

USDA-ARS Identification of Potato Cyst Nematodes in the United States

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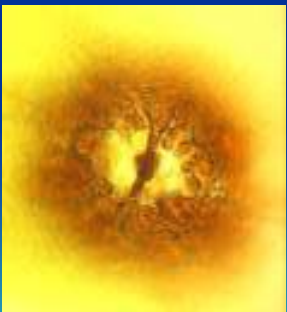
PALE CYST NEMATODE

(*Globodera pallida*)

Morphology

Zafar A. Handoo, Ph. D.

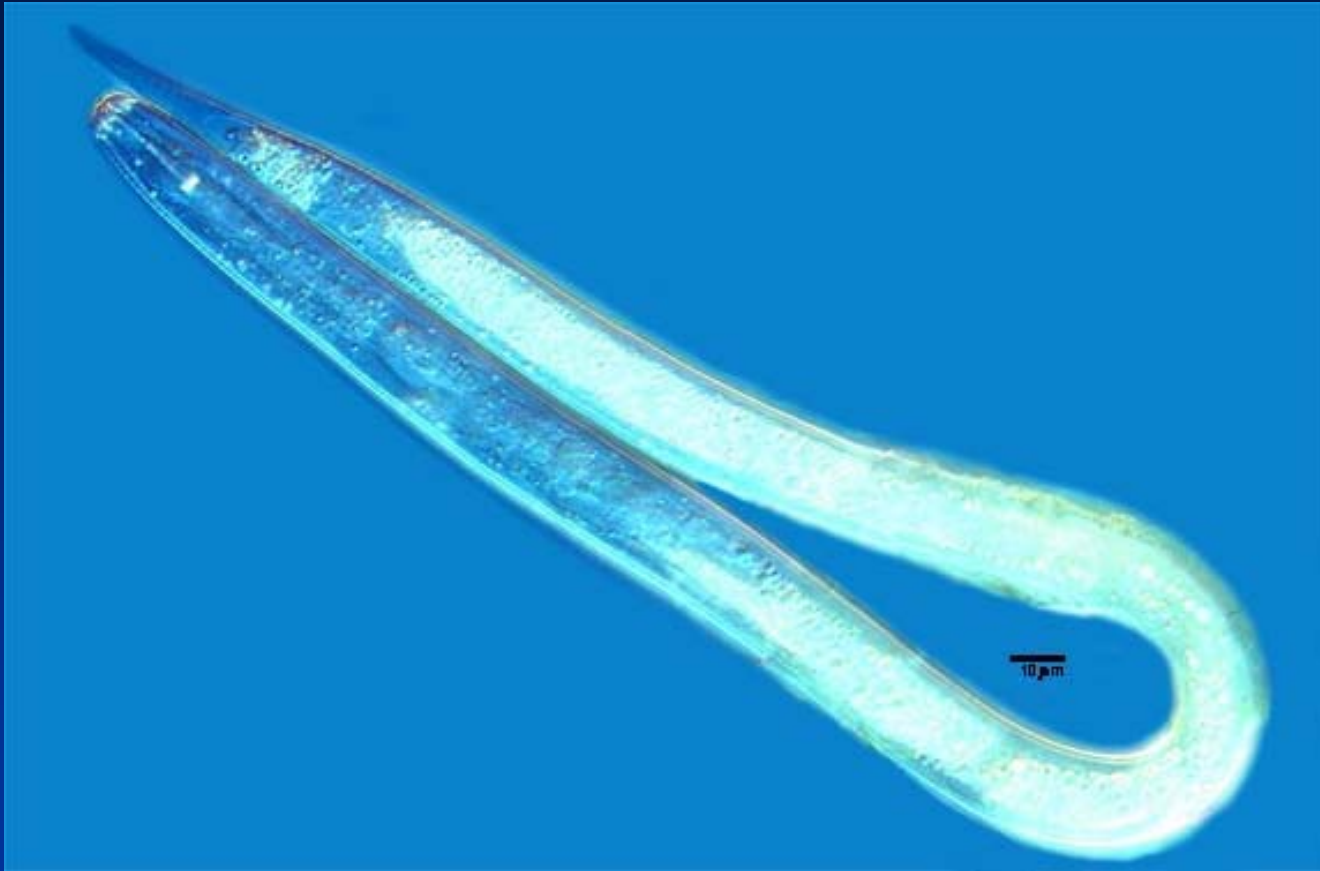
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Morphological characters for identification:

- Cyst shape
- Characteristics of cyst terminal cone including nature of fenestration
- Cyst wall pattern
- Anal-vulval distance
- Number of cuticular ridges between anus and vulva
- Granek's ratio





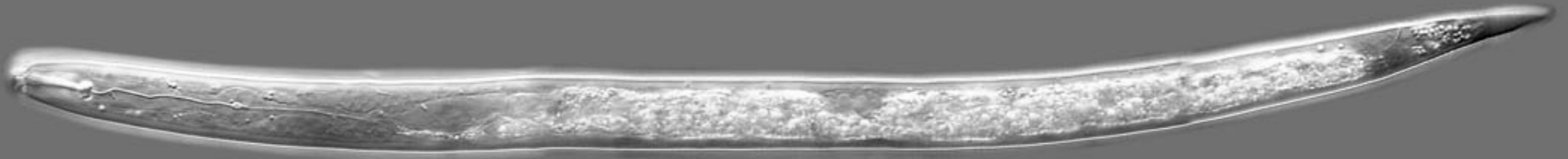
Cyst wall pattern after
Golden & Ellington,
1972

G. rostochiensis juvenile from New York

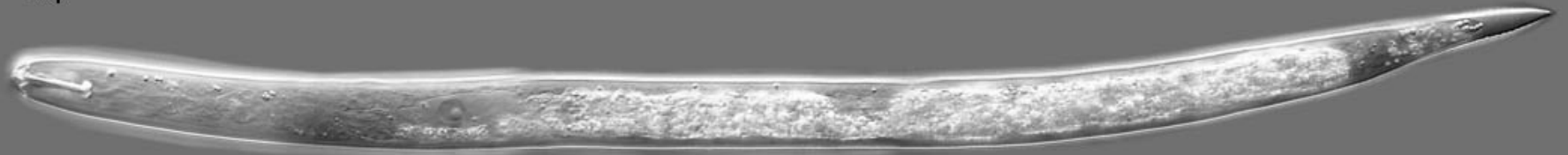


Globodera pallida

cen



sup



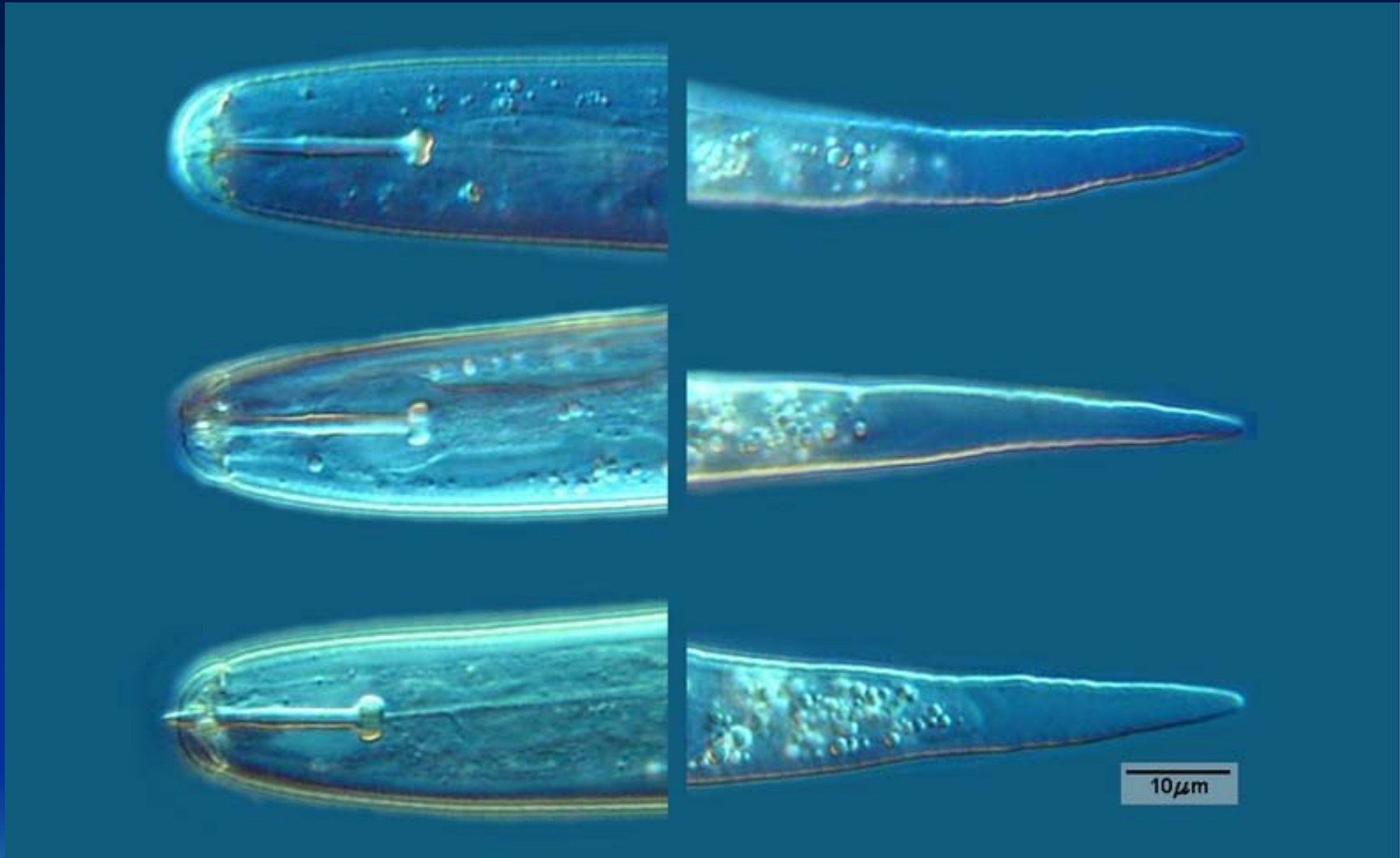
10 μ m

Globodera pallida J2 from Idaho tare soil; differential interference contrast composite images of central and superficial focal planes

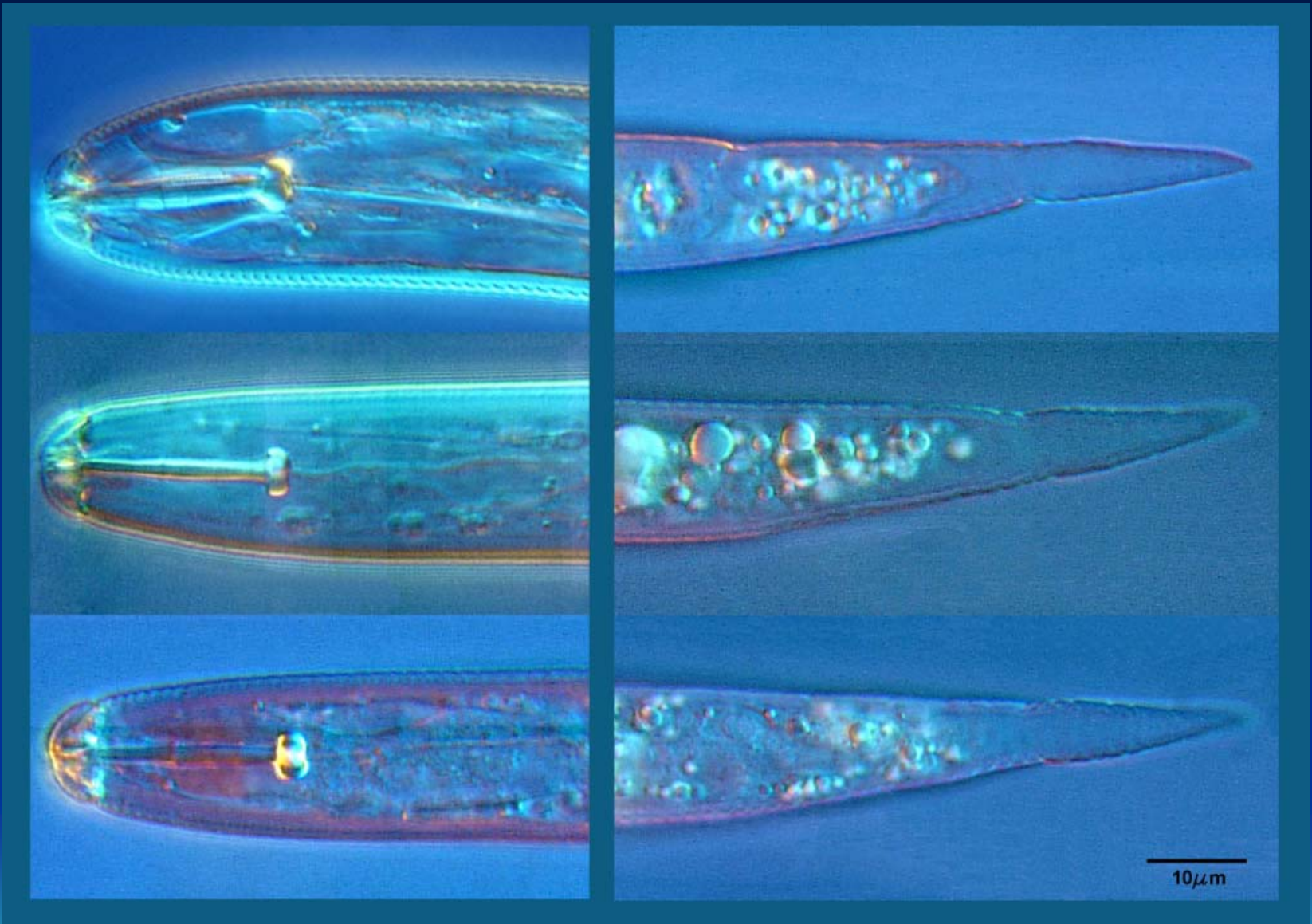
The second-stage juvenile morphology critical for identification:

- **Body length**
- **Stylet length**
- **Shape of stylet knobs**
- **Shape and length of tail**
- **Shape and length of hyaline tail terminus**
- **Number of refractive bodies in the hyaline part of tail**

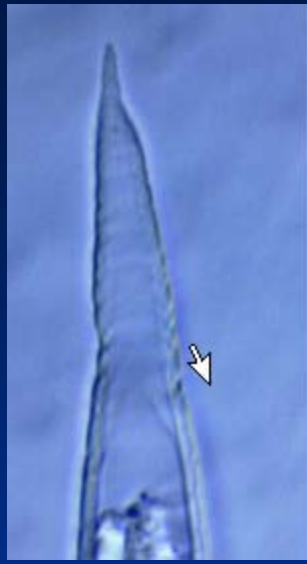




Globodera rostochiensis J2 heads, tails from New York



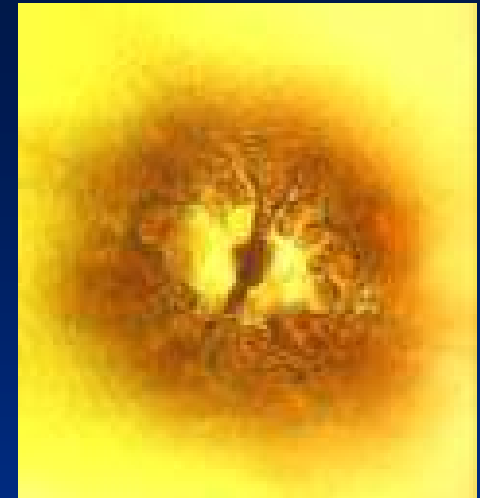
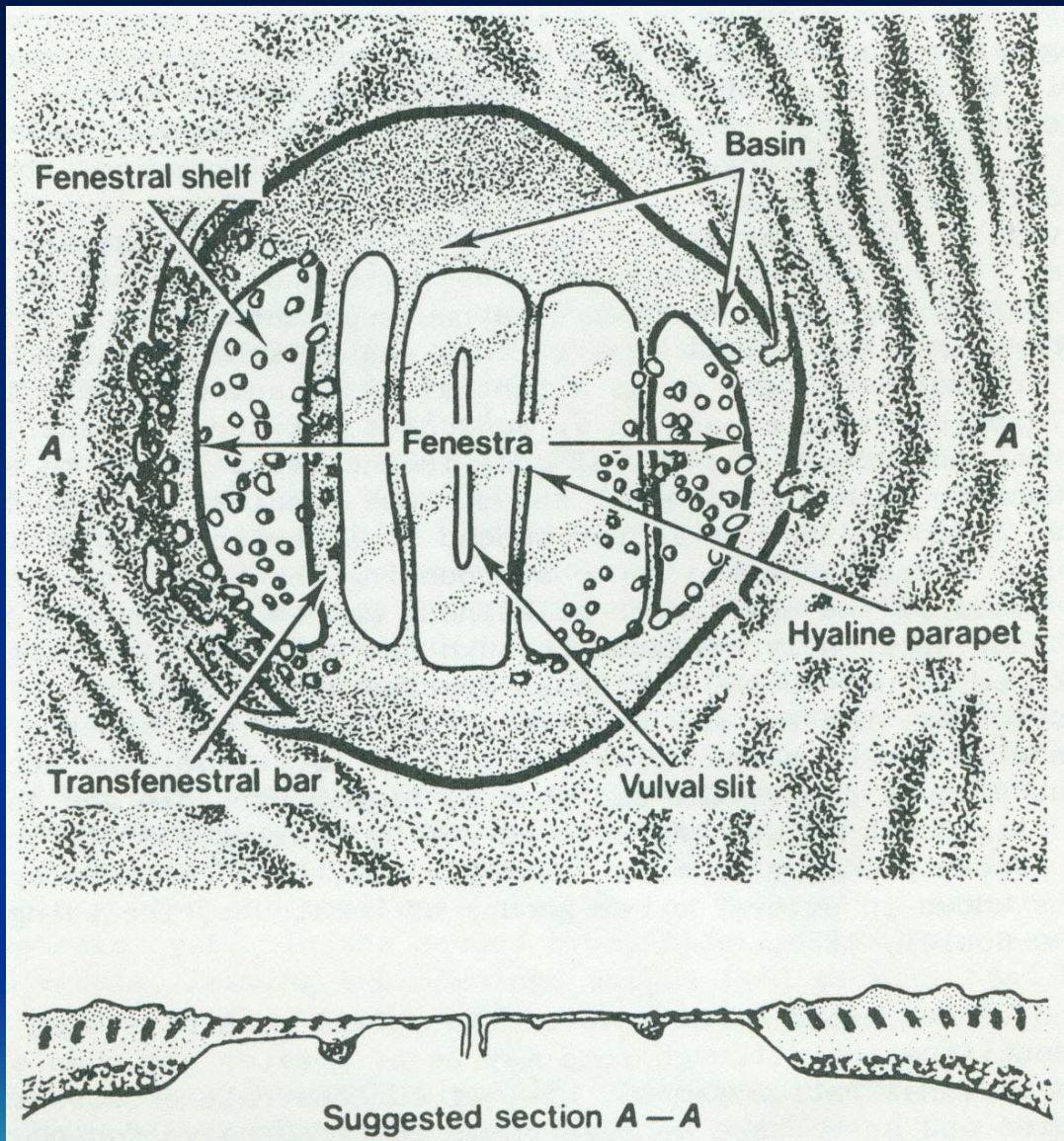
Globodera pallida J2 heads, tails from tare soil, Idaho.



Some tails from the new population are somewhat more acutely pointed than the original population of *G. pallida*.



G. pallida has 4-7 distinctive refractive bodies in the hyaline portion of tail vs. 2 in *G. rostochiensis*.



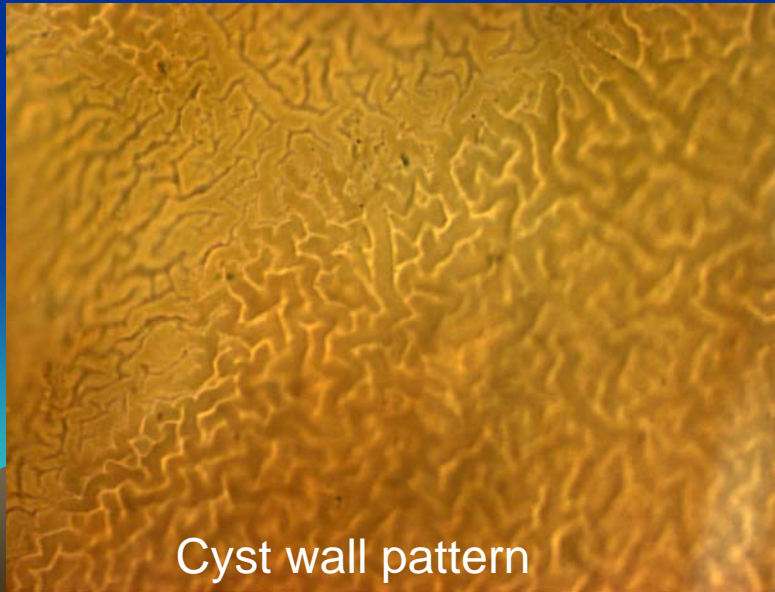
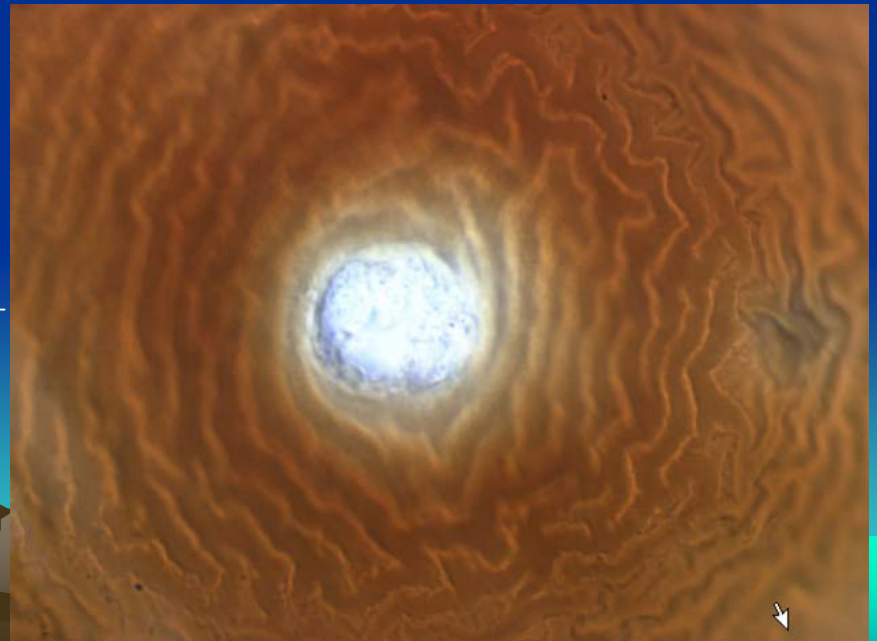
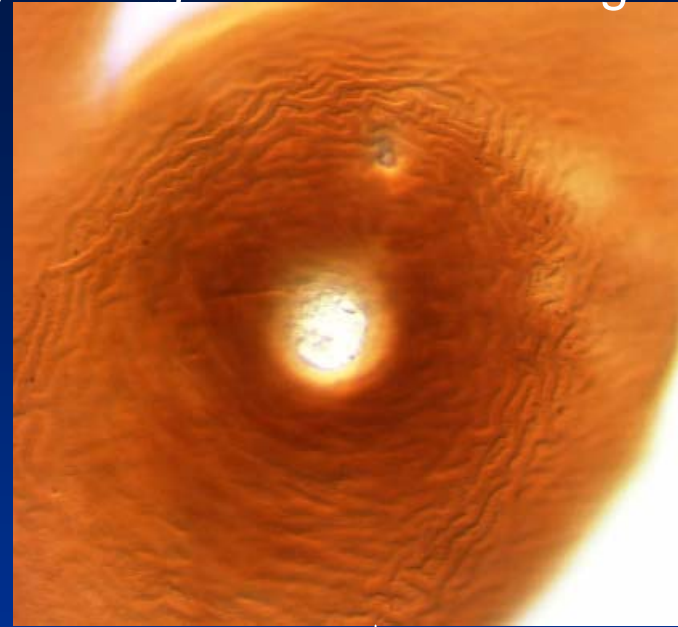
Heterodera avenae



Side view of lemon-shaped cyst cone

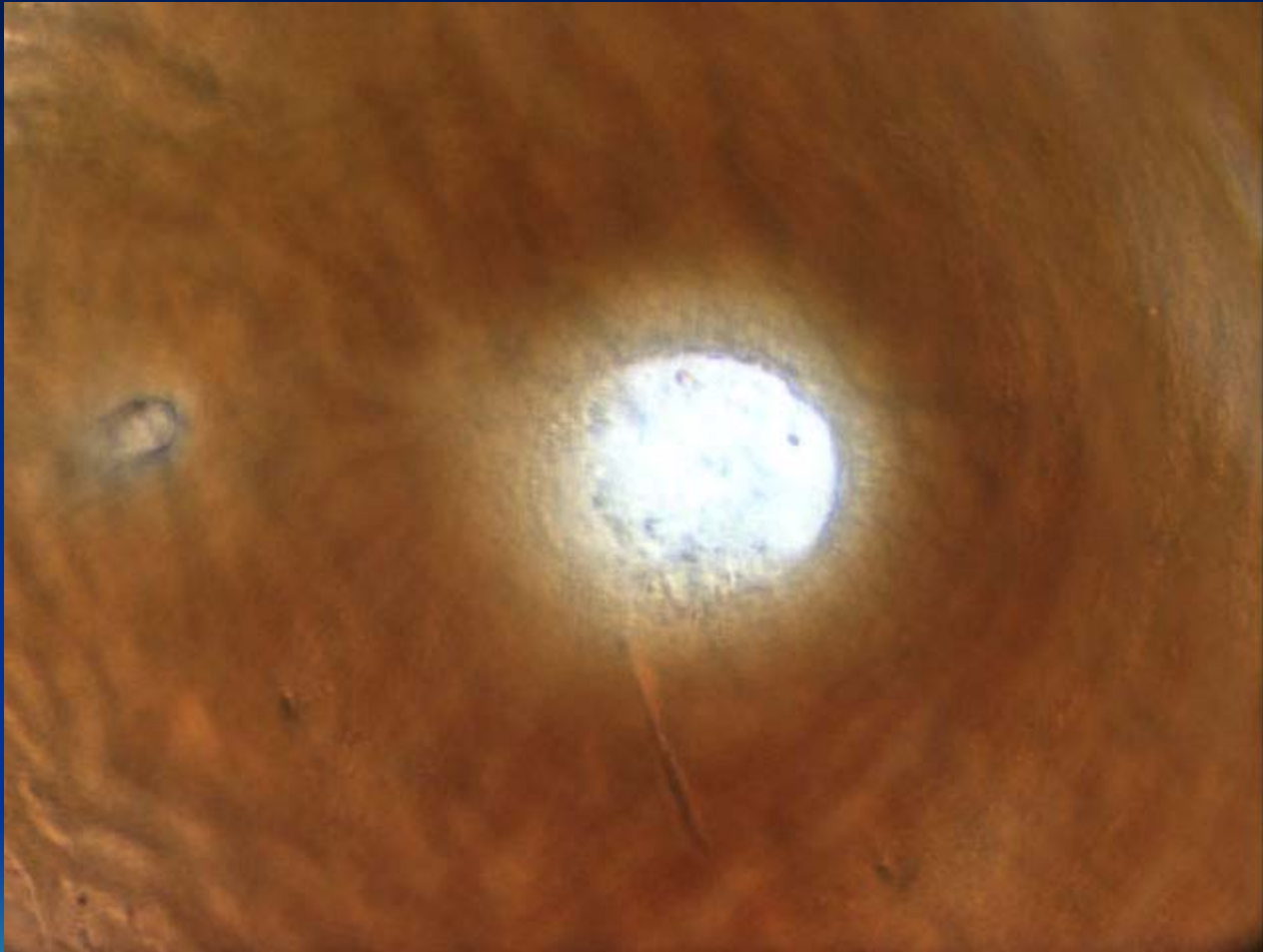
Face View of Perineal Region of Cyst Nematodes after Golden, 1986

Globodera pallida Circumfenestrate Cyst images from Low to High Power



Cyst wall pattern

Information on the 6 cysts we received follows:



Cyst 1

Cyst 1

From this cyst, 9 second - stage juveniles were measured as follows:

Body length = 470.0 (427.0 - 490.0 um)

Stylet length = 23.1 (22.5 - 24.0 um)

Stylet knobs with forward projection on anterior face in lateral view

Tail length 49.0 (45.0 - 52.5 um)

Hyaline tail terminus length = 26.4 (23.0 - 27.5 um).

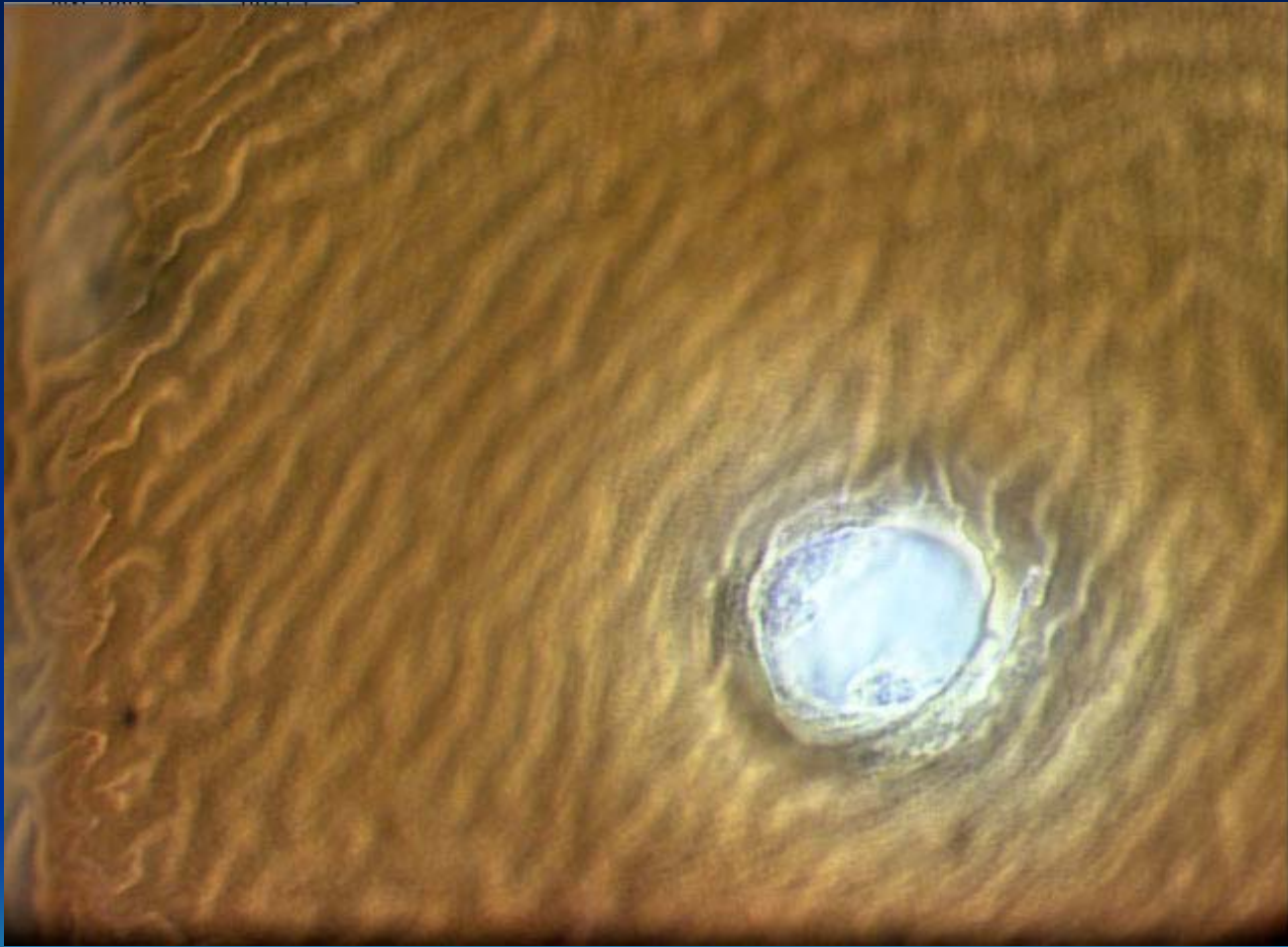
Tail gradually tapers to a finely rounded point, (a few tails had more pointed terminus variation)

Number of refractive bodies on hyaline tail terminus were between 4 to 7 like typical *G. pallida*.

Conclusion: Cyst and juvenile morphology were consistent with *G. pallida*.

Juveniles were given to Lynn Carta and Andrea Skantar for PCR.

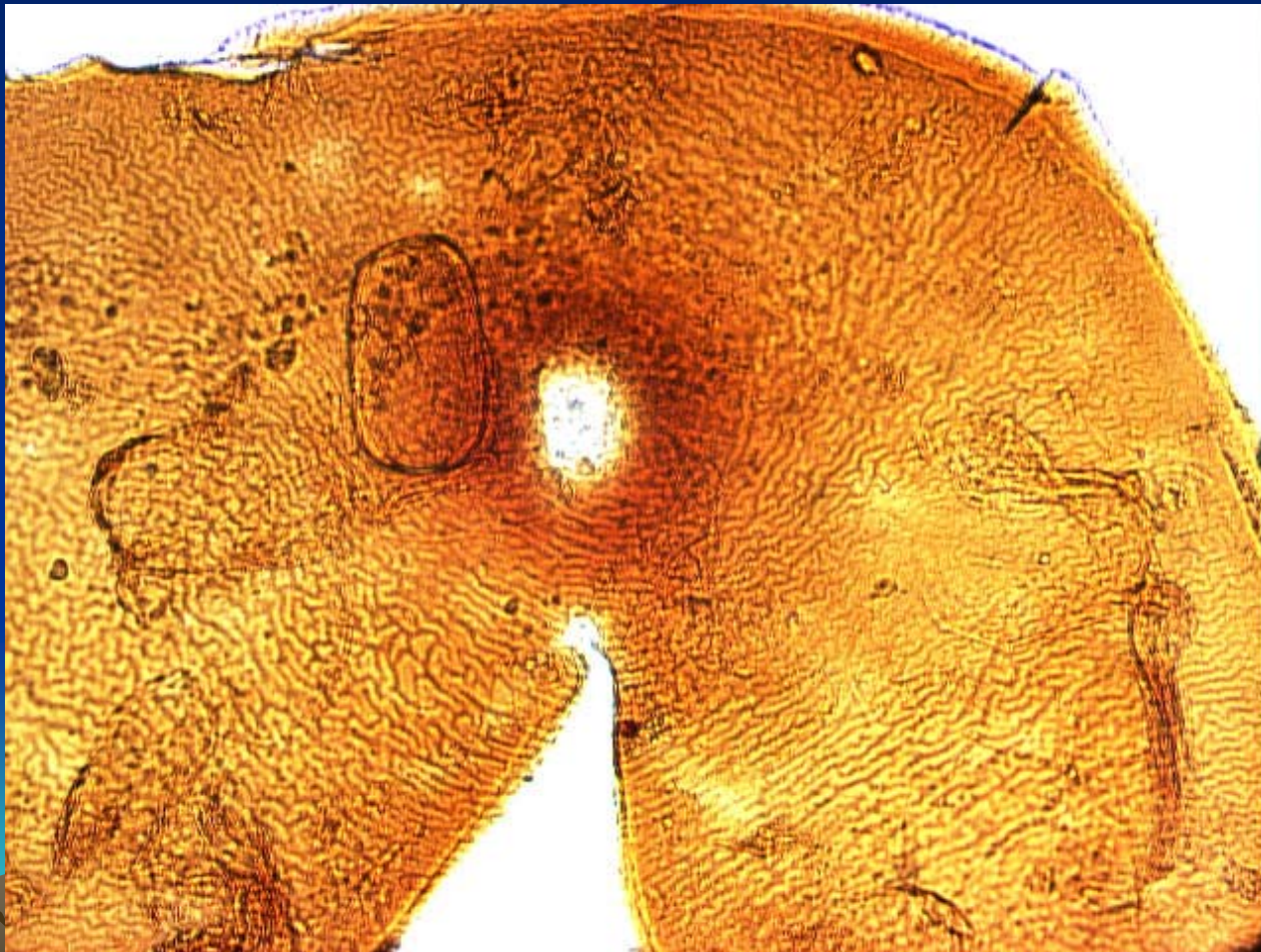
Cyst 2



Cyst morphology consistent with *G. pallida*
Juveniles absent

Cyst 3

Cyst morphology consistent with *G. pallida*.
Juveniles absent, eggs consumed by fungus.



Cyst 4

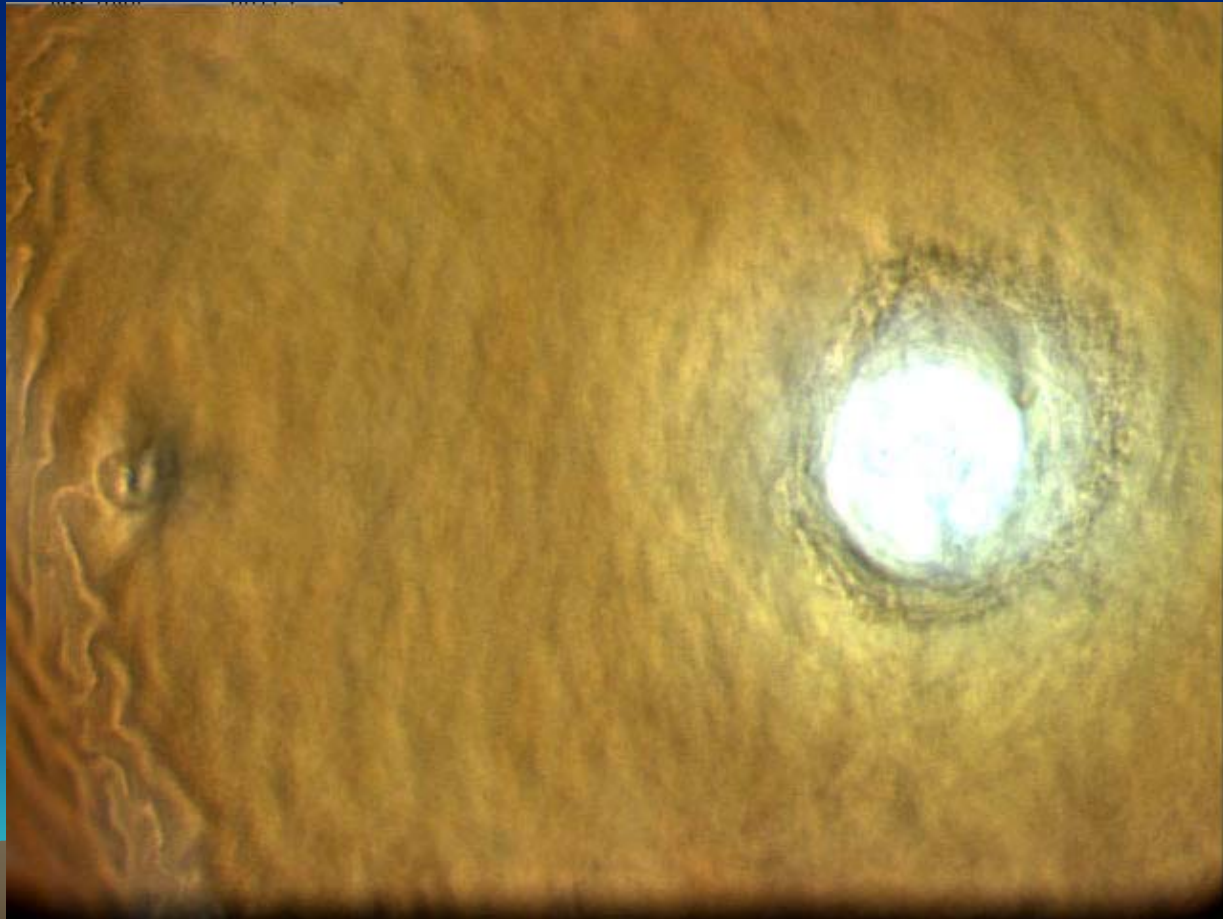
Cyst in poor shape

**Juveniles within eggs had tail
termini consistent with *G. pallida***



Cyst 5

Cyst morphology consistent with *G. pallida*
Juveniles absent



Cyst 6

**Cyst was in very poor shape
Juveniles absent**



- We have assigned the USDA Nematode Collection numbers to these slides as G-15909 to G-15914 cysts, and G-15915 to G-15918 for juveniles. In addition, we put the rest of the material in vials as G-6159f hatched larvae, G-6160f to G-6162f eggs from cysts 2, 3 & 5.



Cyst Features

- Cysts were spherical in shape, circumfenestrate, abulate, punctate, distance from anus to nearest edge of fenestra = 47.5 (35.0 - 65.0), length of fenestra = 23.0 (20.0 - 30.0) number of cuticular ridges between vulva and anus = 12 - 15, mean of Granek's ratio based on four good posterior ends = 2.4 (1.8 - 3.0) as revised by Hesling, 1973.

Globodera pallida Diagnosis

- All the above morphometrics for second-stage juveniles and cysts are consistent with
- Stone, 1972 original description
- Golden, 1986 key
- Baldwin & Mundo-Ocampo, 1991 compendium
- Fleming & Powers, 1998
- EPPO/OEPP, 2004



Summary

The morphology of cysts,
Second-stage juveniles and molecular
analysis
established the identity of the new
population
as the pale cyst nematode species
Globodera pallida (Stone, 1973) Behrens,
1975.



Integration of Morphology and Molecular Characters

Lynn Carta, Ph.D.

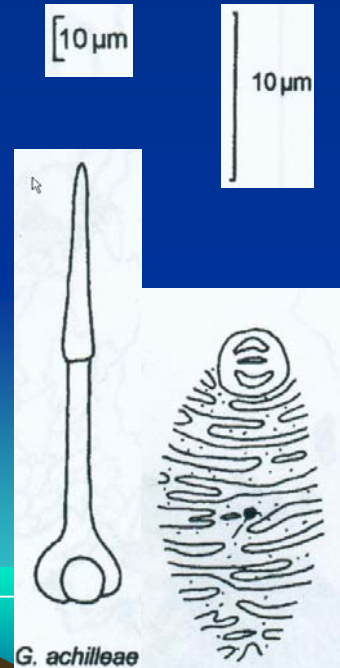
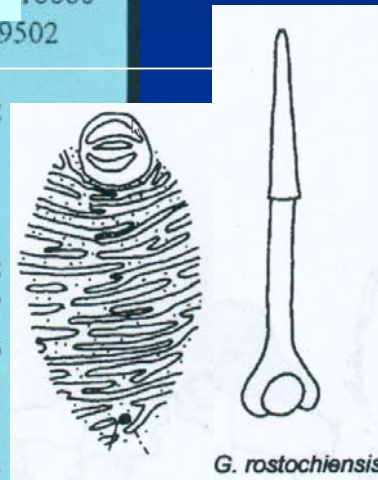
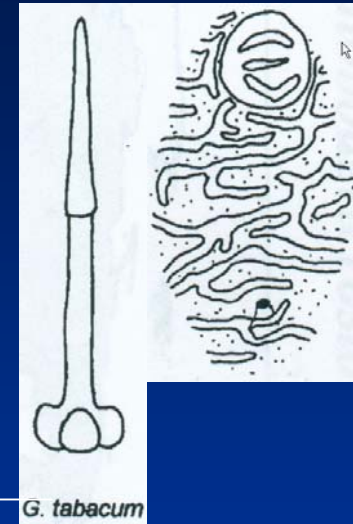
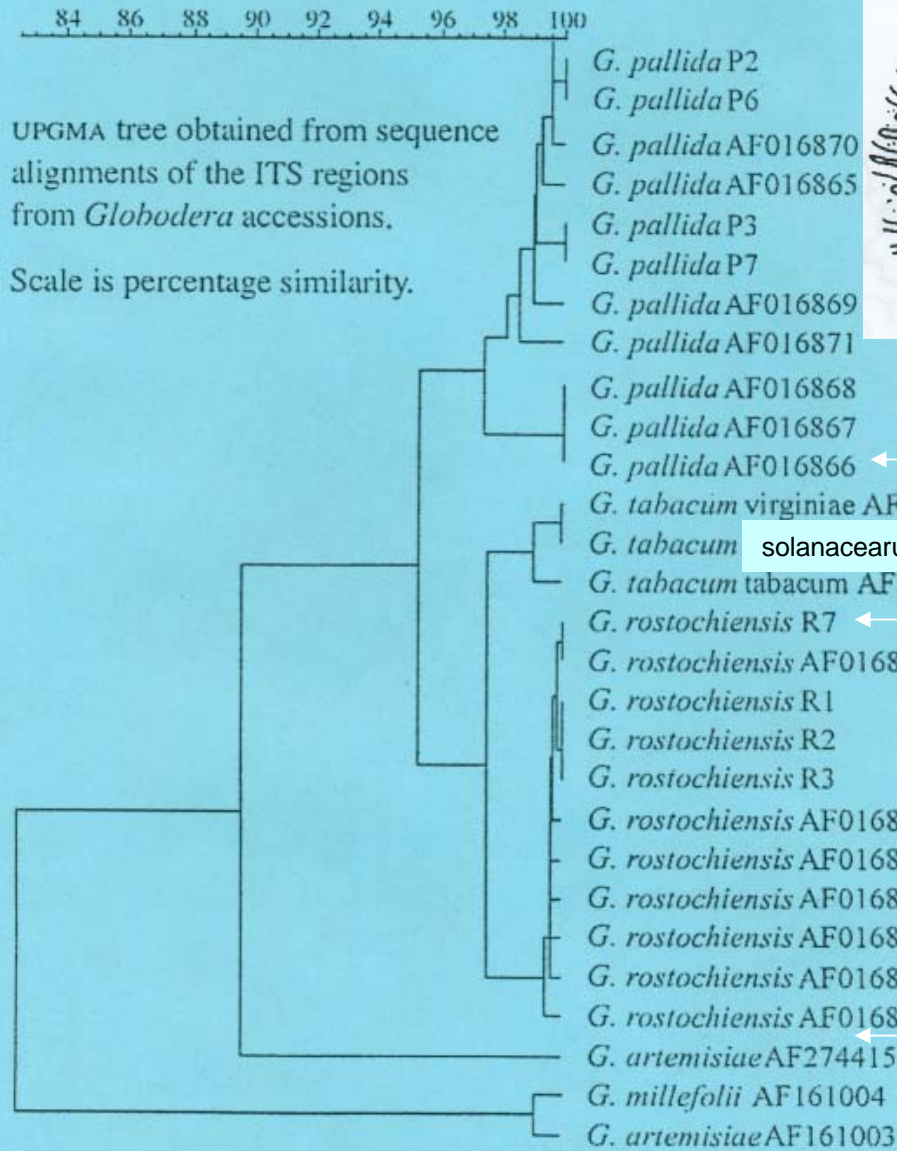
Research Plant Pathologist

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Stylet and Cyst wall pattern of Fleming & Powers, 1986, after Stone, 1973

Distance Tree of Reid and Pickup, 2005



[10 μm]

10 μm

Molecular diagnosis as *G. pallida*

Clear confirmation by two tests:

PCR-RFLP profiles of a ribosomal DNA fragment using restriction enzymes RsaI, TaqI, and AluI were consistent with a *G. pallida* control and not *G. rostochiensis*.

The ribosomal DNA region that extends from the 3' end of the 18S ribosomal subunit and includes all of ITS1, 5.8S, and ITS2, to the 5' end of the 28S ribosomal subunit was used to generate the most accurate species determination. Sequences obtained from three individual juveniles were compared to those from several *Globodera* species, revealing unequivocal similarity to *G. pallida*.

Andrea Skantar, Ph. D.,
Research Molecular Biologist

