

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

LONGIDORUS spp.

XIPHINEMA spp

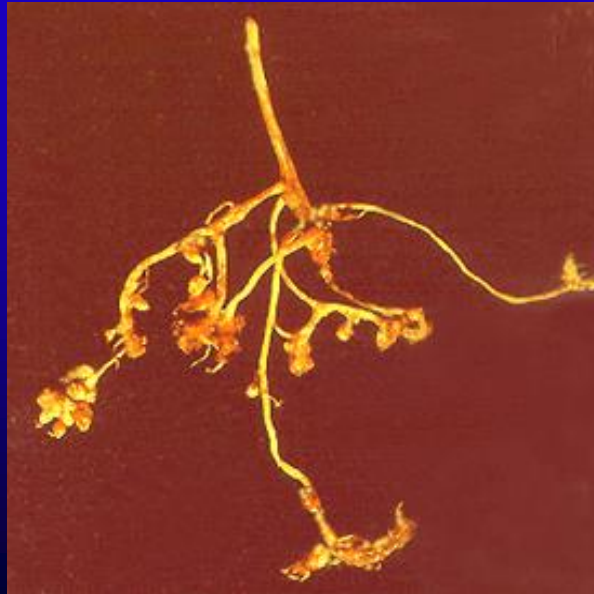
**Extraction and identification basing on
morphological and morphometric
characters**

Symptoms of infestation with *Longidorus* and *Xiphinema* (phot. Adam Szczygieł)



reduction of root system

swellings close to root tip



**Strawberry plants infested with *Longidorus elongatus* (right) compared with healthy plant (left)
(phot. Adam Szczygieł)**



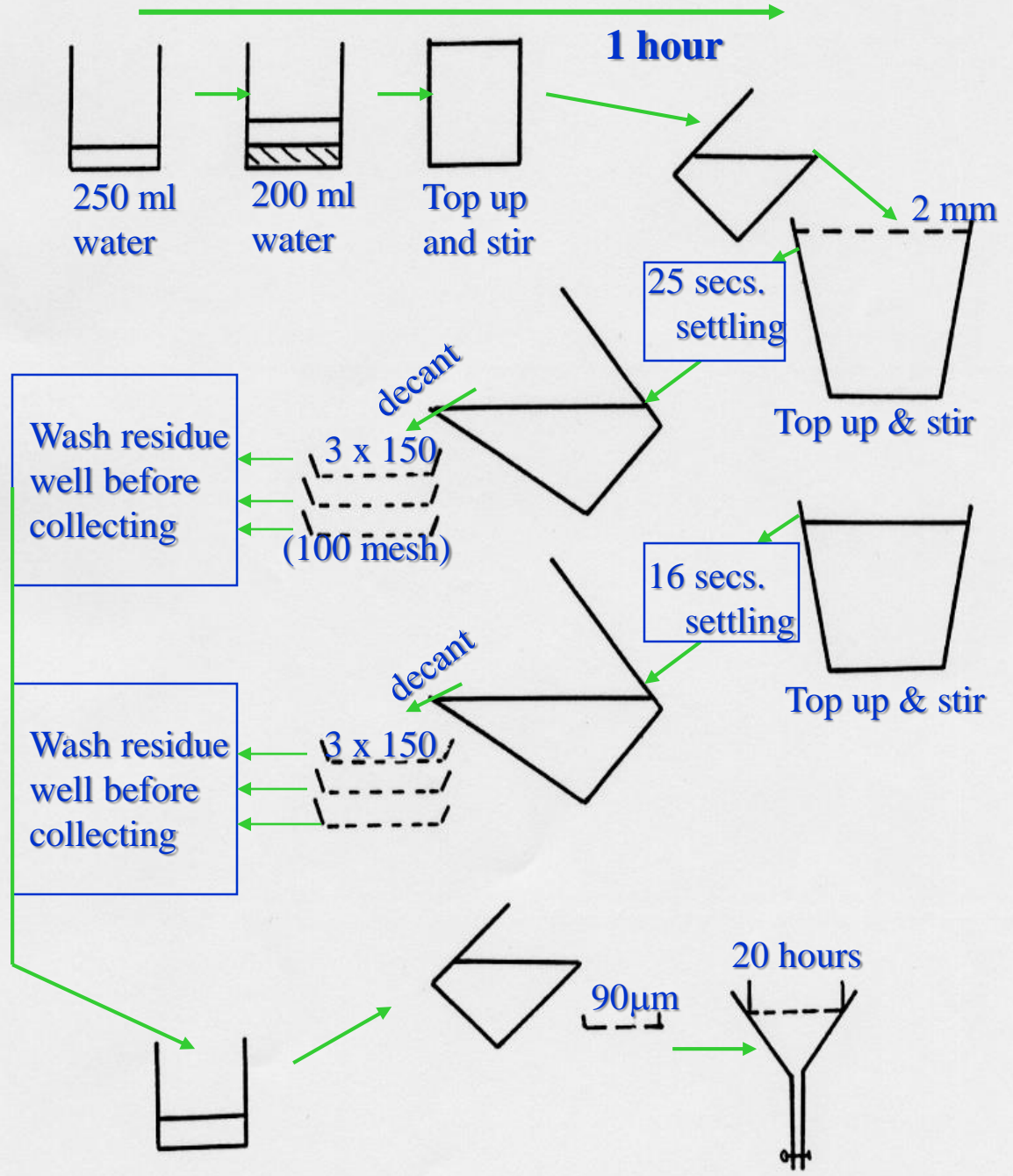
**Many *Longidorus*
and *Xiphinema species*
are vectors of nepoviruses,
so infested plants may have
symptoms of infestation
with these viruses**

**Sample Tobacco Mosaic
Virus of Tomato which
vector are *Xiphinema* spp.**



- *Longidorus* spp. and *Xiphinema* spp. are ectoparasites.
- Many species are vectors of nepoviruses.
- The main method for their detection is analysis of soil samples, which should be taken from root system of tested plants.
- Extraction methods should include Flegg's method, Oostenbrink elutriator, sieving technique, centrifugal flotation, etc.
- The Baermann funnels method is not proper for extraction of these nematodes.

***Flegg's
Method***
-for:
***Xiphinema
Longidorus
Trichodorus
Meloidogyne***
(free living)





Standard Oostenbrink elutriator made of stainless steel (phot. EPPO Website)

**Modified
Oostenbrink
elutriator made of
plastic (producer
MEKU Germany)**

(phot. W.Karnkowski)



**Soil analysis with
modified Oostenbrink
elutriator
(phot. W. Karnkowski)**



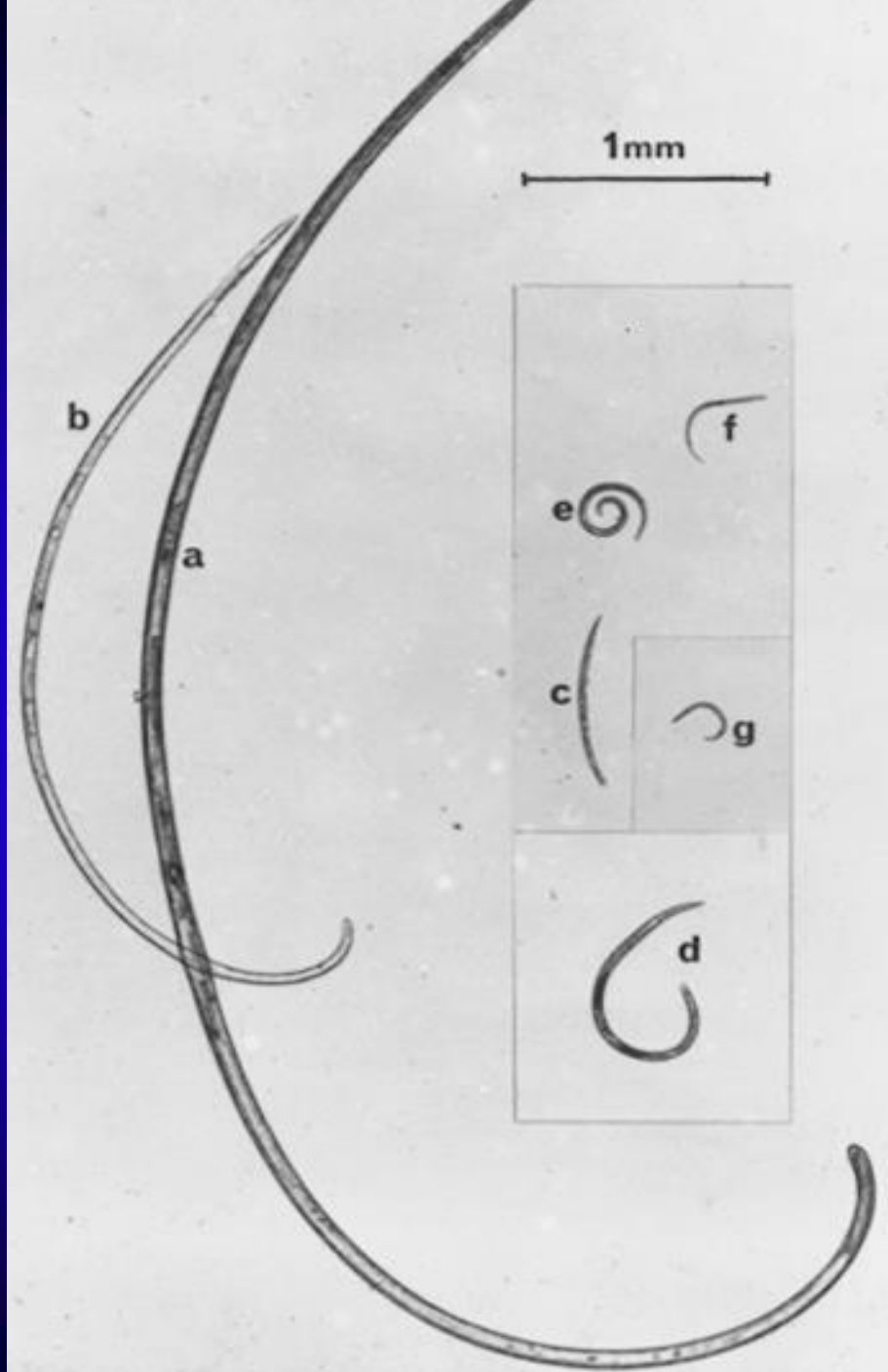
**Analysis of an extract under stereoscopic microscope
(phot. SPHSIS, Voivodship Laboratory in Bydgoszcz, Poland)**



Characters of *Longidoridae* family

- long nematodes 1.5 - 12 mm in length;
- stylet (odontostylet) elongated, not heavily sclerotized, needle-like which comes into odontophore; guiding apparatus with a simple guide ring; stylet knobs are absent;
- oesophagus comprising a narrow, cylindrical anterior section and posterior bulboid expansion which is muscular and glandular; cuticle is smooth
- in anterior part of body there are amphidial apertures in form of small pores which lead back to well developed amphid fovea, pouch-like or stirrup-like;
- nematode juveniles frequently has two stylets – one „active” and the second one „non-active” not removed during moulting.

Size range of plant
parasitic nematodes
(a, b – *Longidoridae*)



Main characters of genus *Longidorus*

- long nematodes 2 - 12 mm in length;
- stylet 58-210 μm in length, elongate, needle-like; guiding apparatus with a simple guide ring situated a couple of head-widths of the anterior end; odontophore about 2/3 of odontostylet in length, thickening slightly in posterior region, but without posterior flanges;
- in anterior part of body there are amphidial apertures in form of small pores which lead back to well developed pouch-like amphid fovea;
- vulva median in position;
- tail short with finely or broadly rounded terminus; male tail without bursa.

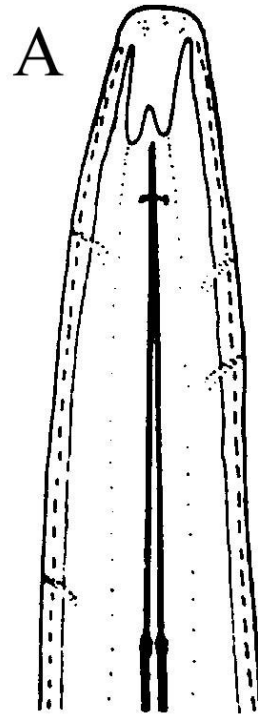
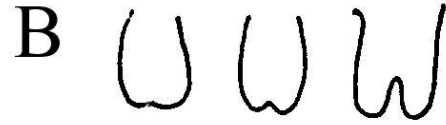
Herbivorous nematode (*Longidorus*)

(source unknown)



Stylet (no knobs)

Longidorus

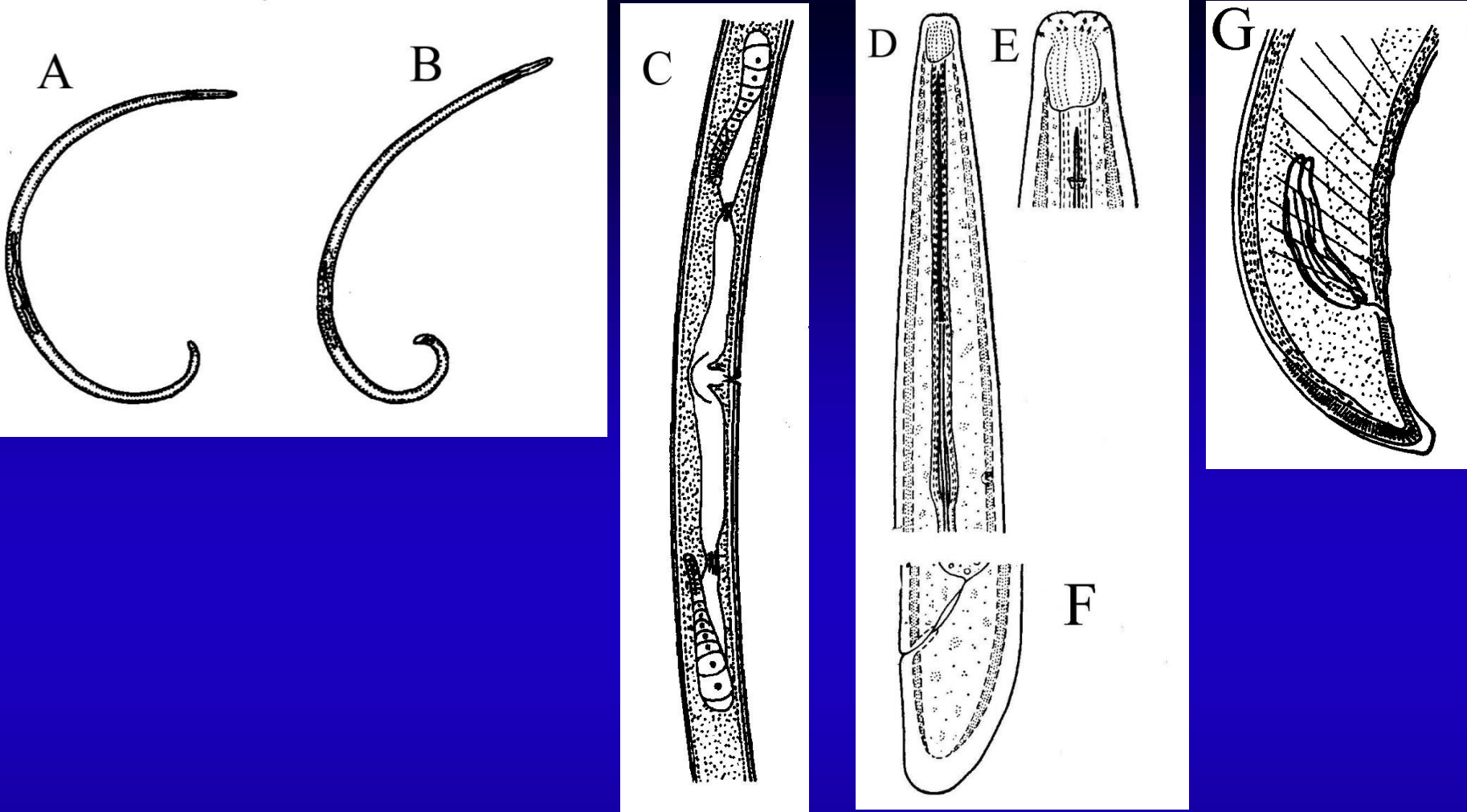


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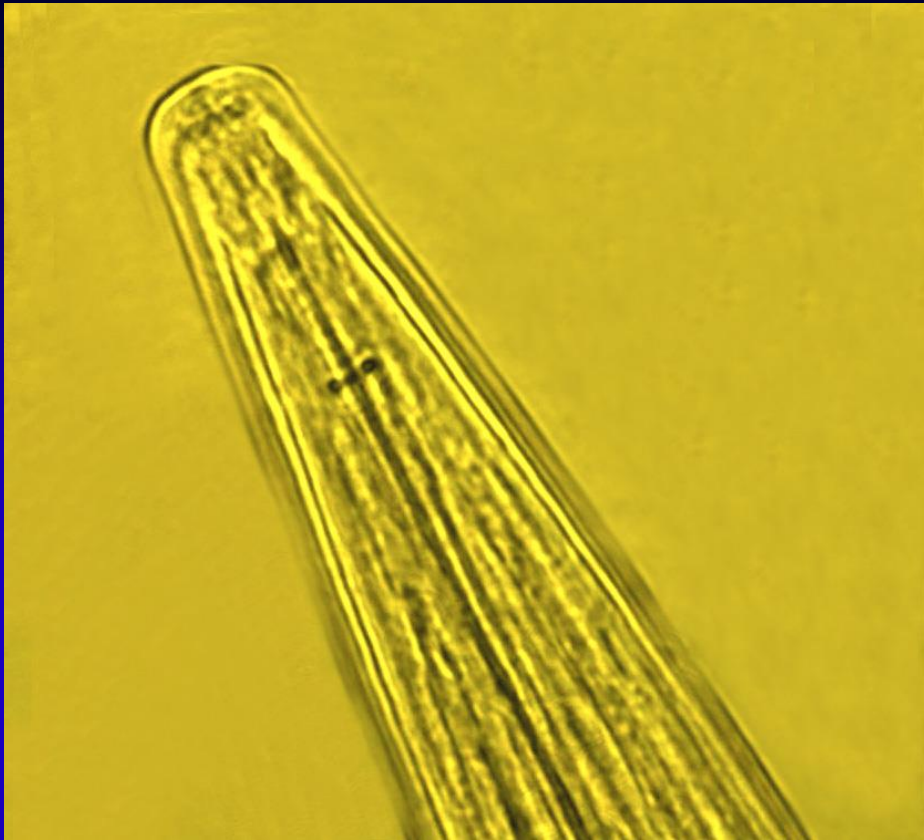
Longidorus:

A. anterior body portion; b. variation of amphid fovea shape; C. odontostylet (1) and odontophore (2).

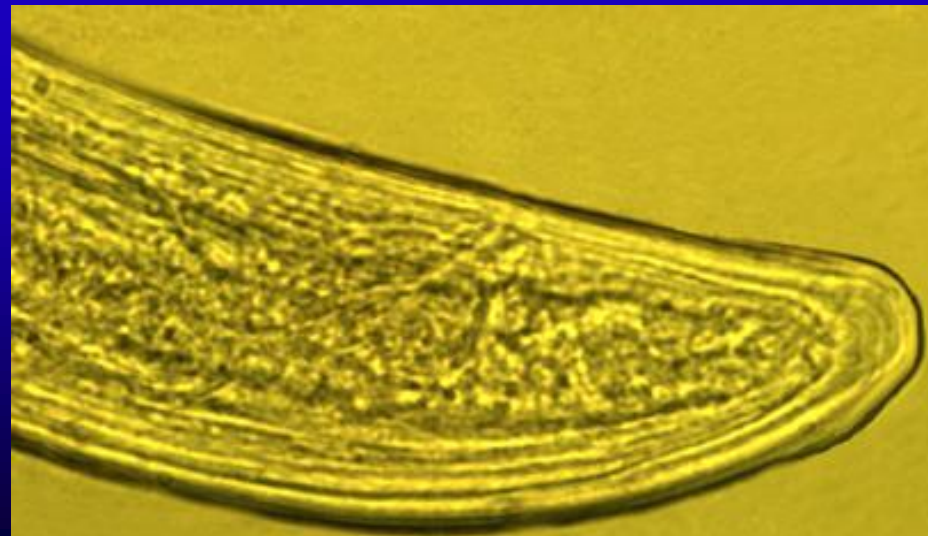


Longidorus elongatus

A. Body shape of female; B. body shape of male; C. female vulval region; D. anterior body portion; E. lip region; F. female tail; G. male tail.



Longidorus elongatus
female head and tail
(phot. W.Karnkowski)



Longidorus diadecturus Eveleigh et Allen –
EU quarantine species (I/A1)

Host plant

Main host plant is peach. This species was also noted on grape and cucumber.

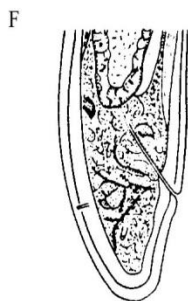
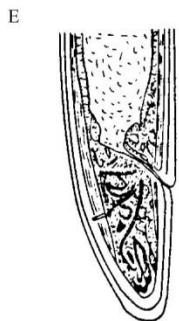
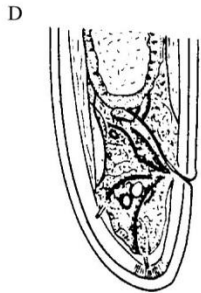
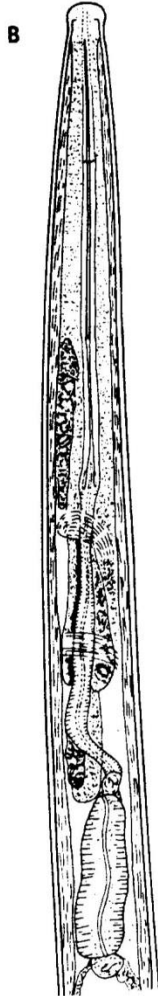
Vector of *Peach rosette mosaic nepovirus* (PRMV) and *Tomato black ring nepovirus* (TBRV).

Geographical distribution

North America: Canada (described as new species in Ontario, in 1982); USA.

Morphological features

- no males have been found.
- female length 3.32-4.02 mm (mean 3.71 mm);
- stylet length 109-121 μm .; odontophore length 56-66 μm ; stylet + odontophore length – 168-187 μm ;
- stylet guide ring 56-66 μm posterior to head end;
- amphid indistinct, pouch-like without lobes;
- tail terminus bluntly rounded;
- main female measurements: a = 74-92; b = 8-13; c=122-177; c' 0.77-0.94; V=44-48%; tail length 25-29 μm ; juvenile tail is more elongated, as c' of J3 juvenile is 1.0-1.2 and J4 juvenile 1.3-1.4.



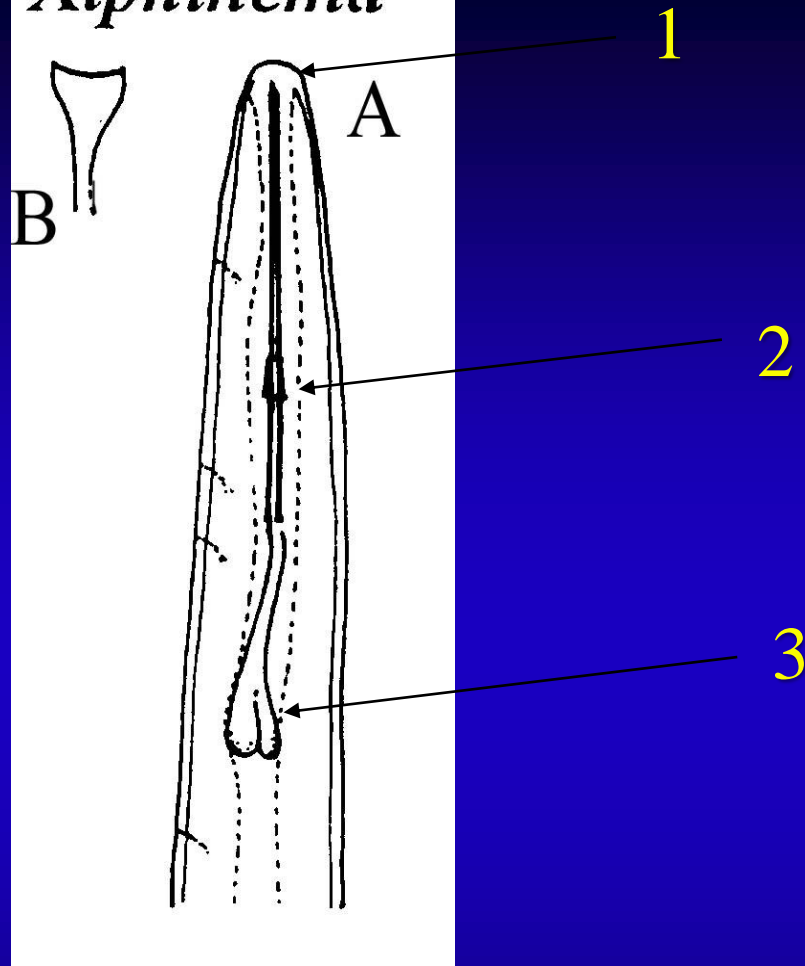
Longidorus diadecturus

A. anterior end of female;
B. oesophageal region of female; **C.** reproductive system; **D.** female tail;
E. third stage larval tail;
F. fourth stage larval tail.

Characters of genus *Xiphinema*

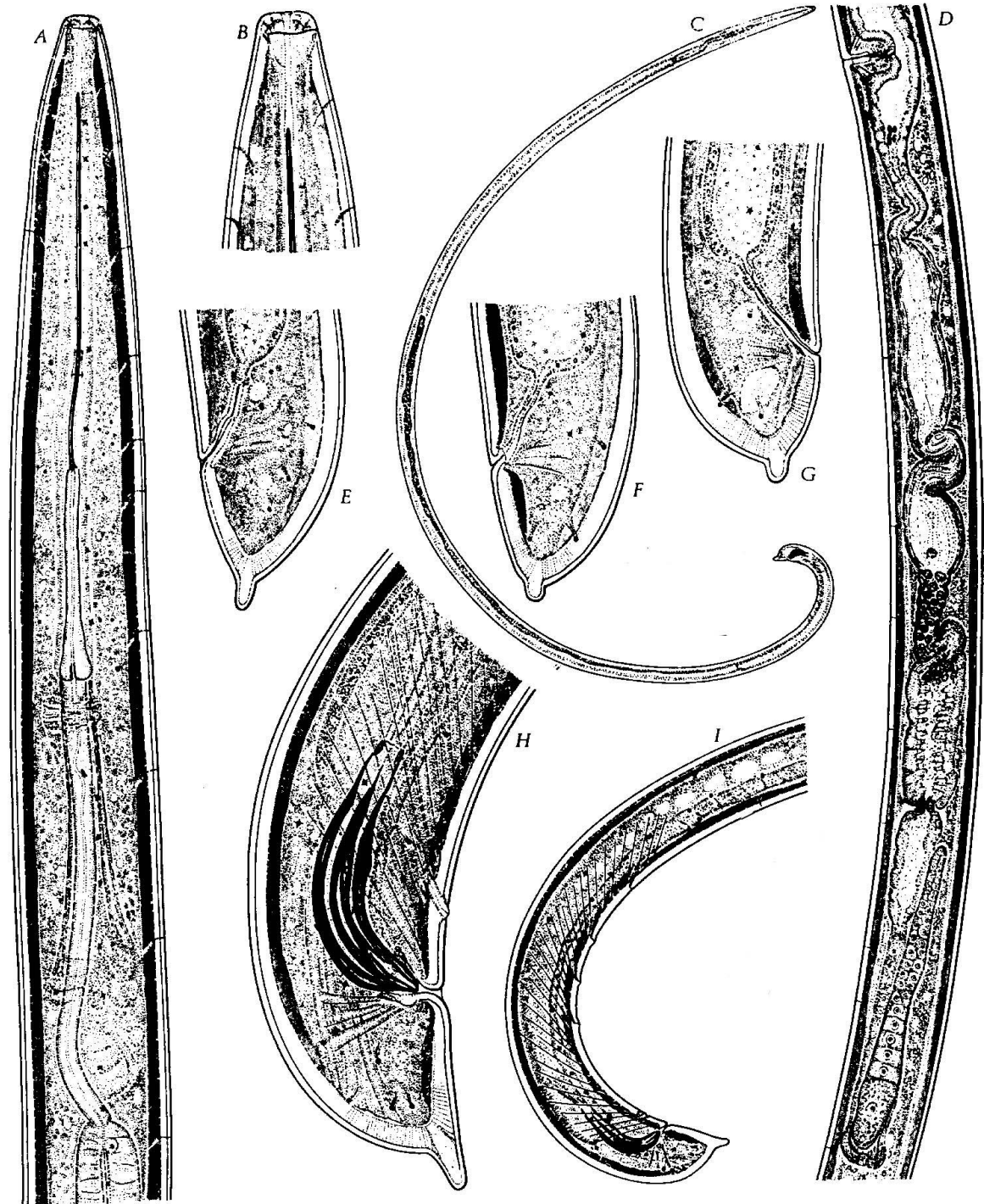
- long nematodes 1.5 - 6 mm in length;
- stylet elongate, needle-like heavily sclerotized; guiding apparatus with a guide ring located posteriorly close to odontostylet/odontophore junction; posterior end of stylet appearing forked at its junction with odontophore; odontophore strongly developed with three massive posterior flanges;
- in anterior part of body there are amphidial apertures in form of small pores which lead back to well developed stirrup-like or funnel-shaped amphid fovea;
- vulva anteriorly to post-median;
- tail form variable from short hemispheroid to long conoid and then attenuating to filiform terminal section; with or without process.

Xiphinema



***Xiphinema*:**

A. anterior body portion: odontostylet (1), guide ring (2) and odontophore (3); B. amphid fovea shape.



*Xiphinema
diversicaudatum*
A–B. anterior body
portion; C. entire
nematode;
D. posterior branch of
female reproductive
system; E–G. female
tail; H–I. male tail.



Xiphinema diversicaudatum
female head and tail
(phot. W. Karnkowski)



Xiphinema americanum sensu lato (non-European populations)

- ▶ **Host plant**
- ▶ Nematodes belonging to the *X. americanum* group have a very wide host range of both herbaceous and woody plants in agriculture, horticulture and forestry.
- ▶ Nematodes belonging to *X. americanum sensu lato* in North America have been shown to be natural vectors of some economically important nepoviruses, such as *Cherry raspberry leaf virus* (Cheravirus) (CRLV), *Peach rosette mosaic virus* (Nepovirus) (PRMV), *Tobacco ringspot virus* (Nepovirus) (TRSV) and *Tomato ringspot virus* (Nepovirus) (ToRSV).

Xiphinema americanum sensu lato (non-European populations)

- ▶ **Geographical distribution**
- ▶ The highest importation as virus vector has species from North America (Canada, Mexico and USA). Some species also occur on other continents (Australia, Belize, Brazil, Chile, Guatemala, India, Japan, Korea (North and South, Mexico, New Zealand, Pakistan, Panama, Sri Lanka, South Africa, Uruguay).
- ▶ Some species occur also in Europe.

Characters of *Xiphinema americanum sensu lato*#

1. body length small to medium (L varies from 1.2 to 3.0 mm)
2. body shape assumes a more or less open C to spiral shape when heat-relaxed .
3. lip region rarely continuous, usually demarcated by a shallow depression or deep constriction
4. guide ring is more anterior and the folded part of the guiding sheath is shorter than in other *Xiphinema* species
5. odontostyle robust, length rarely exceeding 150 μm
6. pharyngeal bulb usually with thick platelet reinforcements of the lumen wall; bulb not offset from the rather wide slender part
7. nuclei in the pharyngeal bulb: dorsal nucleus is often recorded as further from the dorsal orifice and the subventral nucleus is placed more posteriorly than in other *Xiphinema* species
8. V% around or behind the middle of the body (V% = 42–65)
9. female genital branches equally developed but generally short; short or very short uteri without Z-differentiation or spines and usually with weakly developed sphincter muscles
10. compact ovaries, comprising rather few and narrow germ cells and typically associated with verrucomicrobial endosymbionts.
11. tail short, conoid, rounded to slightly digitate, rarely broadly rounded; tail terminus generally pointed or rounded
12. males rare, females devoid of sperm
13. male usually with 5–11 ventromedian supplements, with the most posterior lying closer to the paired precloacal papillae (adanal papillae) than in other *Xiphinema* species (i.e. within spicula range)
14. three or four juvenile stages.

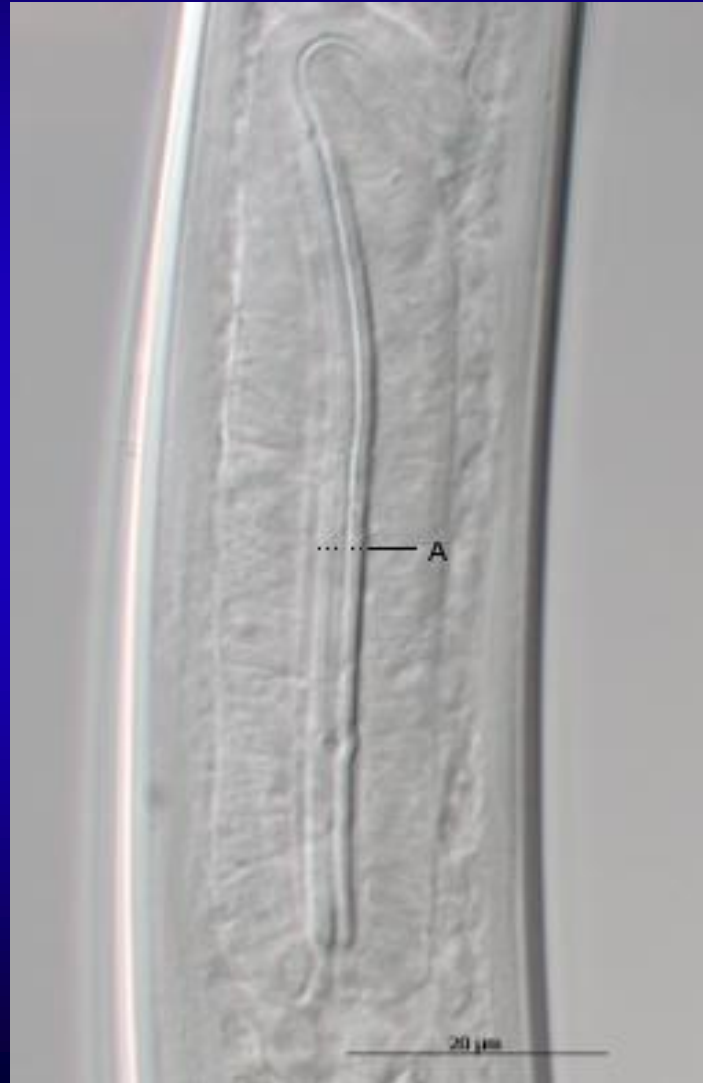
Body shape assuming a more or less pronounced spiral shape when heat relaxed (source: IPPC draft diagnostic protocol)

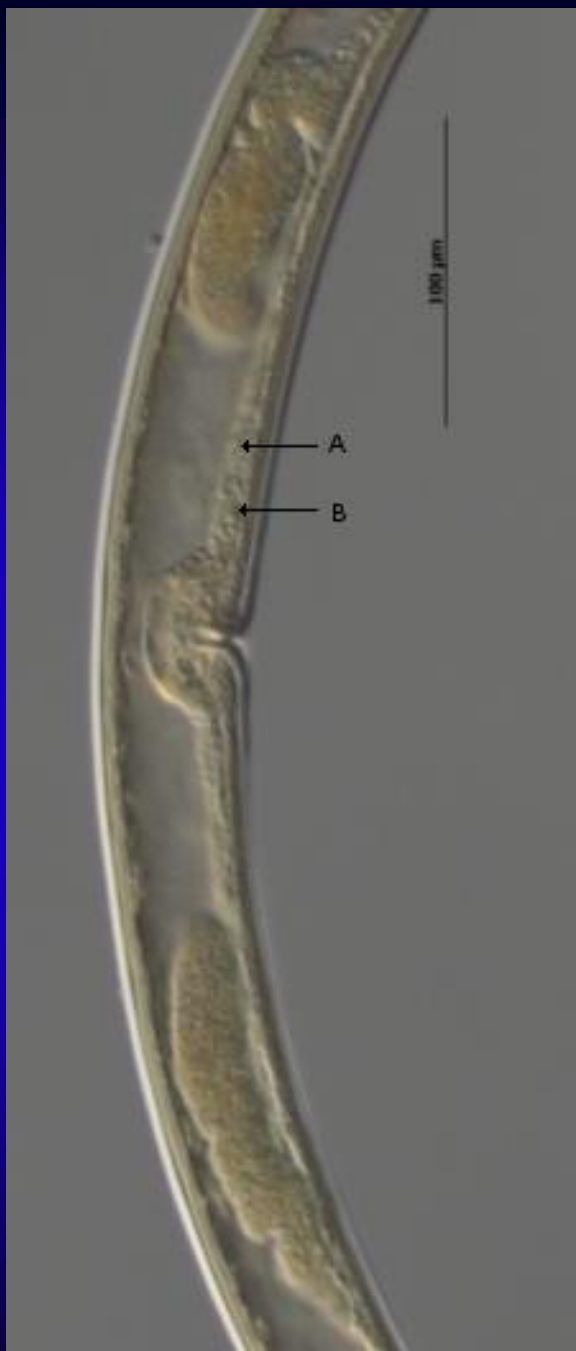


Lip region demarcated by a constriction (A) and relative position of guide ring (B) and anterior part of guiding sheath (C) (source: IPCC draft diagnostic protocol)



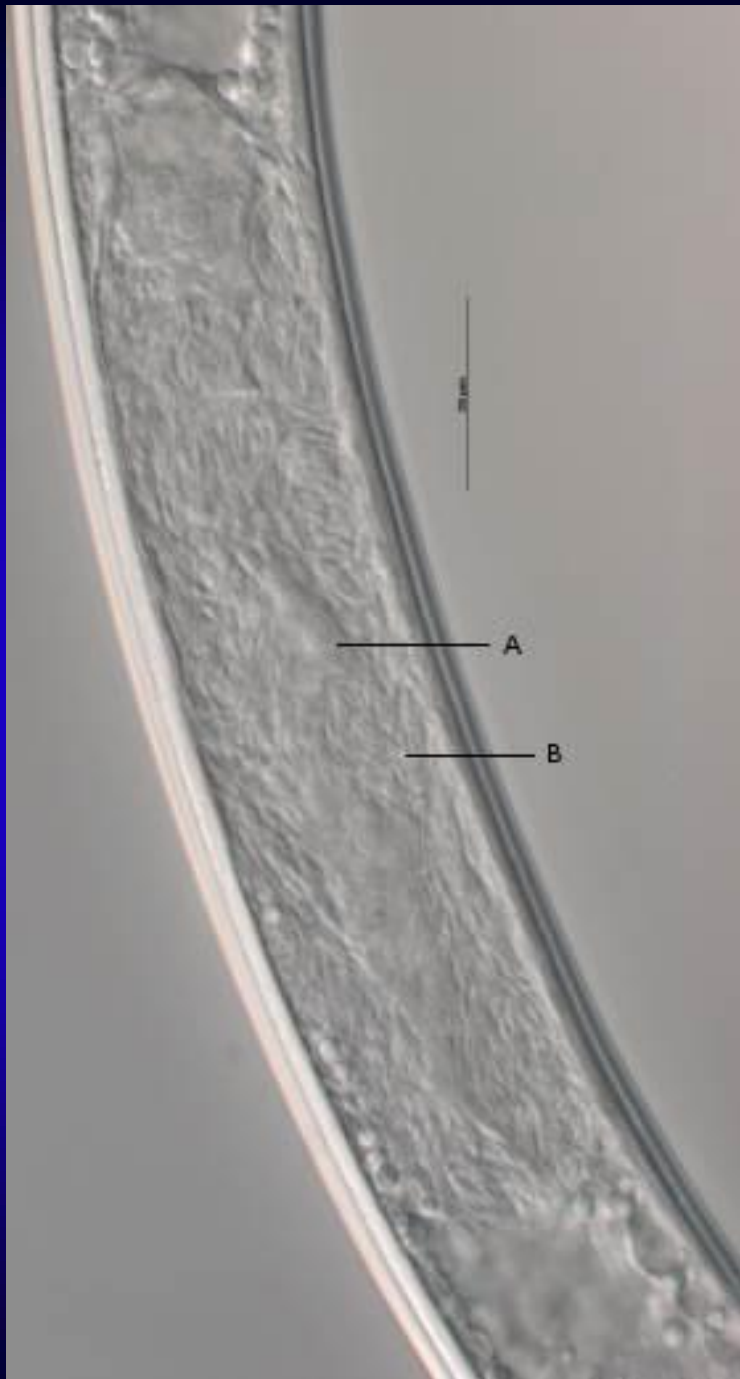
Pharyngeal bulb showing platelet reinforcements of the lumen wall (A). (source: IPPC draft diagnostic protocol)





Female genital branches equally developed but relatively short. Uteri without Z-differentiation or spines (A) and usually with weakly developed sphincter muscles (B) (source: IPPC draft diagnostic protocol)

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Compact ovaries, comprising rather few and narrow germ cells (A) and typically associated with verrucomicrobial endosymbionts (B) (source: IPPC draft diagnostic protocol)

.



Anterior ovary of
Xiphinema non-americanum
group with no
verrucomicrobial bacteria
present
(source: IPPC draft diagnostic
protocol)

Tail terminus generally pointed (fig. 1, 2); or rounded (fig. 3, 4) (source: IPCC draft diagnostic protocol)



1



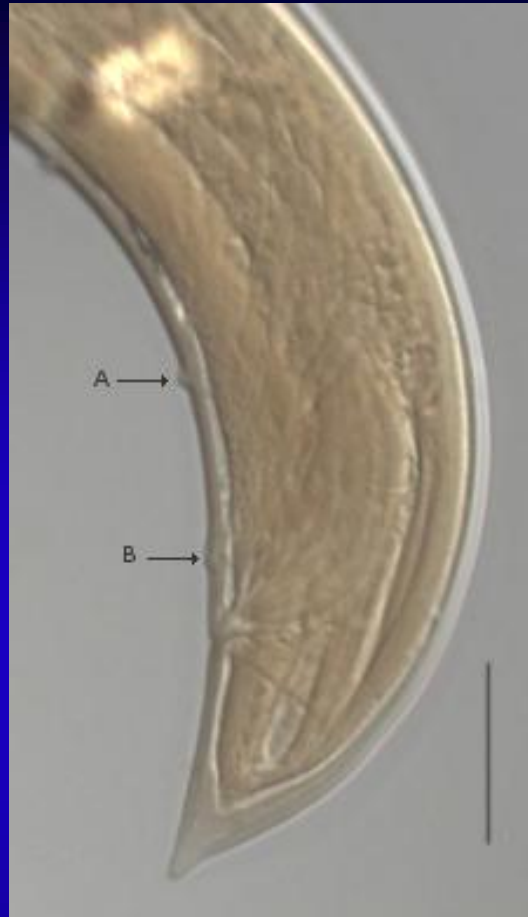
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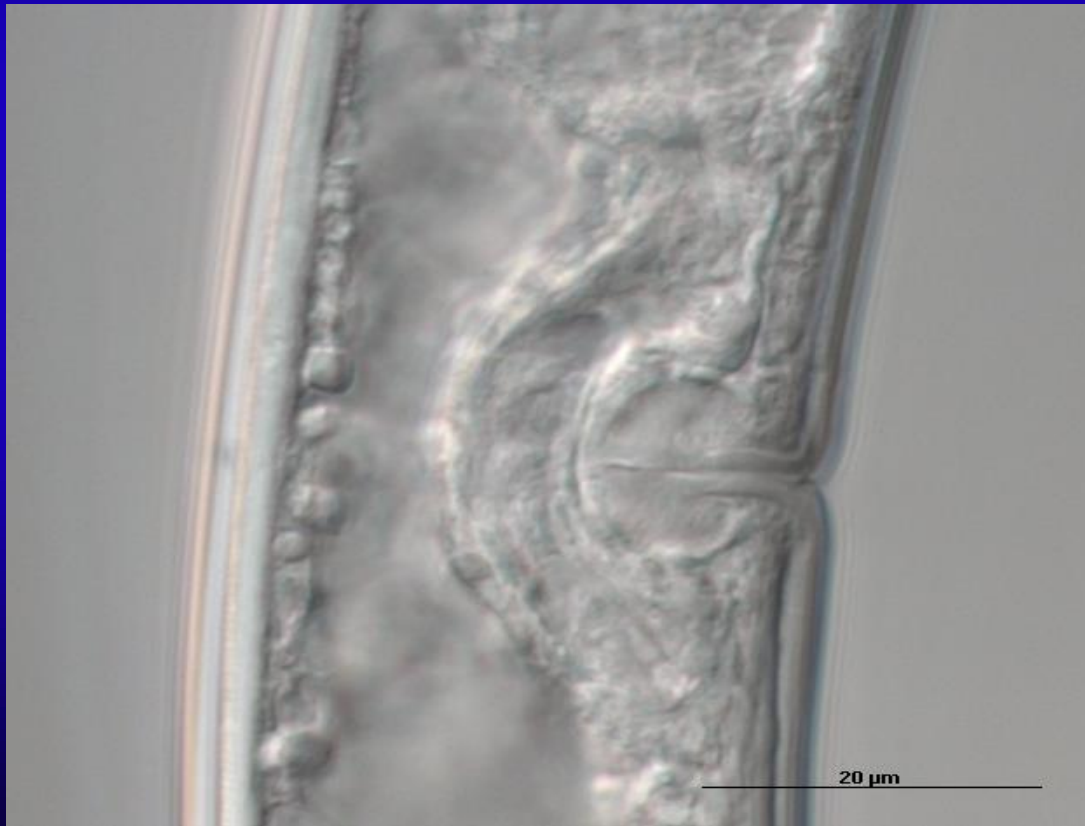


- *Xiphinema americanum* group - male spicular region and posterior ventromedian supplements, with posteriormost (A) lying closer to the precloacal papillae (adanal papillae (B)) (source: IPPC draft diagnostic protocol)

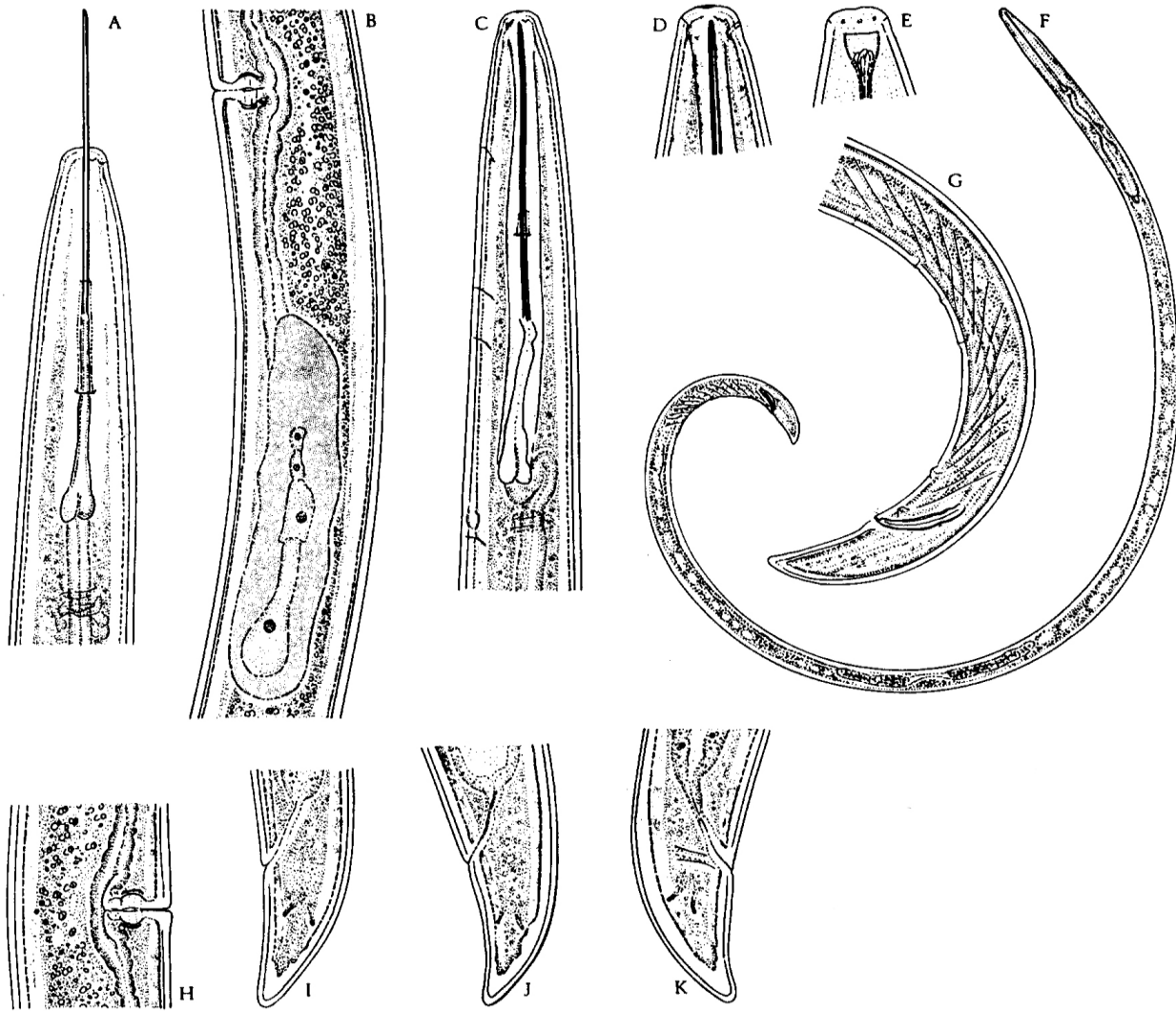


- *Xiphinema non-americanum* groups - male spicular region and posterior ventromedian supplements, with posteriormost (A) lying further from the precloacal papillae (adanal papillae (B)) (source: IPPC draft diagnostic protocol)

An additional character: the vagina has a rounded kidney-like appearance (source: IPCC draft diagnostic protocol)



*Xiphinema
americanum
sensu stricto*



**A, C. anterior
body portion;
D, E. head;
G – male tail;
I-K. female tail,
B. posterior
branch of female
reproductive
system;
F. entire male
H. female vulval
region.**

Xiphinema californicum Lamberti et Bleve-Zacheo - EU quarantine species (I/A1)

- ▶ **Geographical distribution**
- ▶ **North America:** Mexico, USA (California);
- ▶ **South America:** Brazil, Chile, Peru.

Morphology

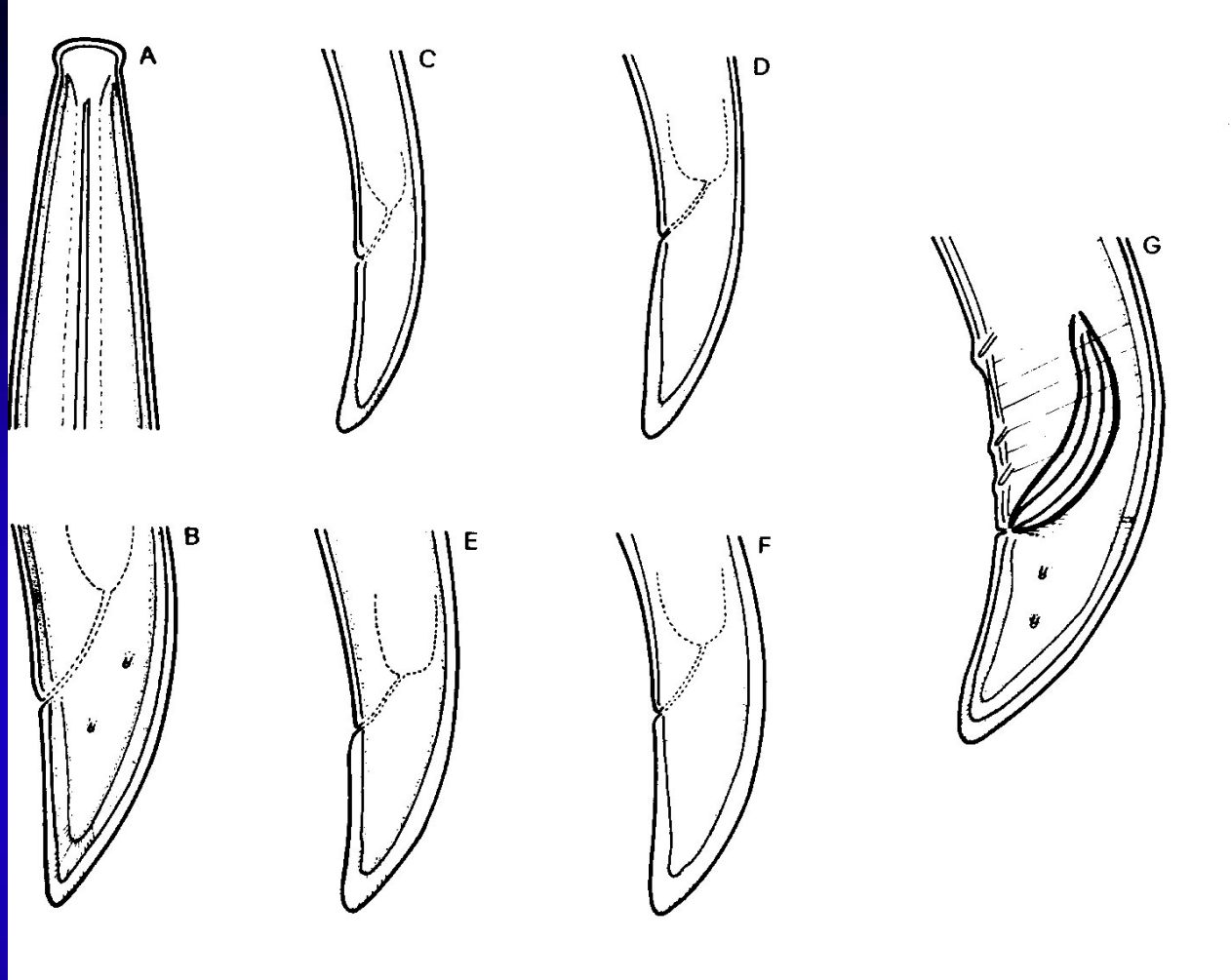
▶Female:

- body slender, C-shaped when relaxed;**
- head high, well expanded and clearly separated from rest of the body by an incisure;**
- vagina occupying $\frac{1}{4}$ – $\frac{1}{2}$ of the corresponding body diameter;**
- tail variable, elongated conical, pointed or narrow rounded from to pairs of papillae**

Morphology

▶Male:

- males are very rare;
- biometrically and morphologically similar to the female but more coiled in the posterior region;
- tail is slightly concave ventrally;
- measurements (one male from type material): L=1,8 mm, a=68; b=5.9; c=61; c'=1,5; odontostylet = 89 μm ; odontophore = 48 μm ; odontophore + odontostylet =137 μm ; oral aperture to guiding ring = 78 μm ; spiculs = 35 μm .



Xiphinema californicum

A. anterior body portion of female; B. female tail; C-F. tails of I, II, II and IV juvenile stages respectively; G male tail.



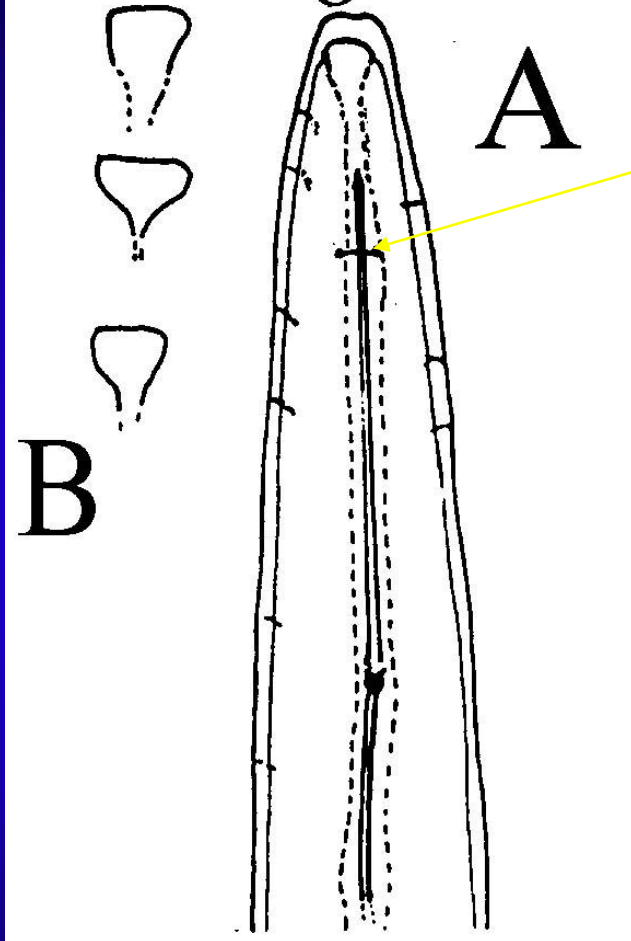
Xiphinema californicum

Variation of female heads (A-D) and tails (E-H) in populations from California (paratypes; A, E, F) and Peru (B, C, D, G, H). (phot. Alkamede and Loof).

Main characters of genus *Paralongidorus*

- **long nematodes up 12 mm in length;**
- **head continuous with body or offset by a constriction;**
- **stylet elongate, needle-like; guiding apparatus with a simple guiding ring situated markedly posterior to head, more than third of stylet length; odontophore without basal flanges;**
- **in anterior part of body there are amphidial apertures in form of transverse slits which lead back to well developed from stirrup-shaped to funnel-like amphid fovea;**
- **vulva median to post-median in position;**
- **tail short, rounded but may be conoid or hemispheroid.**

Paralongidorus



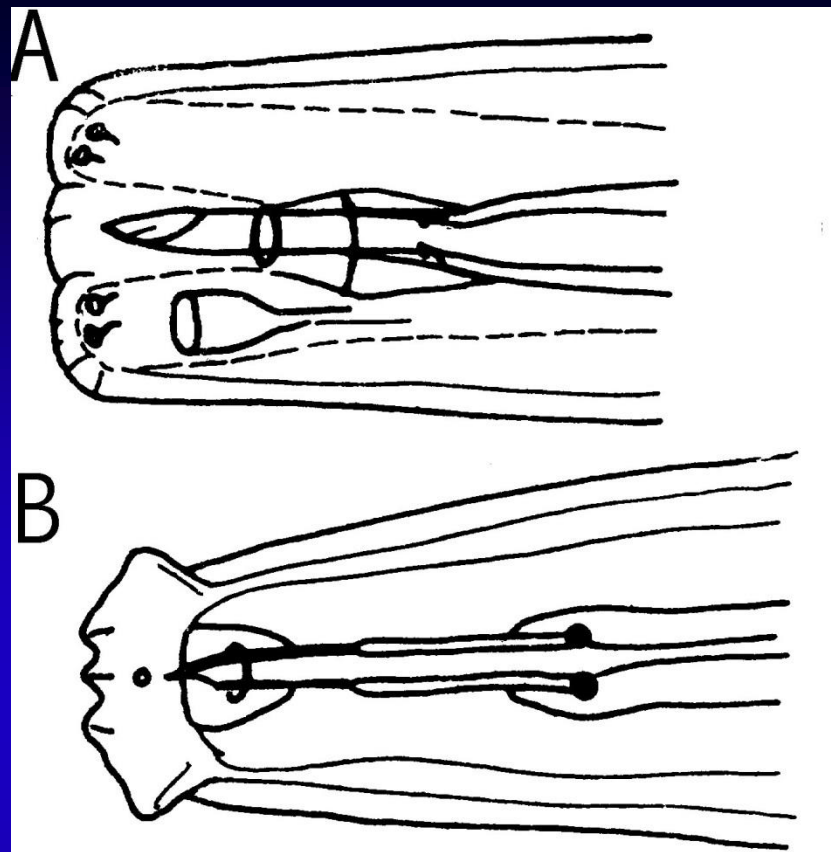
Paralongidorus

A. anterior body portion: 1. guiding ring,

B. amphid fovea shape; C. head of *Paralongidorus maximus*
(phot. Thomas Prior, FERA, UK)

Nematodes from *Dorylaimida*, families other than *Longidoridae*

- long nematodes 0.8 – 8.5 mm in length;
- odontostylet usually strong, not needle-like, usually don't exceed 50 μm in length; guiding ring is present or absent, depending on species; odontophore absent; sometimes stylet with small basal thickenings;
- tail from short, rounded to elongated, filiform.

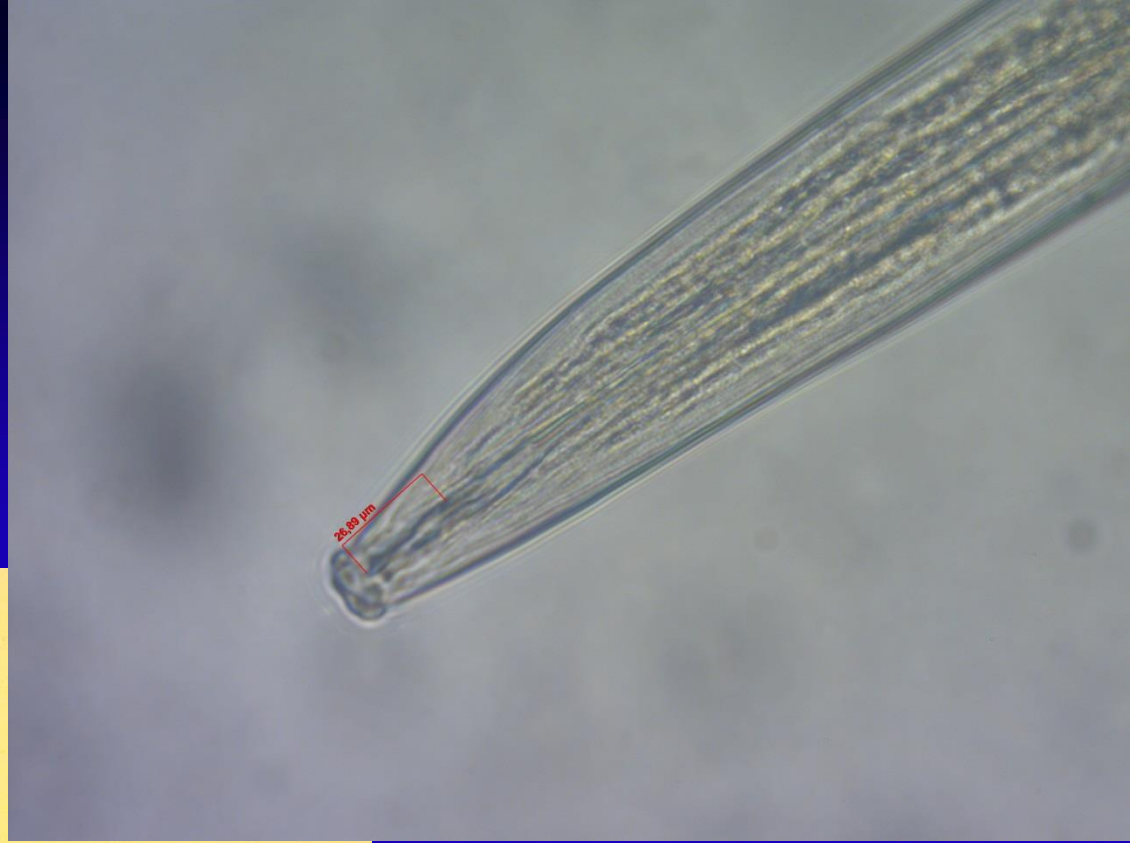


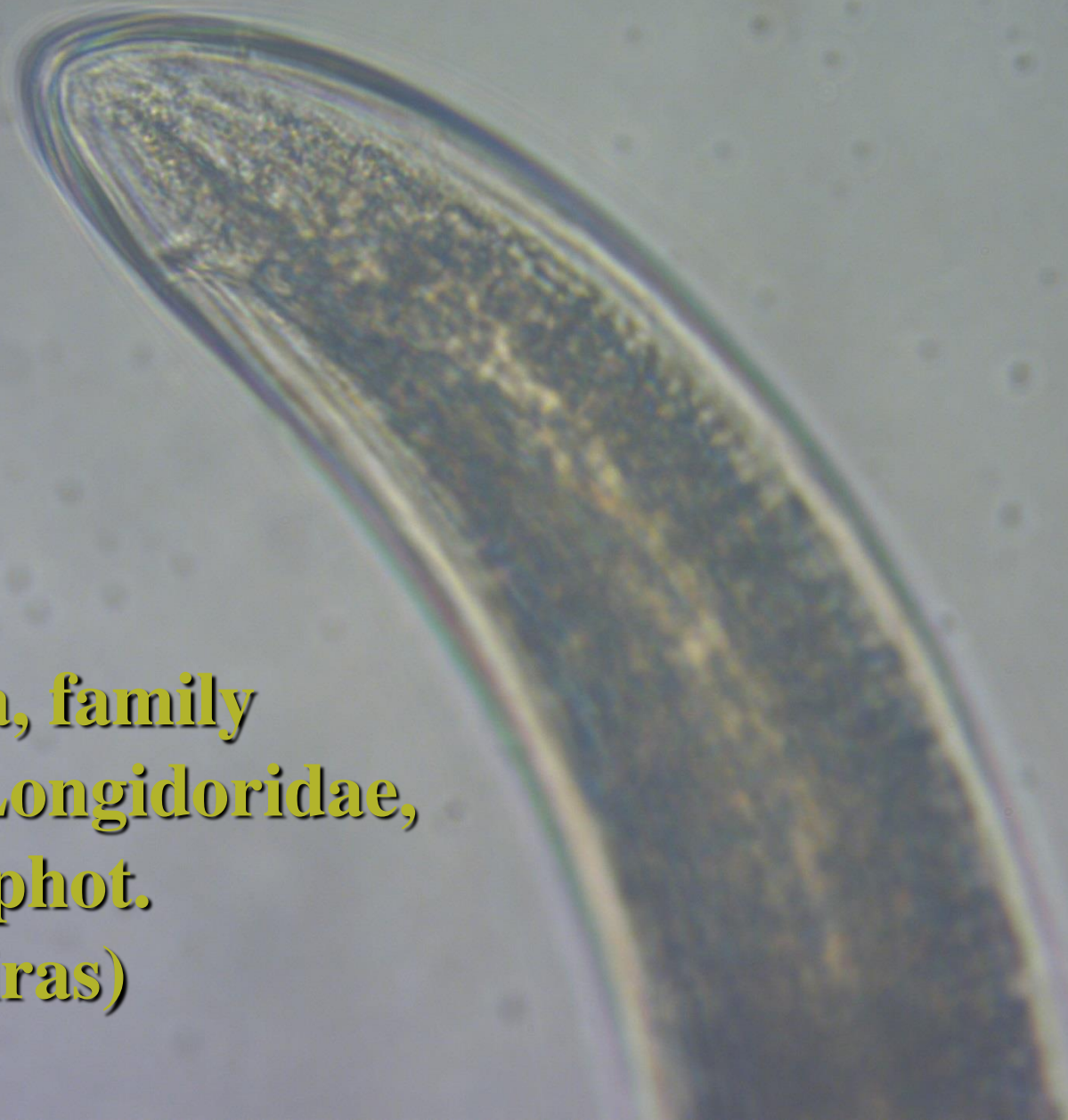
Head of nematodes from Dorylaimida but not from *Longidoridae* family:

A. stylet without basal thickenings (*Dorylaimus* sp.);

B. stylet with basal thickenings (*Tylencholaimus* sp.).

**Dorylaimida, family
other than
Longidoridae,
female head
(phot. Nadejda
Poiras)**



A high-magnification micrograph showing the tail region of a female nematode. The tail is elongated and tapers to a point, with a distinct, textured internal structure. The surrounding background is a light, uniform color with some faint, out-of-focus spots.

**Dorylaimida, family
other than Longidoridae,
female tail (phot.
Nadejda Poiras)**



**Other large nematodes:
saprobiontic nematode,
female head and tail
(phot. Nadeida Poiras)**



Large nematodes may be misidentified with *Oligochaeta* occurring commonly in soil, which may be extracted together with nematodes: length from 1.5 mm even up to 1 m. Important – body is divided into annules (source unknown).