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

***MELOIDOGYNE CHITWOODI* Golden et. al., 1980**

***MELOIDOGYNE FALLAX* Karssen, 1996**

Detection and identification basing on morphological and morphometric characters

GEOGRAPHICAL DISTRIBUTION

M. chitwoodi

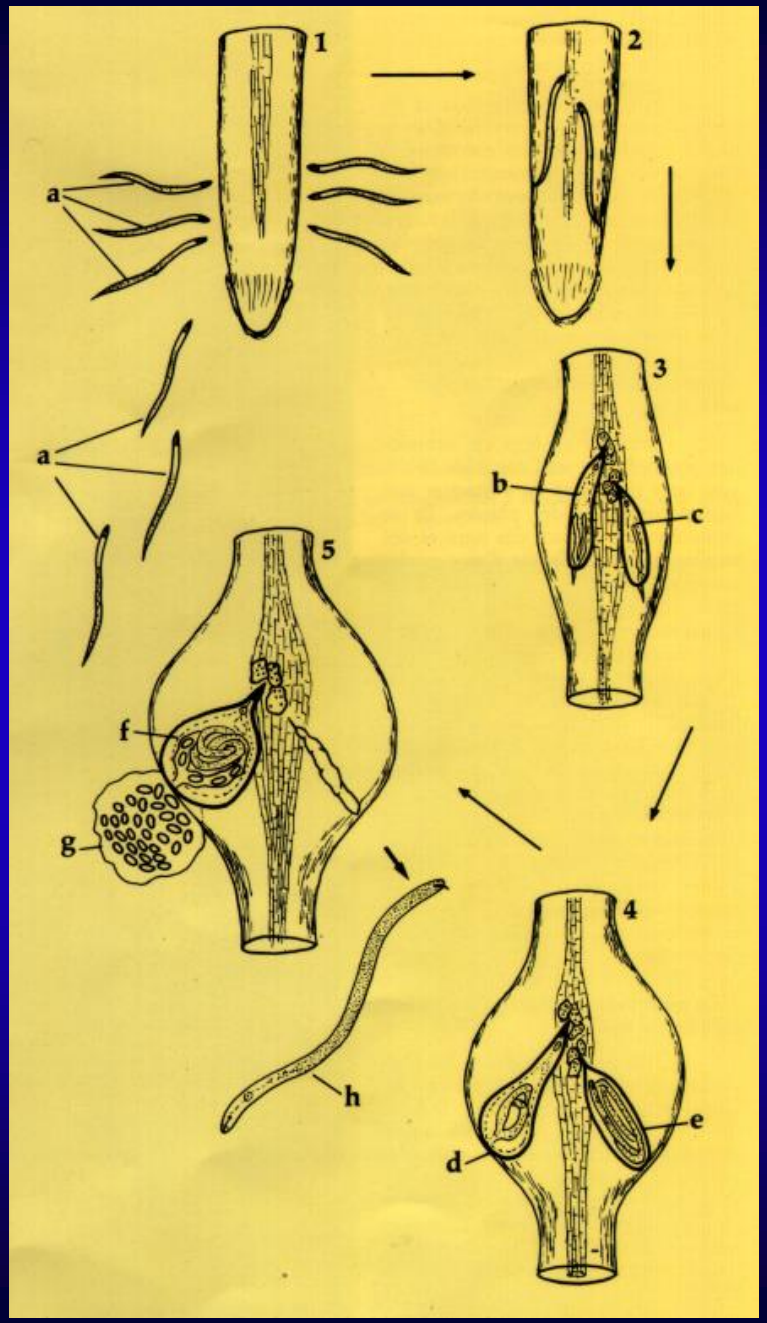
Europe: Belgium, France, Germany, the Netherlands, Portugal, Turkey, the United Kingdom
Africa: South Africa
North America: Canada, USA
South America: Argentina

M. fallax

Europe: Belgium, France, Germany, the Netherlands, Switzerland
North America: USA
Oceania: Australia, New Zealand

HOST PLANTS

- **The root-knot nematodes *Meloidogyne chitwoodi* and *Meloidogyne fallax* parasitize monocotyledons and dicotyledons, including several crop plants such as potatoes, vegetables, such as carrots, beet, black salsify and tomatoes, some ornamental plants, cereals, grasses, maize and lucerne.**
- **Host range of both species is similar, but development of one species is better on some hosts than the second one.**
- **Especially important is development of both species on potatoes as they infest tubers of this plant and may be easily spread with them.**



Developmental cycle of root-knot nematodes on roots of hosts plants

1. J_2 juveniles (a) enter root
2. J_2 juveniles become sedentary
3. J_2 juveniles begin to develop to females (b) and males (c); beginning of forming of galls on the roots.
4. Further development of female (d) and male (e).
5. Adult females, pear-shaped (f) are sedentary in roots; they lay eggs to gelatinous egg sacs; adult males (h) leave roots; they apparently do not feed.

SYMPTOMS OF INFESTATION

- **Above-ground symptoms of heavily infested plants include stunting and yellowing, while below ground galling is typical.**
- **The root galls produced by *M. chitwoodi* and *M. fallax* are comparable to those produced by several other root-knot species, i.e. relatively small galls in general without secondary roots emerging from them (as in *M. hapla*).**
- **On potato tubers, *M. chitwoodi* and *M. fallax* may cause numerous small pimple-like raised areas on the surface. But they are not observed in each case.**
- **In some cases, for instance if some potato cultivars are infested, although heavily infested, potato tubers may be free from visible external symptoms, while the internal potato tissue is necrotic and brownish, just below the peel.**

Black salsify (skorzonera) plants (left) infested with *M. chitwoodi* (left) compared with helthy ones (right).

(source unknown)

Galls caused by *M. chitwoodi* on young sugar beet plant

(source unknown)

Small galls caused by *M. chitwoodi* on tomato roots (phot. Central Laboratory of SPHSIS, Toruń, Poland)

Galls caused by *Meloidogyne hapla* (left) i *M. chitwoodi* (right).
(phot. Renata Dobosz, Institute of Plant Protection - Governmental Research Institute, Poznań, Poland)

Tropical *Meloidogyne* species occurring in Europe in glasshouses and in the south part of the continent outdoors, cause usually much bigger root galls.

Galls on tomato roots caused by *Meloidogyne incognita* (left) dompared with healthy roots (right) (source unknown).

Tomato root system infested with *Meloidogyne* sp. (right) compared with not infested one (left)

(phot. extension.entm.purdue.edu)

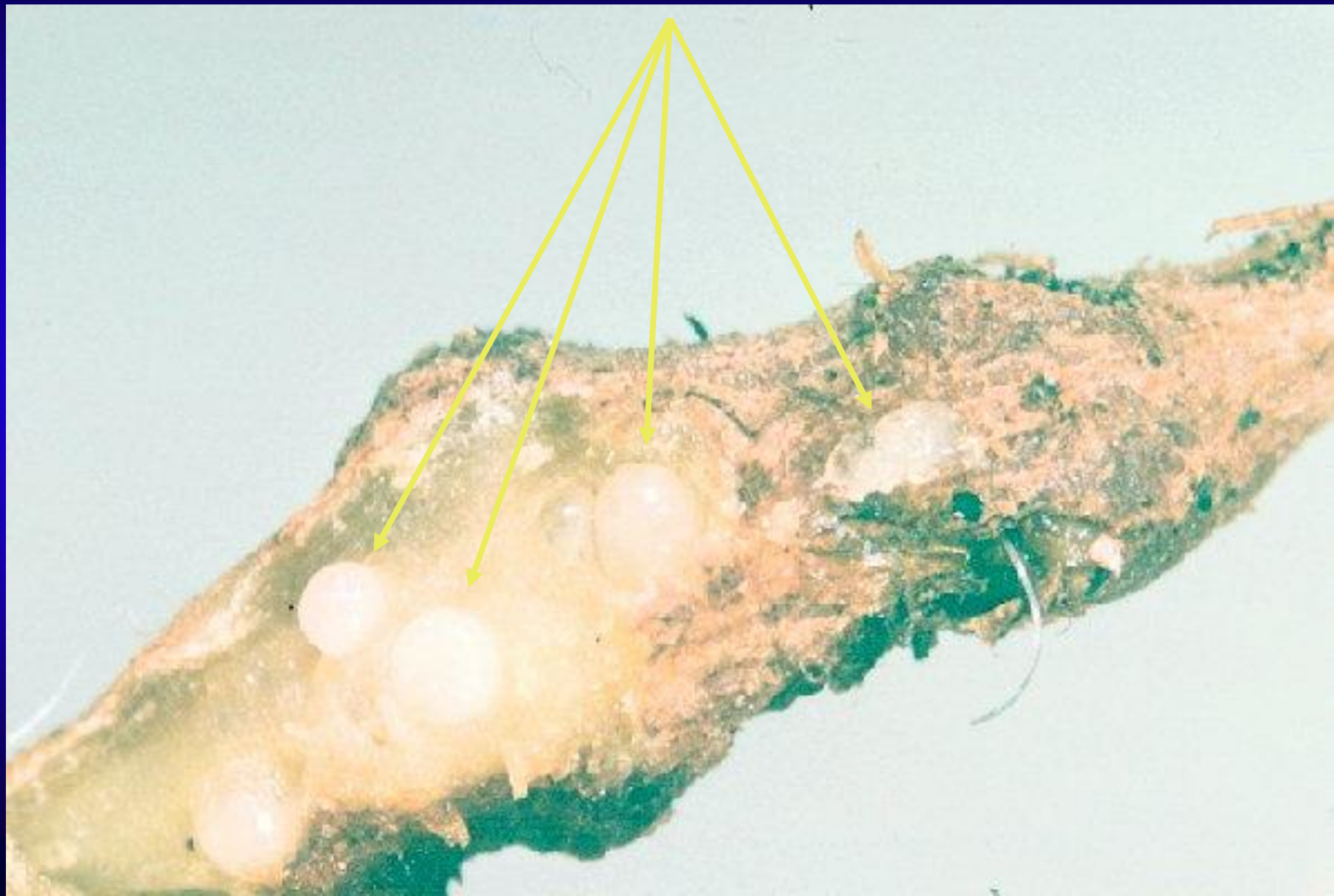
Galls on tomato roots caused by *Meloidogyne ethopica* in Slovenia (phot. Dr S. Širca (EPPO Website))

Galls on tomato roots caused by *Meloidogyne enterolobii* in Switzerland (phot. Agroscope (EPPO Website))

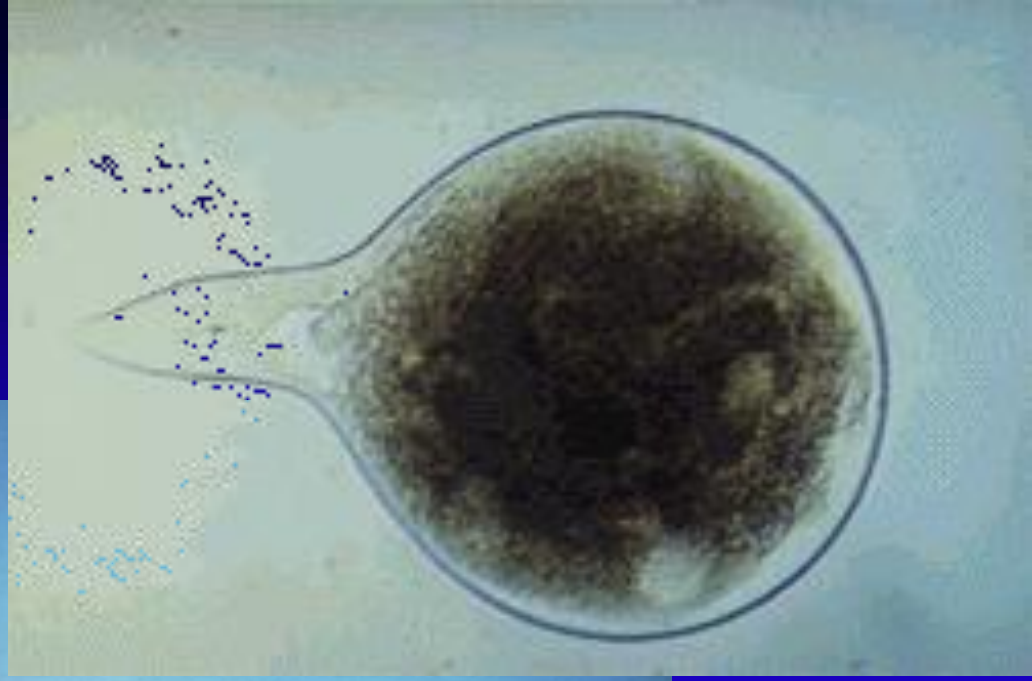
Meloidogyne naasi on wheat root
(phot. Institute of Plant Protection, Poznań, Poland)

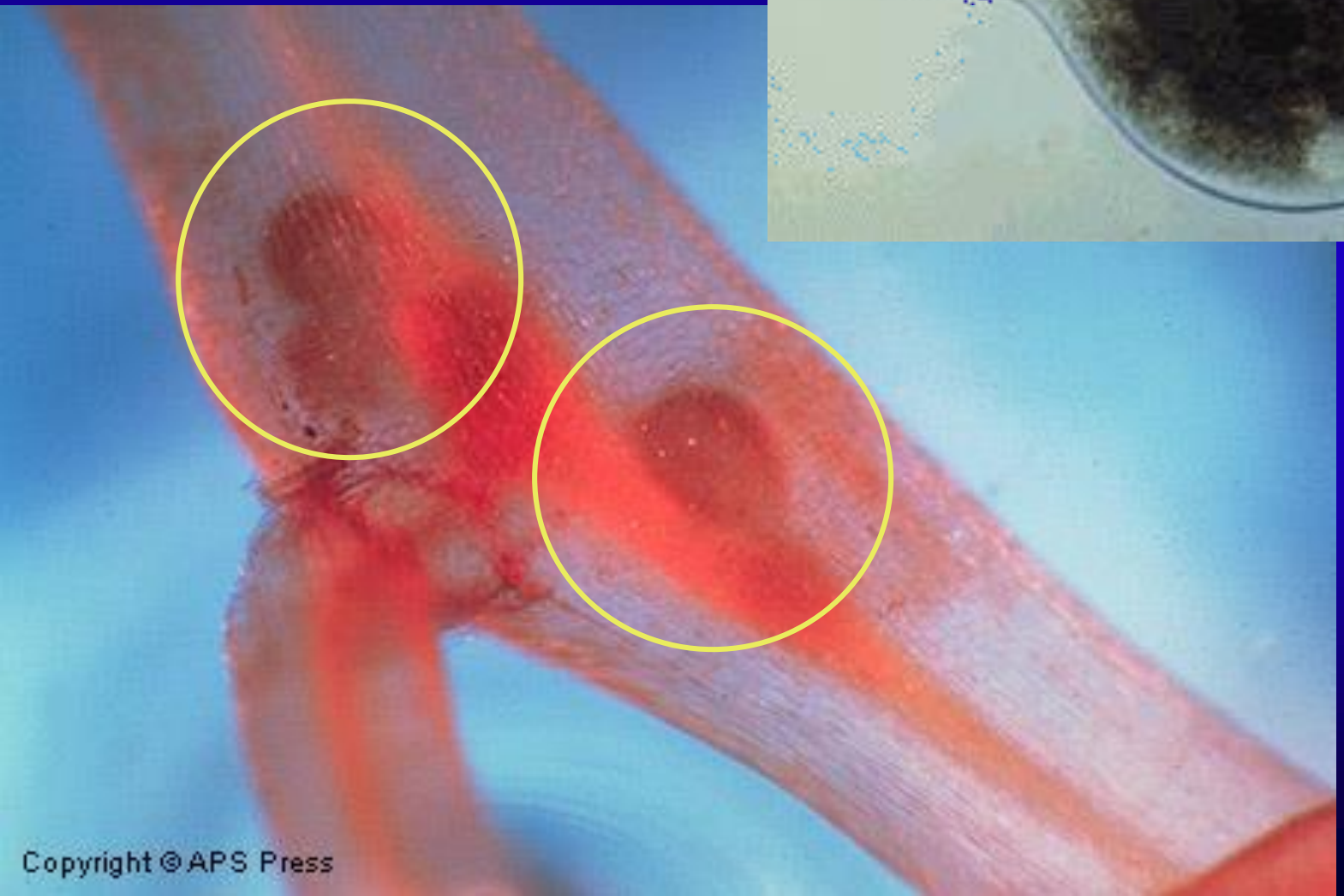
Root nodules caused by nitrogen-fixing bacteria
(*Rhizobium* and *Bradyrhizobium*) (left) compared with *Meloidogyne* galls (right)
(phot. Institute of Plant Protection, Poznań, Poland)

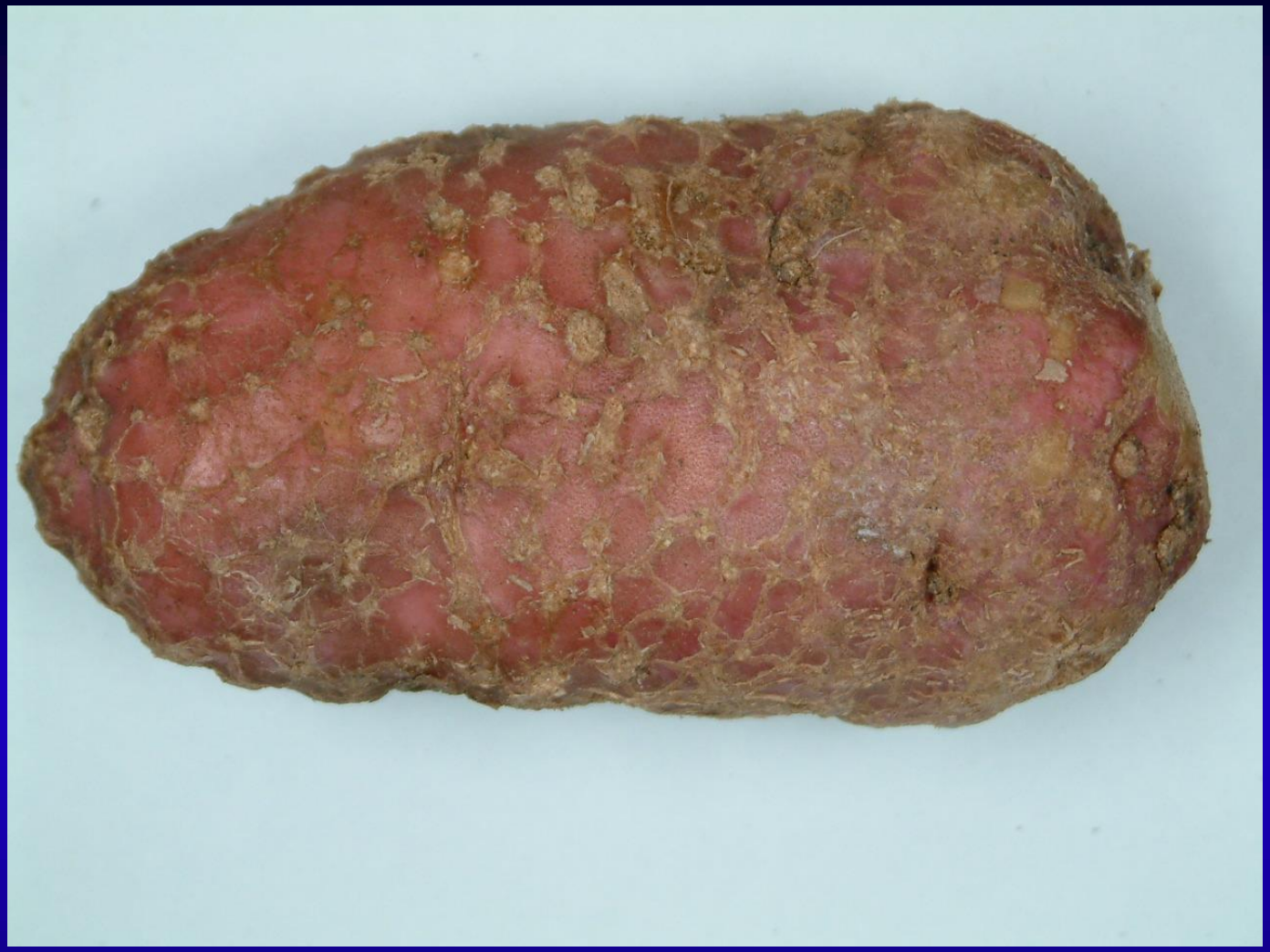
Females of *Meloidogyne hapla* on infested roots (phot. Institute of Plant Protection, Poznań, Poland)



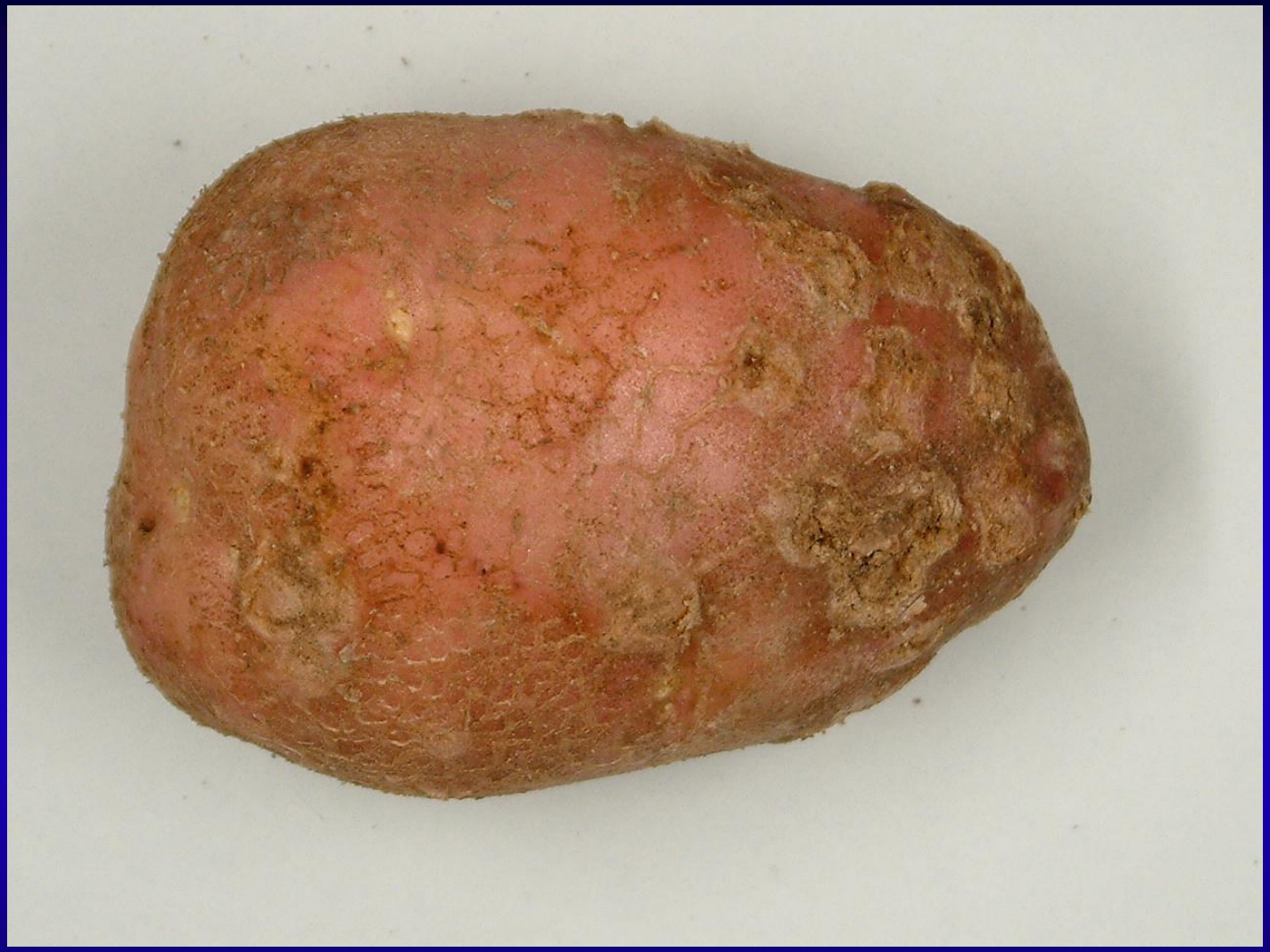
Strawberry root with stained *M. hapla* females and isolated female





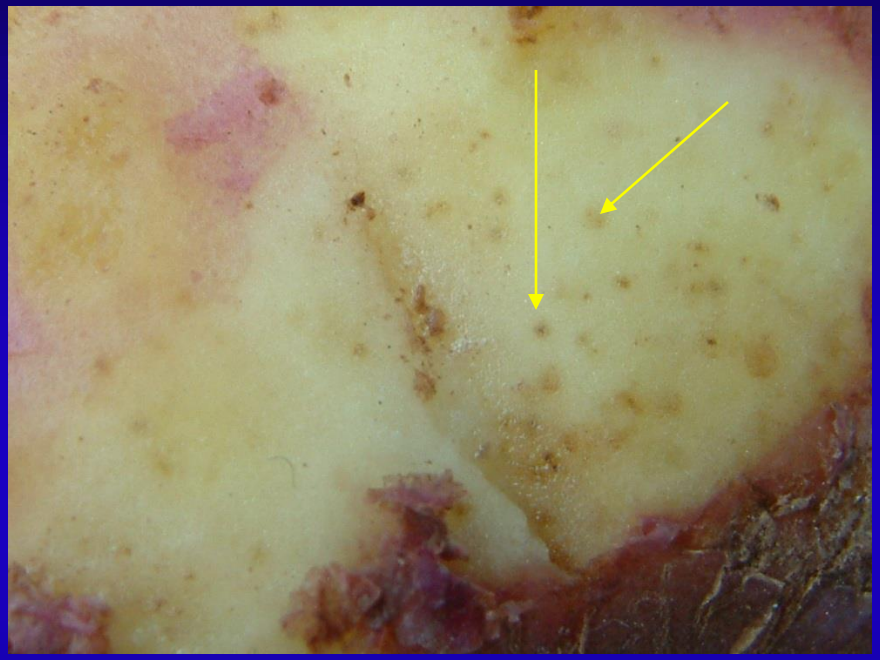


Potato tuber infested with *Meloidogyne fallax* – shortly after harvest (phot. Central Laboratory of SPHSIS, Toruń, Poland)

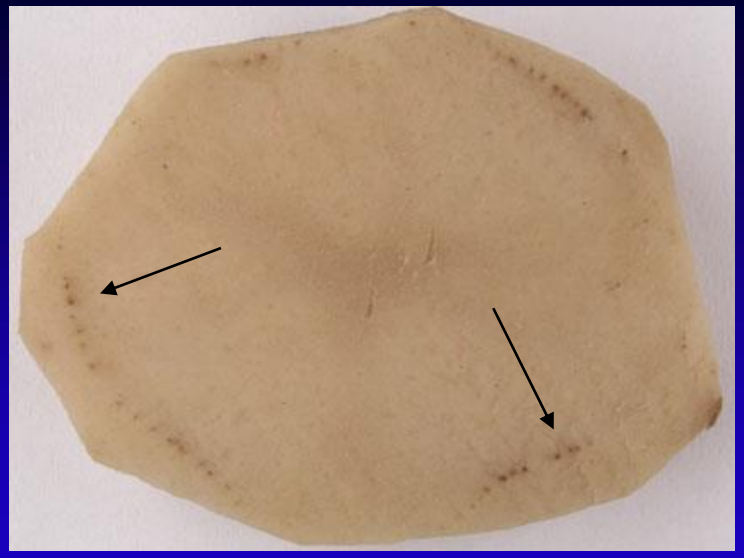


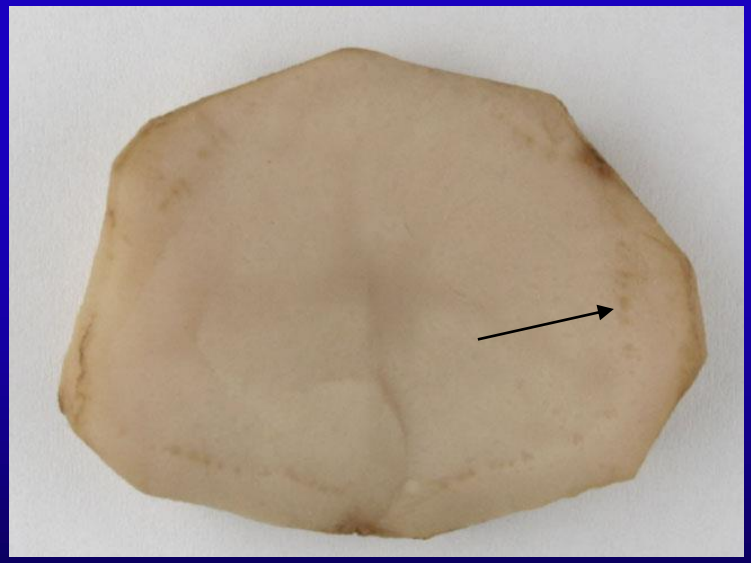
Potato tuber infested with *Meloidogyne chitwoodi* – shortly after harvest (phot. Central Laboratory of SPHSIS, Toruń, Poland)

Potato tuber infested with *Meloidogyne chitwoodi* – after storage at 4°C for a few months (phot. Central Laboratory of SPHSIS, Toruń, Poland)

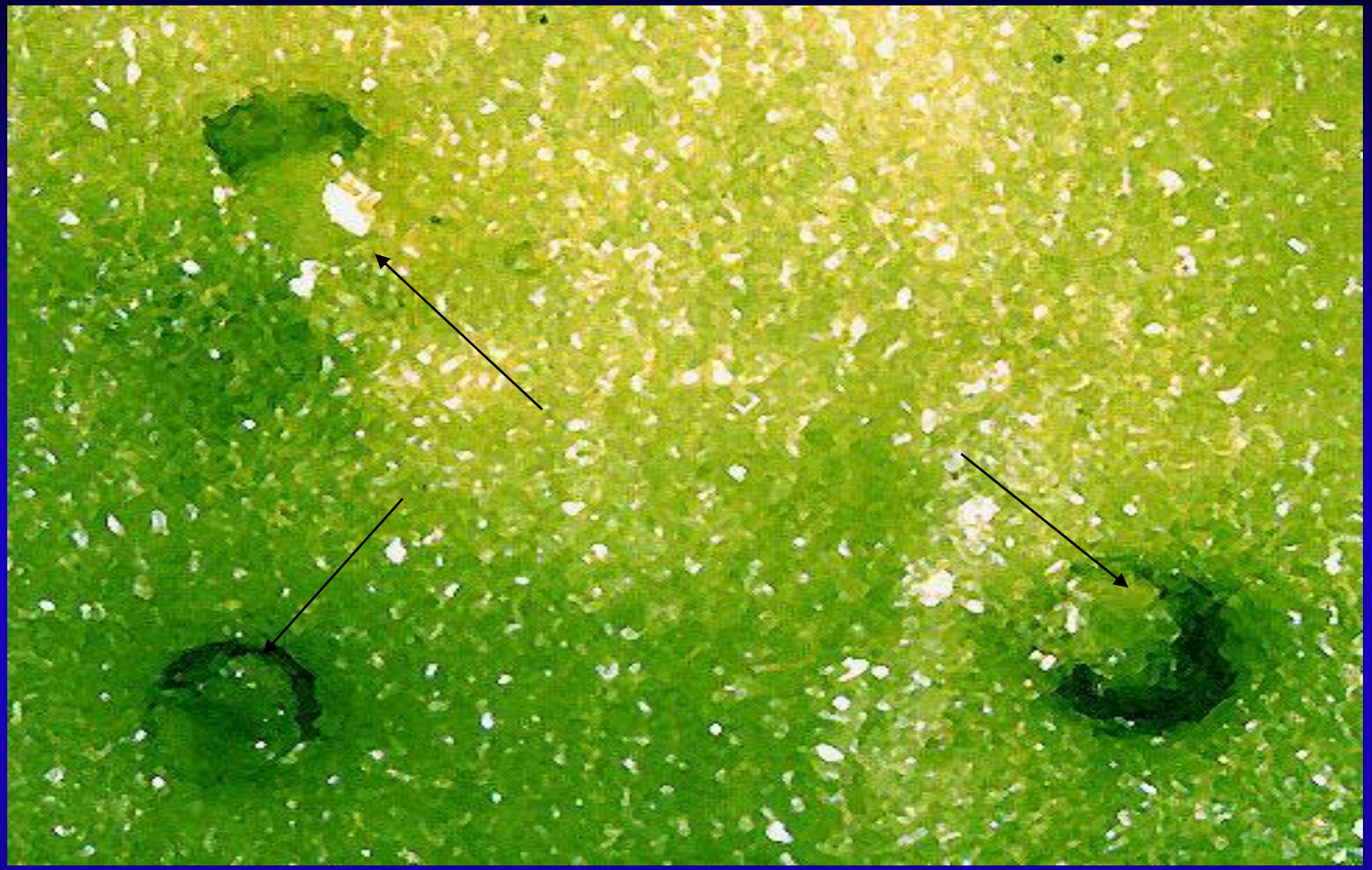


Brown necrotic spots at places of occurrence of *Meloidogyne chitwoodi* females at potato flesh (phot. Central Laboratory of SPHSIS, Toruń, Poland)

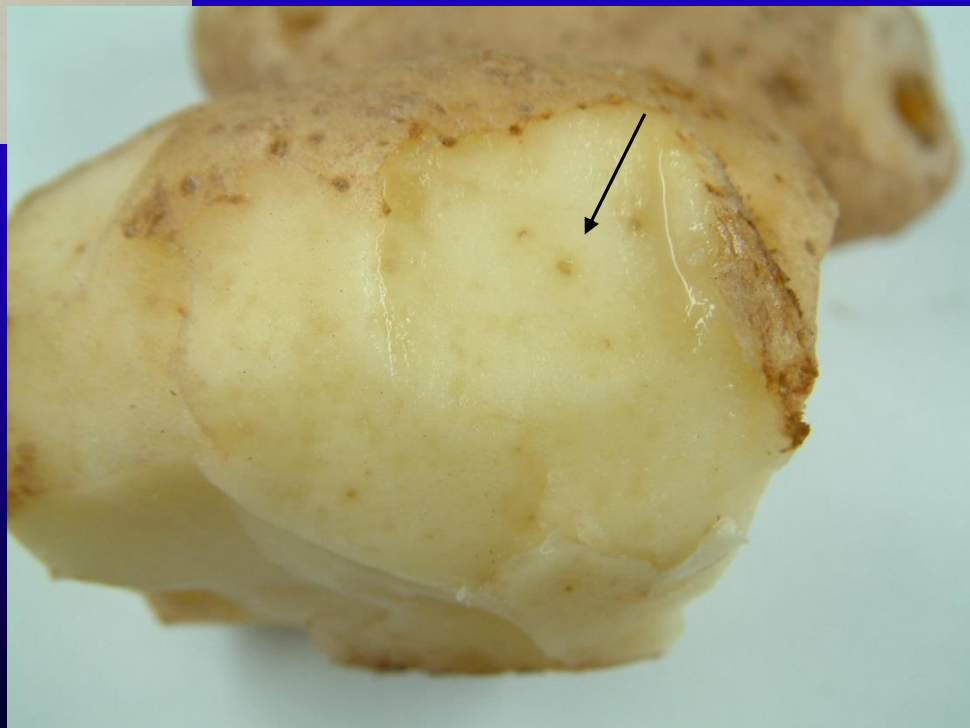




Symptoms of infestation with *Meloidogyne chitwoodi* on crossed potato tubers
(phot. Central Laboratory of SPHSIS, Toruń, Poland)



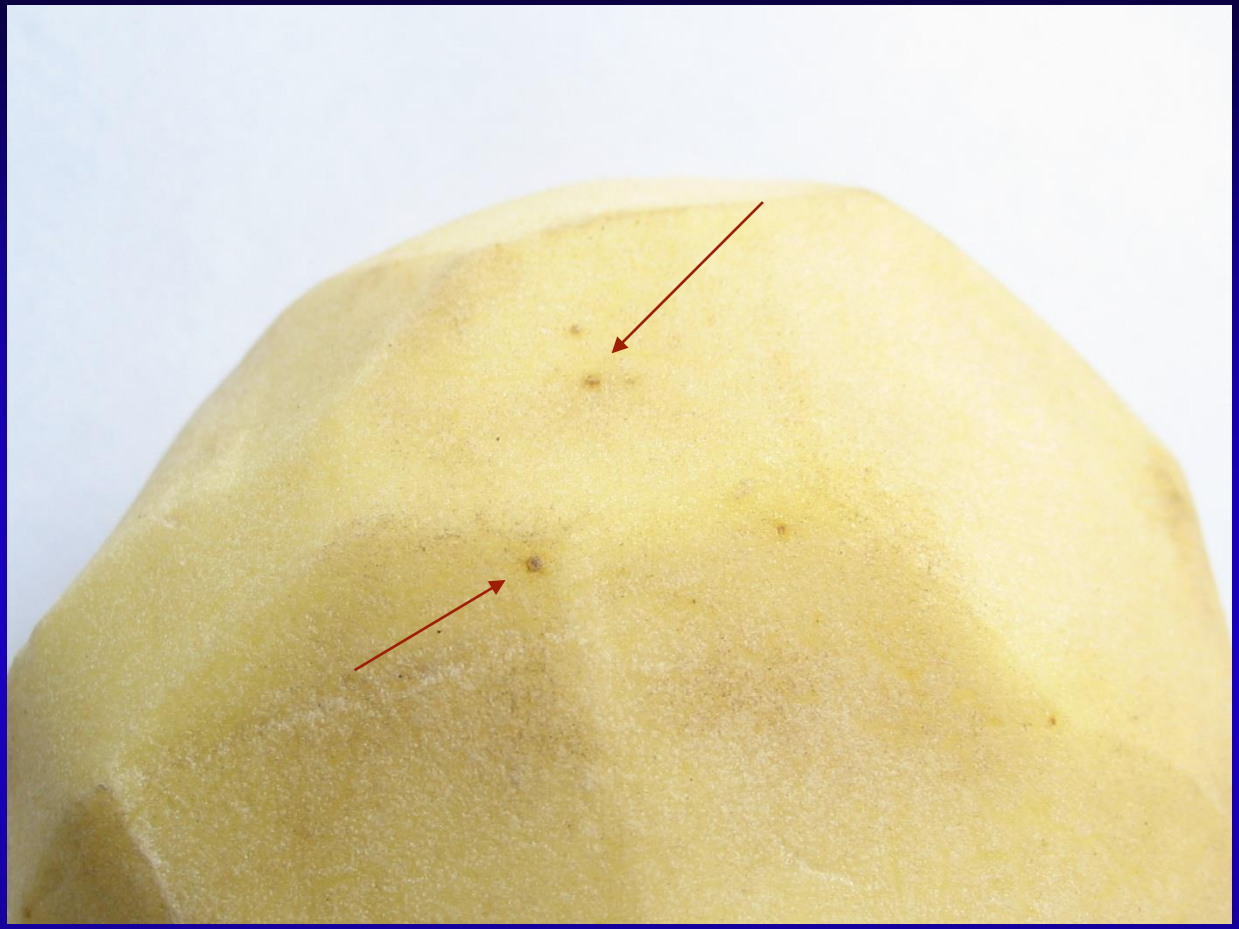
Meloidogyne chitwoodi **females in flesh of potato tubers (source unknown)**



Potato (for consumption) from Egypt infested with *Meloidogyne javanica*

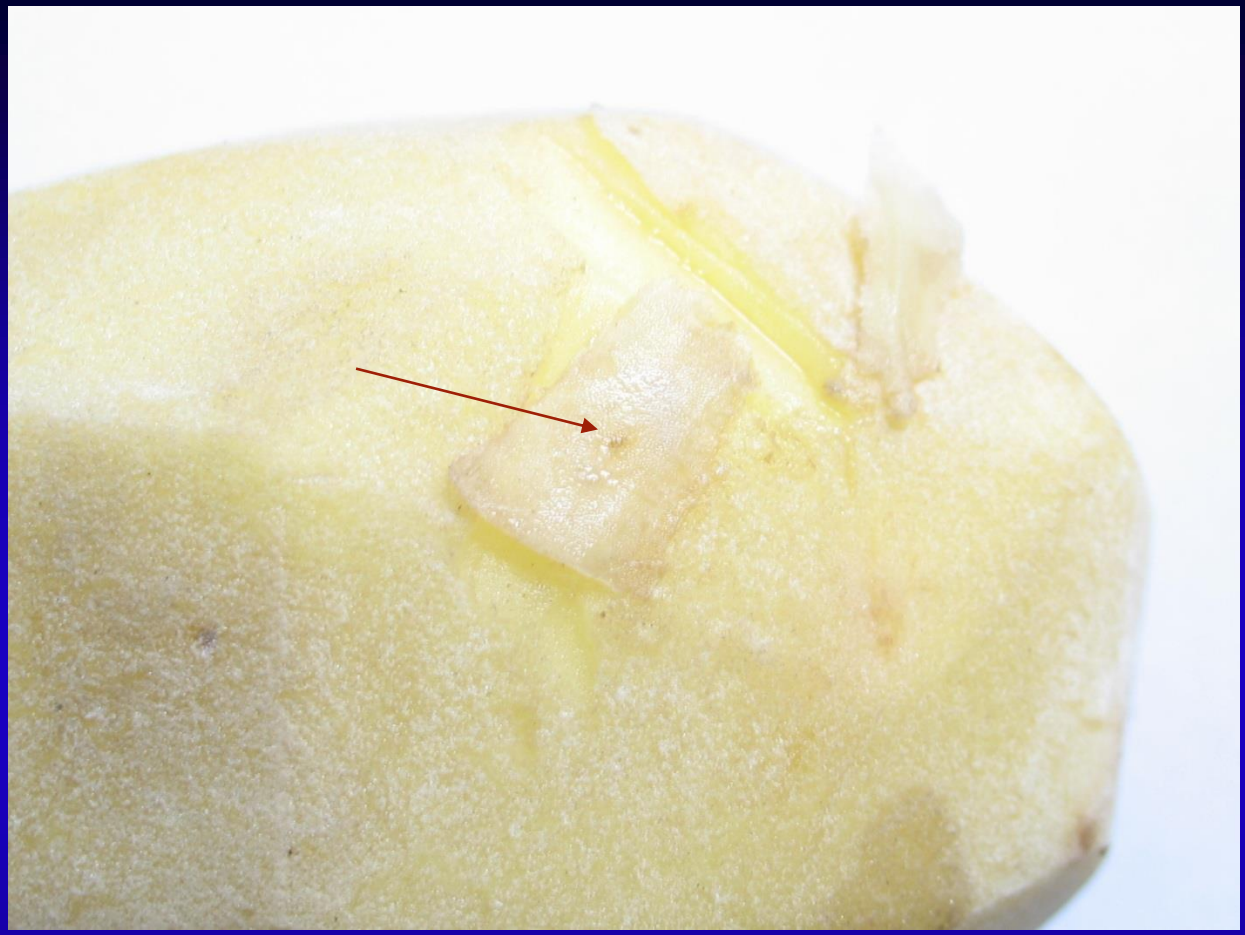
(phot. Central Laboratory of SPHSIS, Toruń, Poland)

Potato tuber infested with *Meloidogyne hapla* – no external signs of infestation (Poland) (phot. Małgorzata Stadnicka, Voivodeship Laboratory in Radzyń Podlaski, Poland)

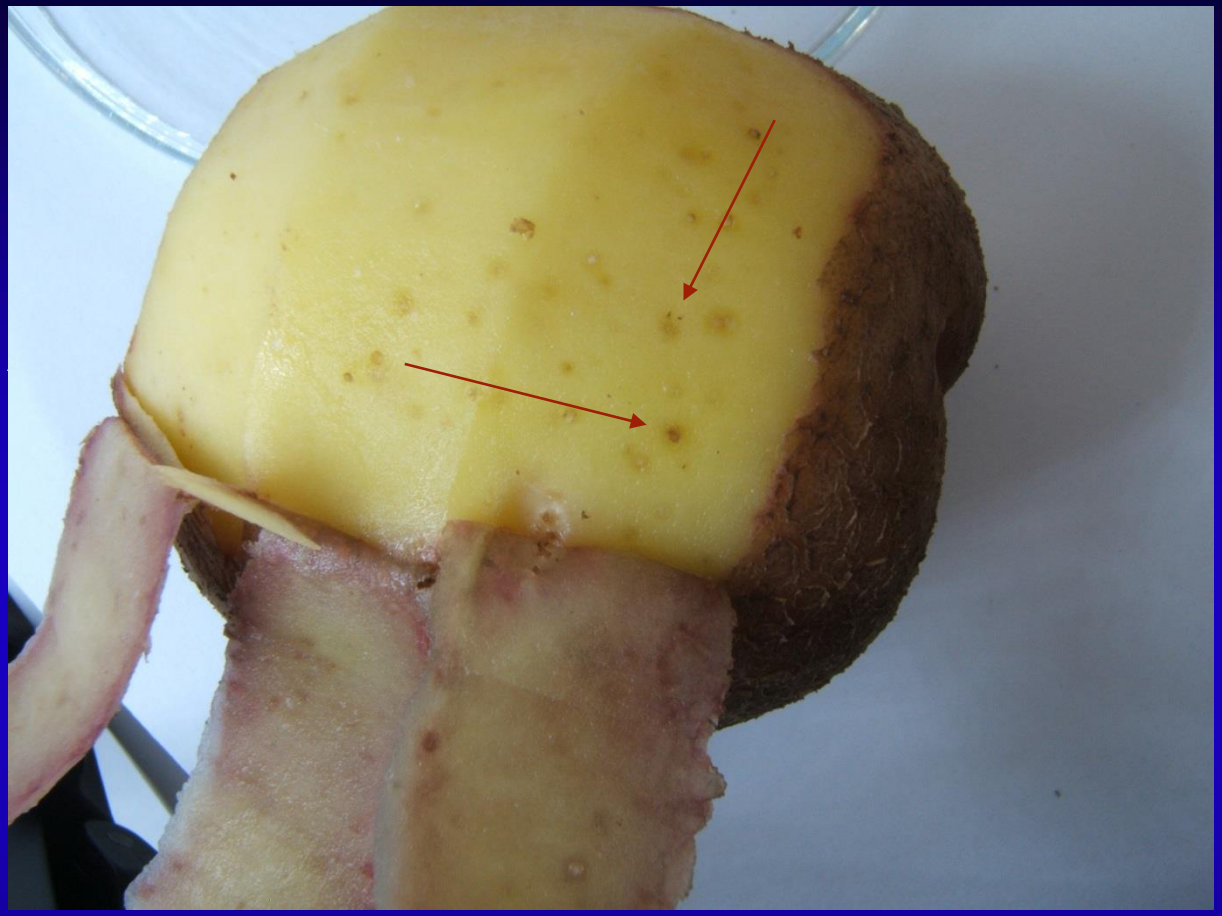


Potato tuber infested with *Meloidogyne hapla* – weakly developed internal signs of infestation (Poland) (phot. Central Laboratory of SPHSIS, Toruń, Poland)

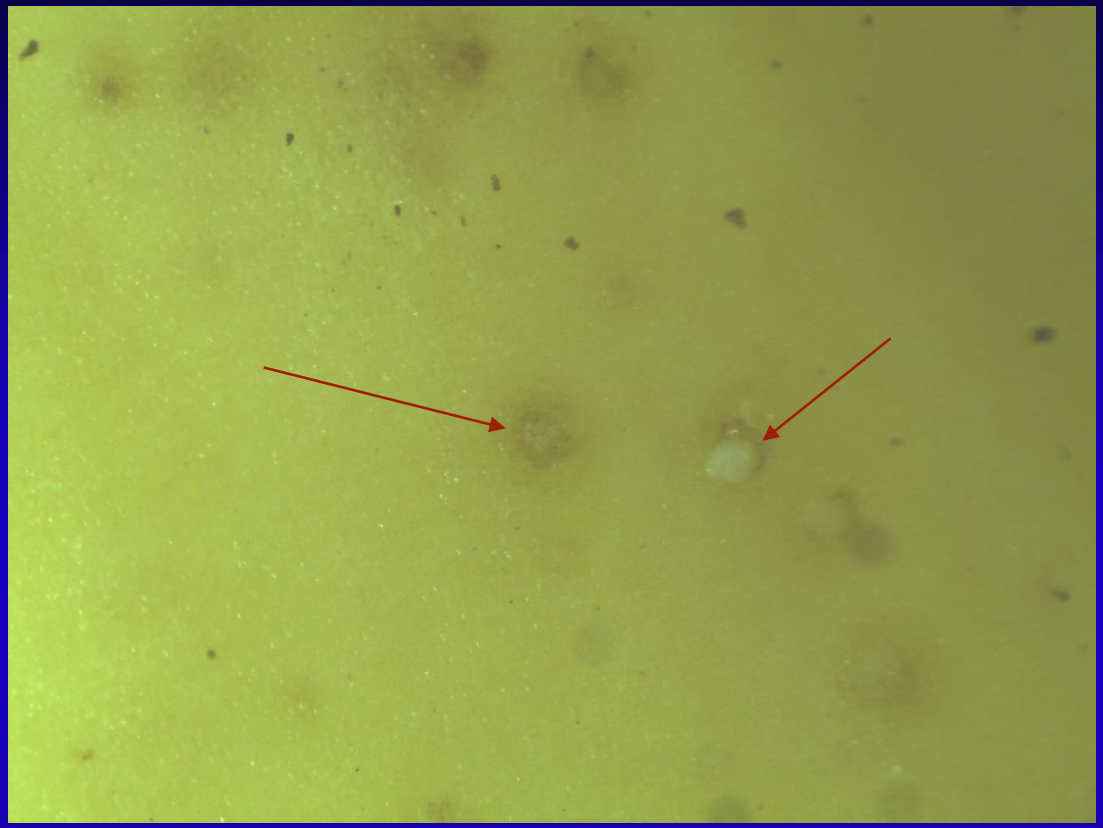
Potato tuber infested with *Meloidogyne hapla* – weakly developed internal signs of infestation (Poland) (phot. Central Laboratory of SPHSIS, Toruń, Poland)



Potato tuber infested with *Meloidogyne hapla* – weakly developed internal signs of infestation (Poland) (phot. Central Laboratory of SPHSIS, Toruń, Poland)



Potato tuber infested with *Meloidogyne hapla* – well developed internal signs of infestation (Poland)
(phot. Małgorzata Stadnicka, Voivodeship Laboratory in Radzyń Podlaski, Poland)

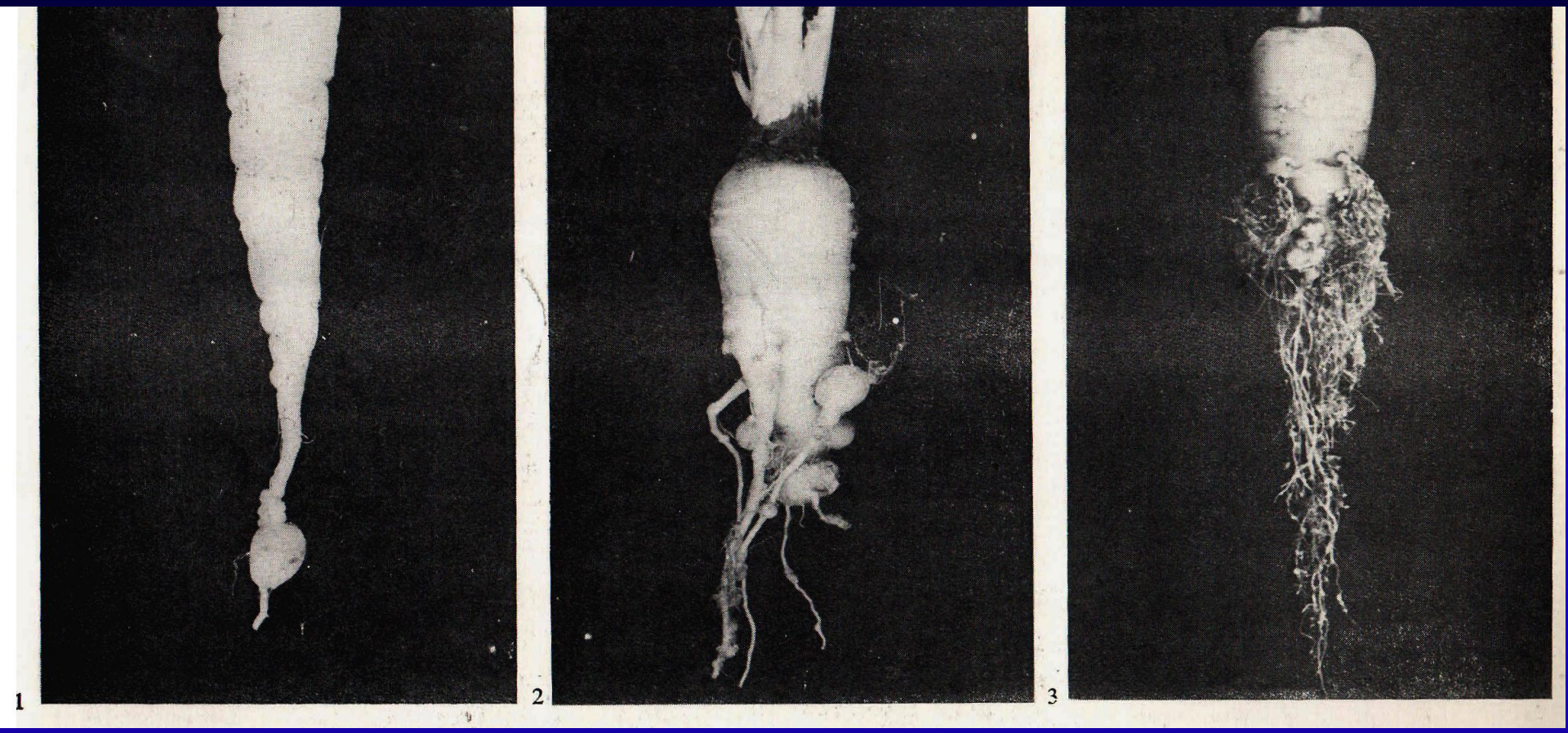


Potato tuber infested with *Meloidogyne hapla* – well developed internal signs of infestation and a nematode female (Poland)

(phot. Małgorzata Stadnicka, Voivodeship Laboratory in Radzyń Podlaski, Poland)

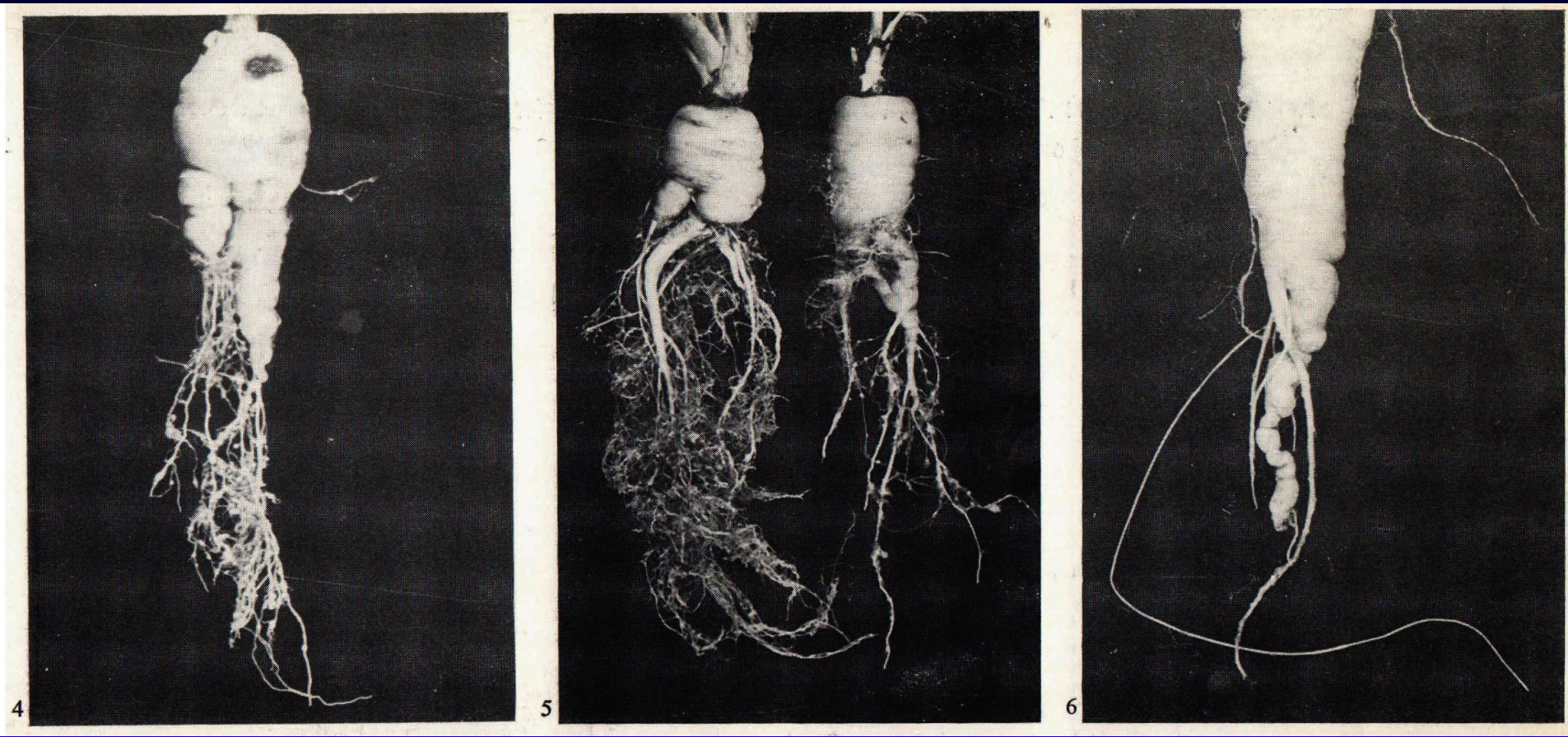
Potato tuber infested with *Meloidogyne hapla* – well developed external internal signs of infestation (Bosnia), as a result of nematode development in hotter climate (phot. Branimir Njezić)

Galls caused by *M. chitwoodi* on storage root of carrot. No deformation of roots typical for *M. hapla* is observed (phot. Central Laboratory of SPHSIS, Toruń, Poland)



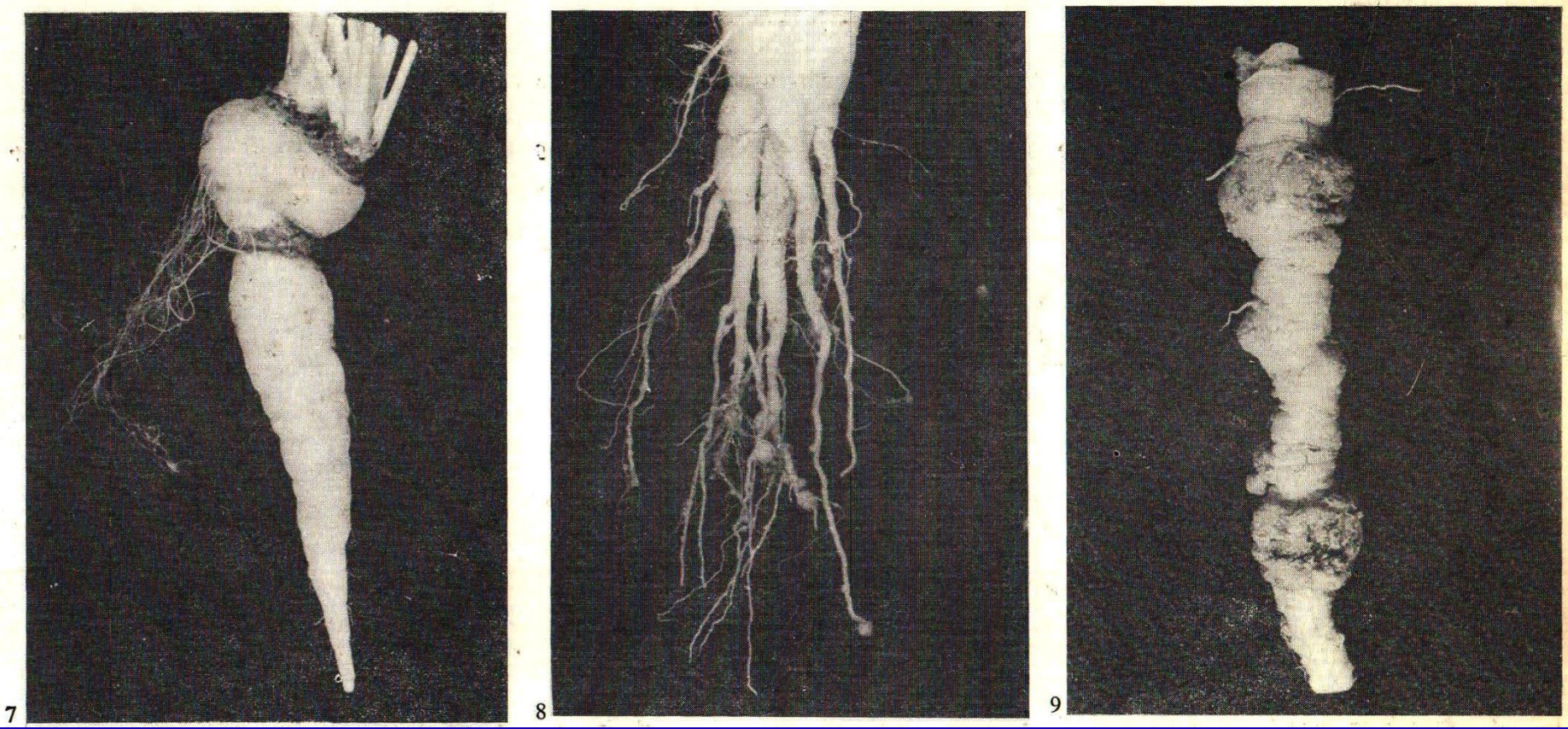
Carrots infested with *M. hapla*
1. Gall on root tip. 2. Galls of various shape on root; 3. Producing of numerous lateral roots.

Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)



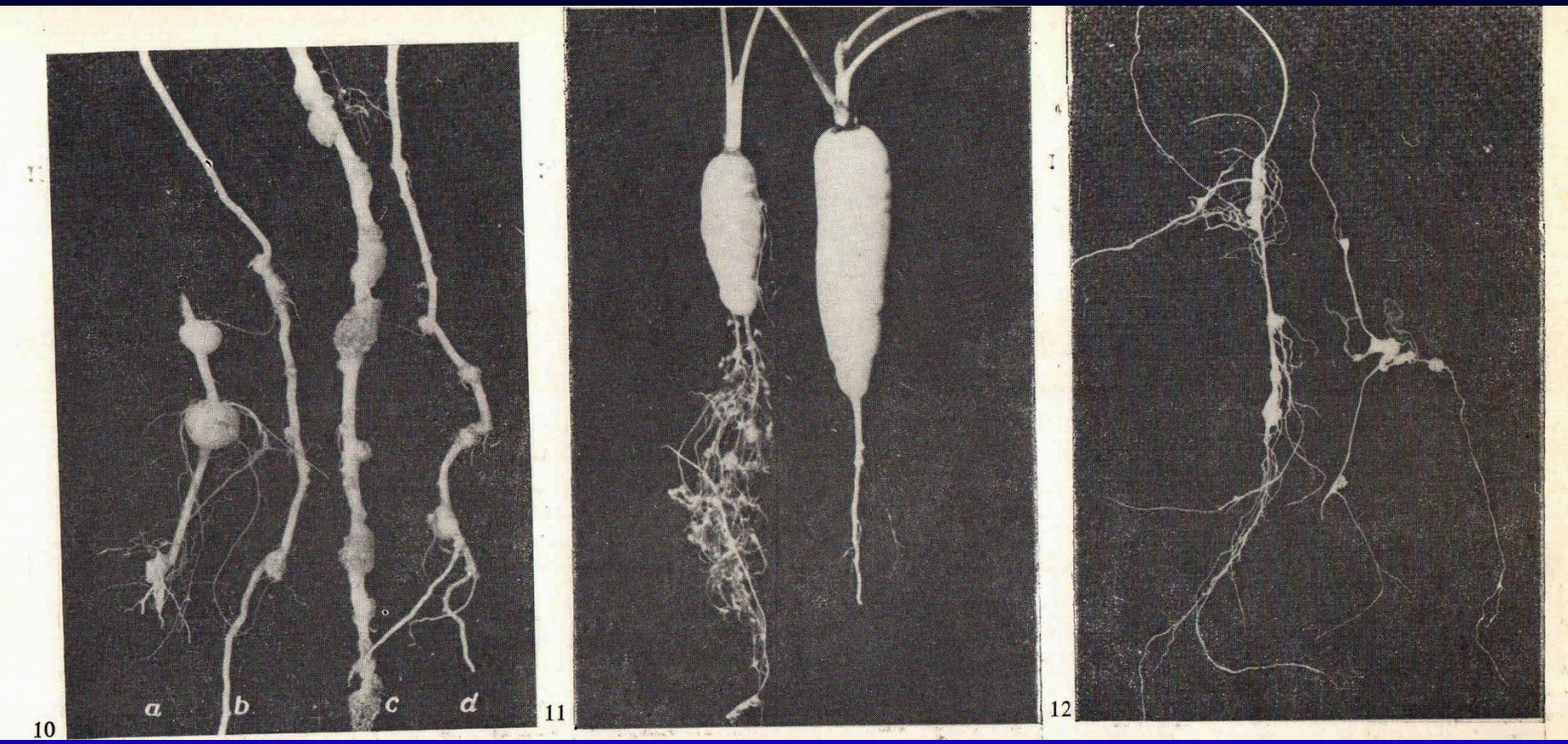
Carrots infested with *M. hapla*
3.4. Divided roots; 5. Deformed root tip.

Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)



Carrots infested with *M. hapla*
7. Narrowed and deformed root; 6. Divided root; 7. Cancerous galls on main root.

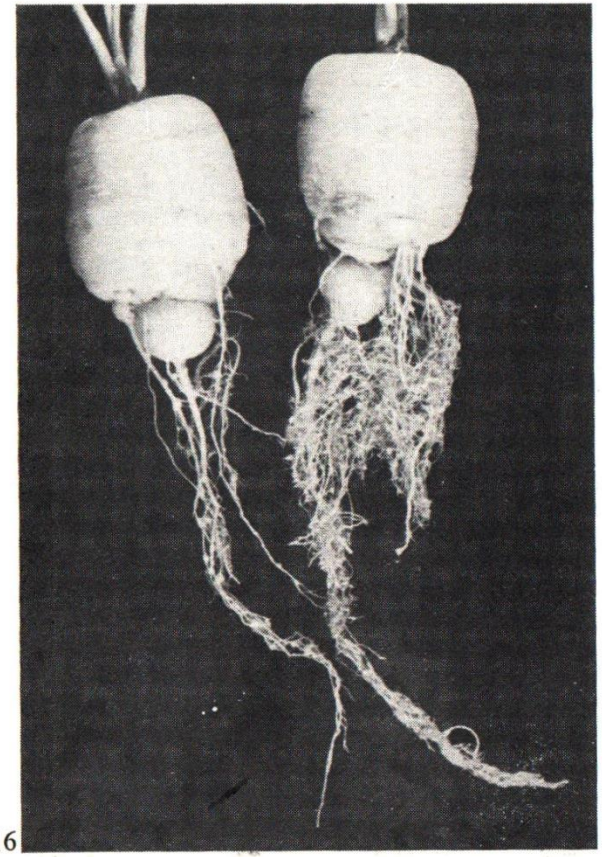
Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)



Carrots infested with *M. hapla*

10. Symptoms of infestation on lateral roots; 11 Infested root (reduced length, numerous additional roots; left) and healthy root (right); 12. Symptoms on young roots.

Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)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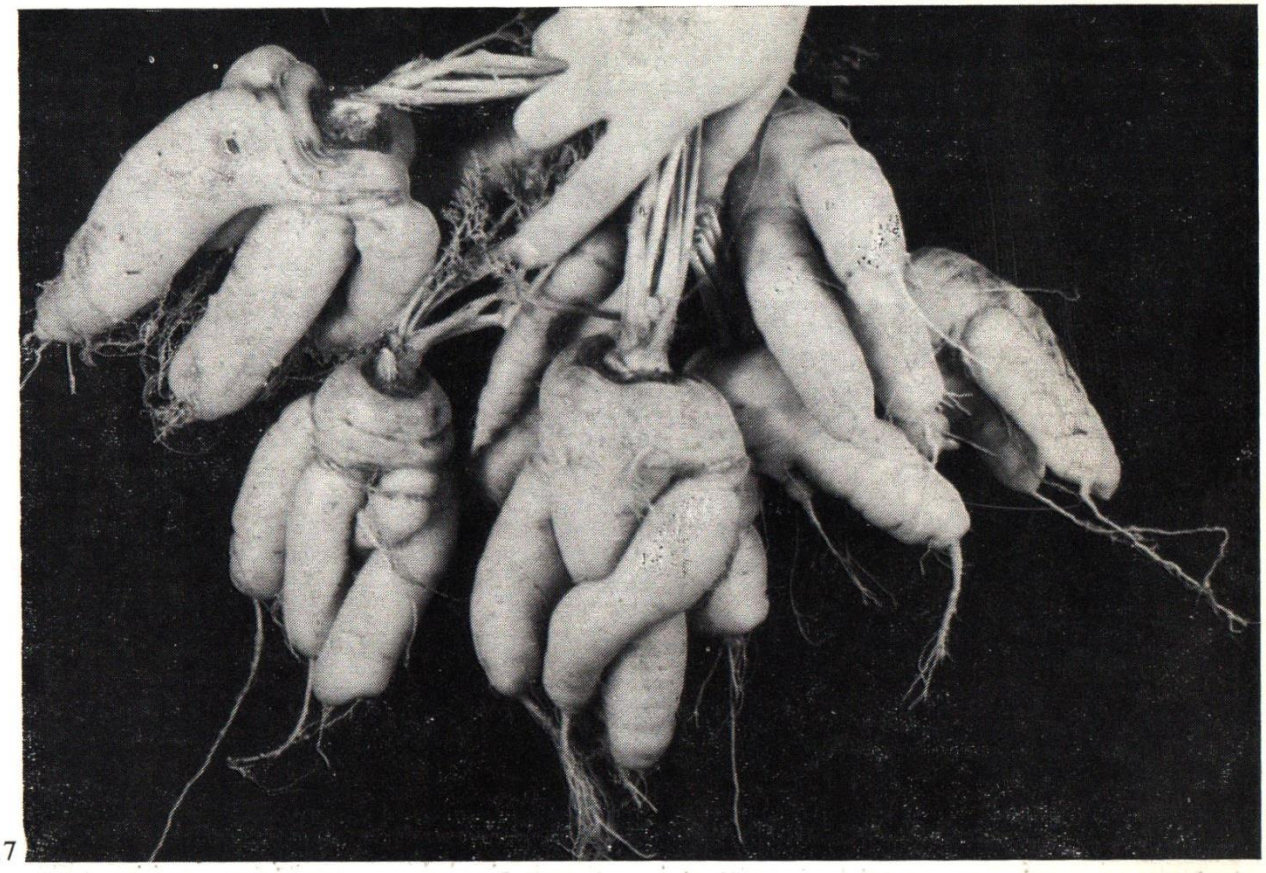
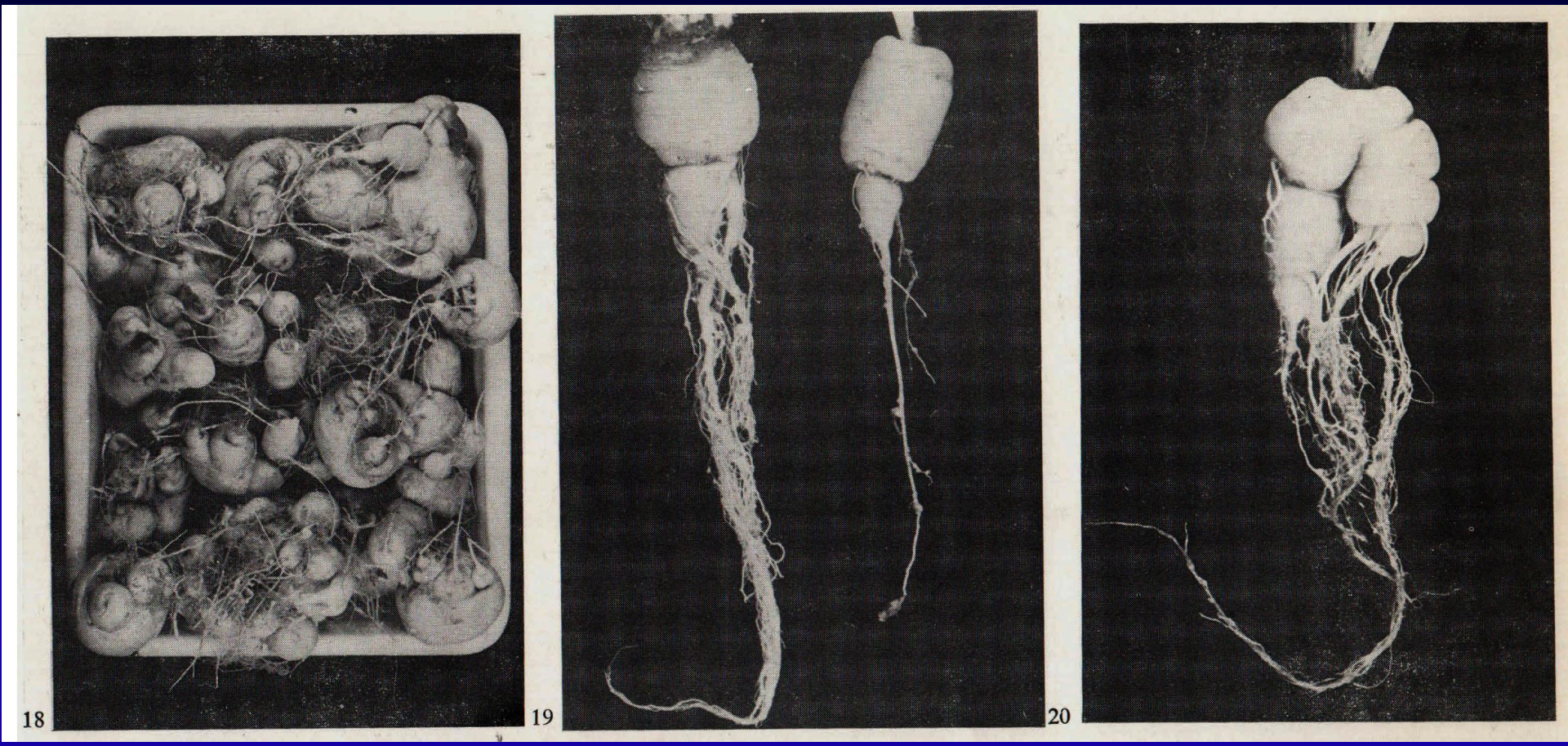

17

Carrots infested with *M. hapla*

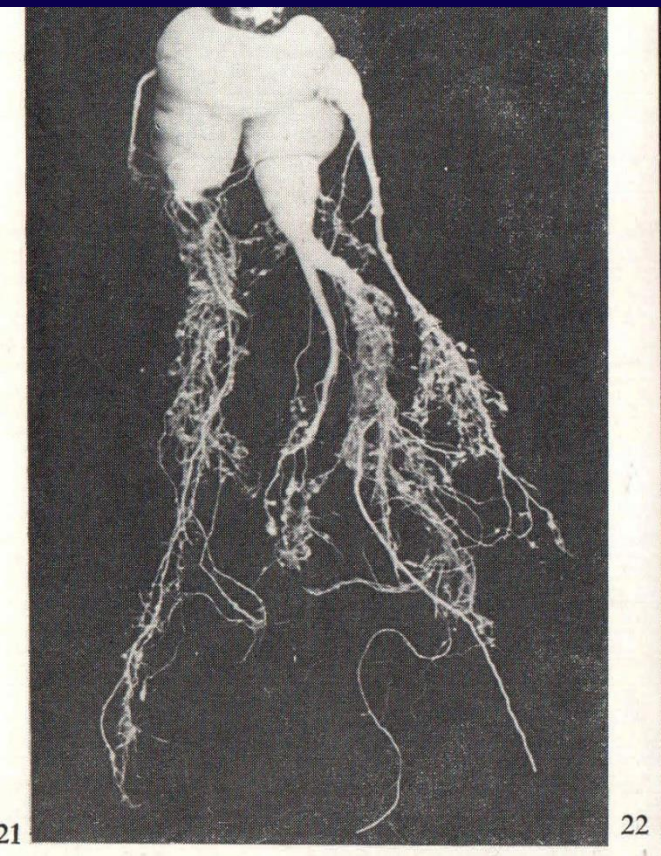
16. Infested root (reduced length, numerous additional roots);
17. Heavily infested root – short roots divided into a few additional roots.

Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)



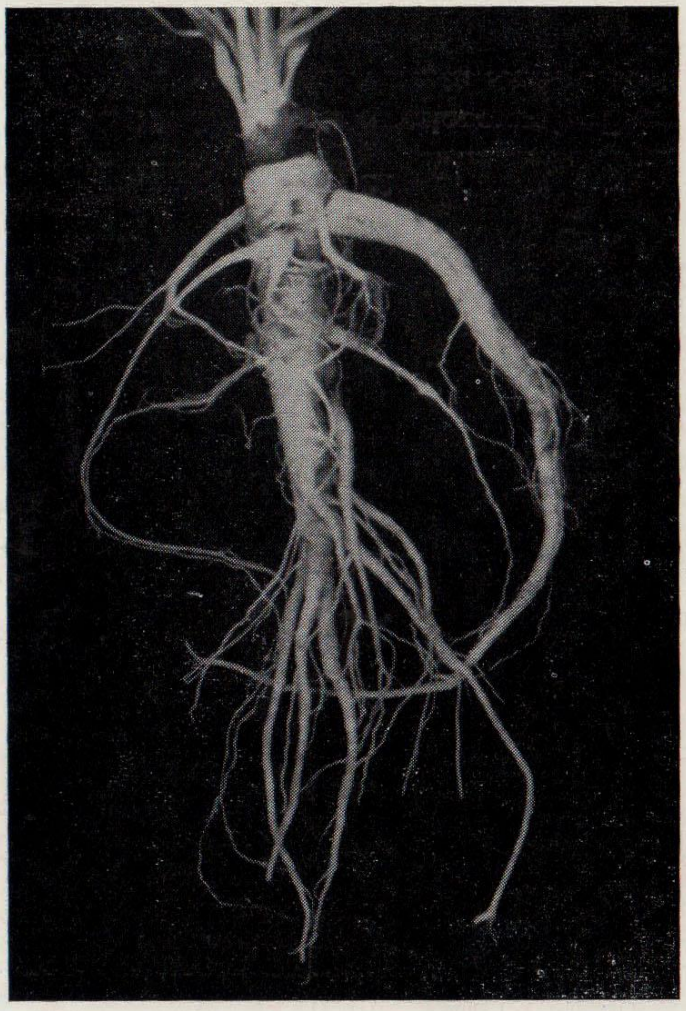
Carrots infested with *M. hapla*
18. Heavily infested roots; 19. Infested roots – short roots with a few additional lateral roots; 20. Infested root – dividing into two roots and additional lateral roots.

Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)



Carrots infested with *M. hapla*
21, 22. Infested roots – dividing into two roots and additional lateral roots.

Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)



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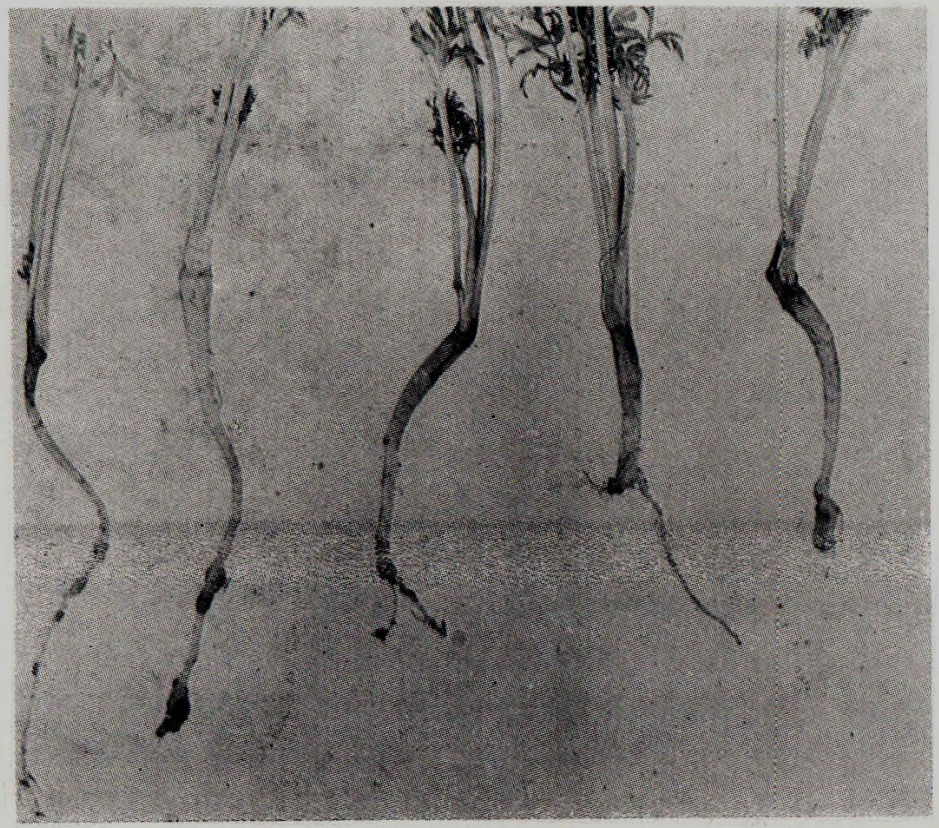
24

Carrots infested with *M. hapla*

23. Infested wild carrot – only small galls visible; 19. Infested young carrot plants (6 week old).

Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)



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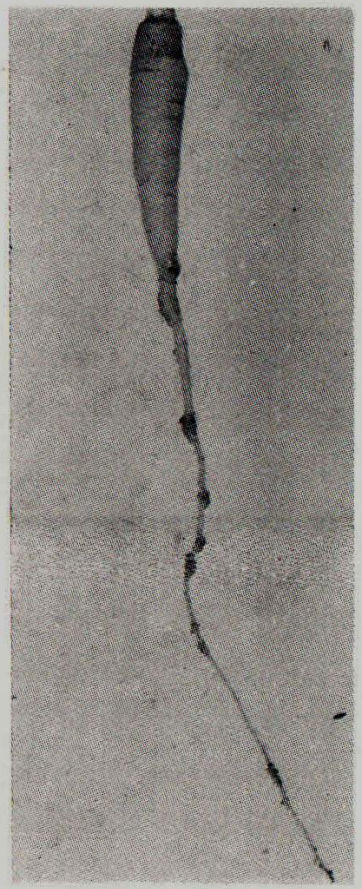

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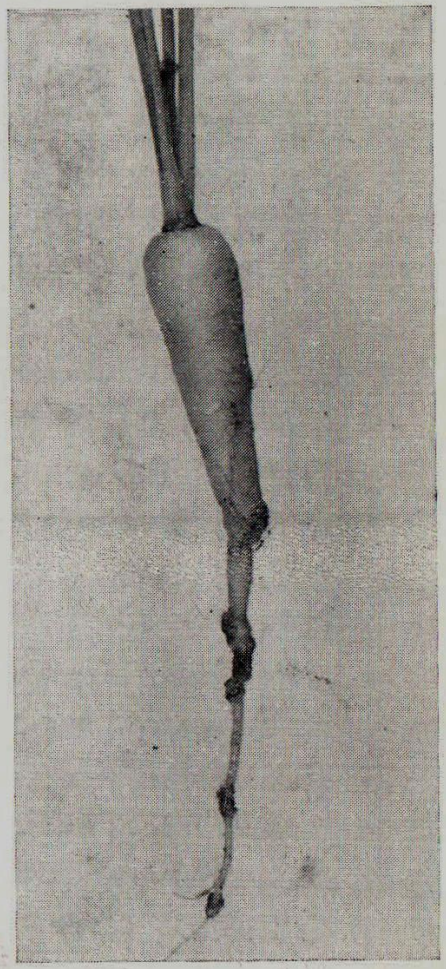

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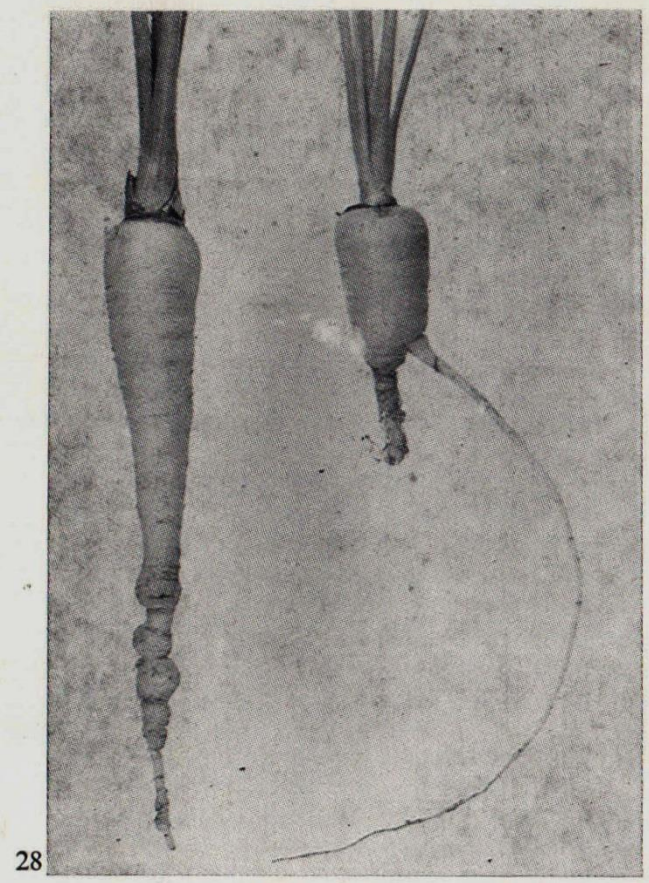
Carrots infested with *M. hapla*
25 – 27. Infested young carrot plants – numerous small galls visible

Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)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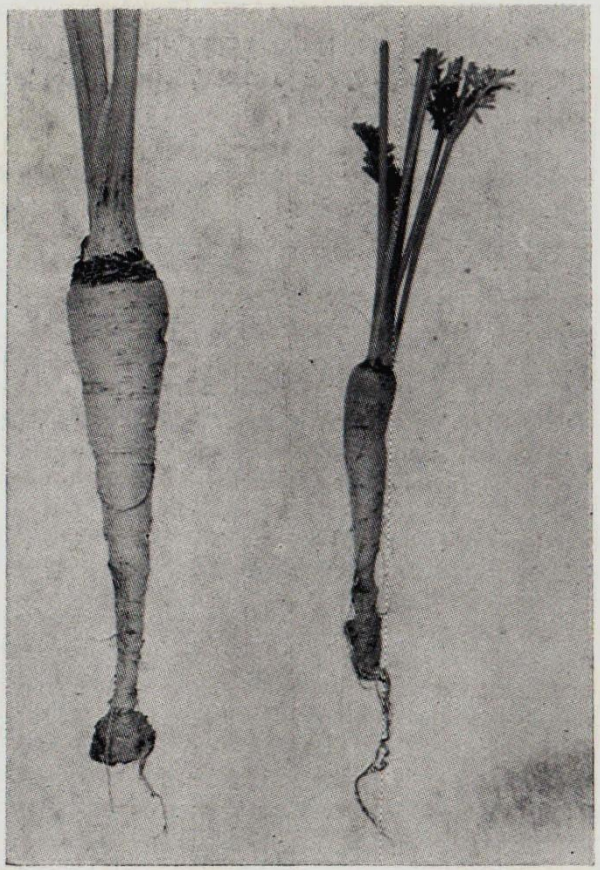
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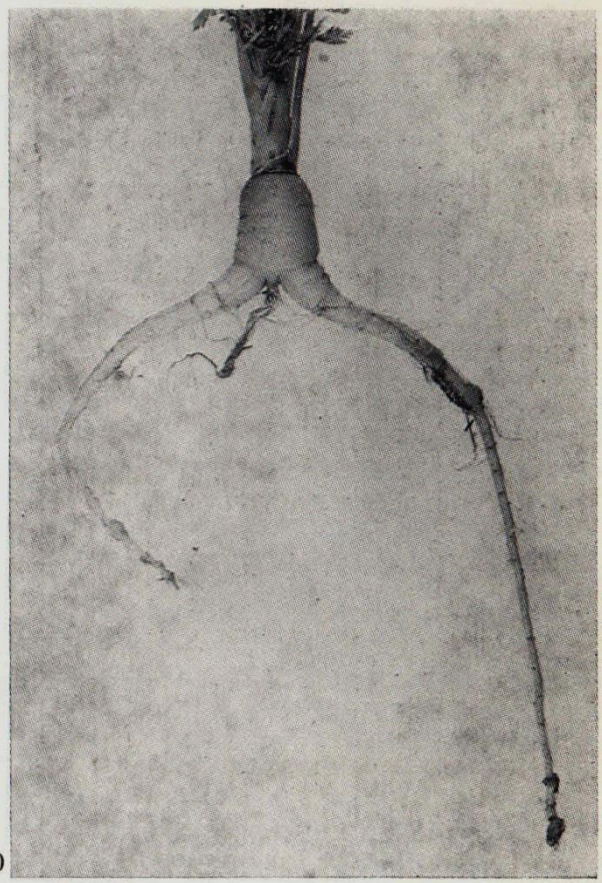
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Carrots infested with *M. hapla*
28 - 29. Infested carrot roots – galls, deformation and dividing of roots

Source: Berbeć E., 1972, [The investigation on appearance and harmfulness caused by northern root-knot nematode *Meloidigyne hapla* Chitwood on carrots}. Prace Kom Nauk. Rolniczych i Biologicznych, IX: 3-45 (in Polish)

Inspection of potato tuber (left) and leek plants (right) for *M. chitwoodi* and *M. fallax* in field conditions (workshop in the Netherlands; phot. W. Karnkowski)

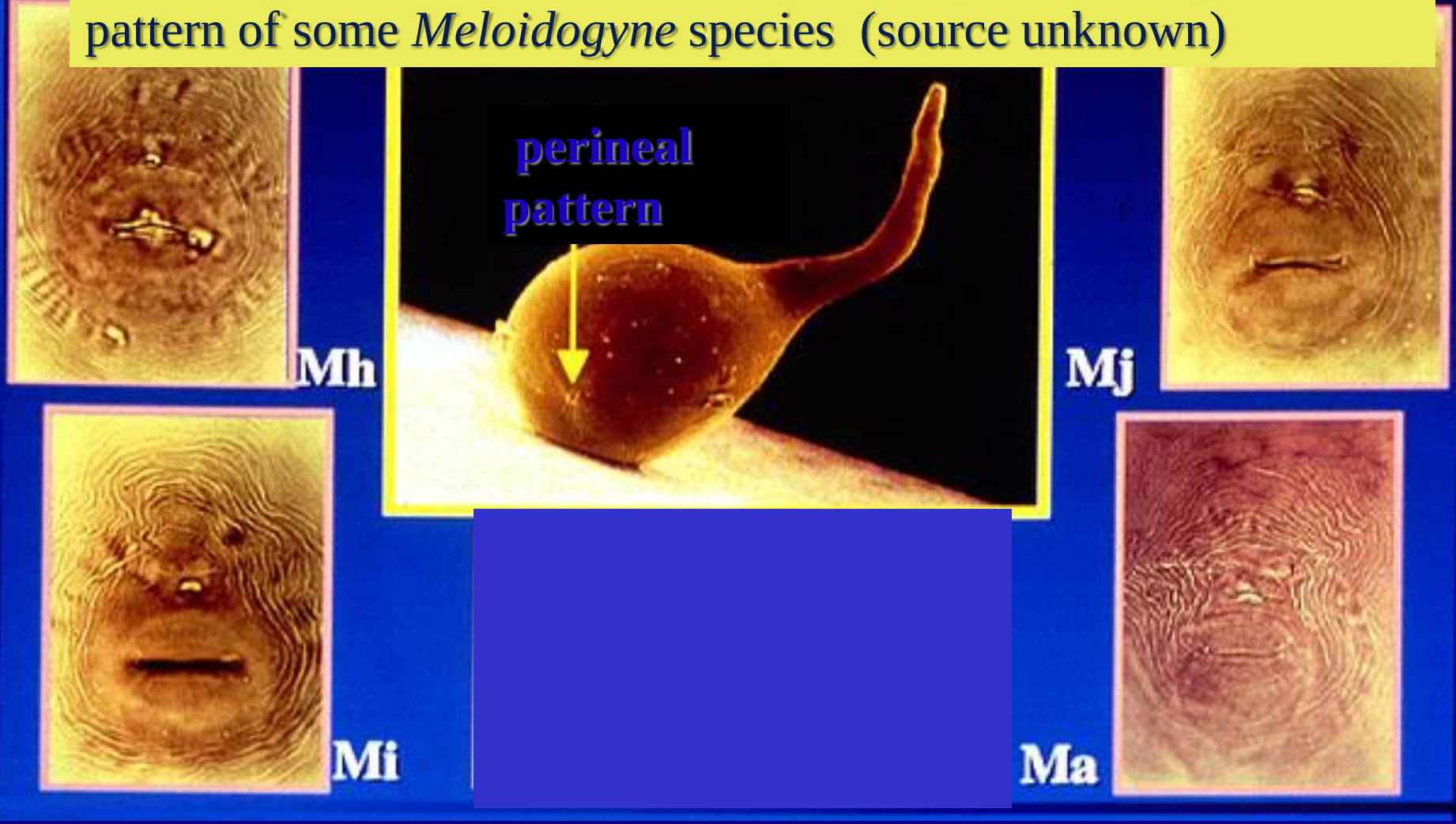
Detection&extraction procedures

- Mature females can be extracted by dissecting apart the tissues but then should be stored in a 0.9% solution of NaCl in order to avoid possible osmotic disruption in plain water.
- Staining of nematodes in plant tissue, maceration & centrifugal flotation and enzymatic digestion of roots and tubers with cellulase and pectinase can be used for the recovery of females and other developmental stages.
- Males and second-stage juveniles should be obtained also from soil by suitable extraction techniques, such as Flegg method, Oostenbrink elutriator, centrifugal flotation, etc.

DIAGNOSTIC CHARACTERS USED DURING IDENTIFICATION OF ROOT-KNOT NEMATODES TO SPECIES

- perineal region which forms a fingerprint-like pattern (perineal pattern)
- stylet length and stylet knobs shape and width;
- J_2 juveniles body length;
- J_2 juveniles whole tail length and hyaline tail part length.

Meloidogyne female with marked perineal pattern and perineal pattern of some *Meloidogyne* species (source unknown)



Mh = *Meloidogyne hapla*, Mj = *Meloidogyne javanica*
Mi = *Meloidogyne incognita*, Ma = *Meloidogyne arenaria*

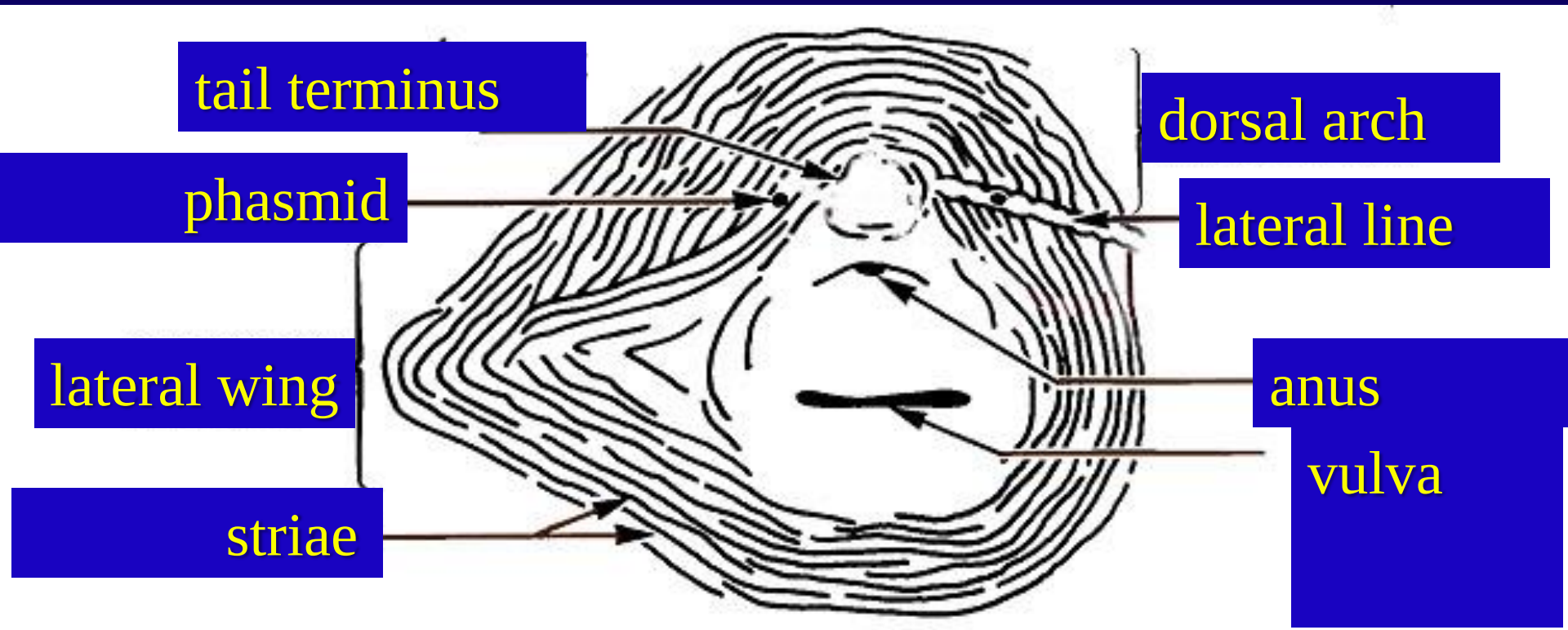
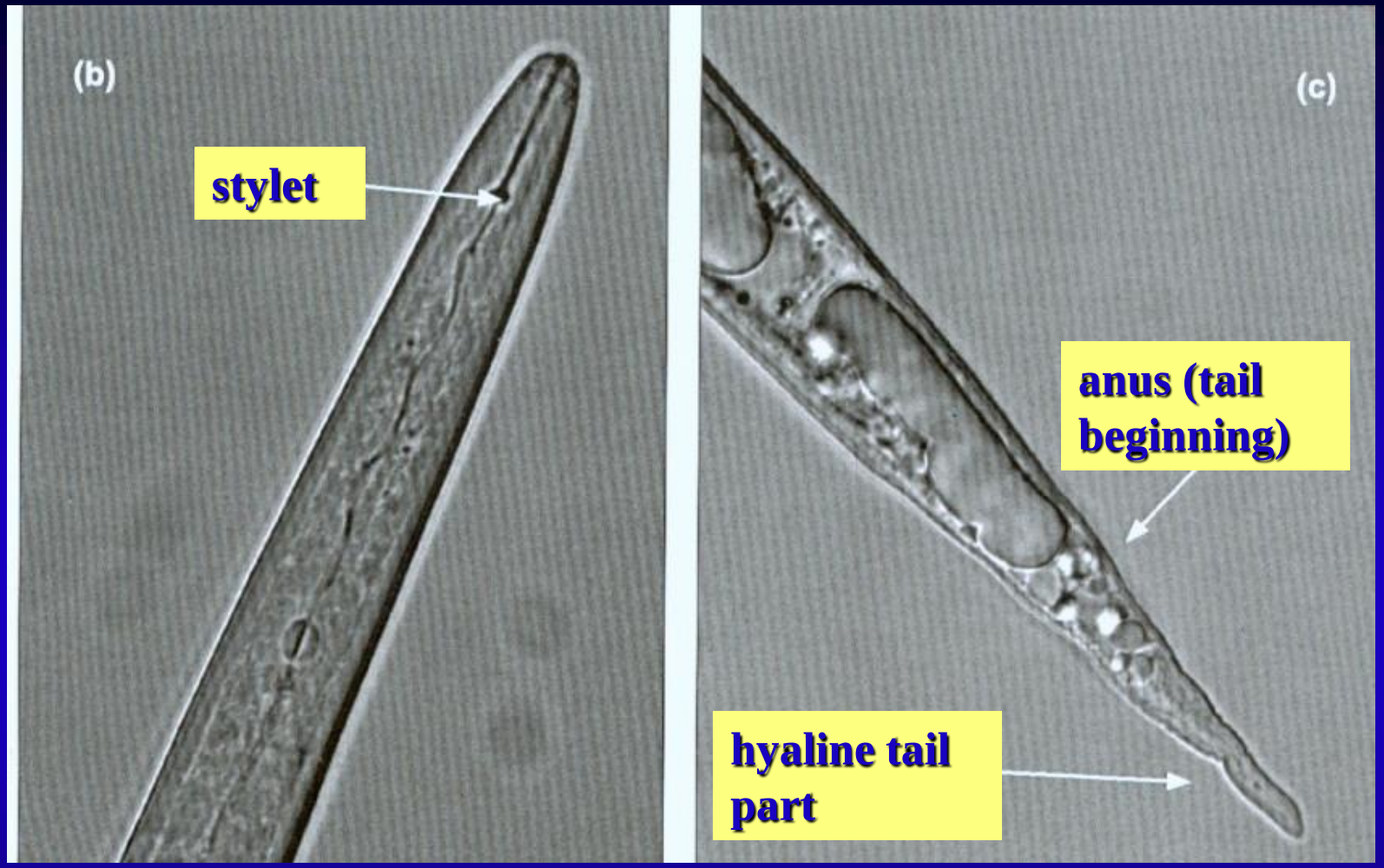


Diagram of the perineal pattern of *Meloidogyne* spp.

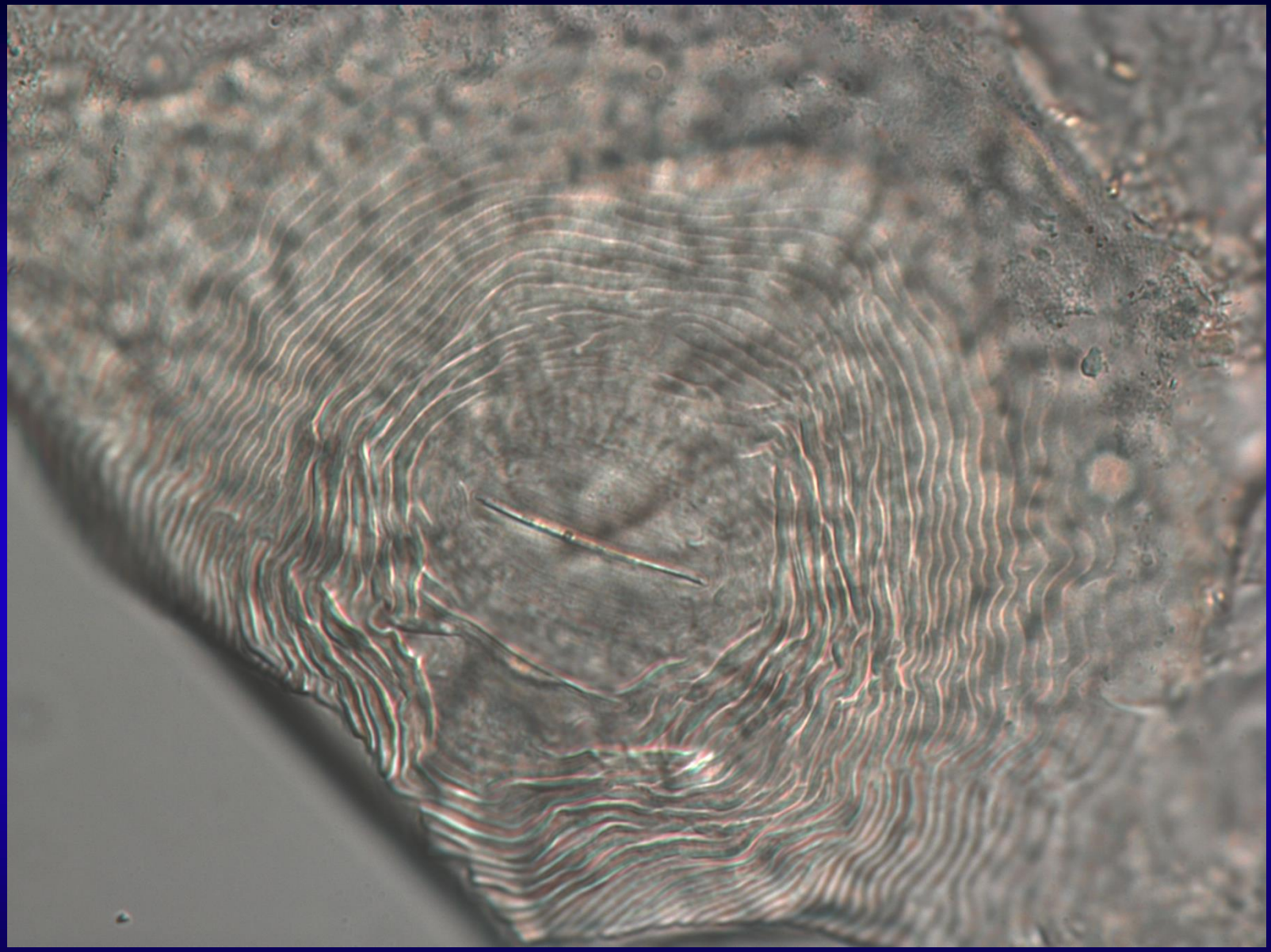


J_2 juveniles and their main diagnostic characters
(phot. Renata Dobosz, Institute of Plant Protection - Governmental Research Institute, Poznań, Poland)

Meloidogyne chitwoodi Golden et al. – morphological characters

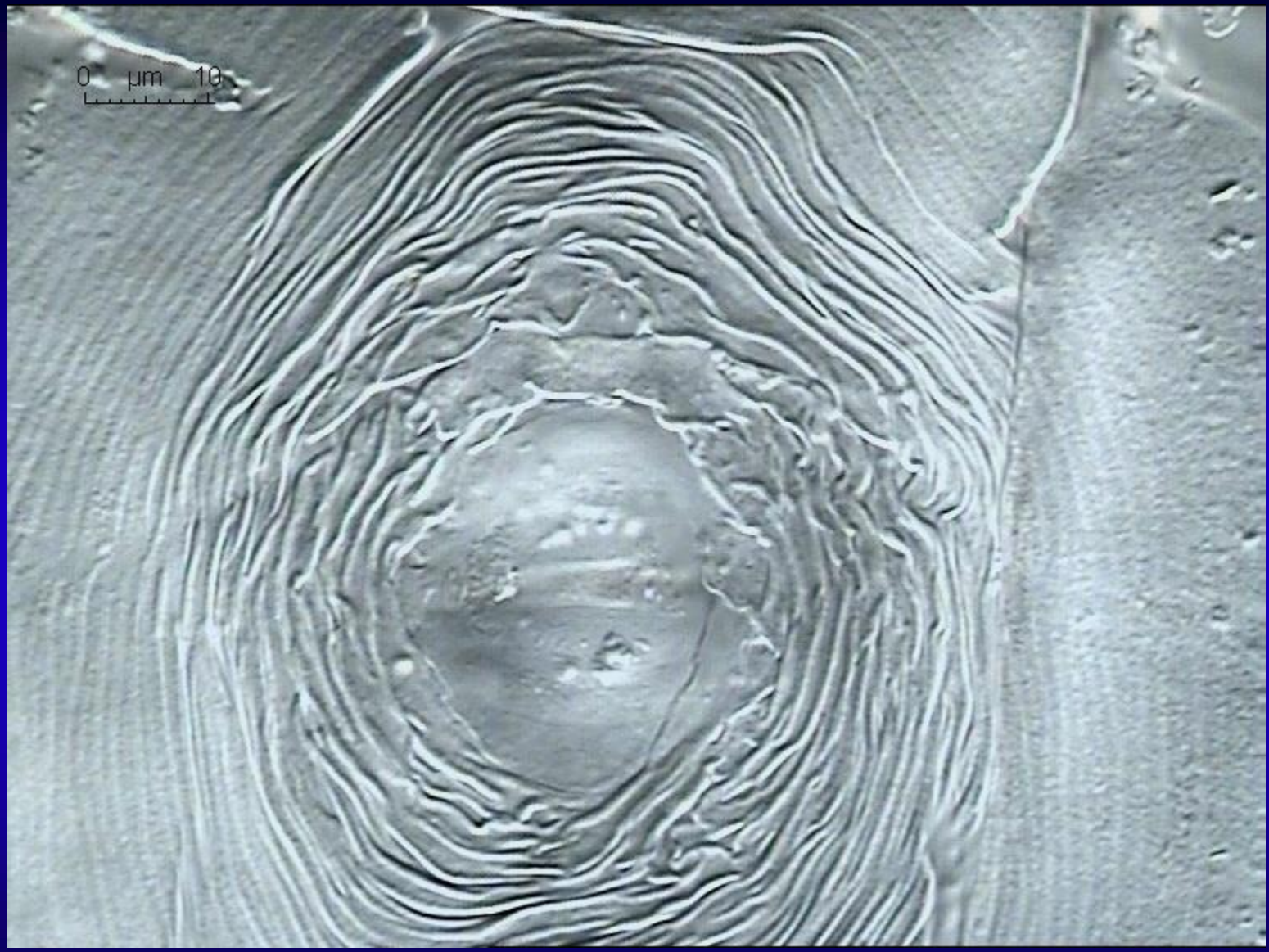
Females

- **body length 0.43 – 0.74 mm (mean 0.59 mm);**
- **body rounded or oval-shaped,white or creamy with short neck;**
- **stylet 11.4-13 µm in length (mean 11.8 µm), delicate; stylet knobs small, oval to irregularly shaped and backwardly sloping; stylet cone slightly curved dorsally;**
- **vulva on small protuberance;**
- **perineal pattern relatively small, rounded to oval-shaped with dorsal arch ranging from low and rounded to relatively high and angular;**
- **lateral field with four lines, weakly visible;**
- **there is no punctation in tail terminus area.**

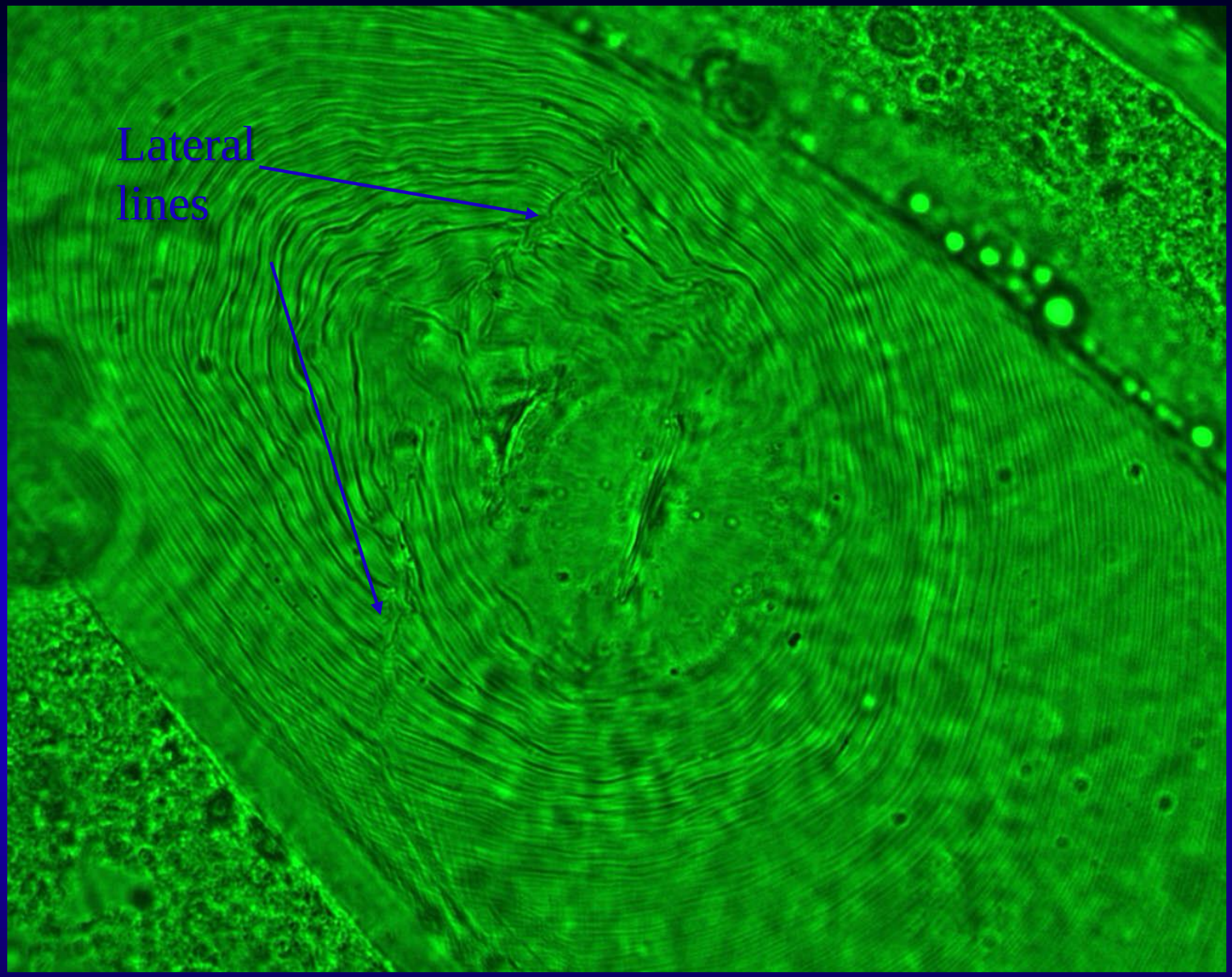


Pertineal pattern of *M. chitwoodi* (phot. Renata Dobosz, Institute of Plant Protection - Governmental Research Institute, Poznań, Poland)

Pertineal pattern of *M. chitwoodi*
(phot. LNPV, Le Rheu, France)



Pertineal pattern of *M. chitwoodi*
(phot. LNPV, Le Rheu, France)



Perineal pattern with distinct lateral lines (*M. javanica)* (phot. W. Karnkowski).

Meloidogyne chitwoodi Golden et al. – morphological characters

Males

- body length 0.9 – 1.07 mm; body worm-like, anteriorly tapering;
- head not set off from the body;
- stylet 16-18.3 µm in length (mean 17.7 µm), delicate; stylet knobs small, oval to irregularly shaped and backwardly sloping;
- lateral field with four lines, weakly developed.

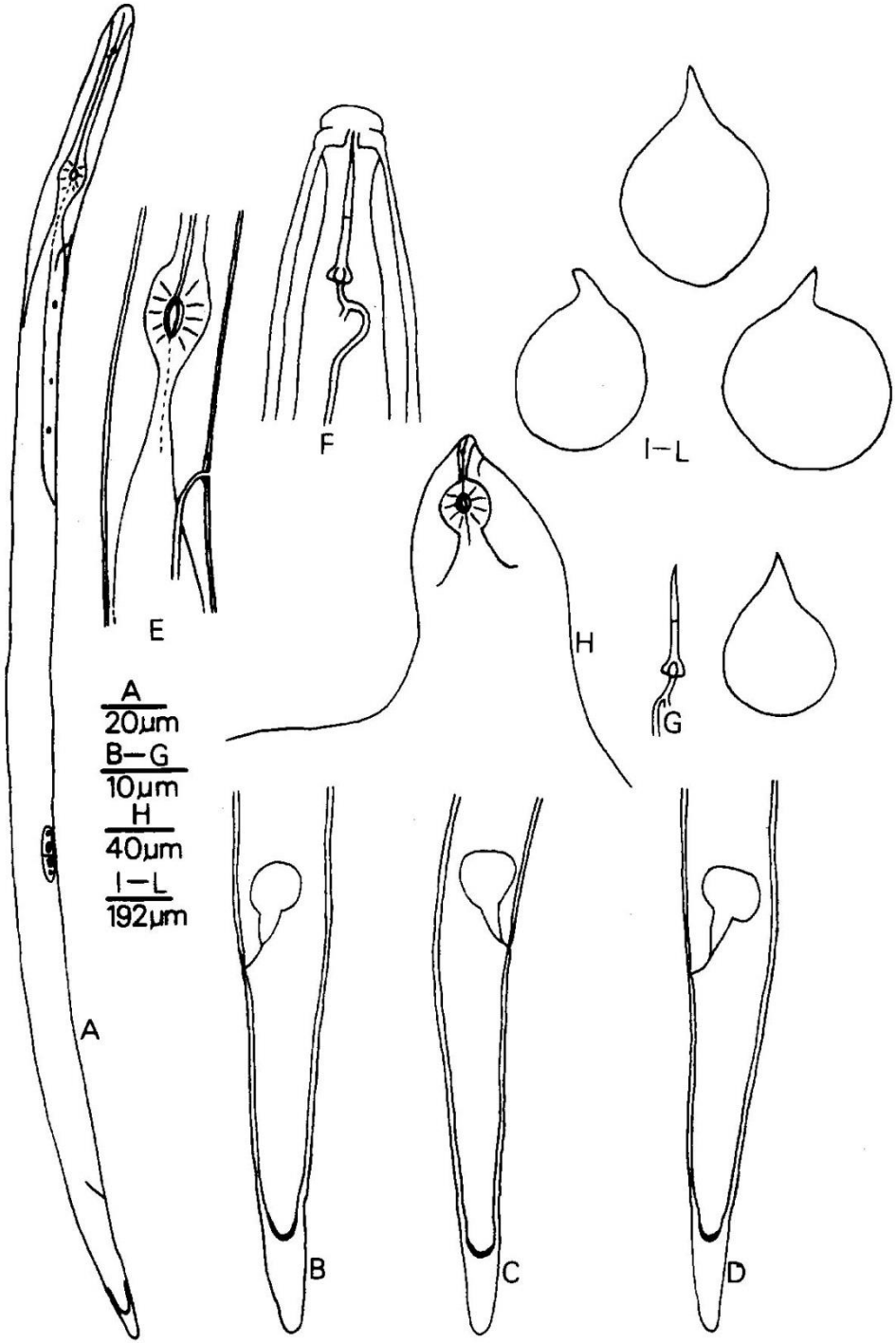
Anterior body portion of *M. chitwoodi* male (phot. LNPV, Le Rheu, France)

Meloidogyne chitwoodi Golden et al. – morphological characters

J_2 juveniles

- **body length 336 - 417 µm (mean 380 µm);**
- **stylet 9.5-14 µm in length, delicate; stylet knobs small, rounded;**
- **tail conical; tail tip bluntly rounded, not irregularly shaped;**
- **tail length 39-47 µm; hyaline tail part length 9 - 14 µm;**
- **hemizonid anterior and adjecent to excretory pore.**

Variation of tail shape of *M. chitwoodi* J_2 juveniles (phot. LNPV, Le Rheu, France)



Meloidogyne chitwoodi:

J_2 juvenile

A. whole body; B-D. tail; E. metacorpus;

Male

F. head;

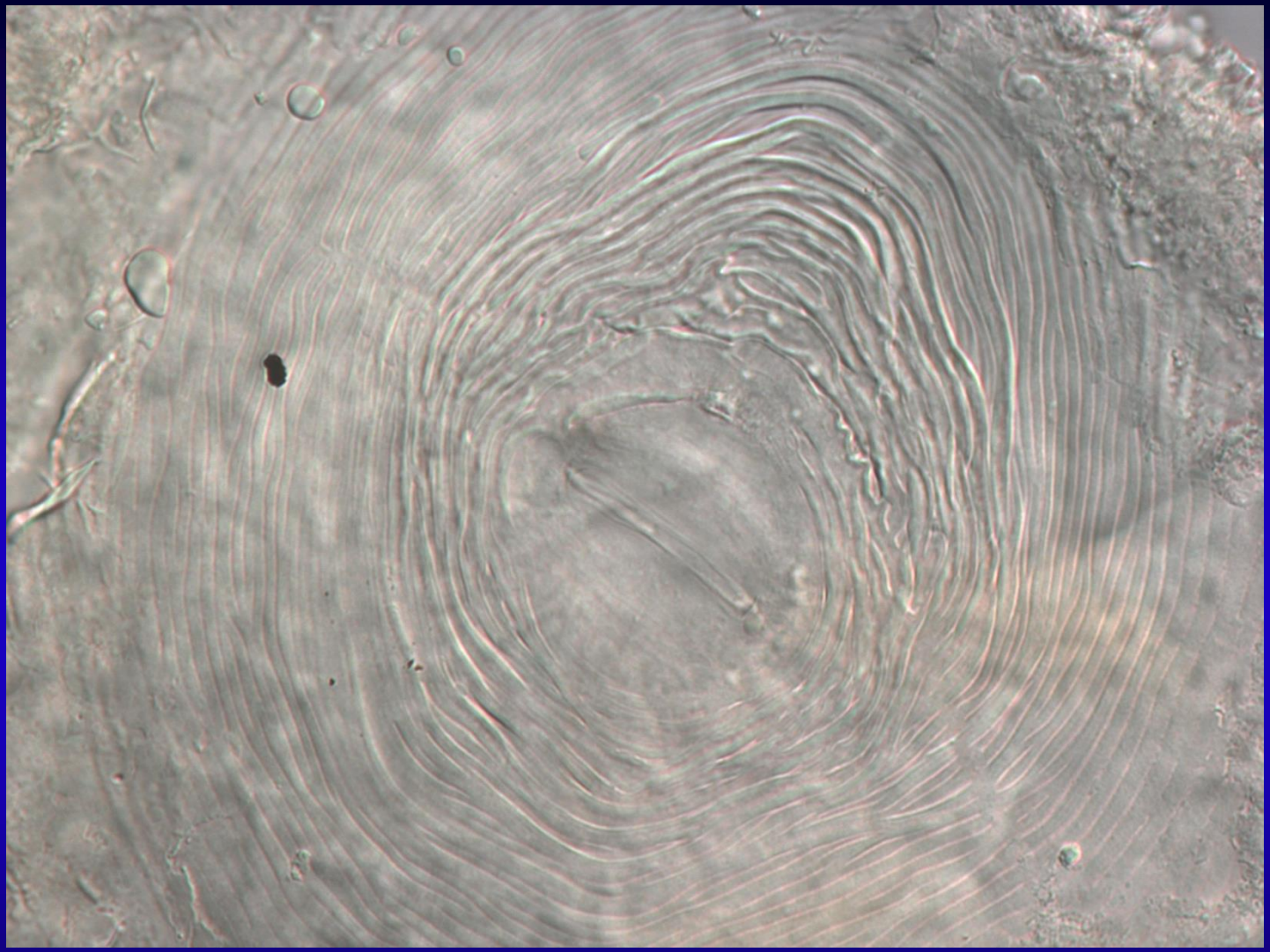
Female

G. stylet; H. anterir body portion; I-L. body shape.

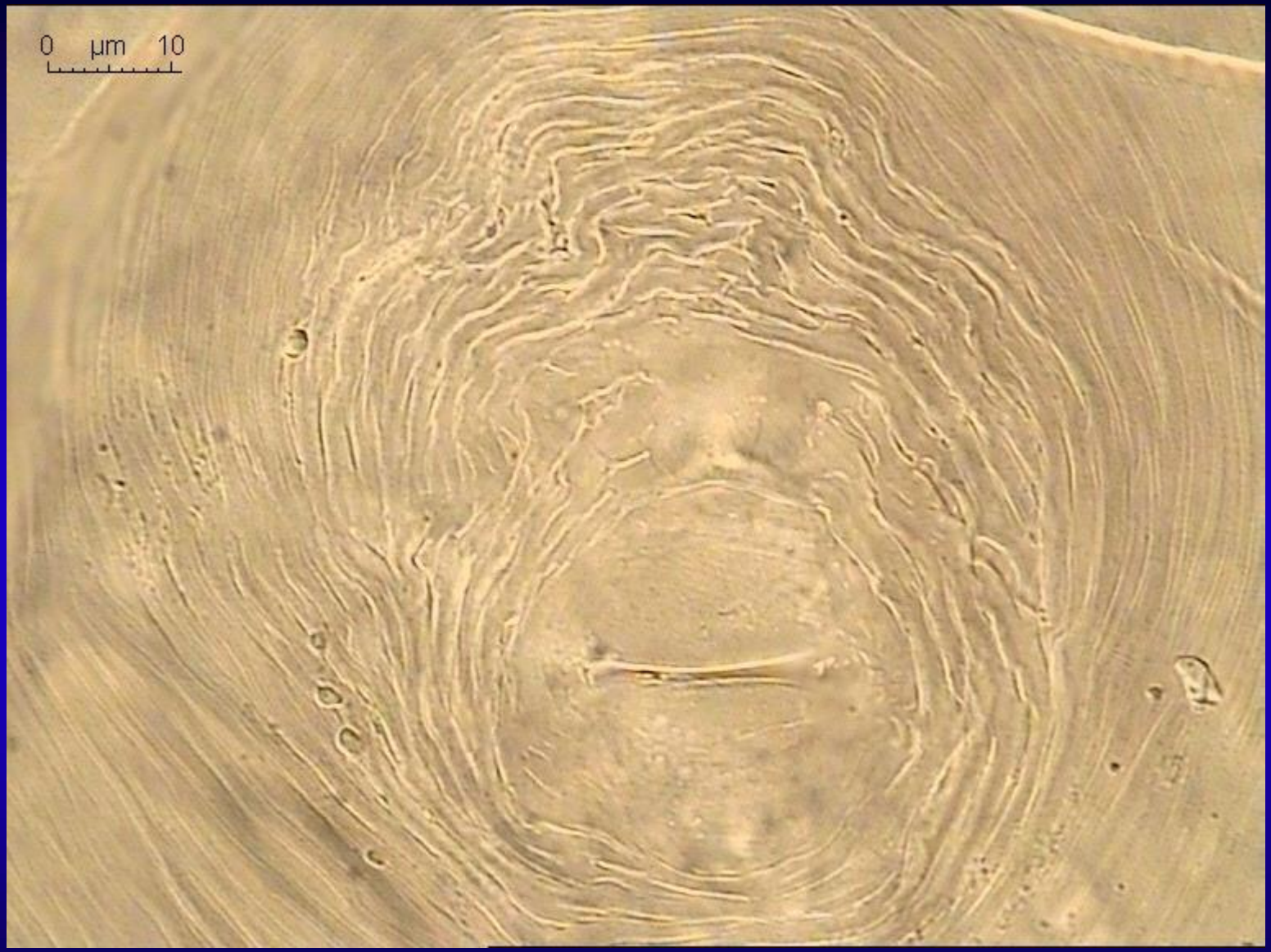
Meloidogyne fallax Fallax – morphological characters

Females

- **body length 0.40 – 0.72 mm (mean 0.59 mm);**
- **body rounded or oval-shaped, white or creamy with short neck;**
- **stylet 13.9-15.2 µm in length (mean 14.5 µm); stylet knobs distinct, rounded and set off; stylet cone slightly curved dorsally;**
- **vulva on small protuberance;**
- **perineal pattern oval-shaped with high dorsal arch and relatively coarse striae;**
- **lateral field with four lines, weakly visible;**
- **there is no punctation in tail terminus area.**



Petineal pattern of *M. fallax*
(phot. Renata Dobosz, Institute of Plant Protection - Governmental Research Institute, Poznań, Poland)



Petineal pattern of *M. fallax*
(phot. LNPV, Le Rheu, France)

Meloidogyne fallax Karssen – morphological characters

Males

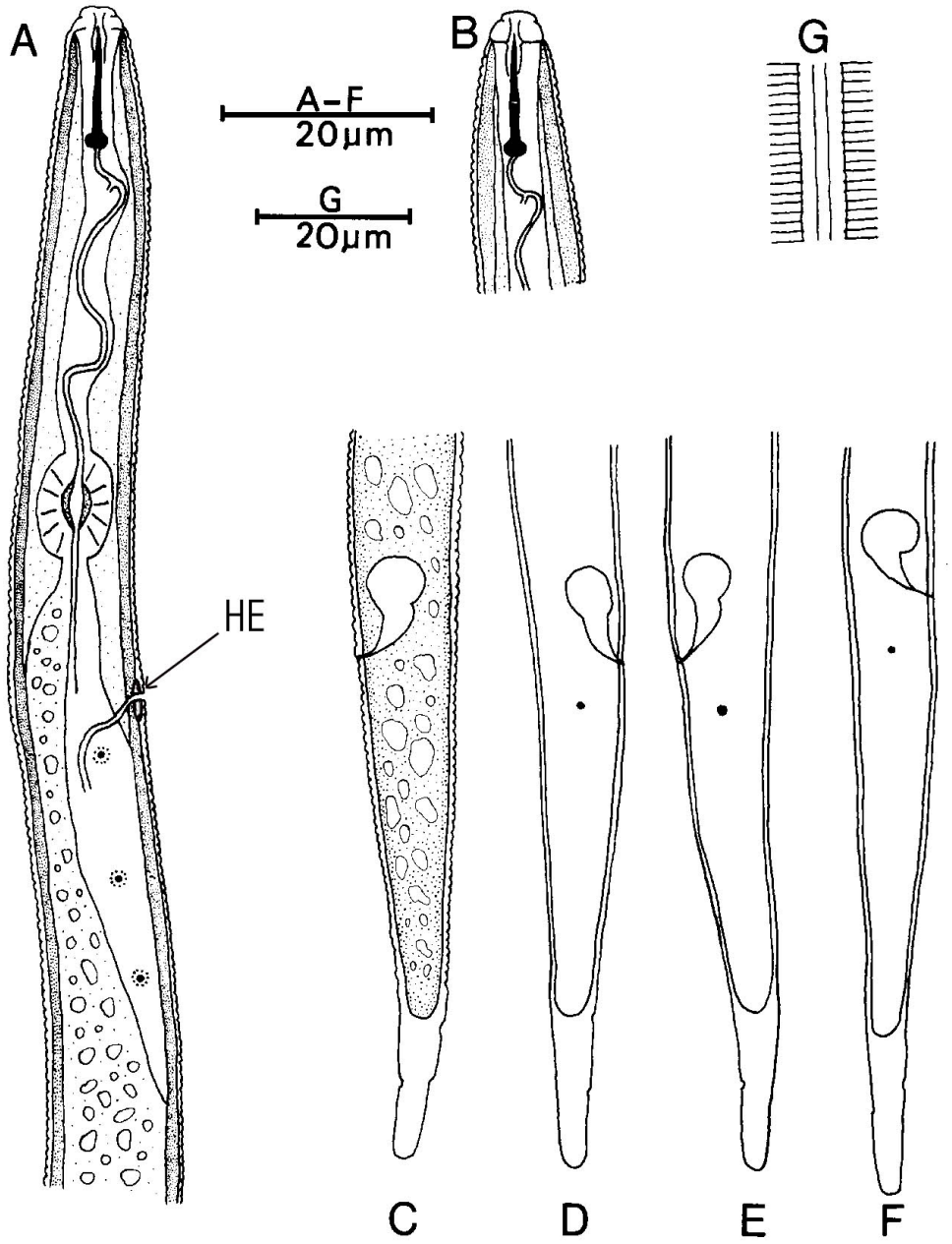
- **body length 0.73 – 1.52 mm; body worm-like;**
- **head slightly set off from the body;**
- **stylet 18.9-20.9 µm in length (mean 19.6 µm); stylet knobs large, rounded and set off.**
- **lateral field with four lines and fifth incomplete line is sometimes present.**

Anterior body portion of *M. fallax male*
(phot. LNPV, Le Rheu, France)

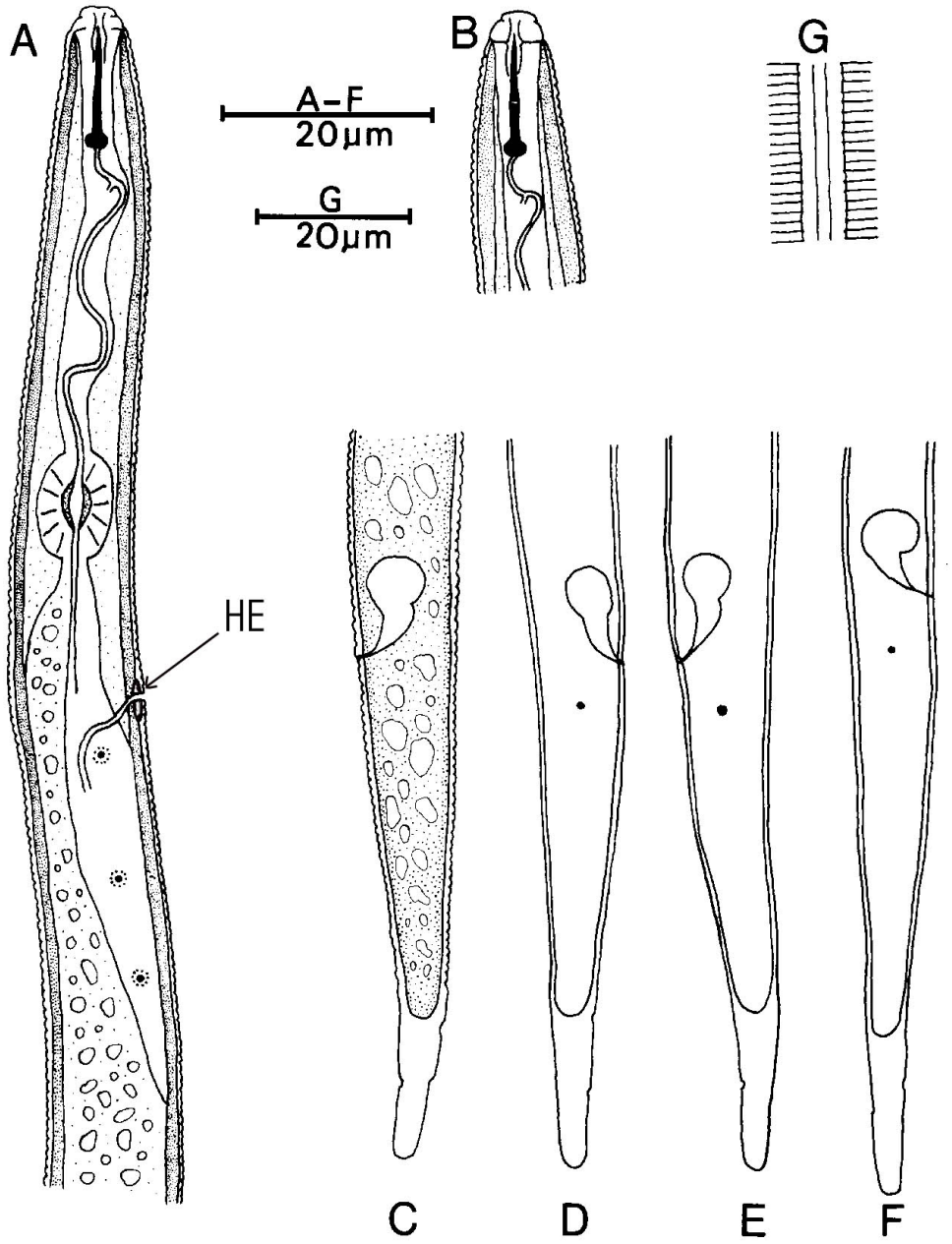
Meloidogyne fallax Karssen – morphological characters

J_2 juveniles

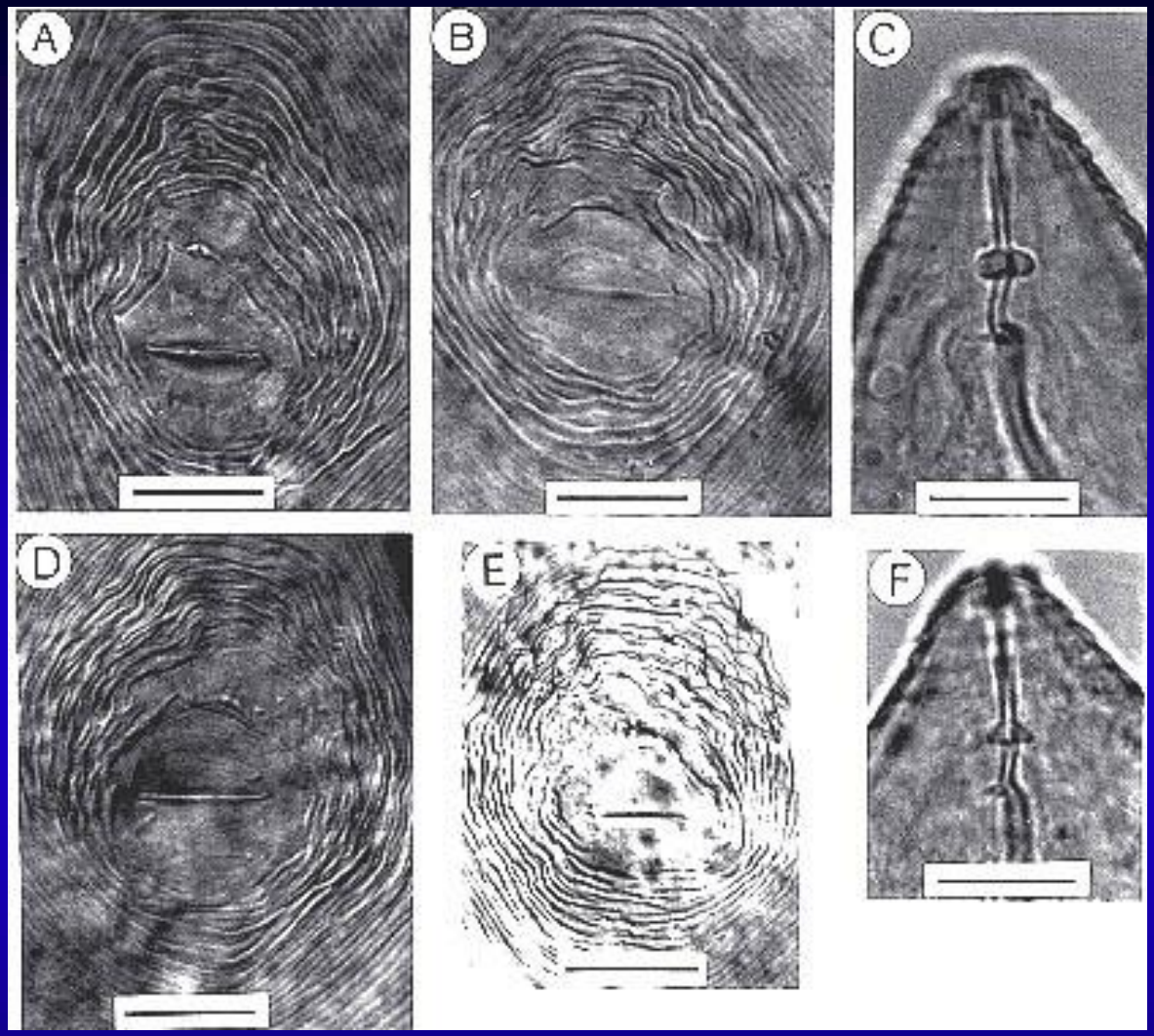
- **body length 381 - 436 µm (mean 400 mm);**
- **stylet 13.9-15.2 µm in length, delicate; stylet knobs large, rounded and set off from the shaft;**
- **tail conical; tail tip broadly rounded, not irregularly shaped;**
- **tail length 49 - 56 µm; hyaline tail part length 12 - 16 µm;**
- **hemizonid at the same level with the excretory pore.**



***M. fallax* J_2 juveniles**
A. anterior body portion (HE – hemizonid);
B. head; C-F tail;
G. lateral field.



***M. fallax* J_2 juveniles**
A. anterior body portion (HE – hemizonid);
B. head; C-F tail;
G. lateral field.



Perineal patterns *Meloidogyne fallax (*A, B, D) and *M. chitwoodi* (E); female head *M. fallax* (C) and *M. chitwoodi* (F)

(phot G. Karssen, the Netherlands)

(scale bar = 25 µ on fig A, B, D, E and 10 µ on fig C and F).

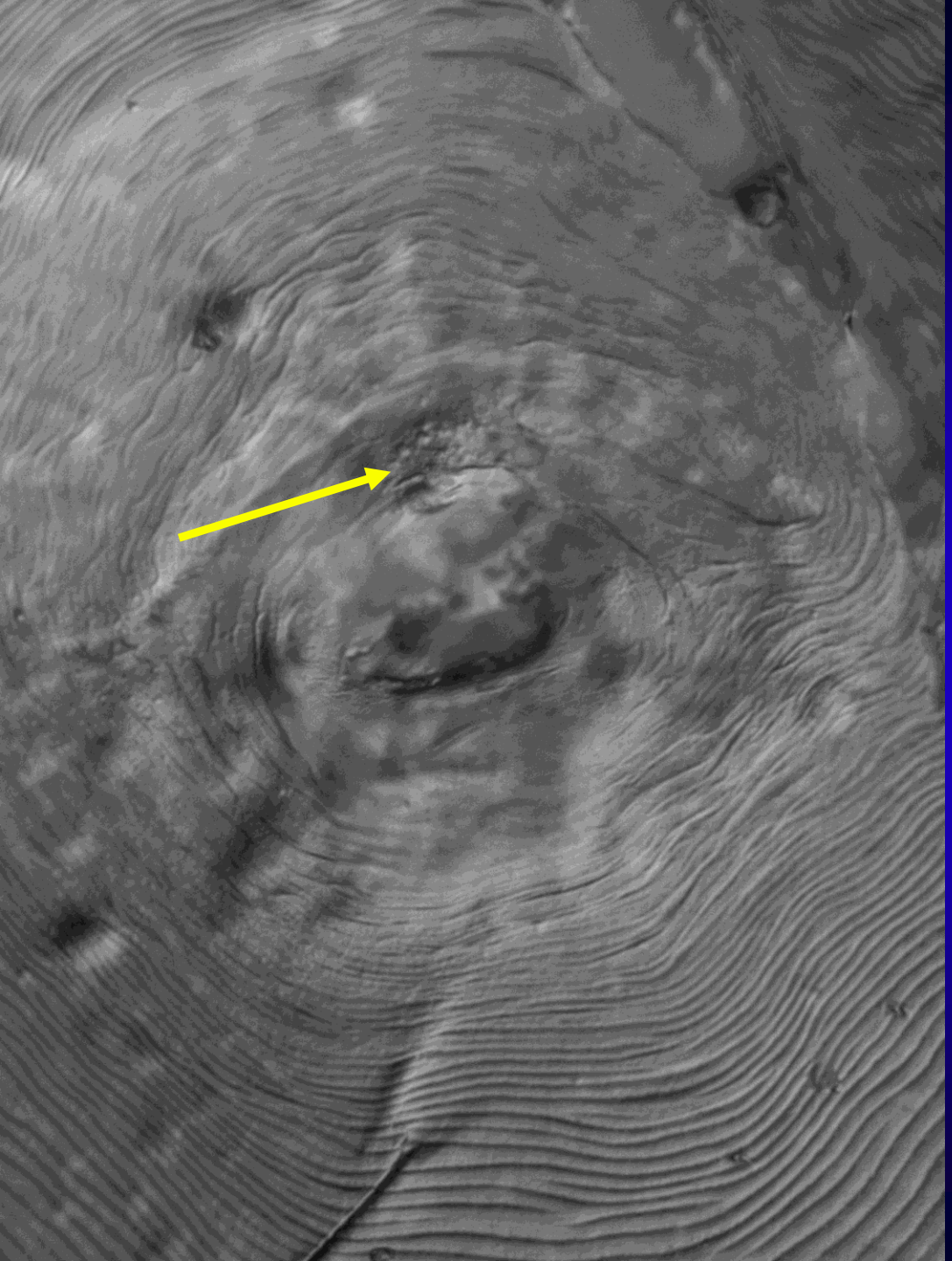
Meloidogyne hapla Chitwood: a common non-quarantine species – morphological characters

Females

- body length 0.42 – 0.85 mm (mean 0.61 mm);
- body pear-shaped,white or creamy with short neck;
- stylet 12.5-17.2 µm in length (mean 14.6 µm); stylet knobs small, rounded and set off; stylet cone slightly straight to slightly curved dorsally;
- perineal pattern rounded with low dorsal arch;
- lateral field with four lines, weakly visible;
- sometimes striae extend laterally, on one side forming a „wing”;
- there is frequently punctation in tail terminus area.

Perineal pattern of *M. hapla* with punctation in tail terminus area (arrow)

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



Perineal pattern of *M. hapla* with punctation in tail terminus area (arrow)

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

Meloidogyne hapla Chitwood – morphological characters

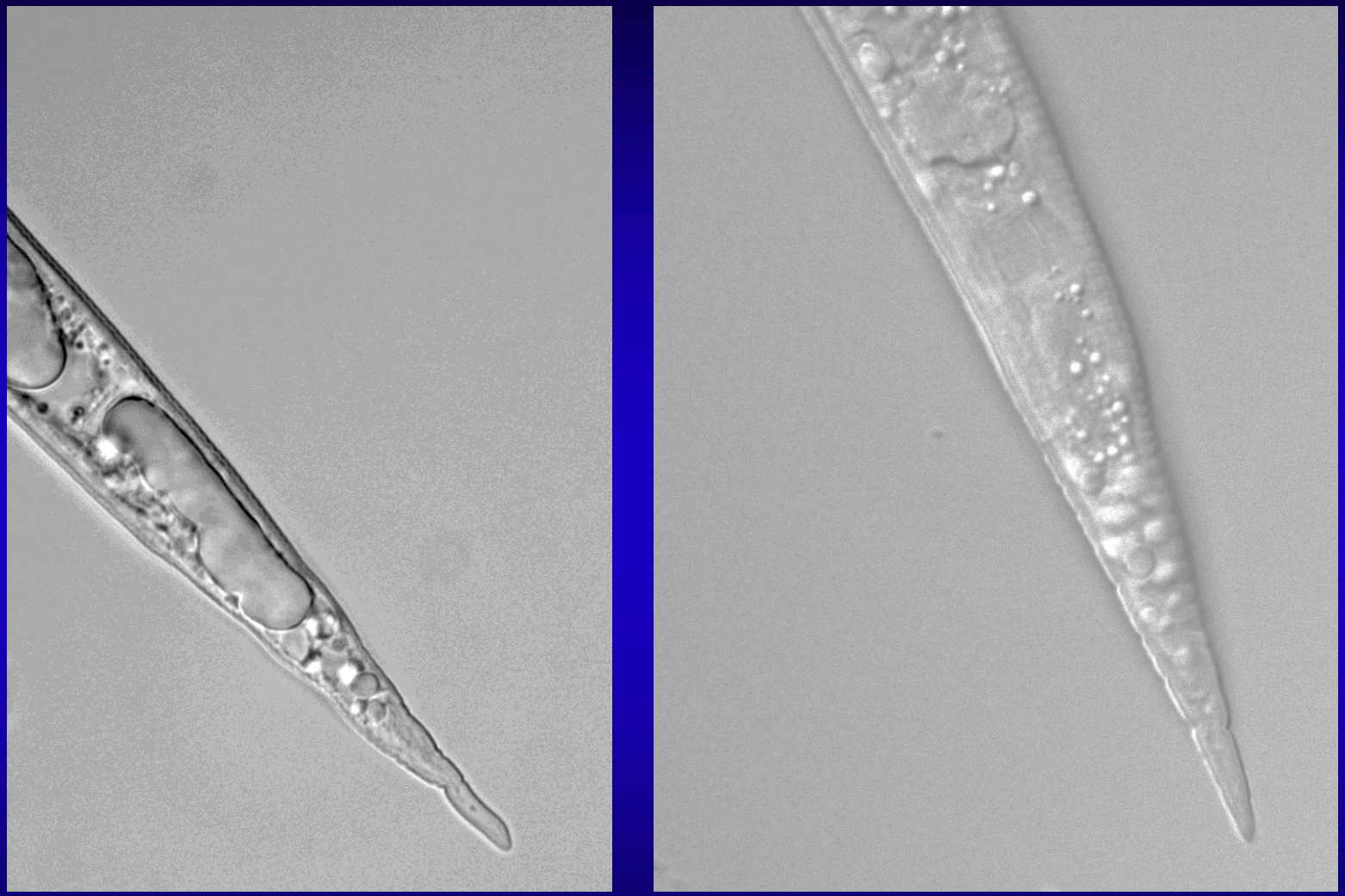
Males

- **head set off from the body;**
- **stylet 19.4-21.6 µm in length (mean 20.4 µm); stylet knobs small, rounded and set off.**
- **lateral field with four lines and fifth incomplete line is sometimes present.**

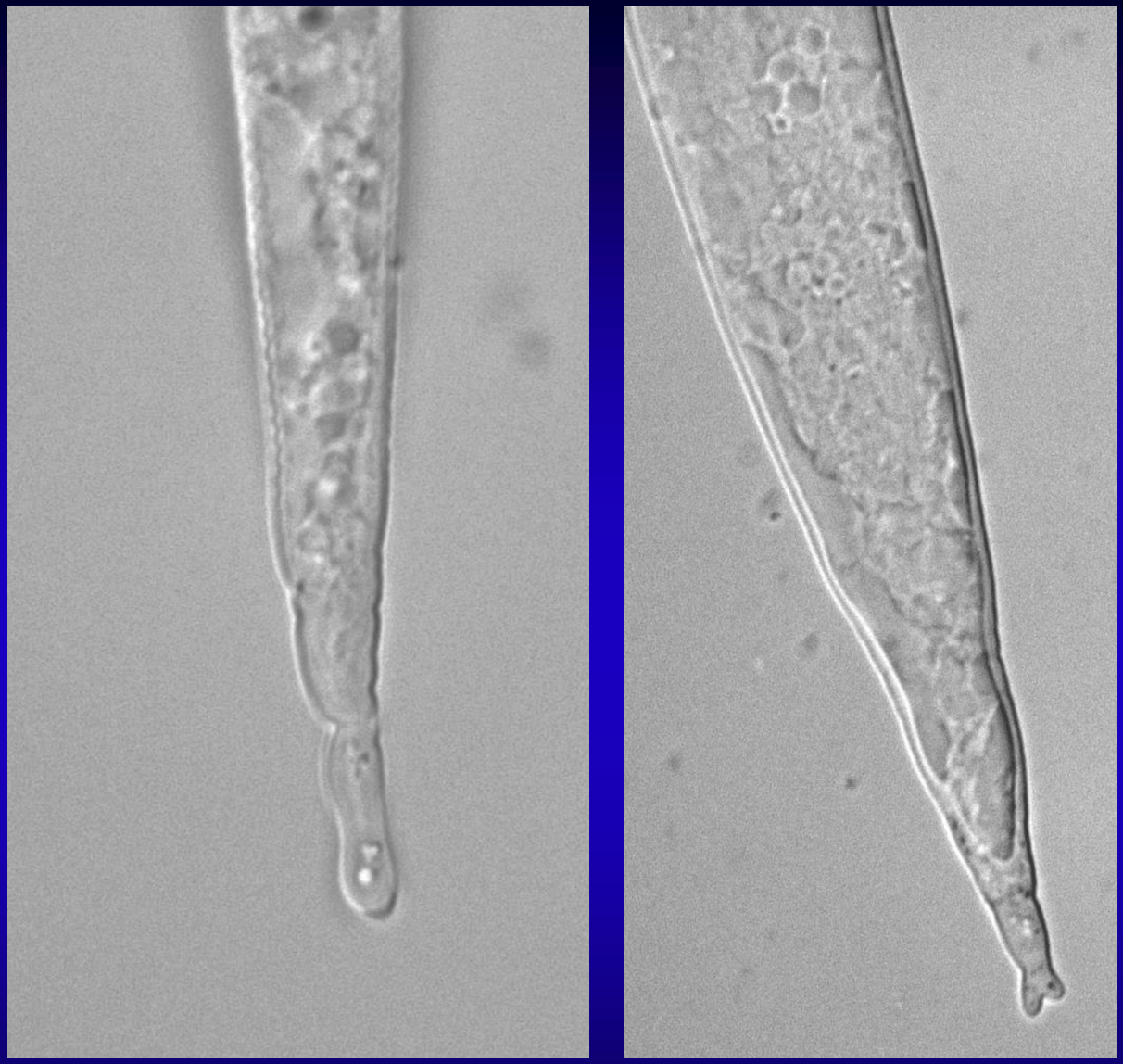
Meloidogyne hapla chitwood – morphological characters

J_2 juveniles

- **body length 305 - 517 µm (mean 410 mm);**
- **stylet 10 - 12 µm in length (mean 11 µm), delicate; stylet knobs large, rounded and set off from the shaft;**
- **tail gradually tapering towards hyaline part; tail tip finely rounded, often irregularly shaped;**
- **tail length 30-69 µm; hyaline tail part length 12 - 16 µm;**
- **hemizonid anterior to the excretory pore.**



Variation of tail shape of *Meloidogyne hapla* J_2 juveniles
(phot. Renata Dobosz, Institute of Plant Protection - Governmental Research Institute, Poznań, Poland)



Variation of tail shape of *Meloidogyne hapla* J_2 juveniles

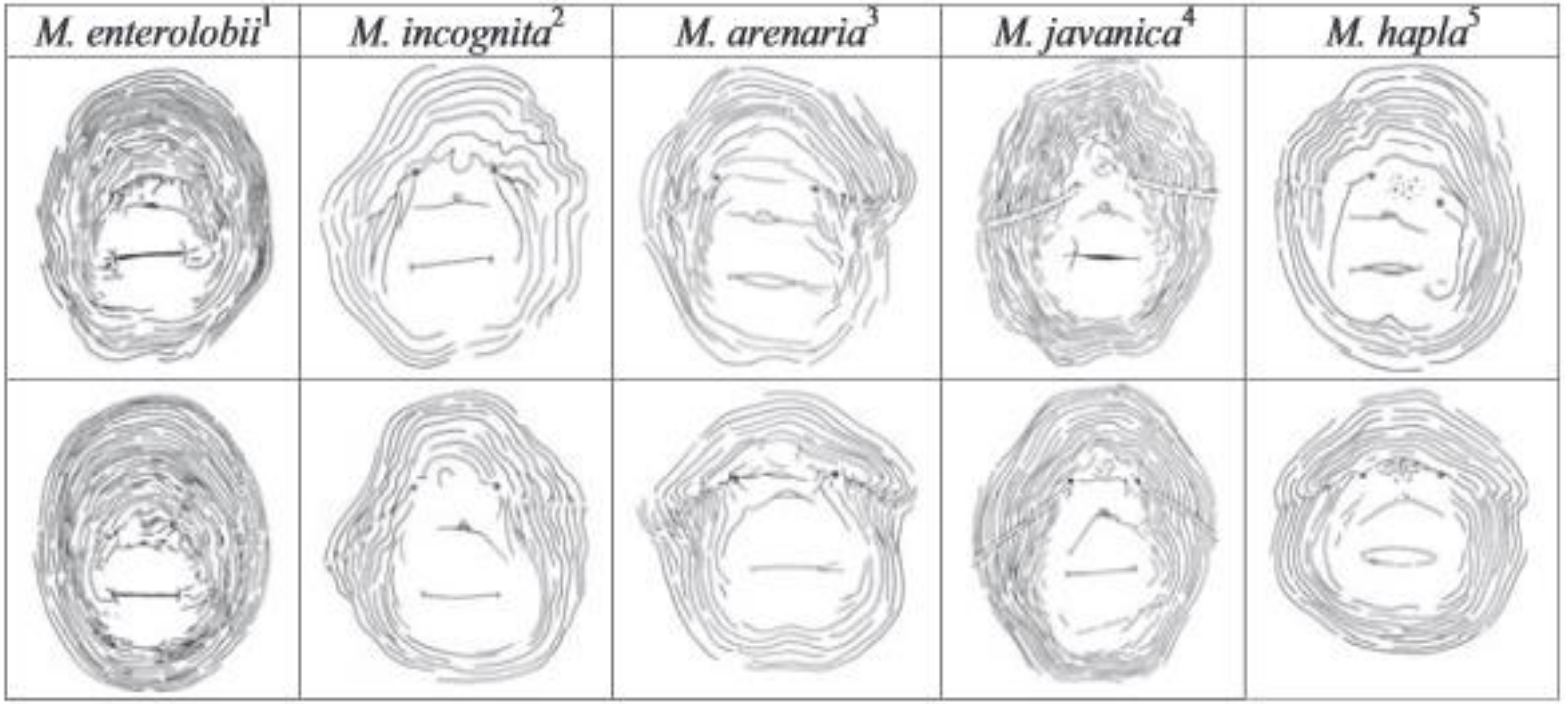
(phot. Renata Dobosz, Institute of Plant Protection - Governmental Research Institute, Poznań, Poland)

Most important measurements and characters of three *Meloidogyne species*

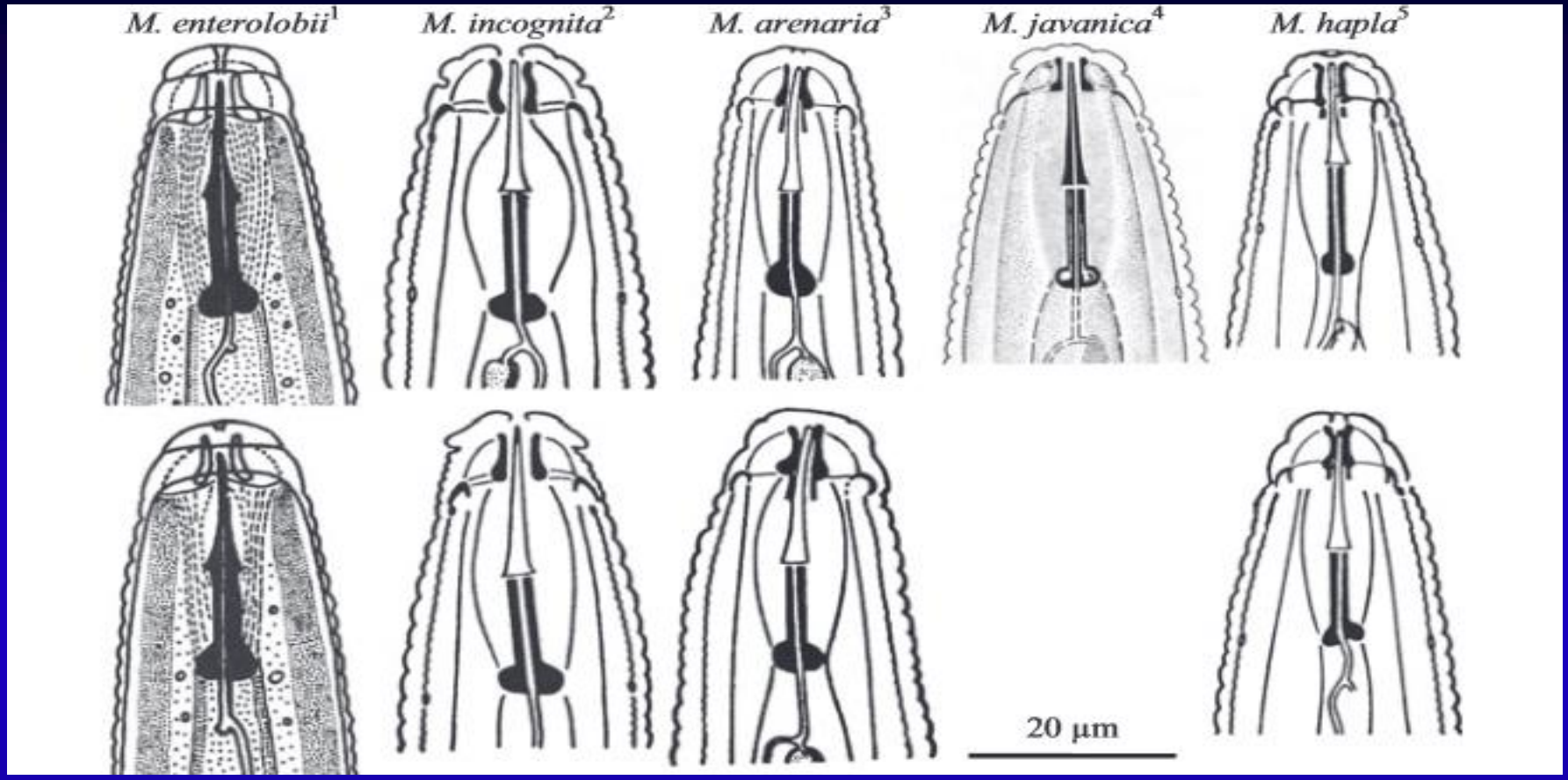
Character	*Meloidogyne hapla*	*Meloidogyne chitwoodi*	*Meloidogyne fallax*
Females Stylet length (µm)	13 - 17	11 - 13	14 - 15
Males Stylet length (µm)	19-22	16 - 18	18 - 21
J_2 juveniles			
Body length	305 - 317	336 - 417	381- 436
Tail length	30 - 69	39 – 47	49 – 56
Hyaline tail part length	6 – 20	9 – 14	12-16
Stylet knobs shape	rounded	rounded	rounded

Meloidogyne **from the former Yugoslavia found in Bosnia and Herzegovina**

1. **M. arenaria (Neal, 1889) Chitwood, 1949 :** Serbia and in Vojvodina, Bosnia and Herzegovina, Slovenia, Macedonia
2. **M. hapla Chitwood, 1949 :** Serbia and in Vojvodina, Bosnia and Herzegovina, Slovenia;
3. **M. incognita (Kofoid & White, 1919) Chitwood, 1949 (syn. M. acrita**) Serbia and in Vojvodina, Bosnia and Herzegovina, Slovenia, Macedonia
4. **M. javanica (Treub, 1885) Chitwood, 1949** Serbia and in Vojvodina, Bosnia and Herzegovina



Perineal pattern of some EU non-quarantine *Meloidogyne* species found in Europe.



Male lip regions of some non-quarantine *Meloidogyne* species found in Europe.