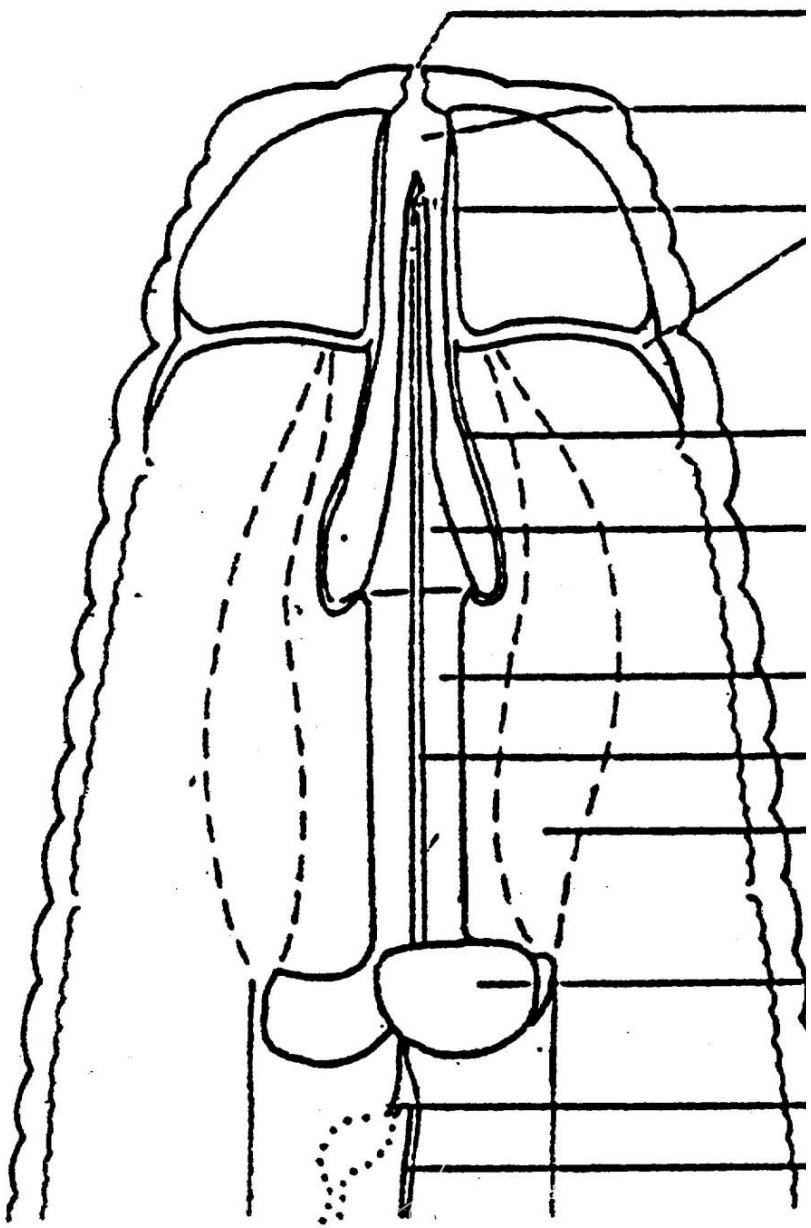
Median bulb oval, elliptic, frequently but not always with central valves, continuous with anterior and posterior parts of pharynx. Spicules tylenchoidal

Tylenchida

Amphids & papillae *(Meloidogyne hapla)* **(source unknown)**



Head structure of tylenchid nematode



oral opening (mouth)

prestoma

cephalic framework

enlargement of cephalic framework

stylet cone

stylet shaft

stylet lumen

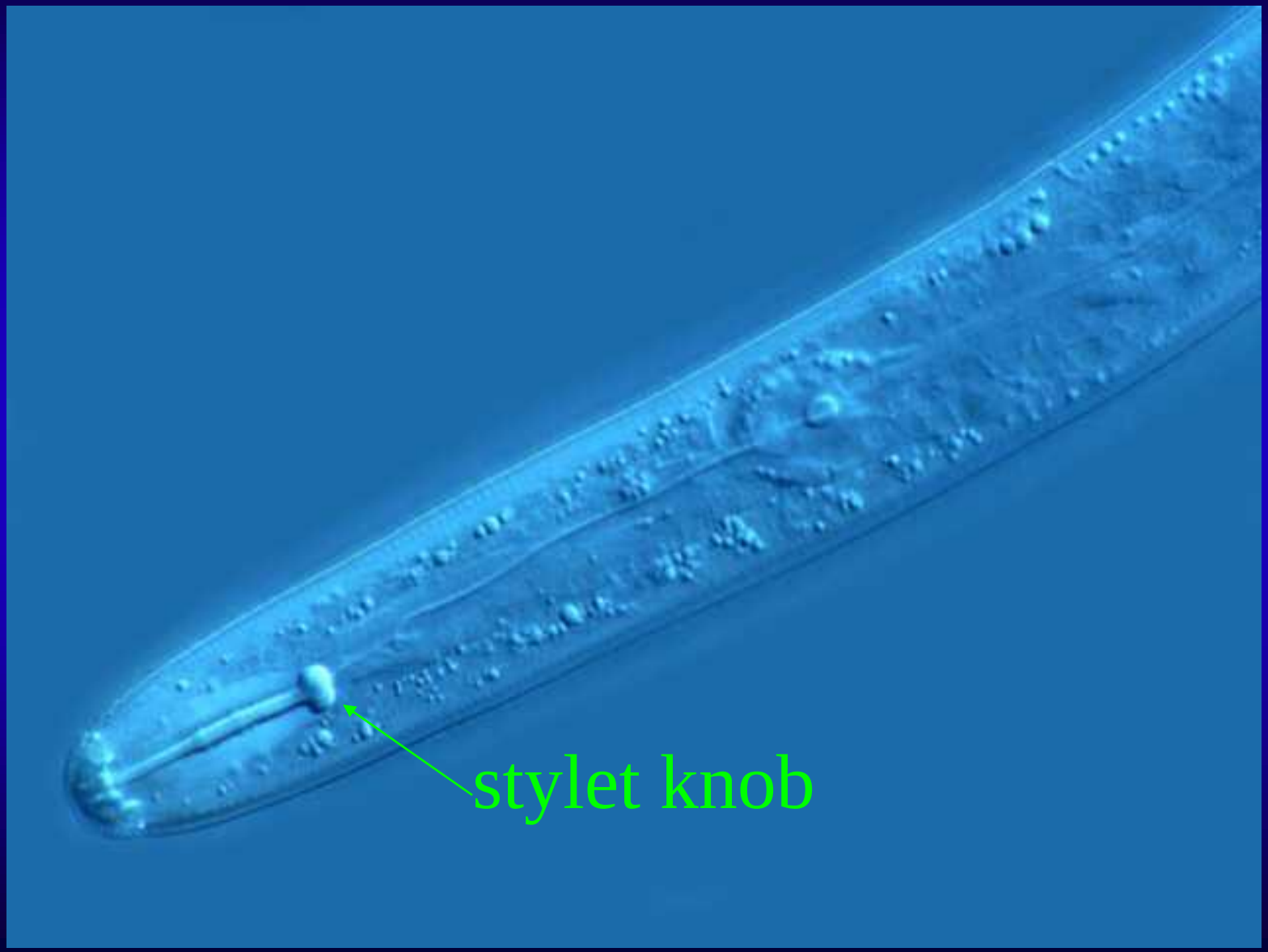
stylet protractor muscles

stylet knobs

dorsal gland orfice

pharynx lumen

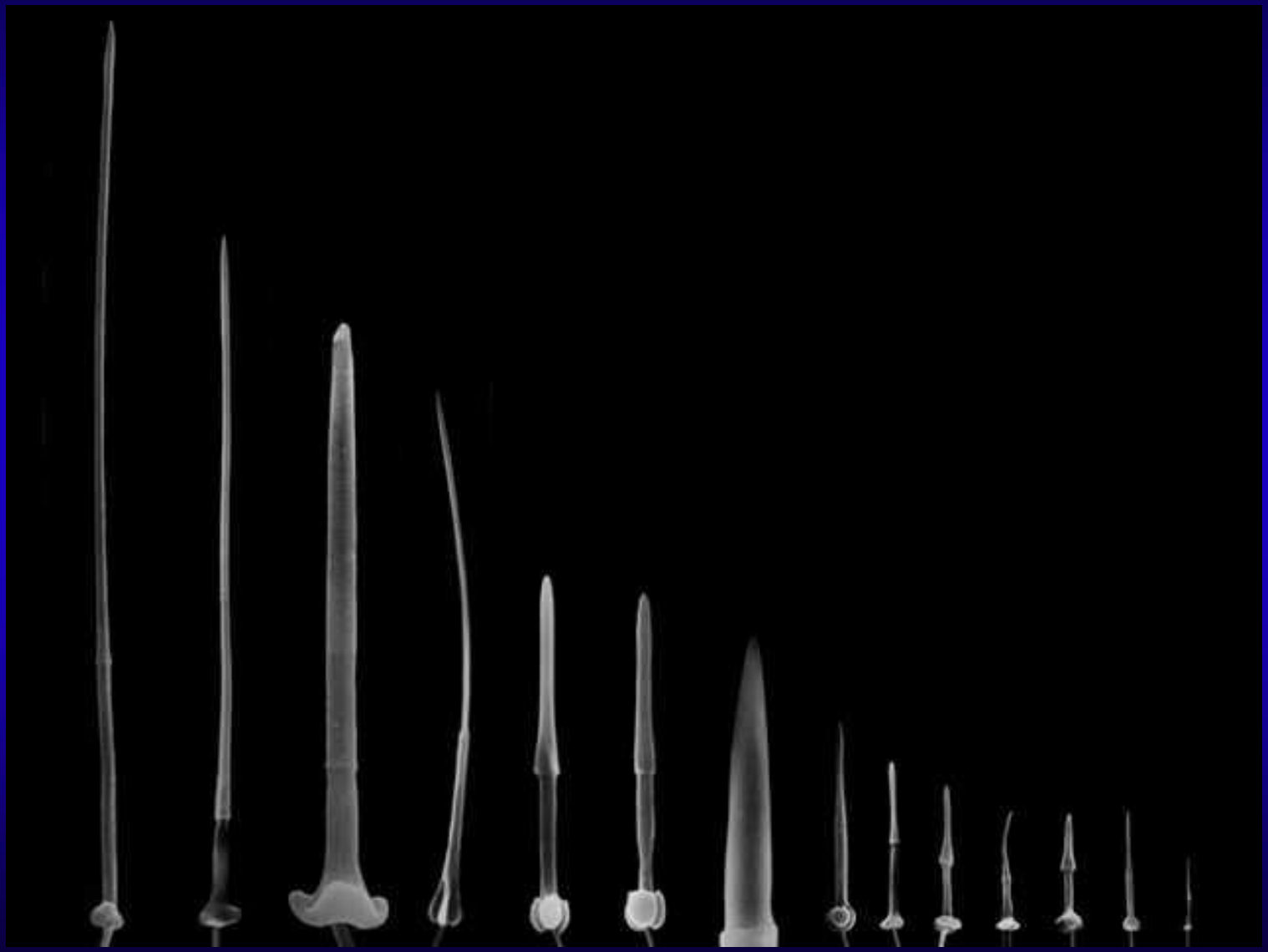
Herbivorous nematode *(Tylenchida)*
(source unknown)



Excised stylet of *Meloidogyne hapla*

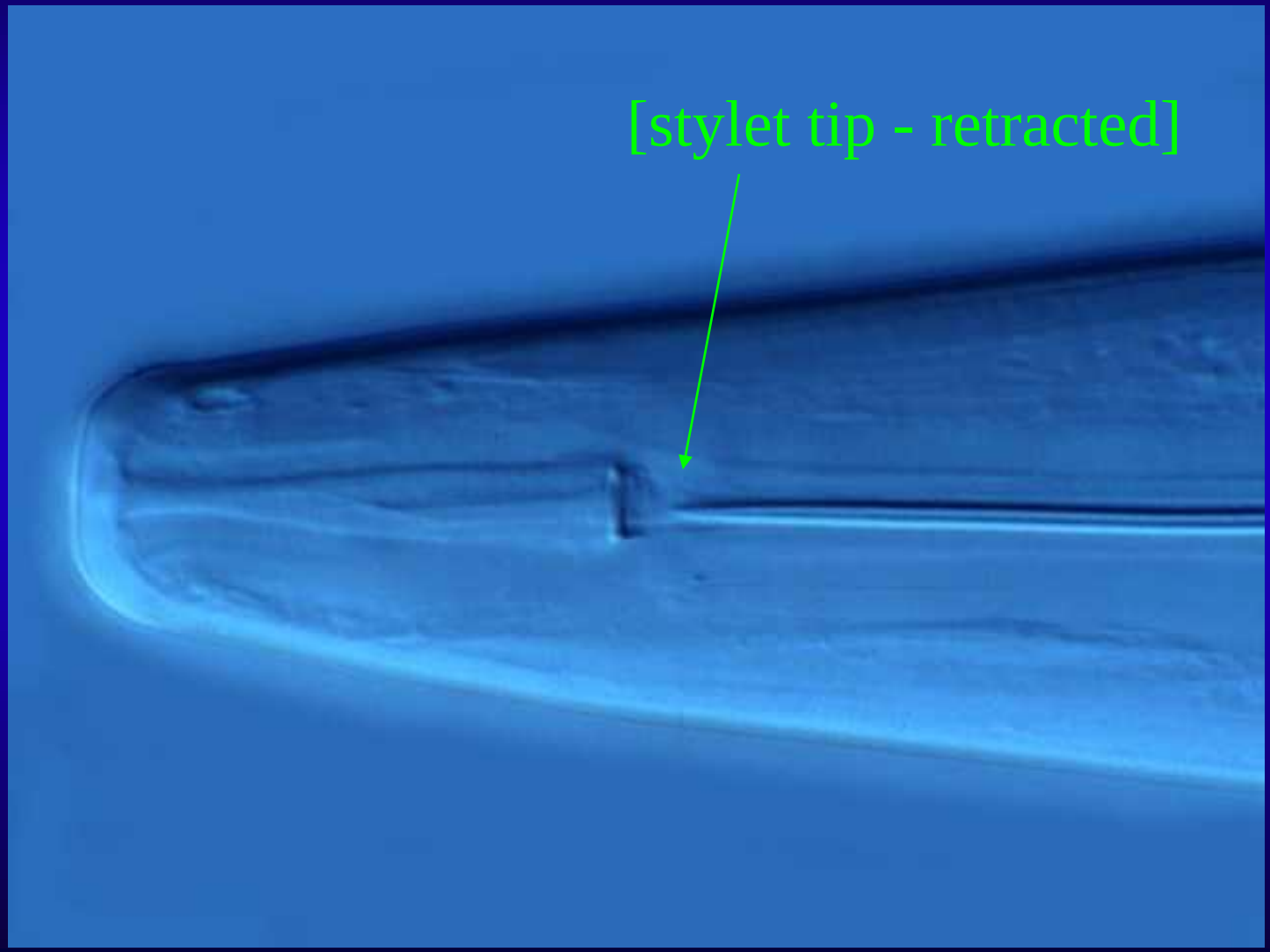
(source unknown)

Range of Tylenchida stylets (source unknown)

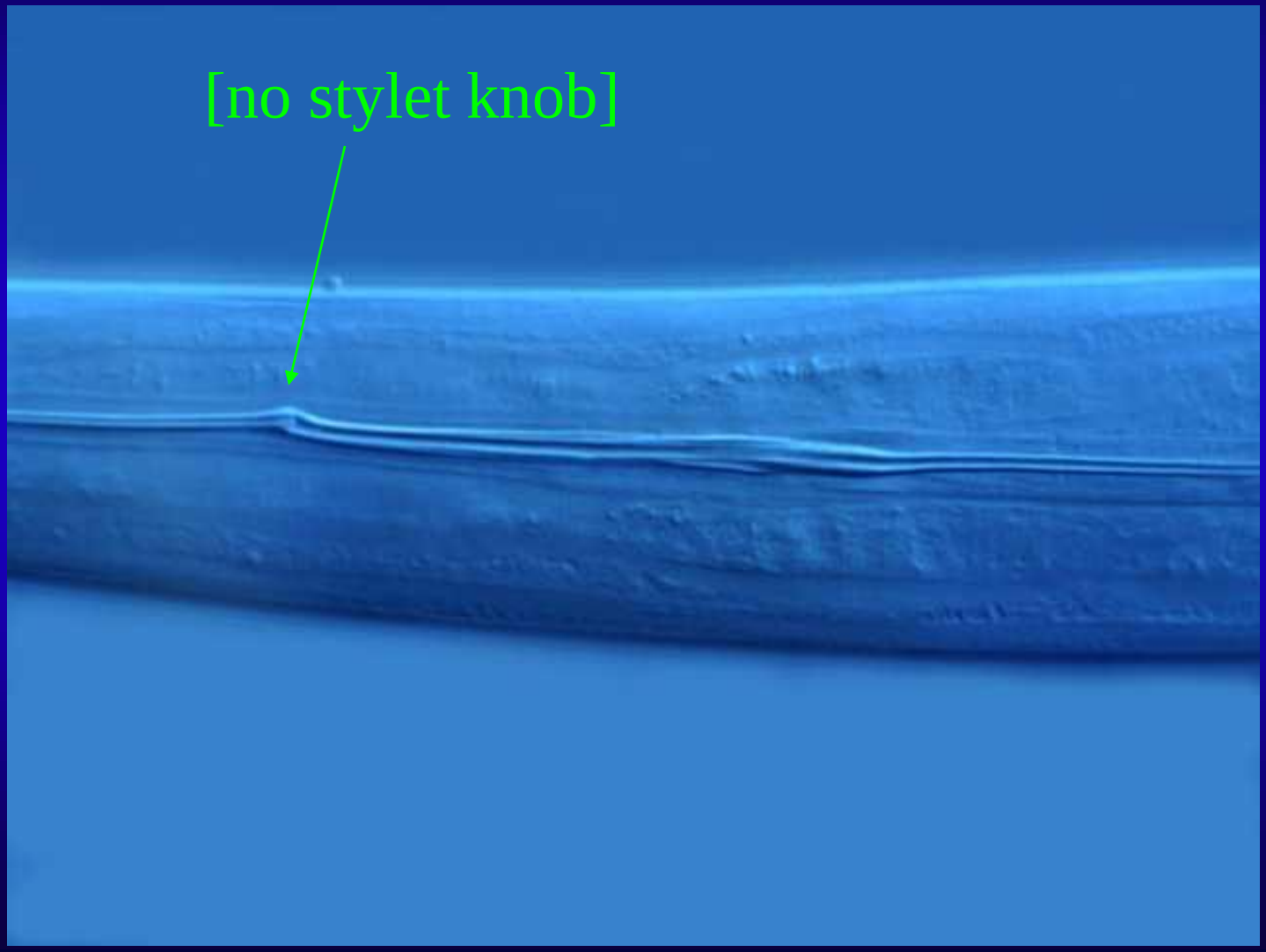


Herbivorous nematode (*Longidorus*) **(source unknown)**

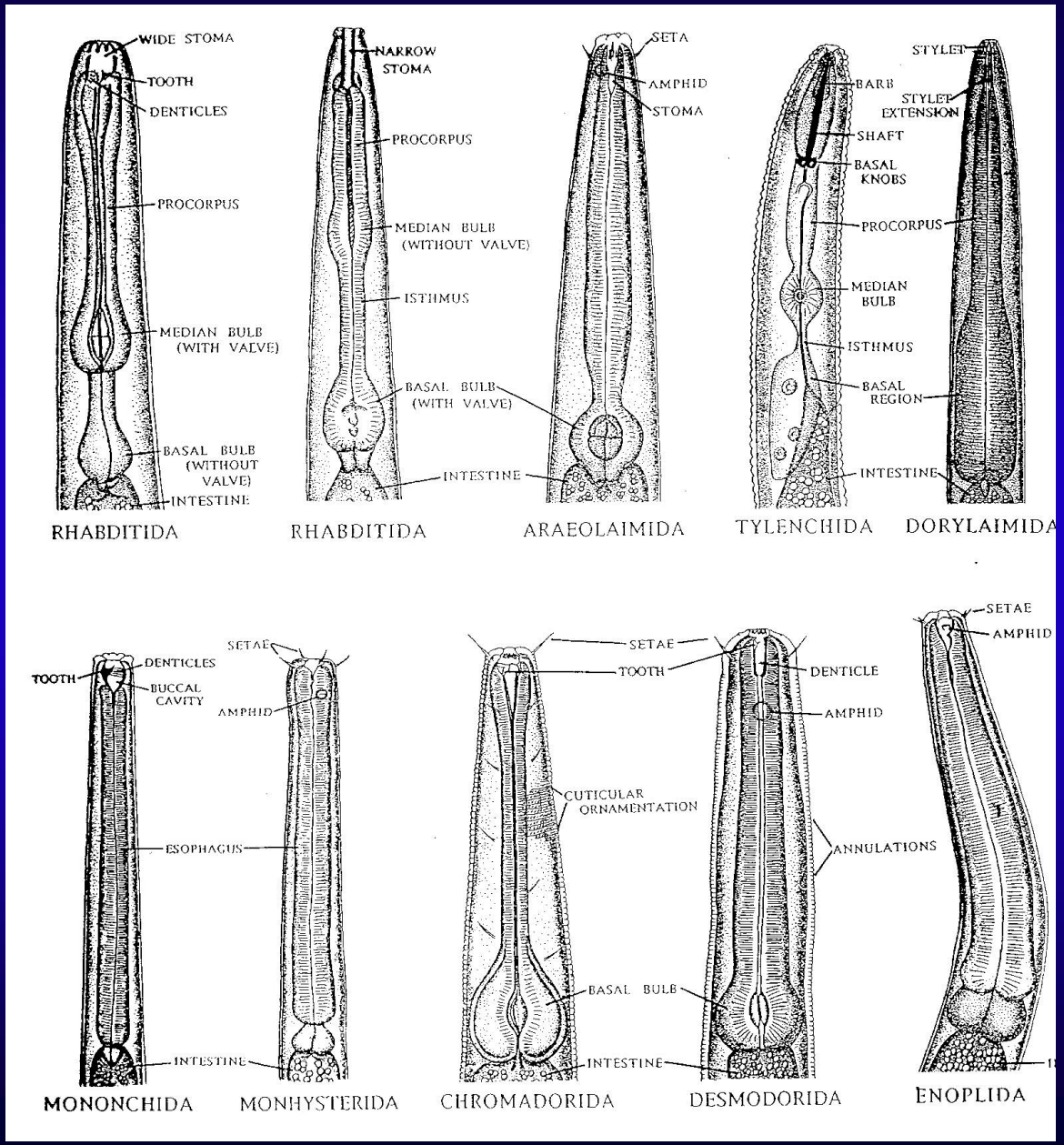
Longidorus sp. (Dorylaimida)
(source unknown)

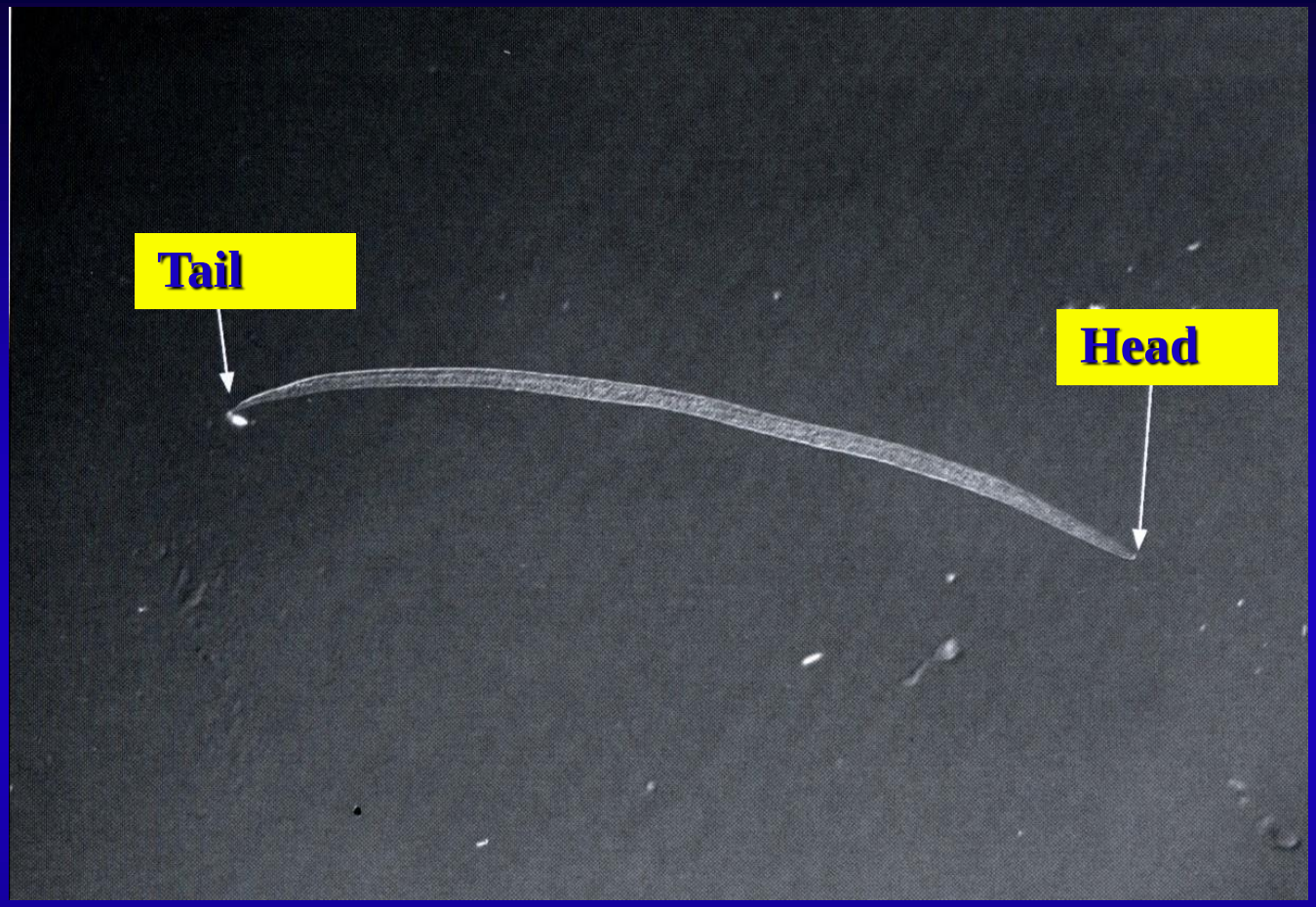


Longidorus sp. (Dorylaimida) (source unknown)



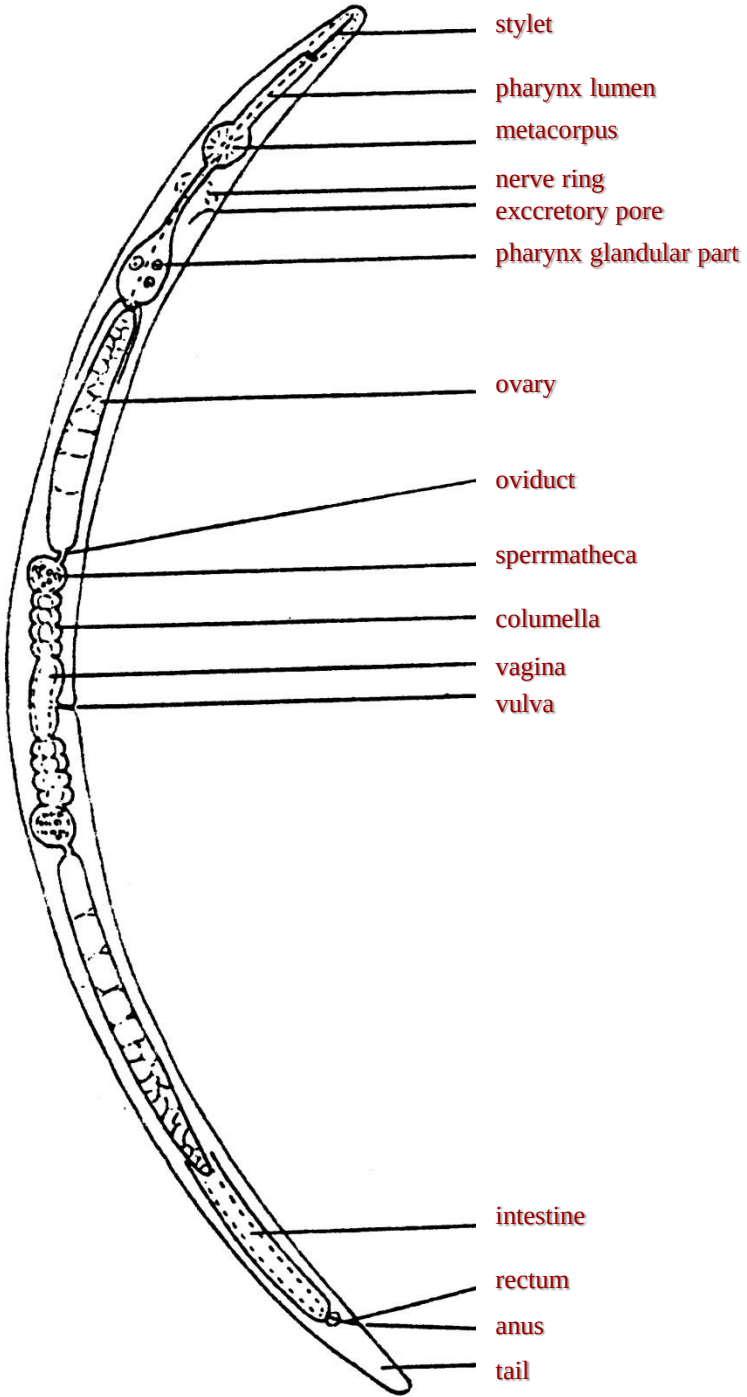
Nematode esophageal types



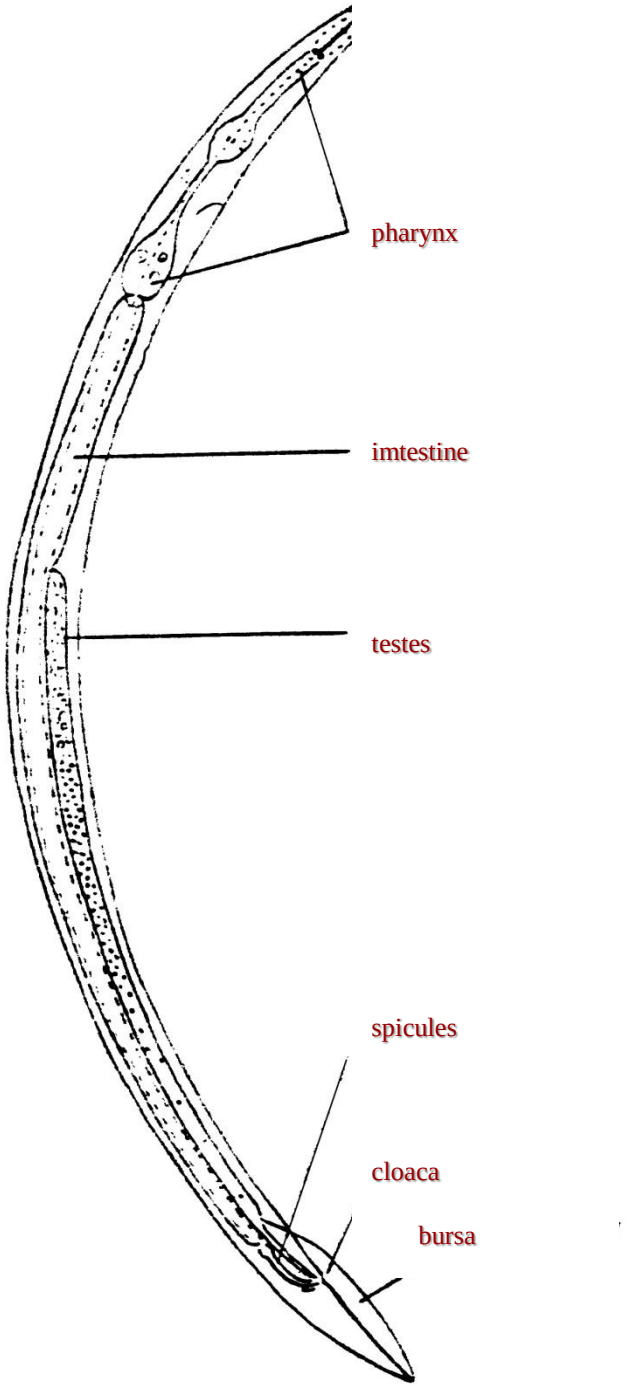


***D. dipsaci* female under stereoscopic microscope**

(phot. Renata Dobosz, Institute of Plant Protection, Poznań, Poland)

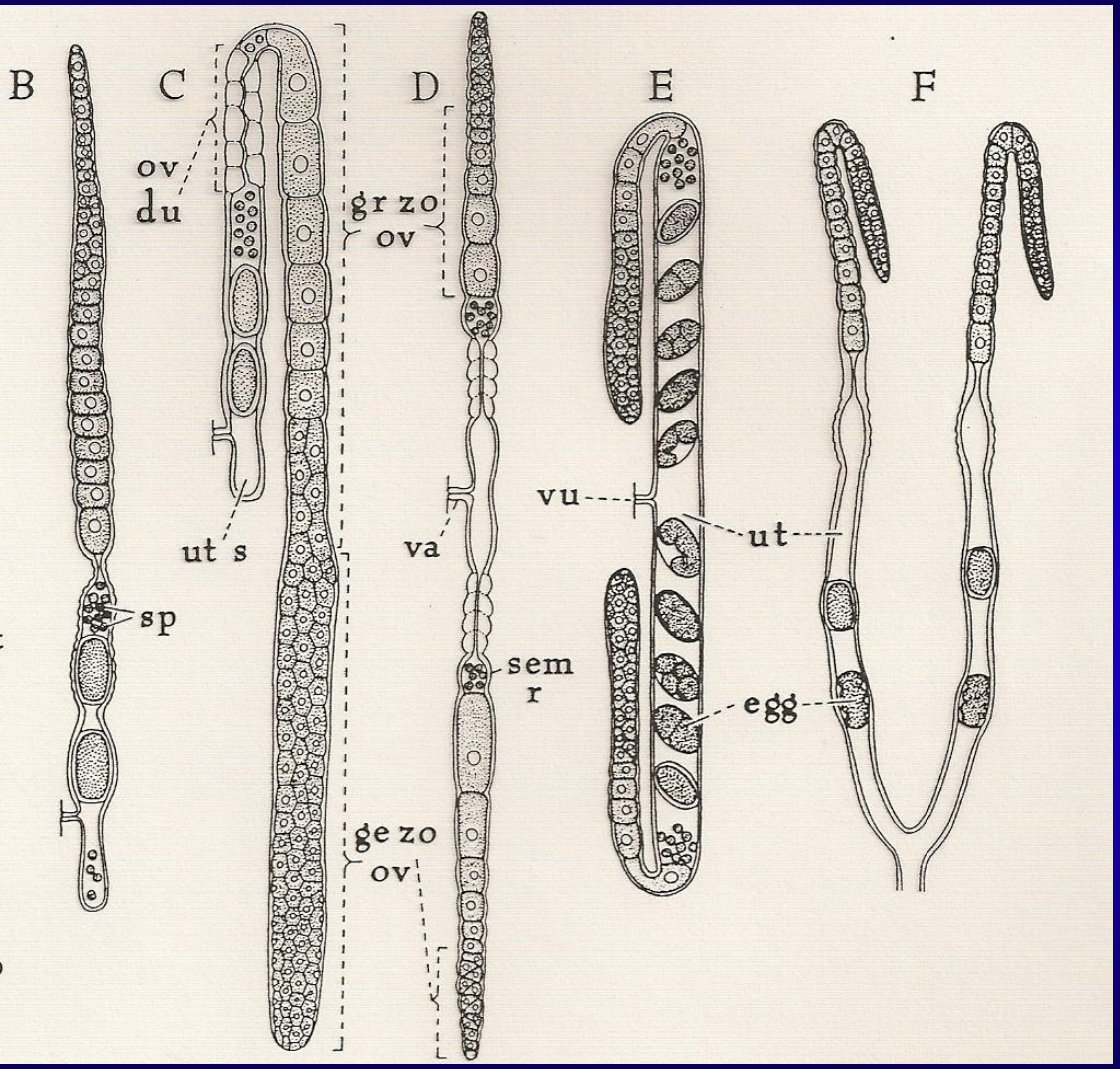


Scheme of nematode female morphology



Scheme of nematode male morphology

Female Reproductive System

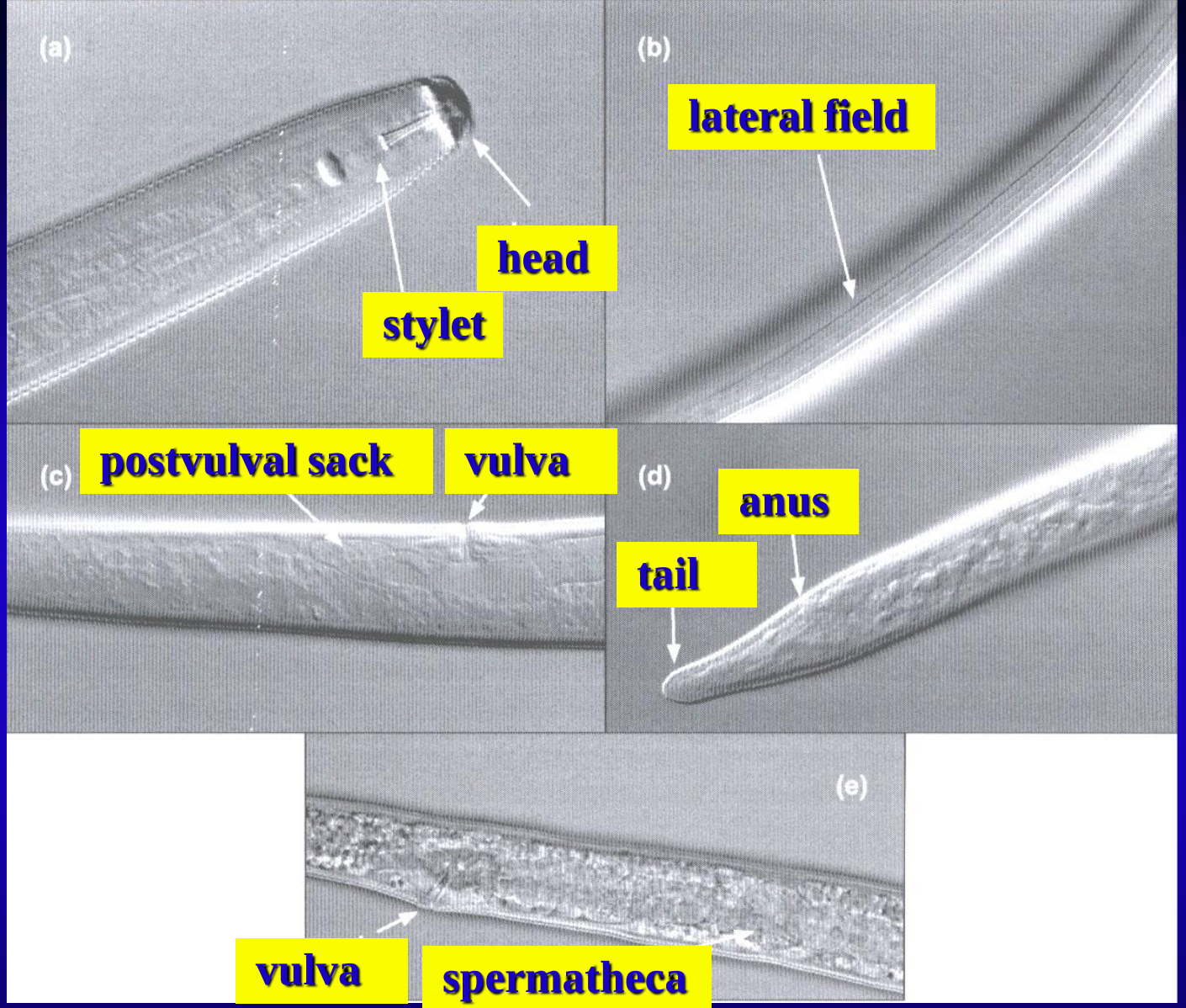


Number of ovaries

Monodelphic = one ovary (B, C)
Didelphic = two ovaries (D, E, F)

Position of ovaries

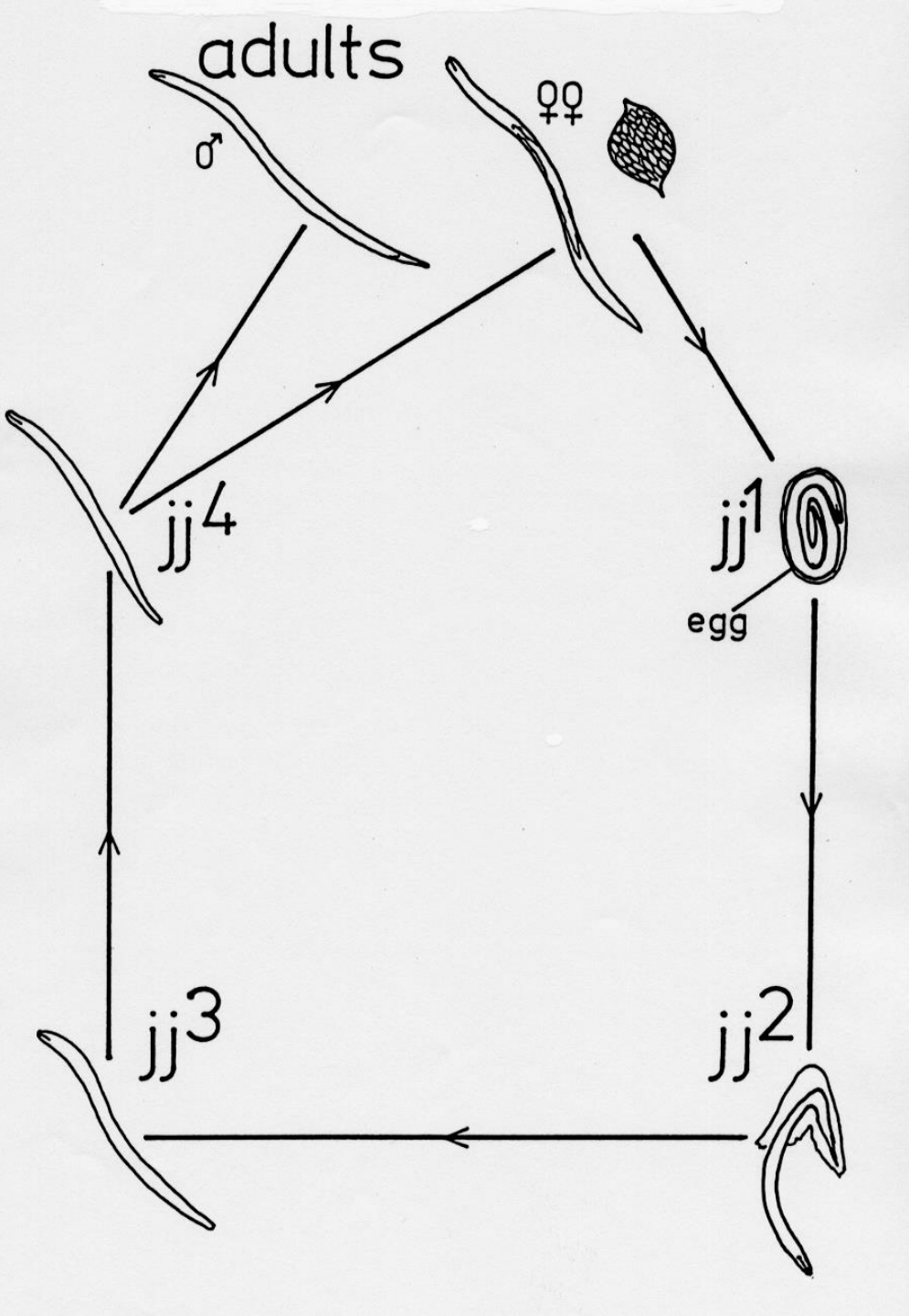
Amphidelphic = opposed ovaries, one anterior; one posterior to vulva (D, E)
Prodelphic = ovary(s) anterior to vulva (B, F)
Opisthodelphic = ovary(s) posterior to vulva (C)
Reflexed = ovaries folded (E, F)
Post uterine sac = vestigial 2nd ovary



Pratylenchus penetrans: (a) head; (b) lateral field (c) postvulval sack; (d) tail, (e) spermatheca. (phot. Renata Dobosz, Institute of Plant Protection, Poznań, Poland)

Typical nematode life-cycle

- egg
- 4 juvenile stages
- adult (male & female

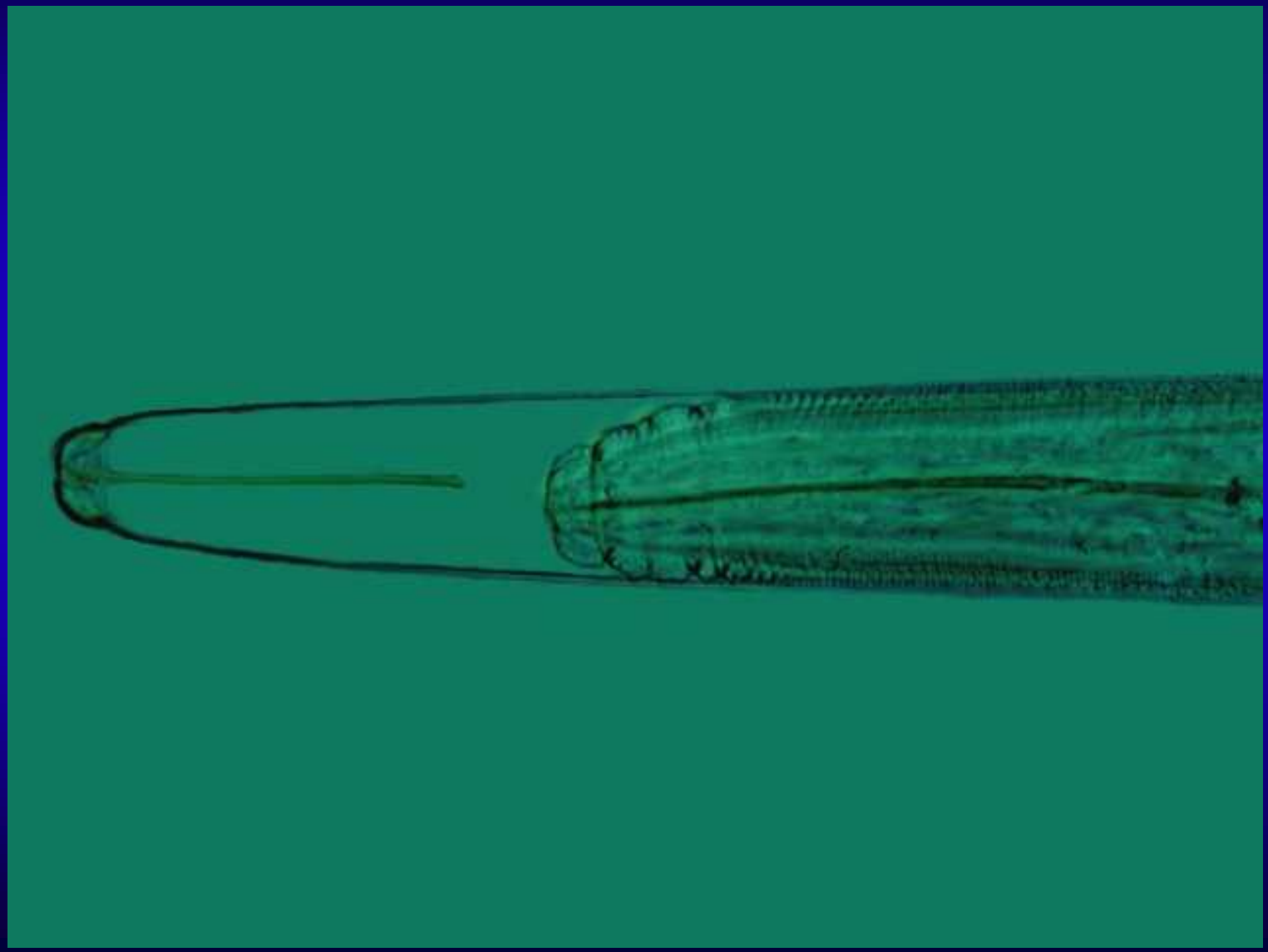


2nd stage juvenile inside egg
(source unknown)

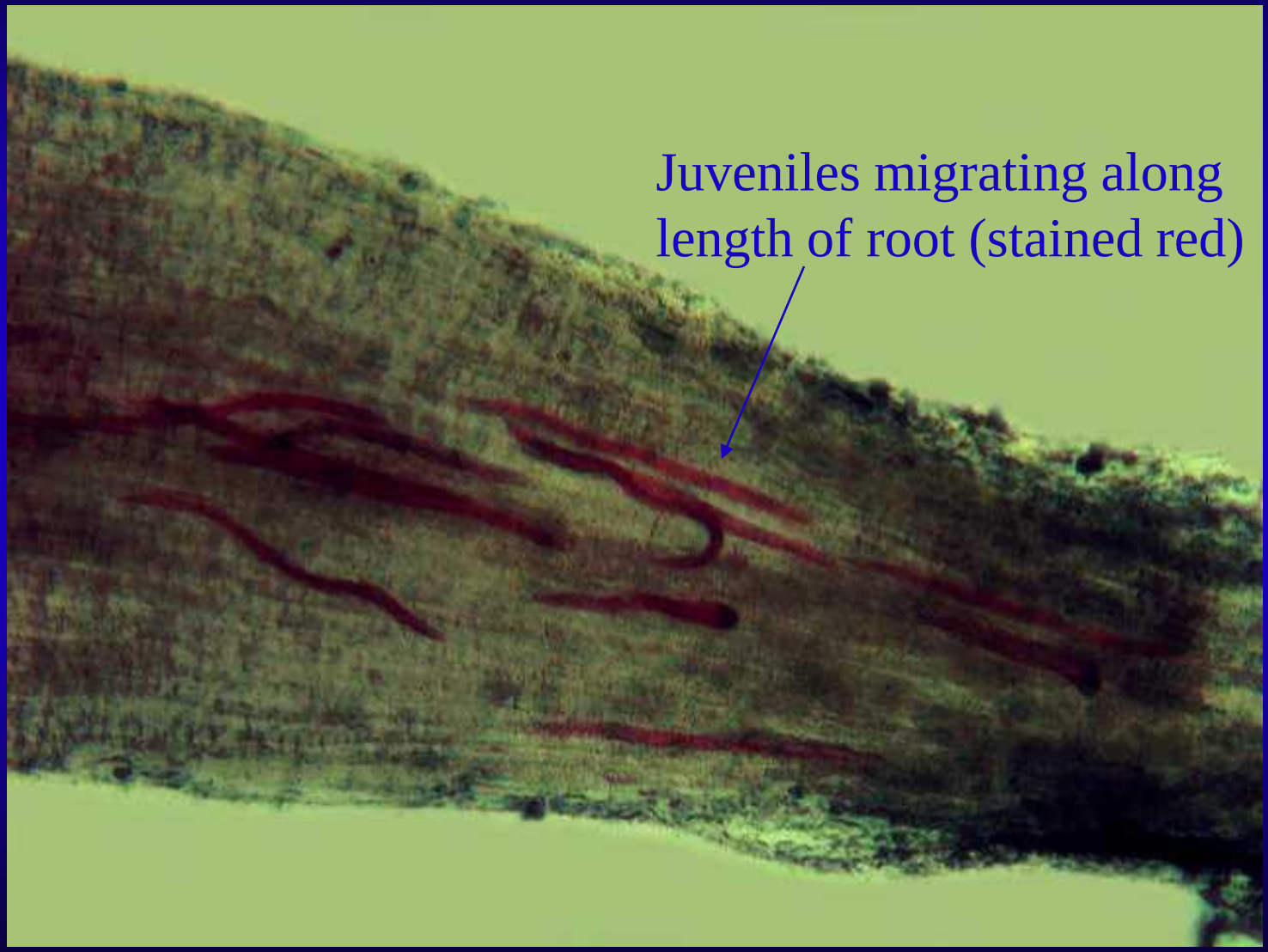
2nd stage juvenile hatching from egg
(source unknown)

Hatched 2nd stage juveniles
(source unknown)

Molting cuticle *(Belonolaimus longicaudatus)* **(source unknown)**

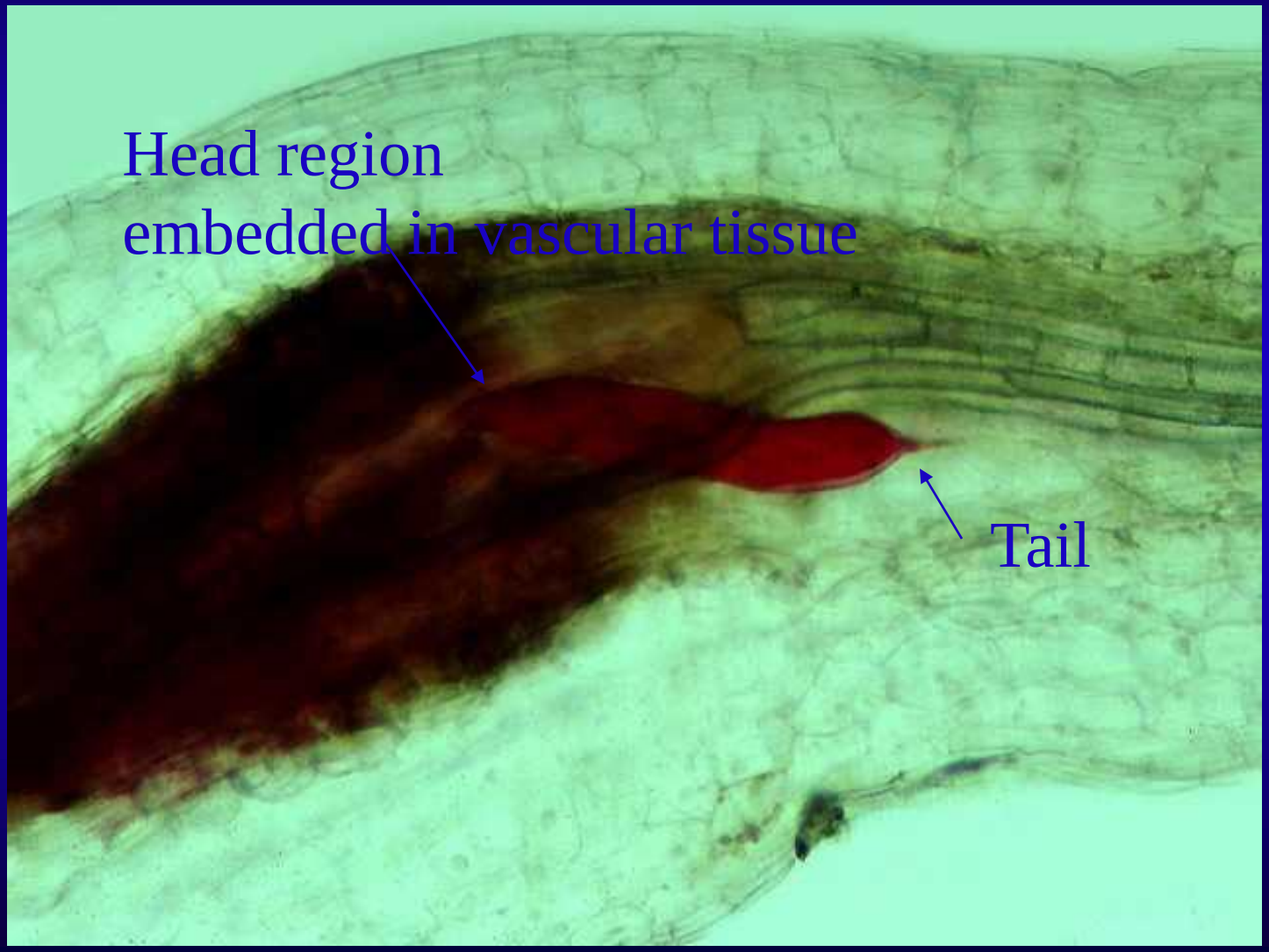


2nd stage juveniles invading root tip
(source unknown)



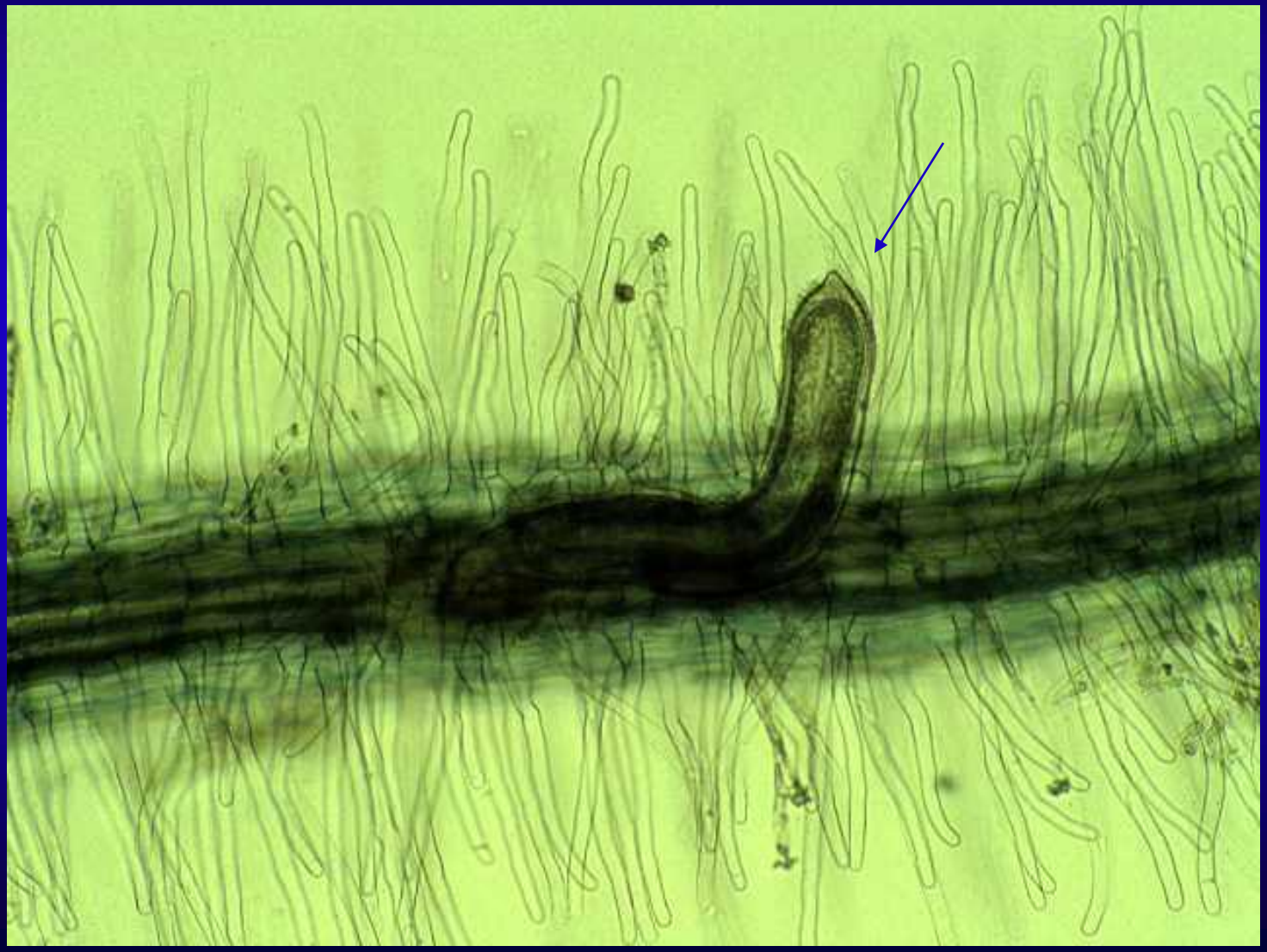
Late 2nd stage juvenile established in root system

(source unknown)



Feeding site established by adult Meloidogyne *female* (source unknown)

3rd - 4th stage juvenile male emerging from root system (source unknown)



Adult male emerging from 4th stage juvenile cuticle (source unknown)

Feeding Habits

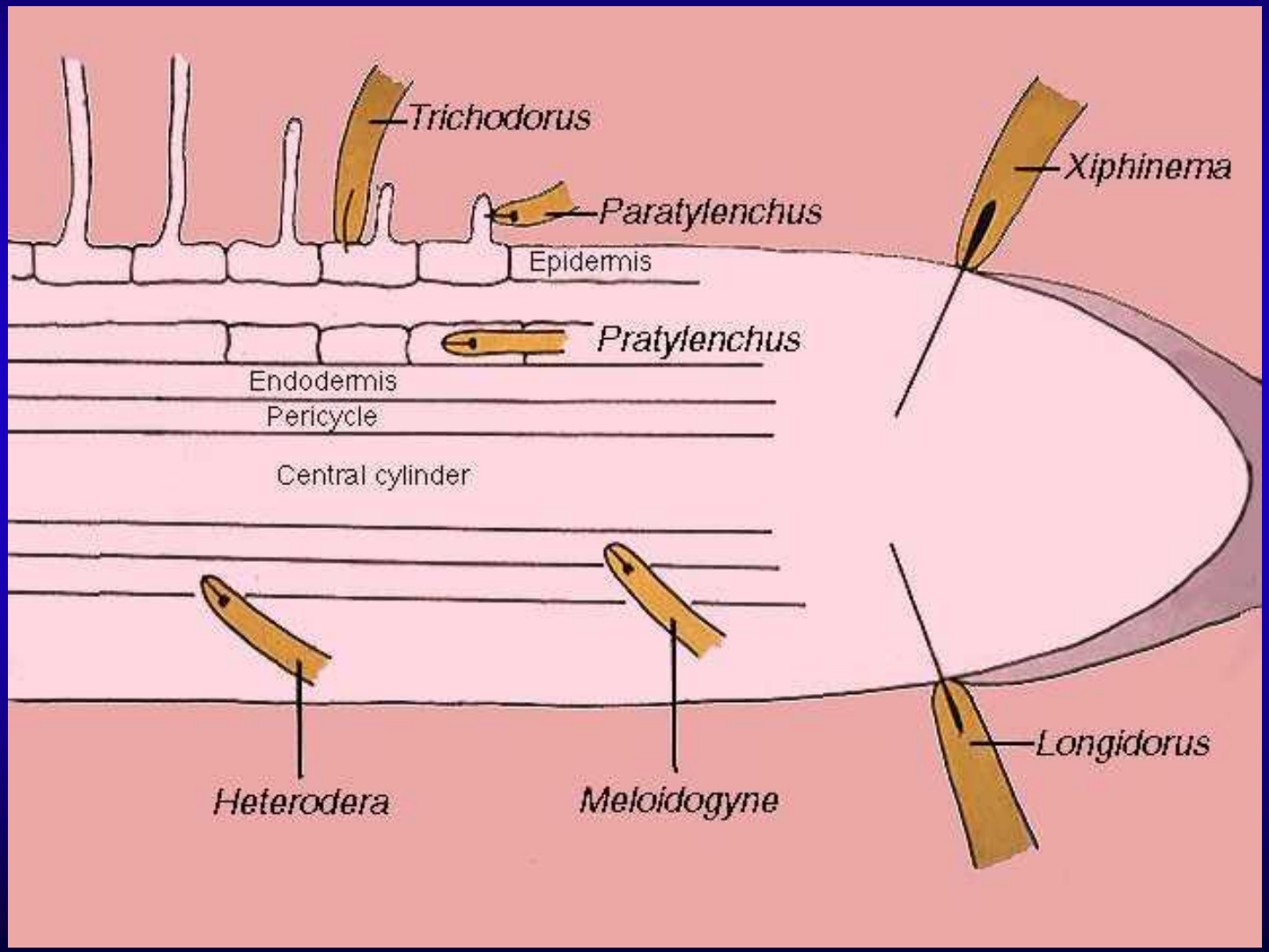
Nematodes may be grouped by feeding habit as:

- Endoparasitic– entire body inside the root.
- Ectoparasitic– entire body outside the root.
- Semi-endoparasitic- part of body inside root.

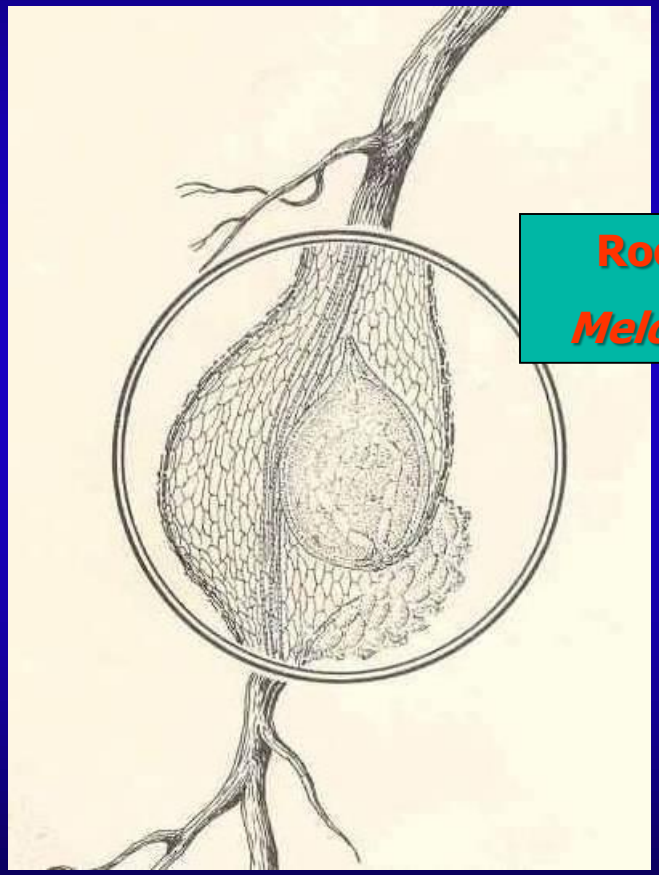
By movement when feeding, they are called:

- Sedentary – mostly immobile during their life.
- Migratory – mobile for all their life.

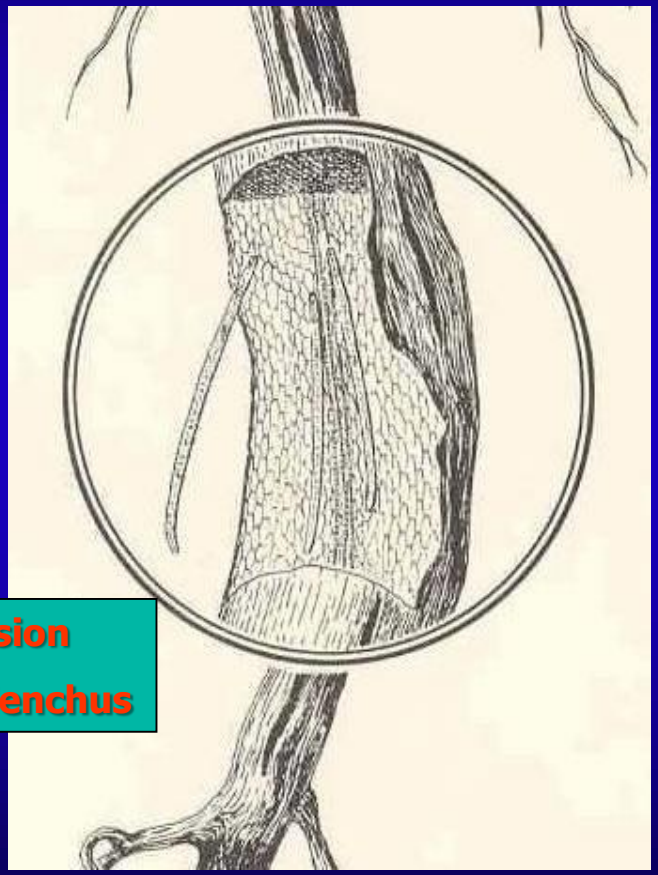
Root feeding sites



Feeding Habits of Some Plant-parasitic Nematodes

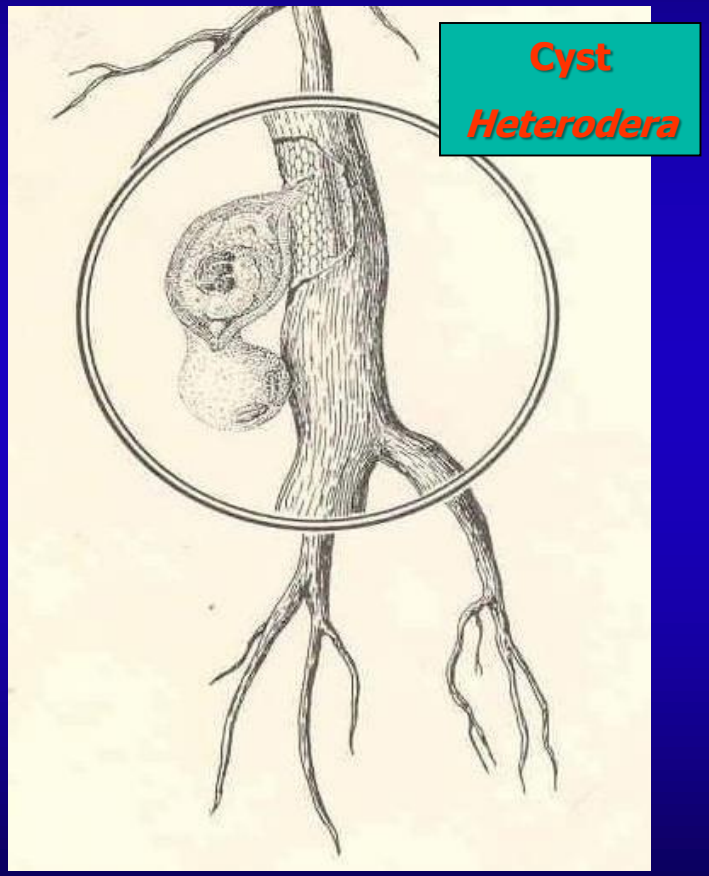


Root-knot
Meloidogyne

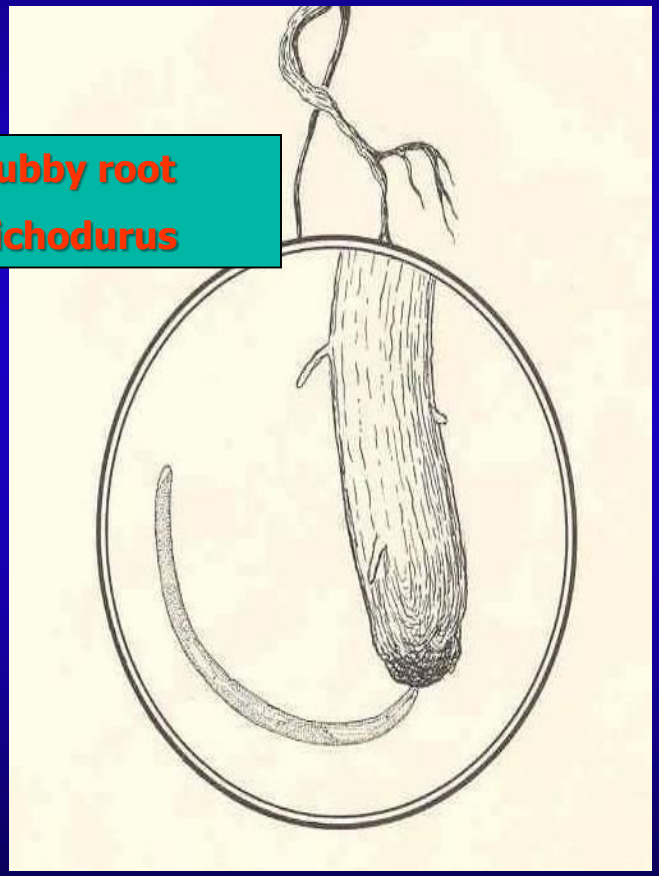


Lesion
Pratylenchus

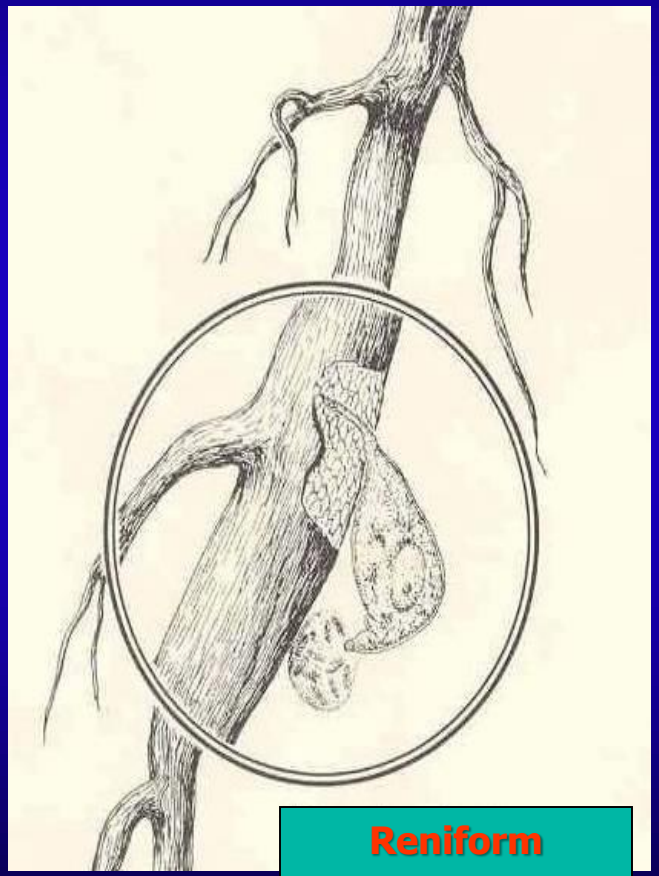
Feeding Habits of Some Plant-parasitic Nematodes



Feeding Habits of Some Plant-parasitic Nematodes

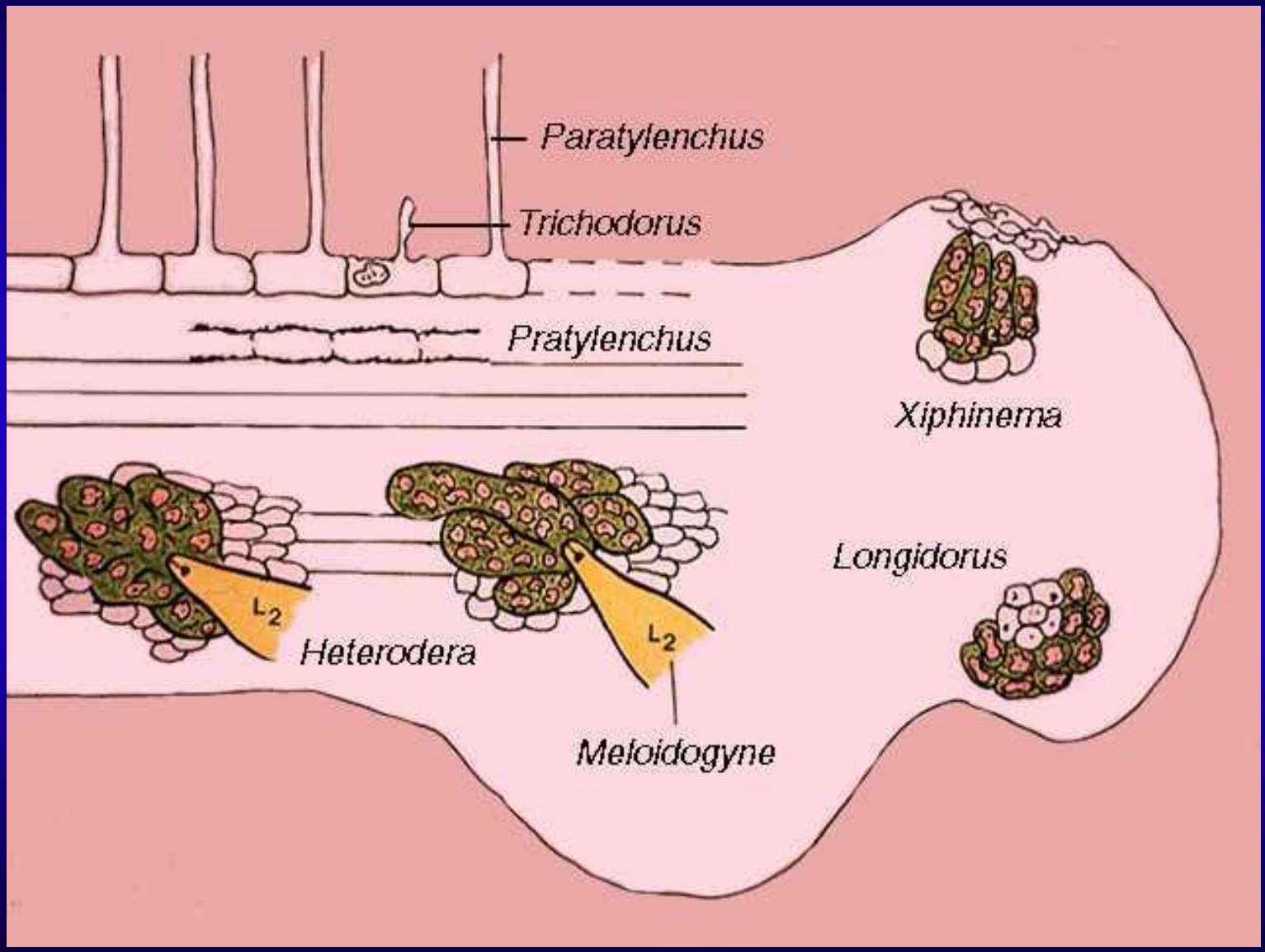


Stubby root
Trichodurus



Reniform
Rotylenchulus

Root damage by plant nematodes



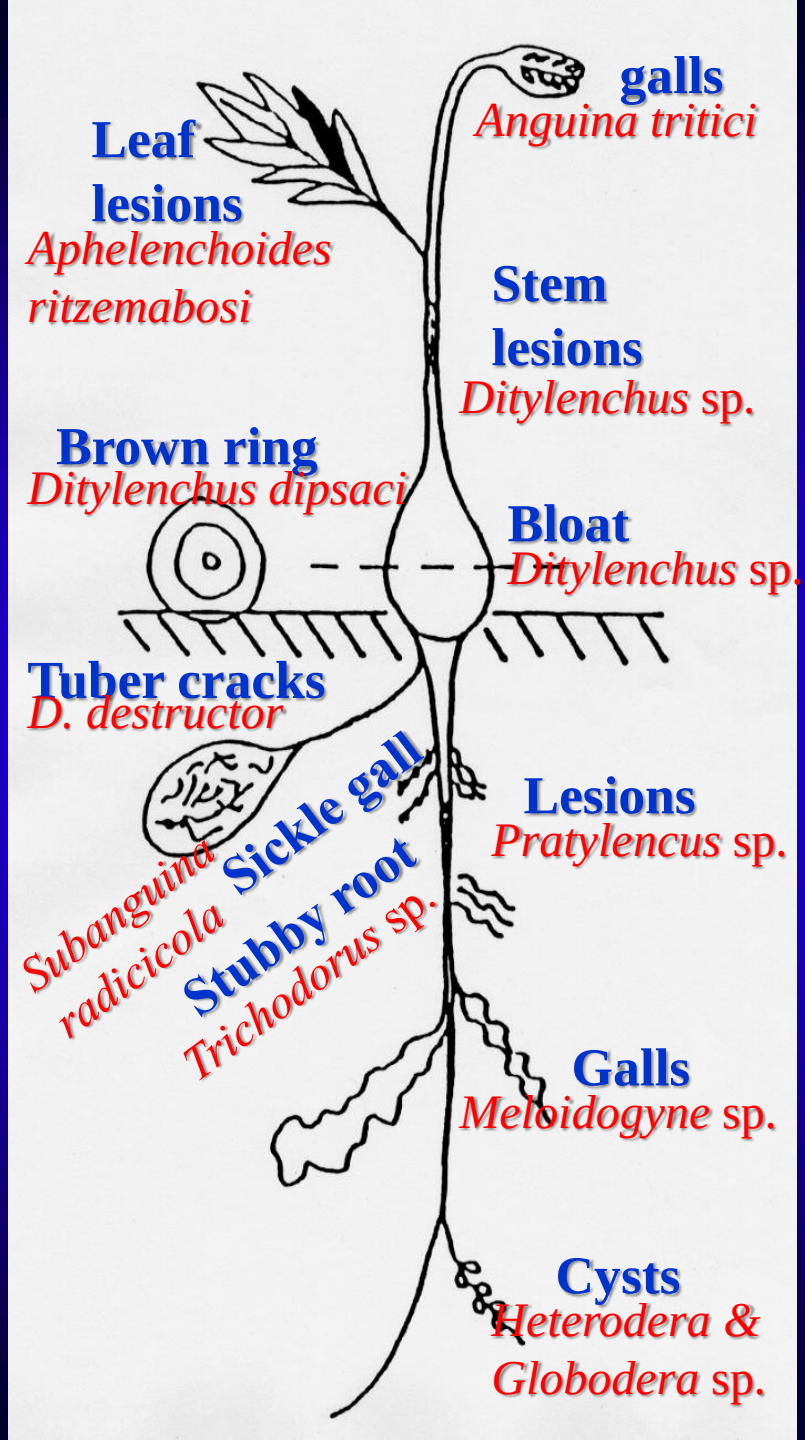
Nematode Damage

- Nematodes damage plants by reducing or modifying root mass.
- Root cells are killed or modified to serve as food for the nematode.
- Typical root and foliar symptoms result.

SYMPTOMS CAUSED BY PLANT-PARASITIC NEMATODES

Composite plant

Nematodes causing symptoms



Initial identification of plant parasitic nematodes is by symptoms of attack **(source unknown)**

Ditylenchus dipsaci-infested oat field (source unknown)

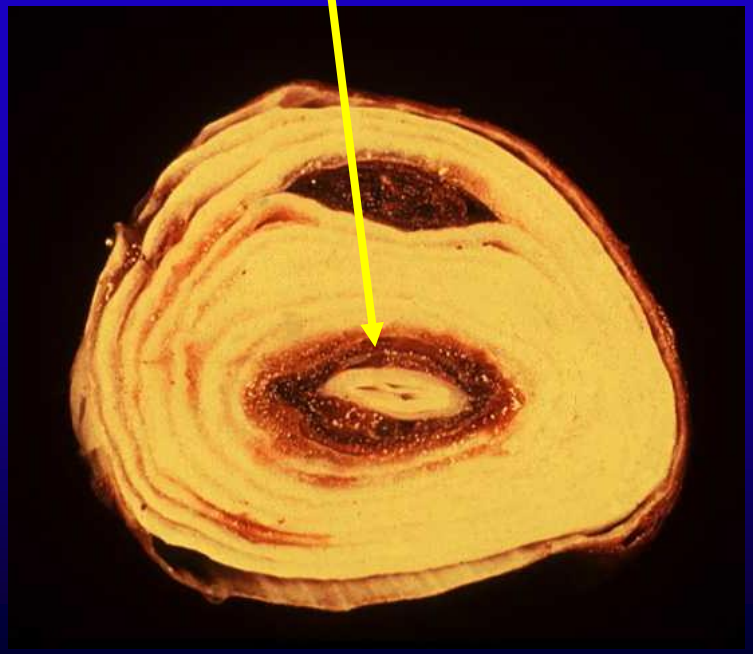
Clover field infested with *Ditylenchus dipsaci* (source unknown)

Ditylenchus dipsaci- infested tulip stems **(source unknown)**

Ditylenchus dipsaci infested narcissus leaf **(source unknown)**

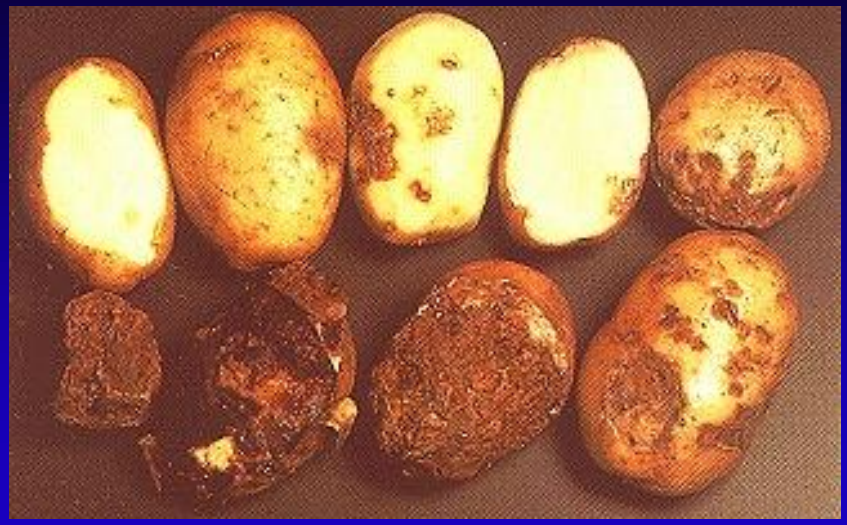
necrosis

‘brown ring’



Ditylenchus dipsaci on garlic **(source unknown)**

Garlic infested with *D. dipsaci* (source unknown)



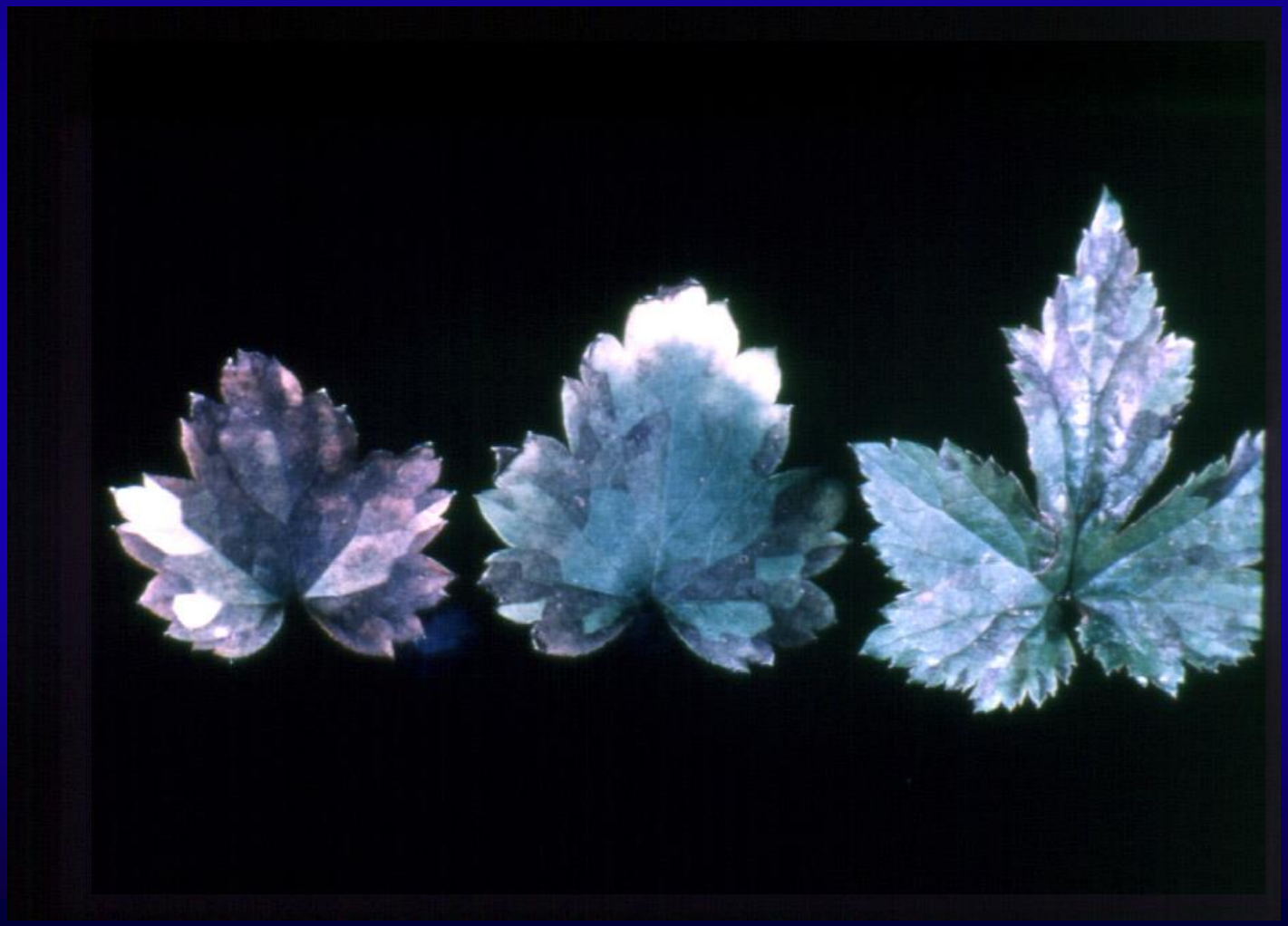
Symptoms of infestation of potatoes with *D. destructor* (phot. H. Andersen, Denmark)

Aphelenchoides ritzemabosi

[Chrysanthemum leaf]

(source unknown)

Aphelenchoides ritzemabosi [anemone leaf] **(source unknown)**

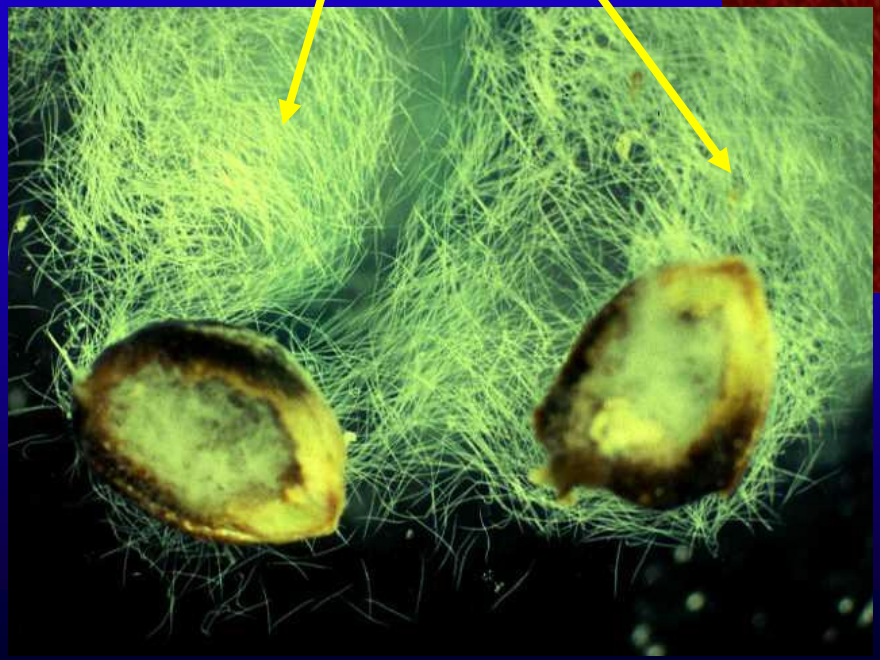


Anguina tritici - infested wheat plant

(source unknown)

Wheat seed infested with *Anguina tritici* (source unknown)

Juveniles released from infested galls



Symptoms of pine wilt disease on *Pinus pinaster* in Portugal

(phot. Central Laboratory of SPHSIS, Toruń, Poland)

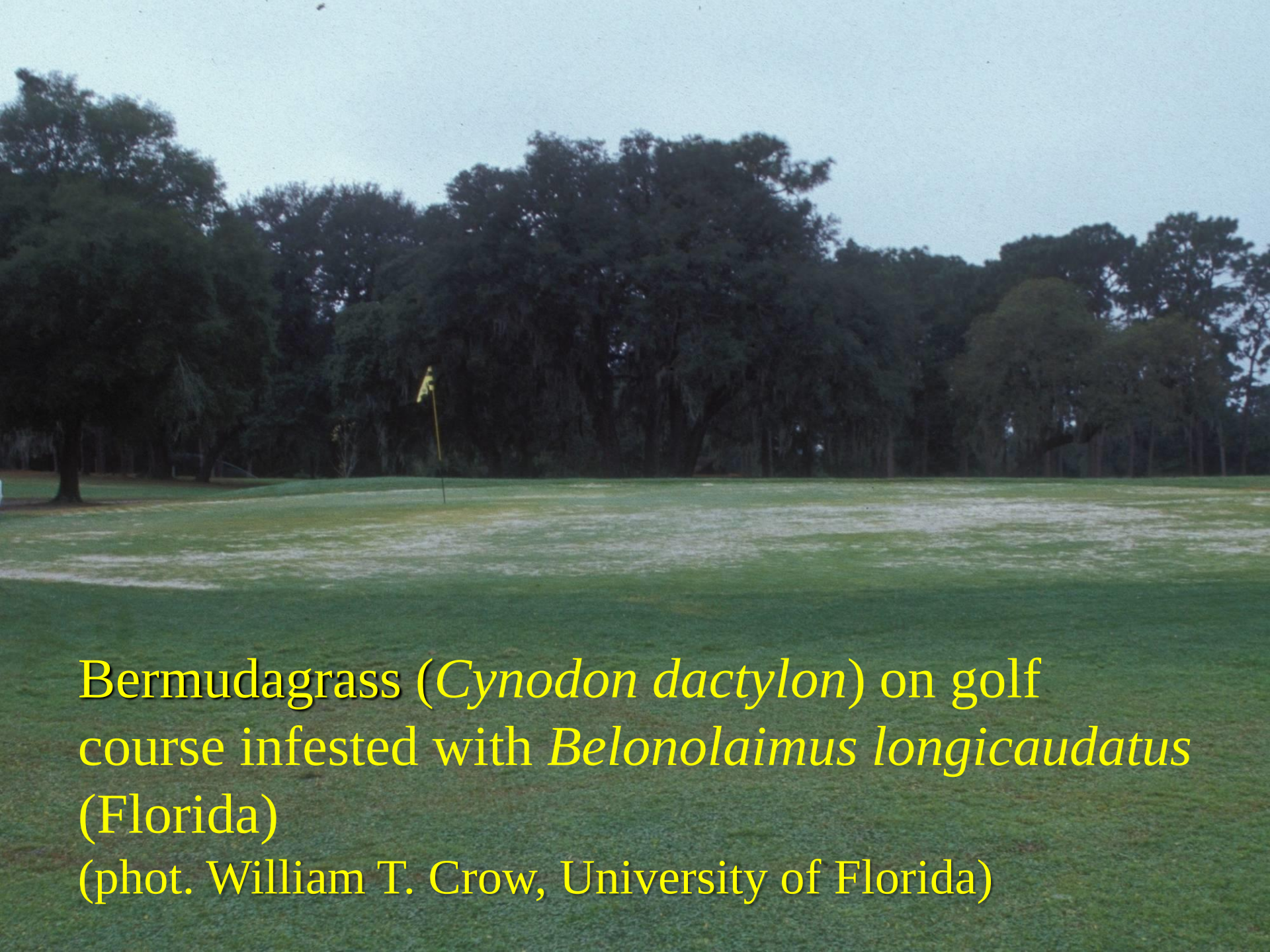
Symptoms of pine wilt disease on *Pinus pinaster* in Portugal

(phot. Central Laboratory of SPHSIS, Toruń, Poland)

Symptoms of pine wilt disease on *Pinus pinaster* in Portugal

(phot. Central Laboratory of SPHSIS, Toruń, Poland)

Symptoms (stubby roots) caused by *Paratrichodorus minor* on maize roots (source unknown)



Bermudagrass (*Cynodon dactylon*) on golf course infested with *Belonolaimus longicaudatus* (Florida)
(phot. William T. Crow, University of Florida)

Bermudagrass (*Cynodon dactylon*) destroyed by feeding of *Belonolaimus longicaudatus* (left) compared with healthy one (right) (Florida)
(phot. William T. Crow, University of Florida)

Symptoms (sickle galls) caused by *Subanguina radicicola* on grass roots (source unknown)

Meloidogyne incognita (root-knot nematode)

(source unknown)

Infested Uninfested

Symptoms (lesions) caused by *Radopholus similis* on ornamental plants (EPPO Website)

Lesions on roots

Reduction of root system

Symptoms (lesions) caused *Pratylenchus* on ornamental plants

(source unknown)

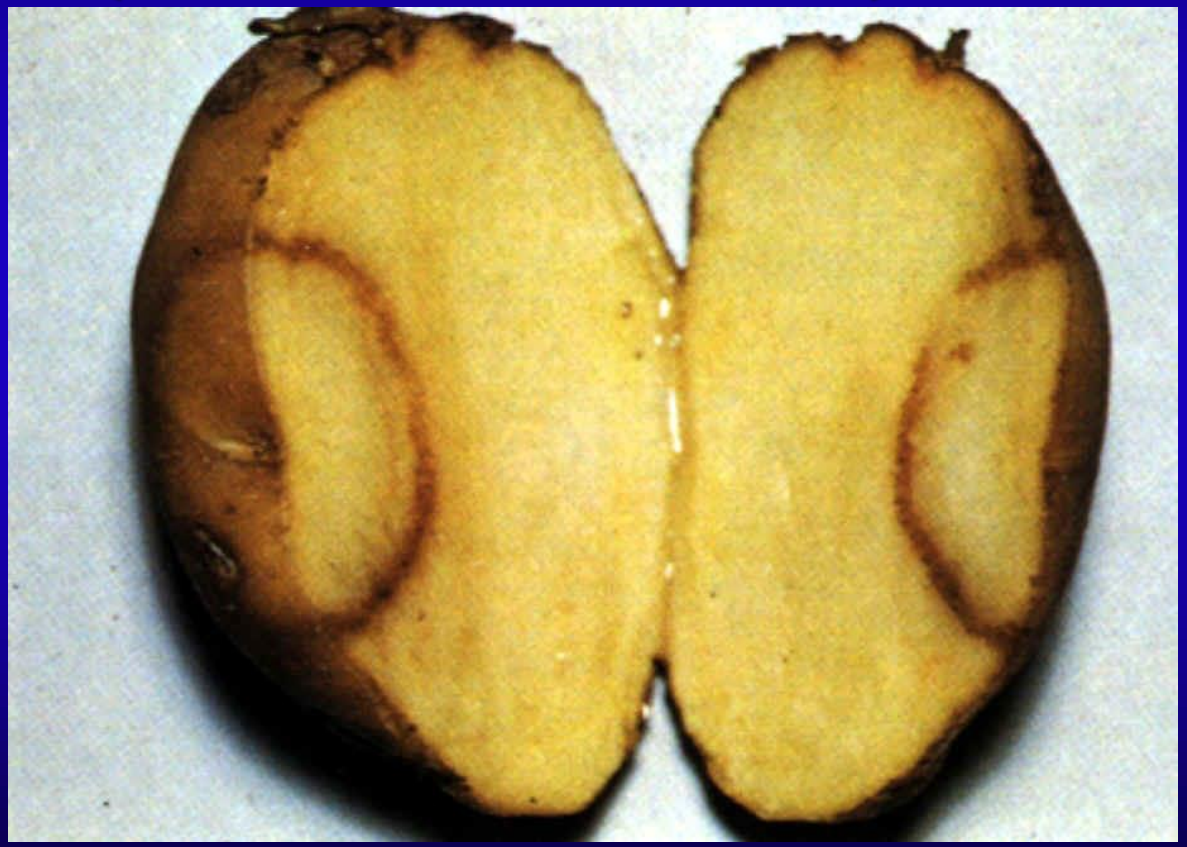
Tobacco Mosaic Virus of Tomato

Host plants:
Tomato, pepper, eggplant, tobacco, spinach, petunia, marigold

Vector:
Xiphinema sp.

Potato plant infested with Tobacco Rattle Virus (source unknown)

Internal tuber necrosis caused by Tobacco Rattle Virus ['spraing'] (source unknown)

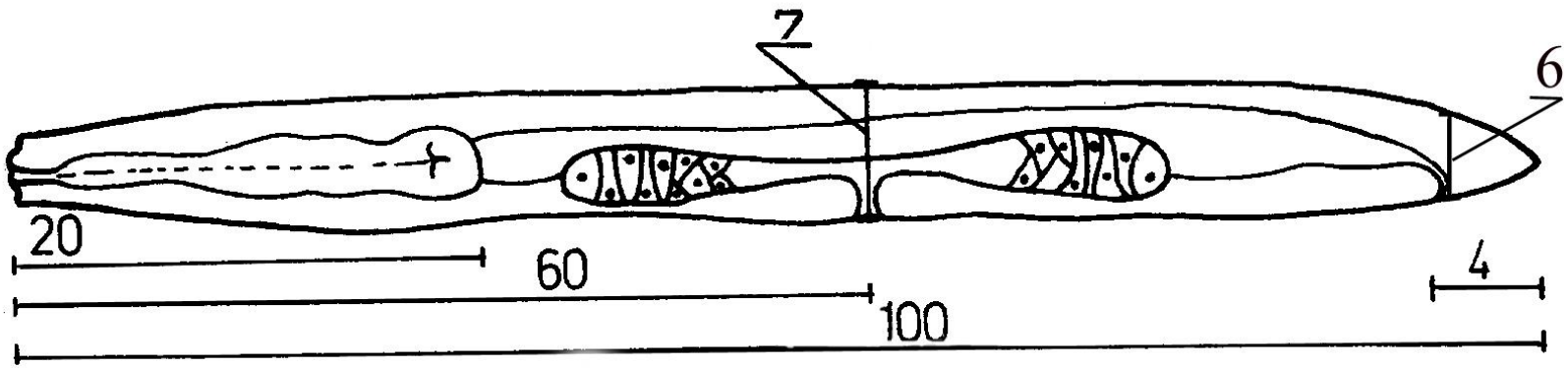


BASIC NEMATODE MEASUREMENT FORMULAE

THE FOLLOWING MAIN NEMATODE MEASUREMENTS ARE IN USE (APART FROM CYSTS)

n = number of specimens on which measurements are based
L = overall body length (in mm or μm)
V = % distance of vulva from anterior
a = body length / greatest body diameter
b = body length / distance from anterior to esophago-intestinal valve
b' = body length / distance from anterior to base of esophageal glands
MB% = % distance from anterior to median bulb relative to length of esophagus
c = body length / tail length
c' = tail length / tail diameter at anus or cloaca
stylet = stylet length in μm
stylet knobs = stylet knobs width in μm
s = stylet length / body diameter at base of stylet
T = % length of male gonad relative to body length
o = % distance of dorsal esophageal gland opening from stylet knobs in relation to stylet length
P = % distance of phasmid from anus in relation to length of tail
P^a = % distance of anterior phasmid from anterior of nematode in relation to body length
P_p = % distance of posterior phasmid from anterior of nematode in relation to body length
G^1 = % length of anterior female gonad in relation to body length
G_2 = % length of posterior female gonad in relation to body length
Tail length = portion of body from anus or cloaca to posterior terminus length in μm
h = hyaline tail length in μm
lateral lines = number of lateral lines

Main nematode measurements - female



L = 100 x ocular division length

a = 100 : 7 = 14;

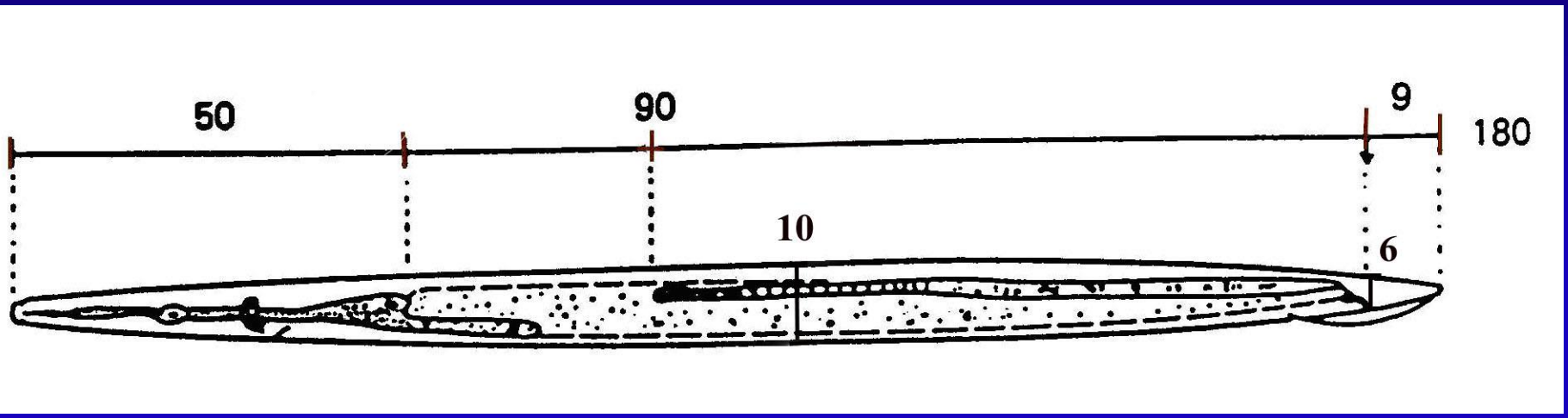
b = 100 : 20 = 5;

c = 100 : 4 = 25;

c’ = 4 : 6 = 0.67;

V = 60 : 100 x 100 % = 60%.

Main nematode measurements - male



L = 180 x ocular division length

a = 180 : 10 = 18;

b = 180 : 50 = 3.6;

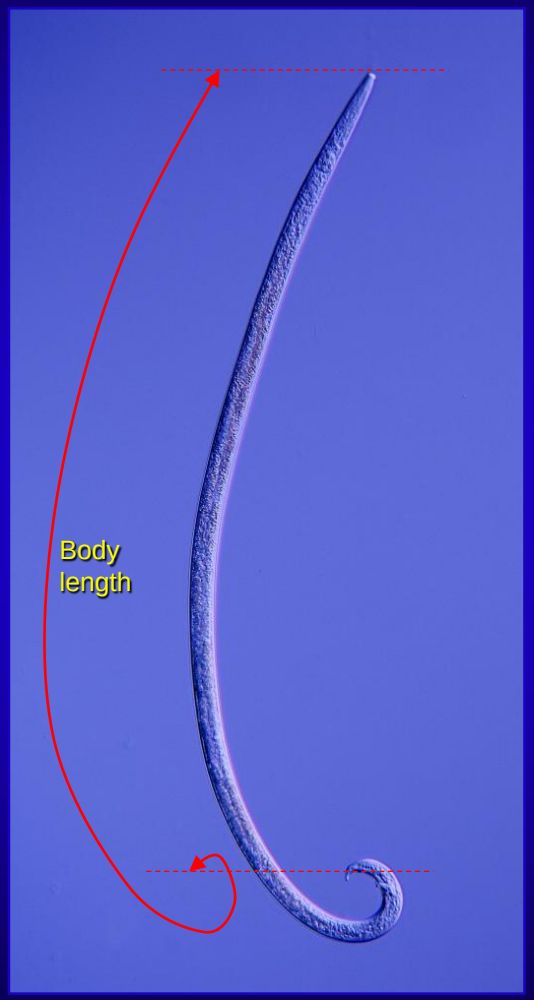
c = 180 : 9 = 20;

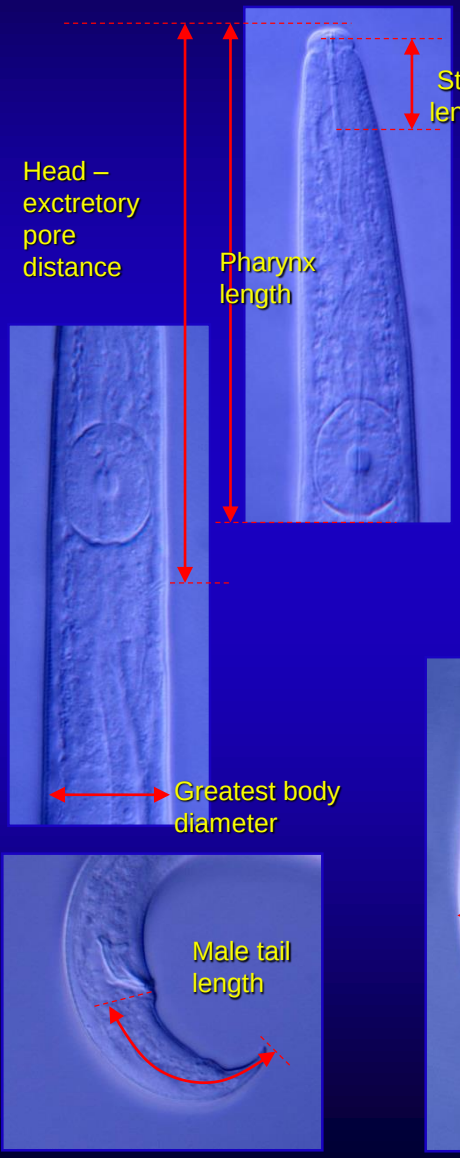
c’ = 9 : 6 = 1.5.

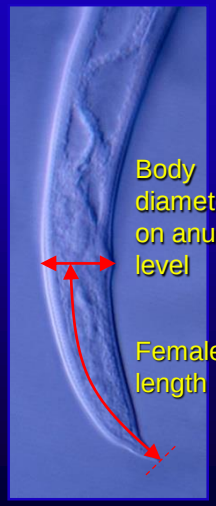

Ditylenchus destructor
male lateral field 1000X
Wisconsin
Potato
in culture
© Peter Mullin/2001

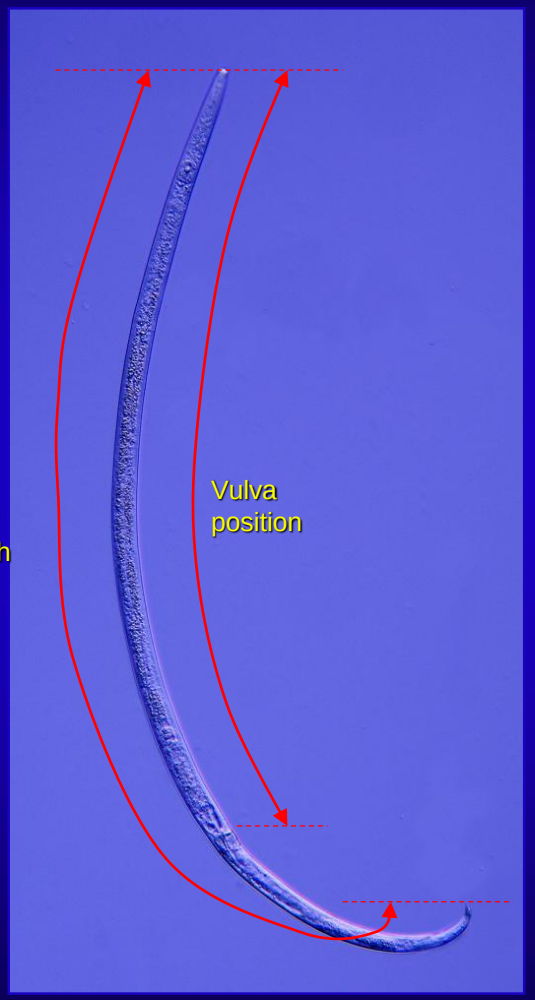
Performance of body measures

Slide prepared by prof. Marek Tomalak, Institute of Plant Protection – Governmental Research Institute, Poznań, Poland









Sample key for nematode identification (EPPO PM 7/4)

Table 1 The identification of genus *Bursaphelenchus* (extracted from wood and bark) (NBS: not *Bursaphelenchus* species):

1.	Tylenchid stylet, pharynx with a metacorpus	2
	Dorylaimid stylet no metacorpus.	NBS
2	Metacorpus with metacorpus plates	3
	No metacorpus plates in metacorpus	NBS
3	One gonad vulva posterior	4
	Two gonads vulva median	NBS
4	Metacorpus strongly developed, distinct at lower magnifications; especially clear in fixed specimens, ovoid-rounded-rectangular in shape; in lateral perspective no sign of a dorsal pharyngeal gland opening or a ventral curvature of pharyngeal lumen behind stylet knobs	5
	Metacorpus smaller, fusiform to rounded; in lateral perspective dorsal pharyngeal gland opening and a ventral curvature of pharyngeal lumen behind stylet knobs	NBS
5	Pharyngeal gland overlaps intestine dorsally	6
	Pharyngeal gland bulb abuts intestine	NBS
6	Stylet knobs present (knobs may be small)	7
	Stylet knobs absent	NBS
7	Male tail tip enveloped by a small bursa (best seen in the dorso-ventral aspect, and even visible using a stereomicroscope)	8
	Bursa absent	NBS
8	Vulva 70-80 % of body length from anterior end; male tail tip strongly recurved	9
	Vulva 85-90 % of body length from anterior end; male tail tip not strongly recurved	NBS
9	Lateral field with 4 lines; vulva with prominent flap; spicules strongly arcuate	Bursaphelenchus xylophilus group (BXG)
	Characters different	Non Bursaphelenchus xylophilus group

ADAPTATIONS OF BASIC LIFE-CYCLE

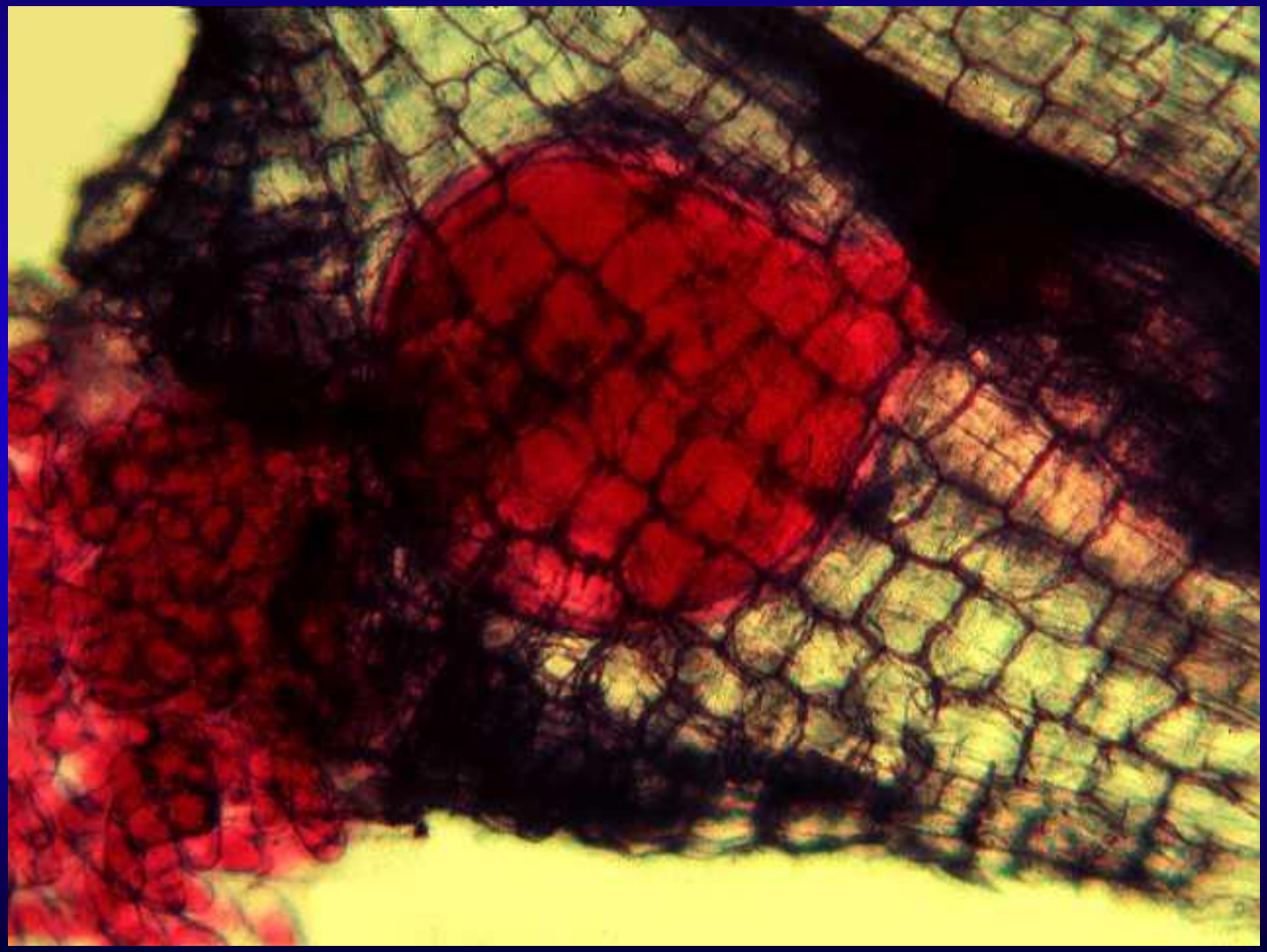
- slow down or stop activities while hazardous conditions prevail
- modify its physiology
- escape to other location
- protect itself

Anhydrobiotic coiled position

(Aphelenchus avenae) (source unknown)

Meloidogyne sp. in tomato root gall

(stained adult female) (source unknown)



PCN *(Globodera rostochiensis)*
on potato roots (source unknown)