Molecular and Morphological Identification of Nematode Isolate from China

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Outline of the Presentation

- **The pupose of this study**
- **Background**
- **Molecular Identification**
- **Morphological Identification**
- **Profile of classification characters**
- **Conclusion and discussion**
- **Acknowledgement**

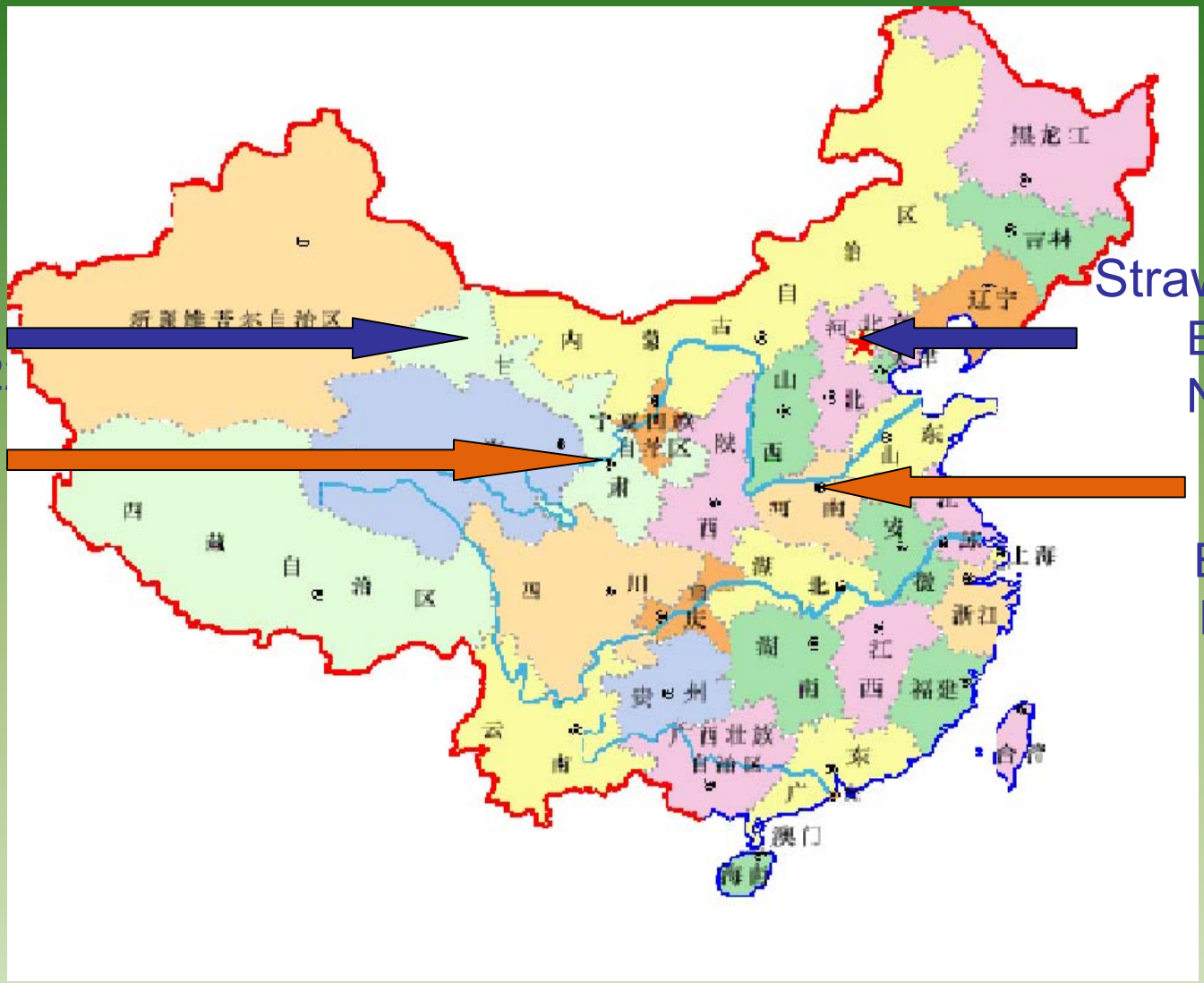
The purpose of this study:

- To make sure whether these nematode isolate are EPNs
- To identify the species of the isolates

Background (1)

Gaotai-Merlot
E99°5’ N 39°2

Gan1
E104°53’
N34°44’



Strawberry3
E116°28’
N 39°54’

H2
E114°12’
N 34°24’

4 isolations from dessert, Beijing and Yangze River, China

Background (2)

- The isolates kill *Galleria* and *Tenebrio* within 2-4d

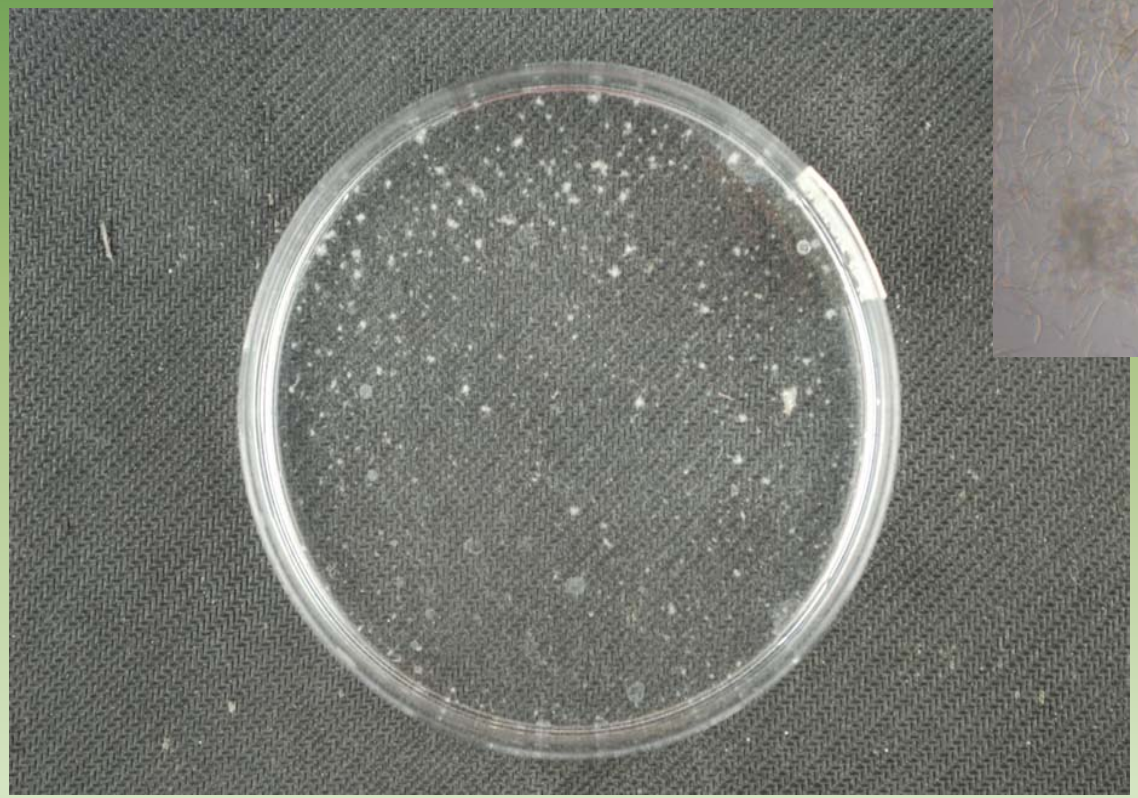
- Can not grow on dissected *Galleria* (dead)

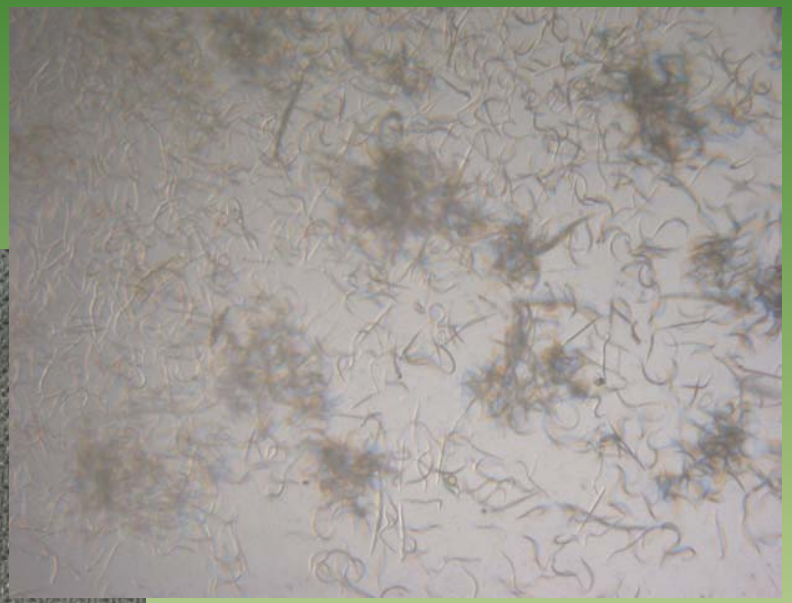
Background (3)

- Kill and grow in the cadaver of *Galleria* and *Tenebrio*

Background (4)

- Accumulate in clump





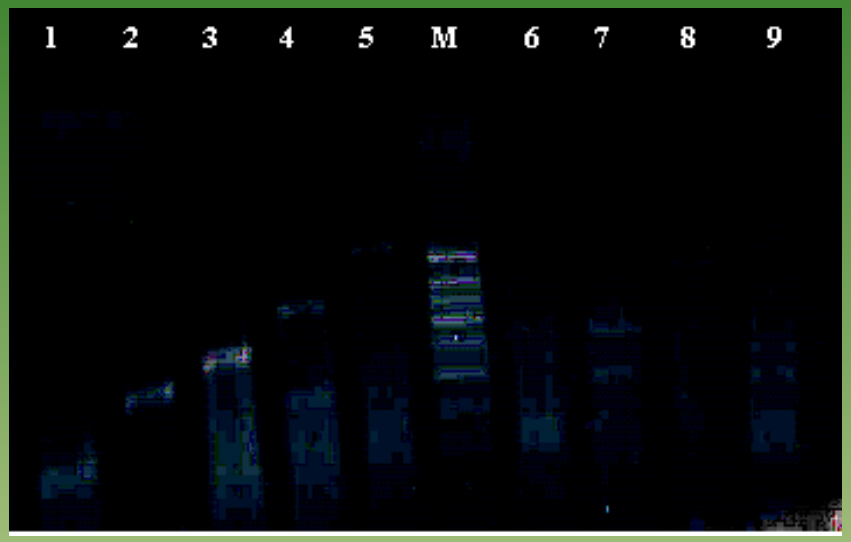
Molecular Identification

*DNA extraction (DNeasy Tissue Kit-Qiagen, Handbook)

*PCR amplification for ITS region
Primer: (Vrain et al.,1992)
Forward 18S (5`-TTGATTACGTCCCTGCCCTTT-3`)
Reverse 26S (5`-TTTCACTCGCCGTTACTAAGG-3`)
Programme: (Reid and Hominick, 1998).
DNA at 95oC- 94oC – 94oC (40 cycl.)- 55oC- 72oC, 4oC

*RFLP (Restriction Enzymes) : Alu I, Dde I, Hae III, Hha I, Hind III, Hinf I, Hpa II, Rsa II, Sau 3 AI

Results (RFLP Patterns)



Gaotai-merlot, Strawberry 3

Gan1 and H2 (8 isolate)

Lanes 1-9 indicate the following enzymes:

M. Marker (1000, 800, 700, 600, 500, 400, 300, 200 and 100 base pairs)

1. Alu I,
2. Dde I,
3. Hae III
4. HhaI,
5. Hind III,
6. Hinf I
7. Hpa II
8. Rsa II
9. Sau 3 AI

Morphological Identification (1)

IJ of Gaotai-merlot(10x)

IJ of Strawberry3 (10x)

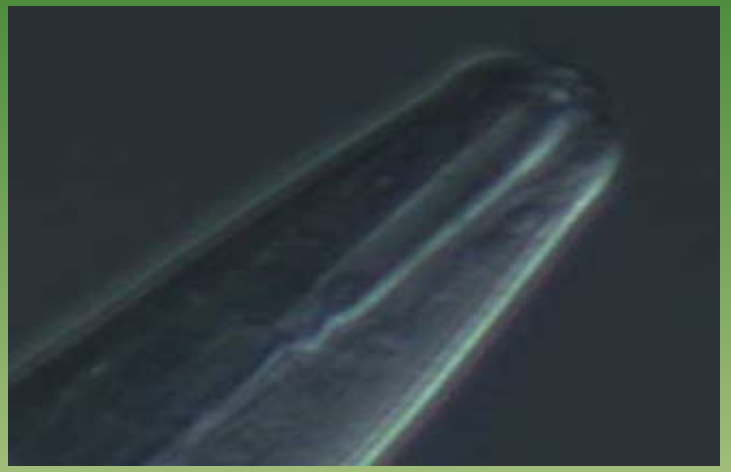
Gan1(10x)

H2 (10x)

Morphological Identification (2)

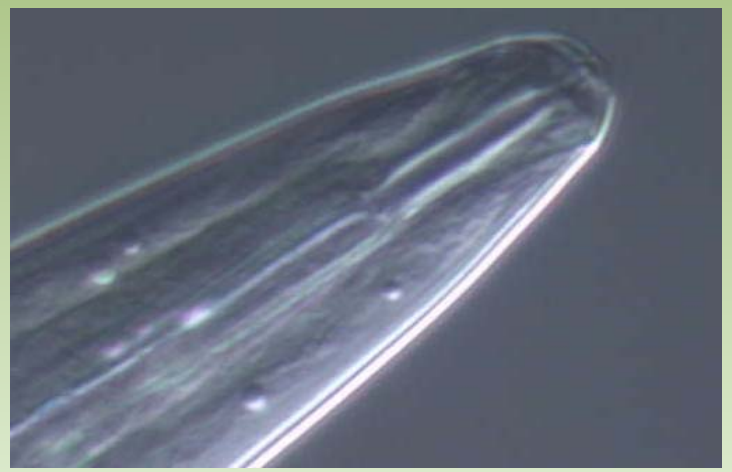
IJ Head of Gaotai-merlot

IJ Head of Strawberry3



IJ Head of Gan1(40x)

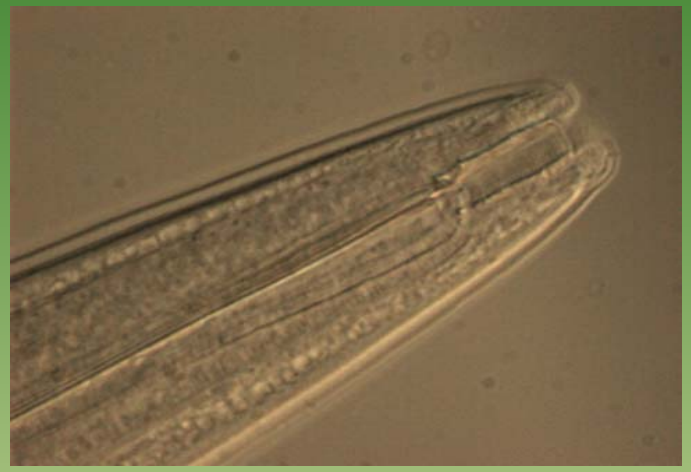
IJ Head of H2 (40x)



Morphological Identification (3)

Mouth of Gaotai-merlot

Mouth of Strawberry3 (100x)

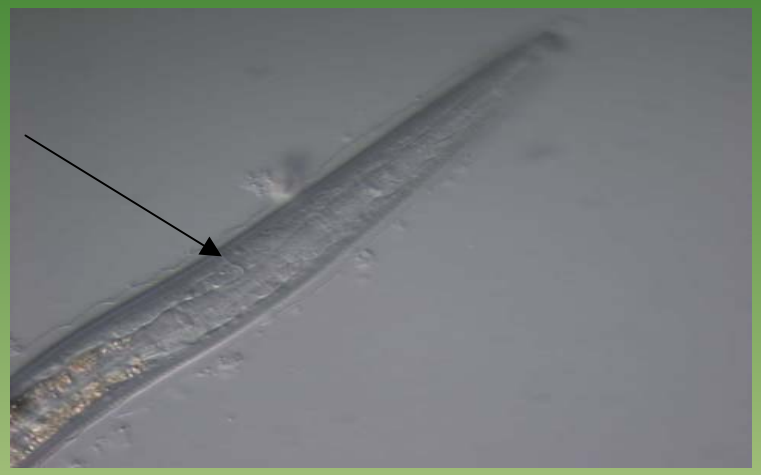


Mouth of Gan1(100x)

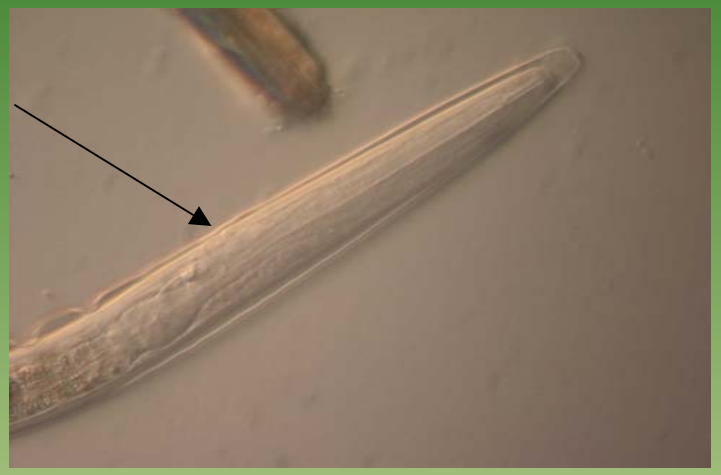
Mouth of H2 (100x)

Morphological Identification (4)

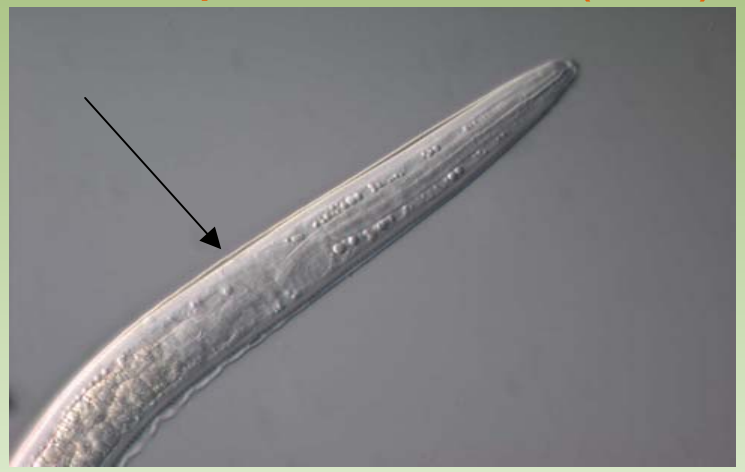
Excr.pore of Gaotai-merlot



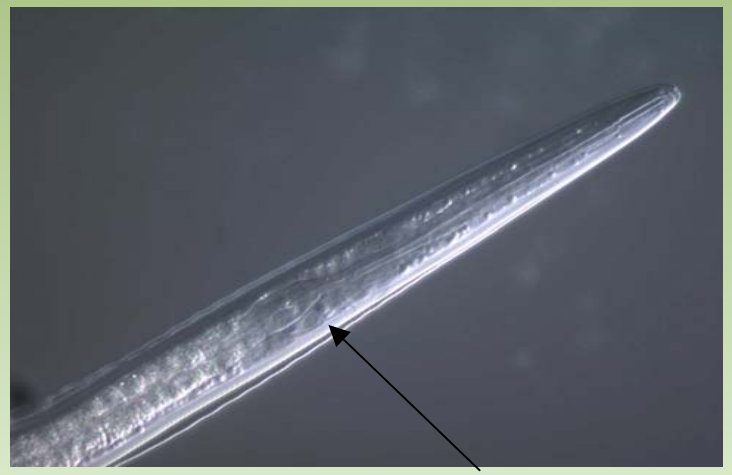
Excr.pore of Strawberry3



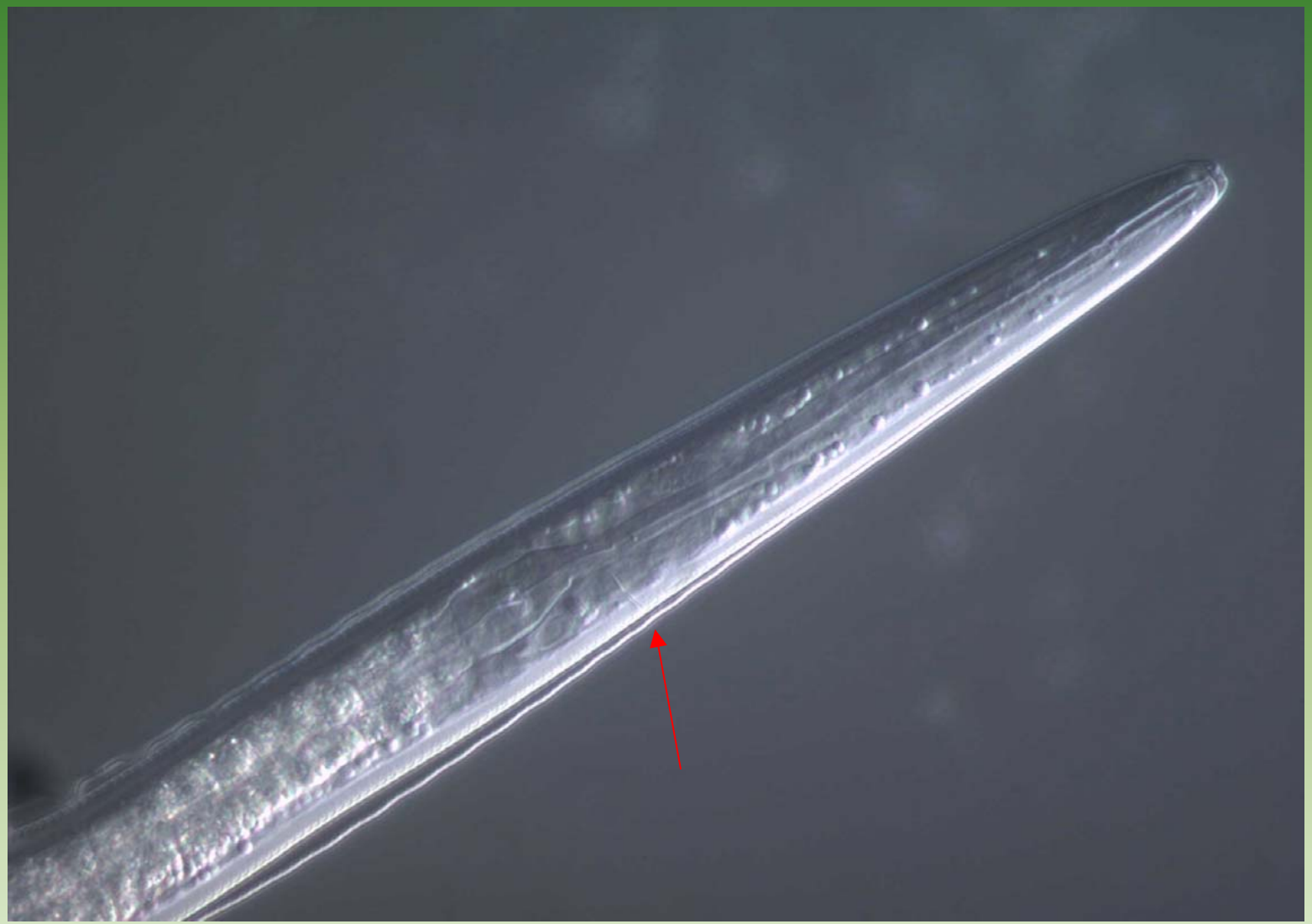
Excre. pore of Gan1(40x)



Excretory pore of H2 (40x)

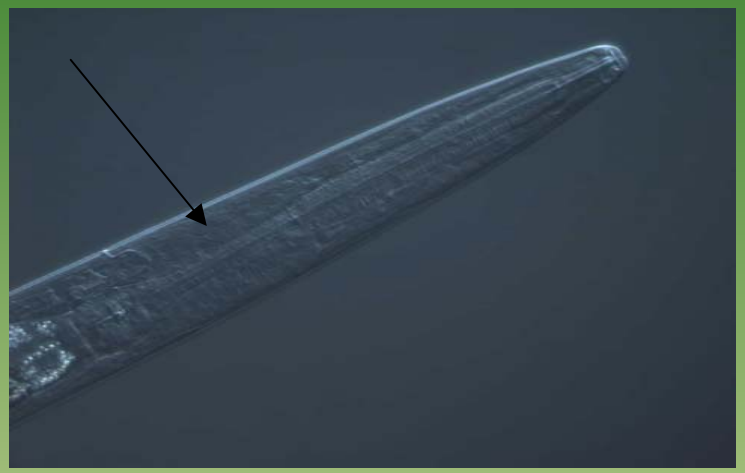


IJ Excretory pore of H2 (40x)

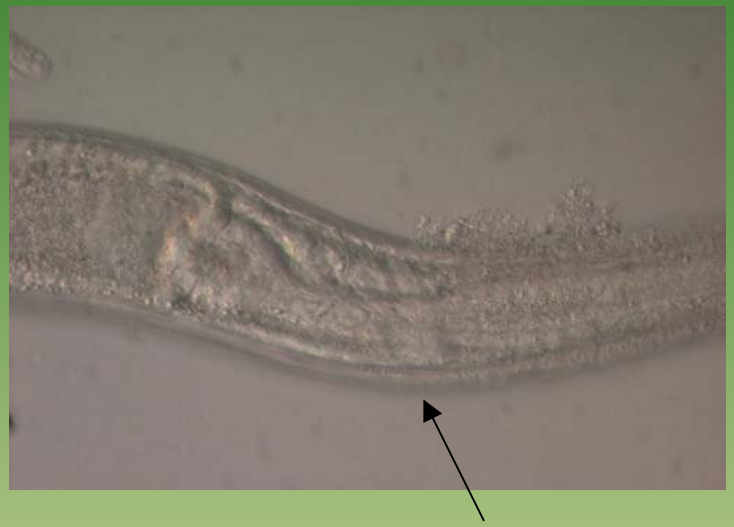


Morphological Identification (4)

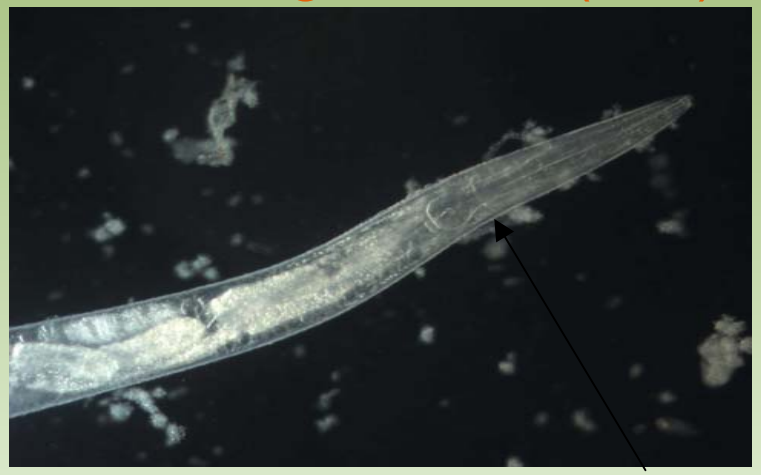
Nerve ring of Gaotai-merlot



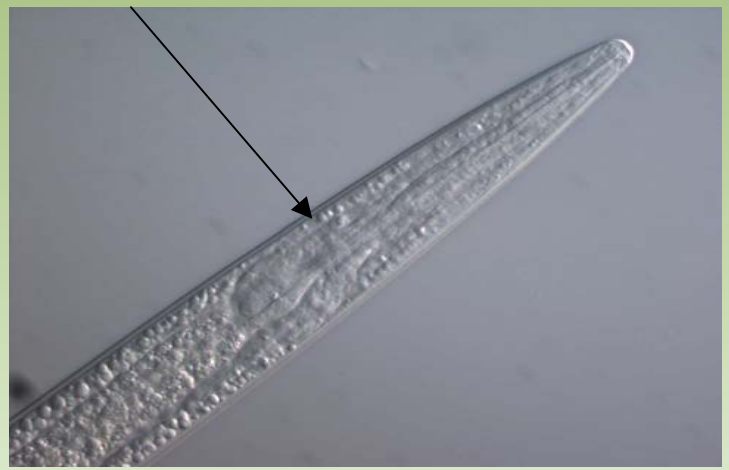
Nerve ring of Strawberry3



Nerve ring of Gan1(10x)



Nerve ring pore of S (40x)

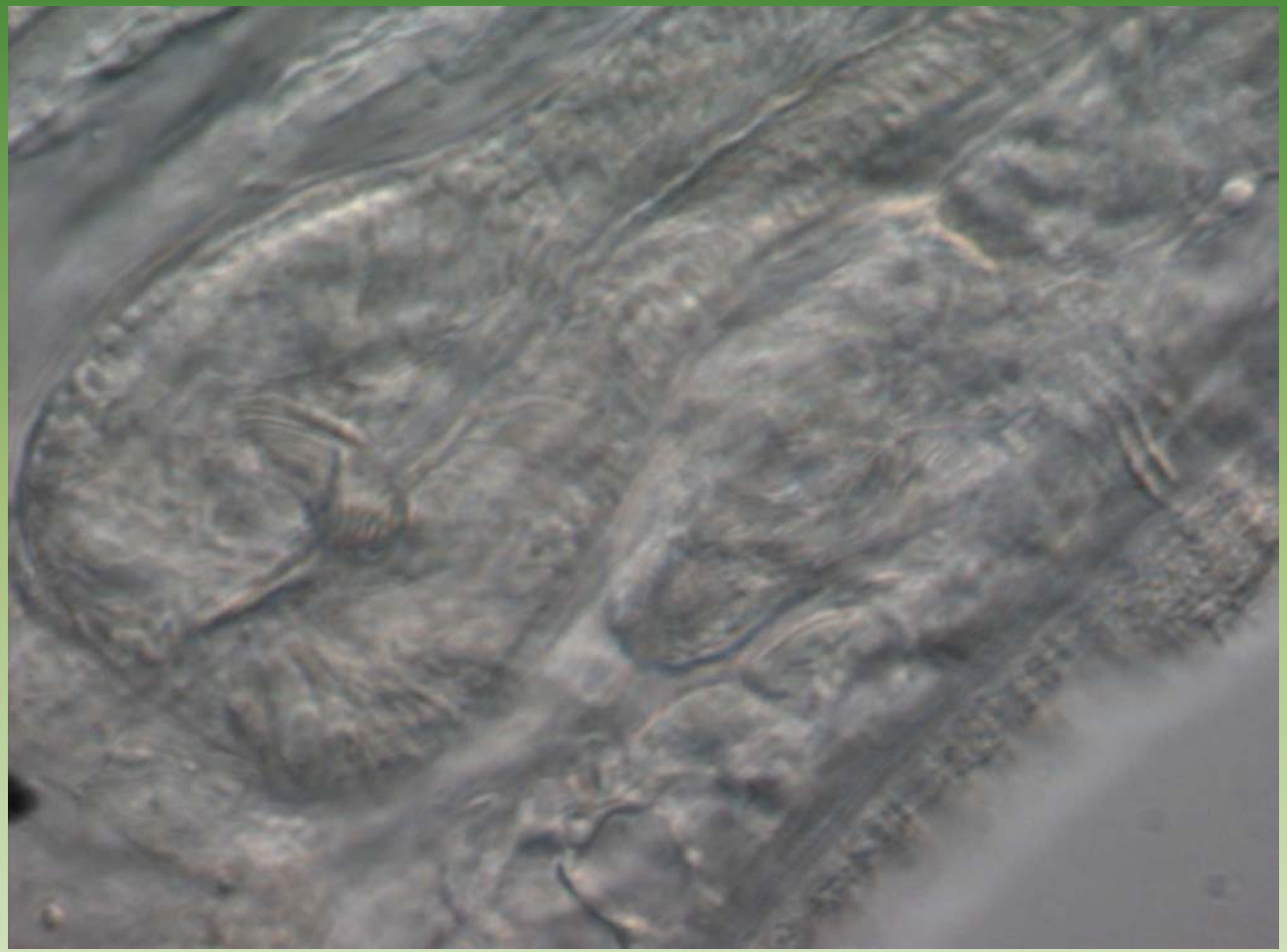


Morphological Identification (5)

Bulb of Strawberry3 (100x, Female)

Morphological Identification (5)

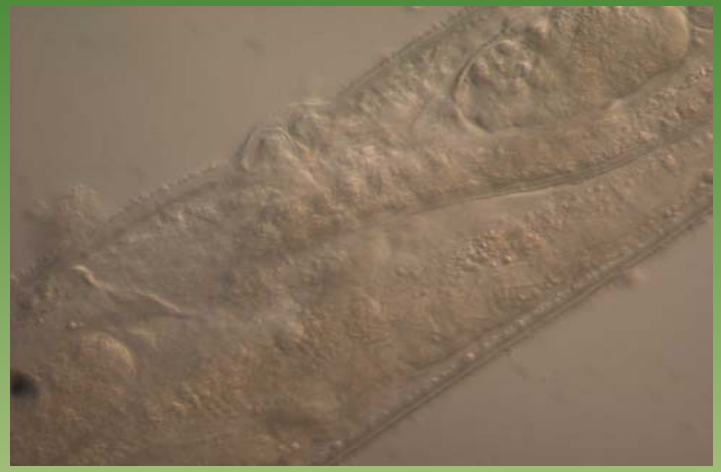
Bulb of H2 (100x, female)



Morphological Identification (6)

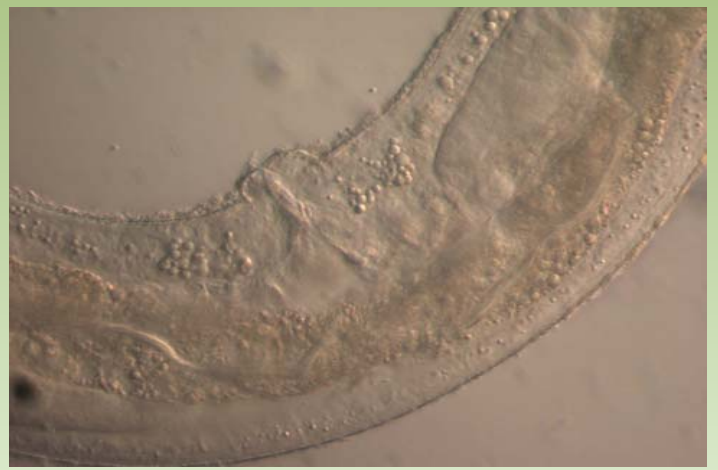
Vulva of Gaotai-merlot

Vulva of Strawberry3 (40x)



Vulva of Gan1(40x)

Vulva of S (100x)

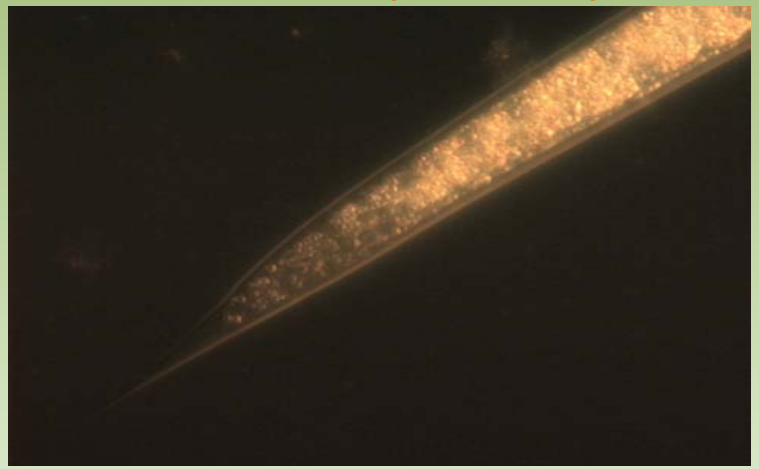


Morphological Identification (7)

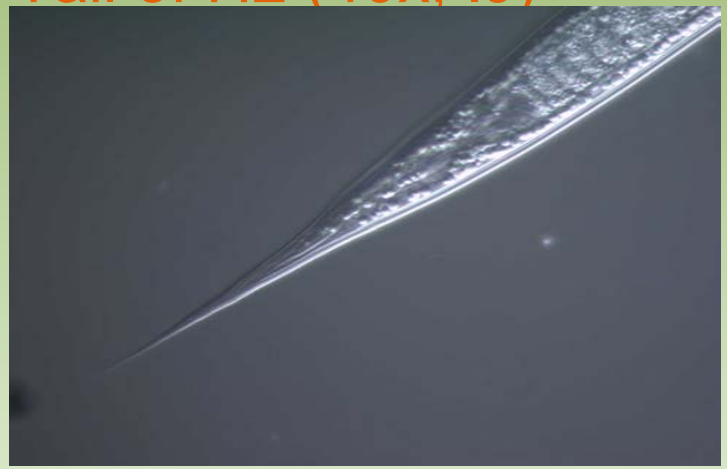
Tail of Gaotai-merlot(40x, IJ)

Tail of Strawberry3 (40x, IJ)

Tail of Gan1(32x, IJ)



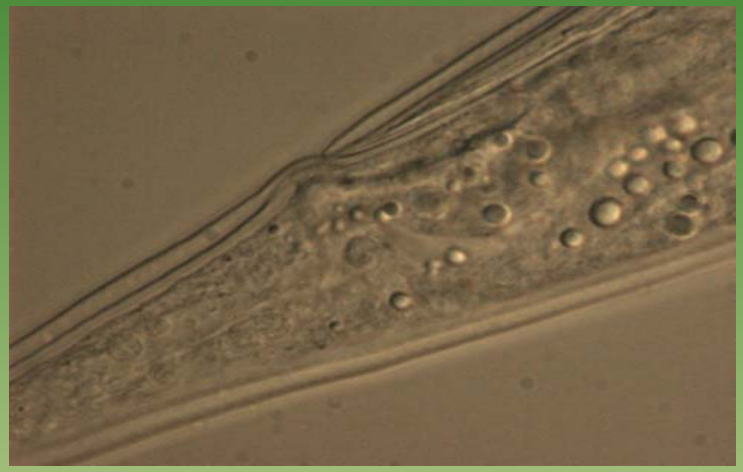
Tail of H2 (40x, IJ)



Morphological Identification (8)

Anus of Gaotai-merlot (40x, F)

Anus of Strawberry3 (40x,F)



Anus of Gan1(40x, Female)

Anus of H2 (100x, Female)

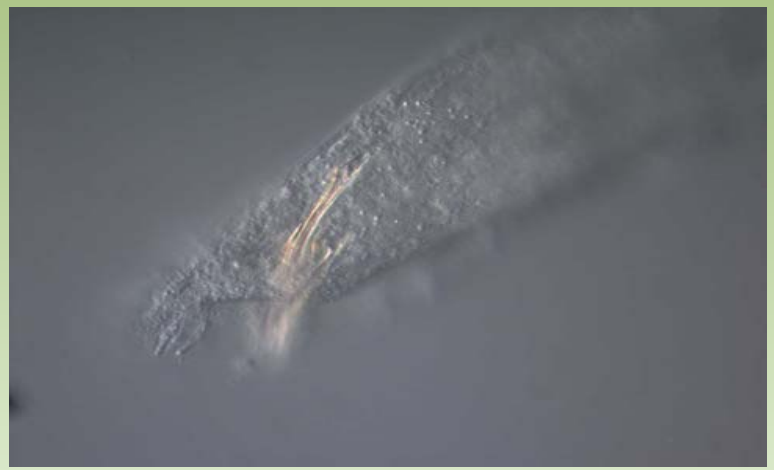
Morphological Identification (9)

Spicule of Gaotai-merlot (40x)

Spicule of Strawberry3 (40x)

Spicule of Gan1(40x)

Spicule of H2 (40x)

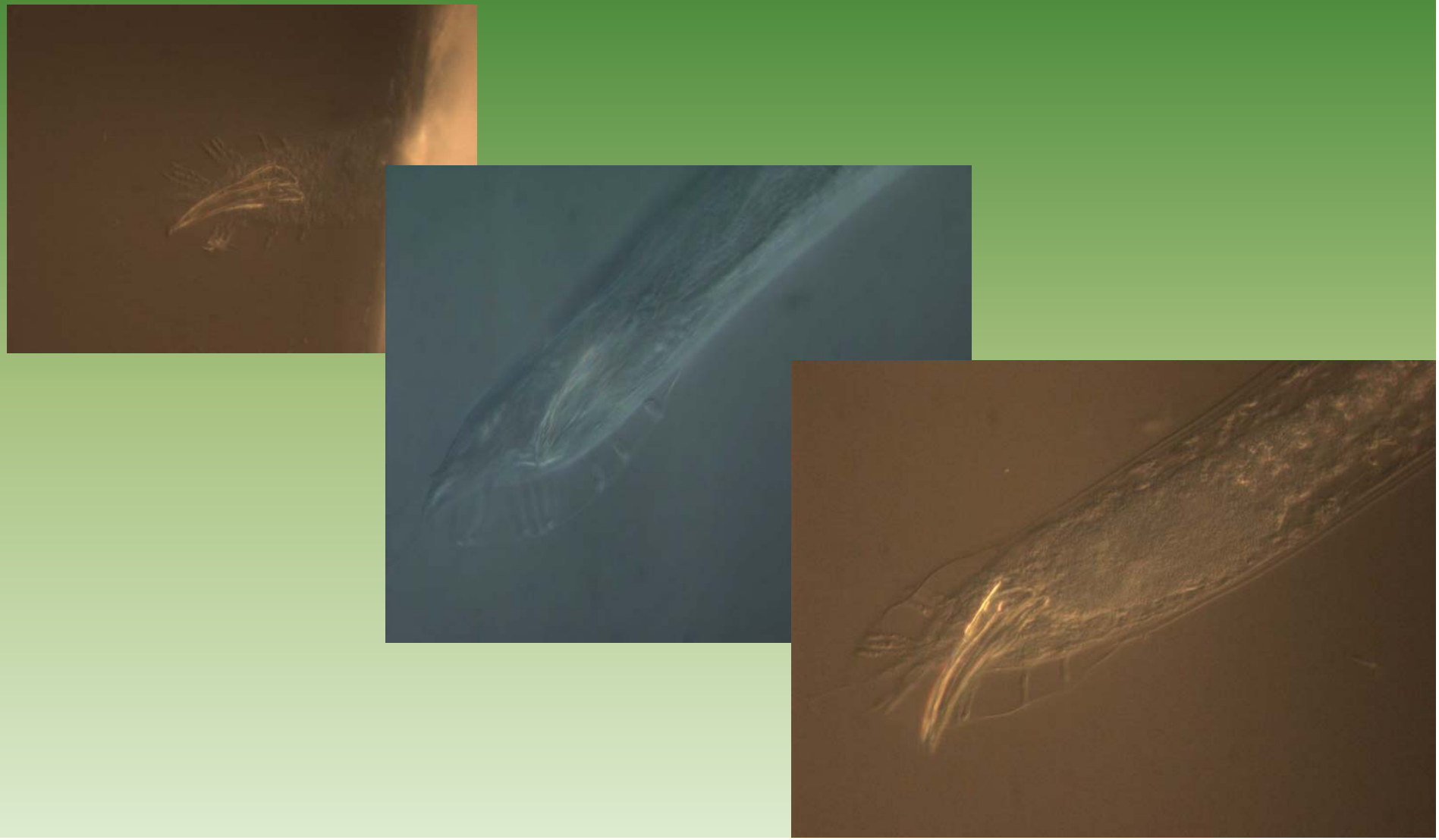


Morphological Identification (9)

Spicule of H2 (1000x)

Morphological Identification (10)

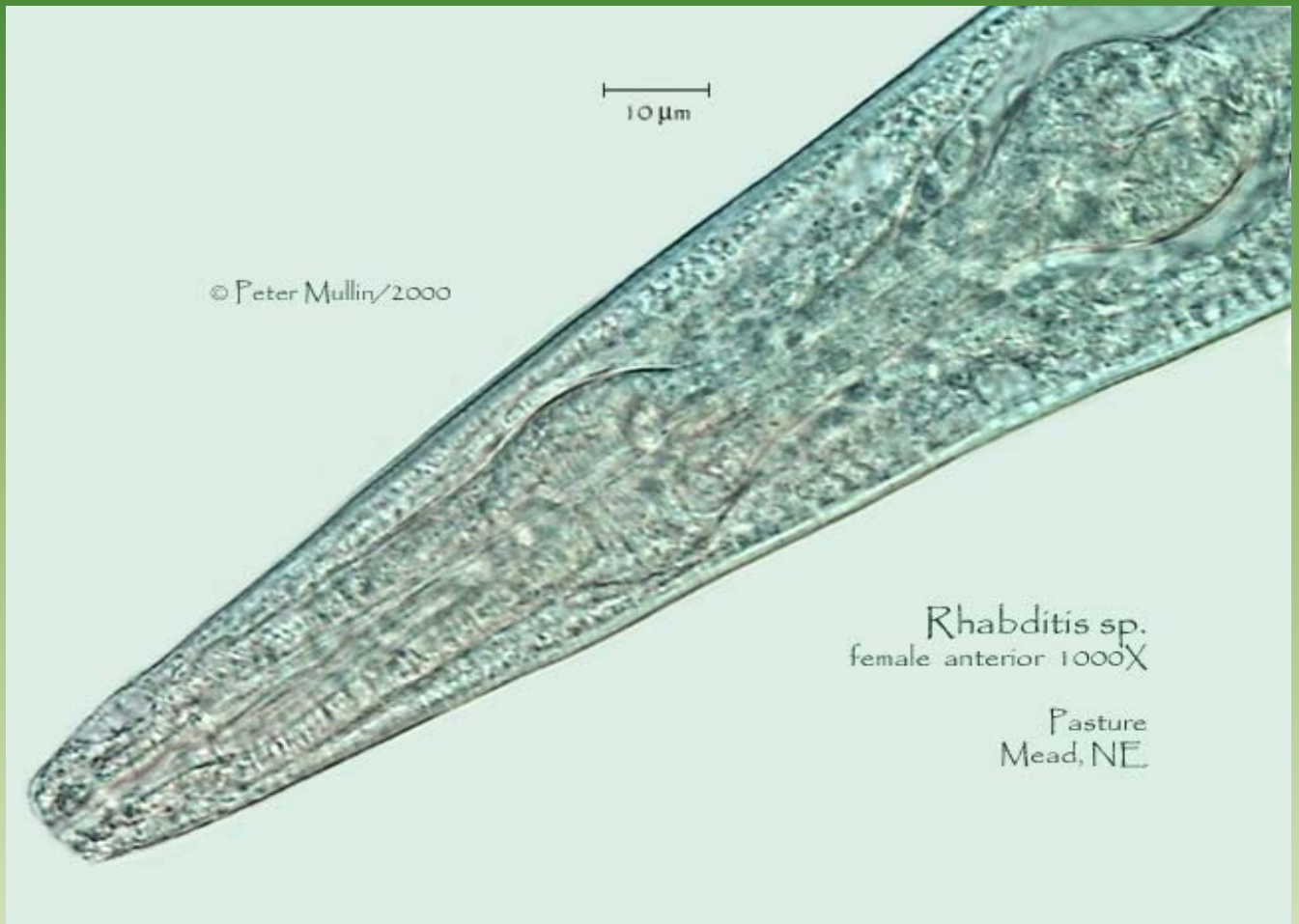
Bursa of Gan1 (400x)



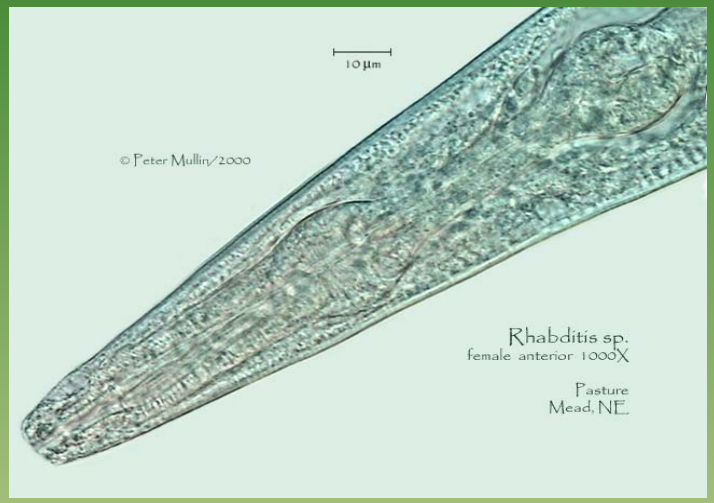
Morphological Identification (10)

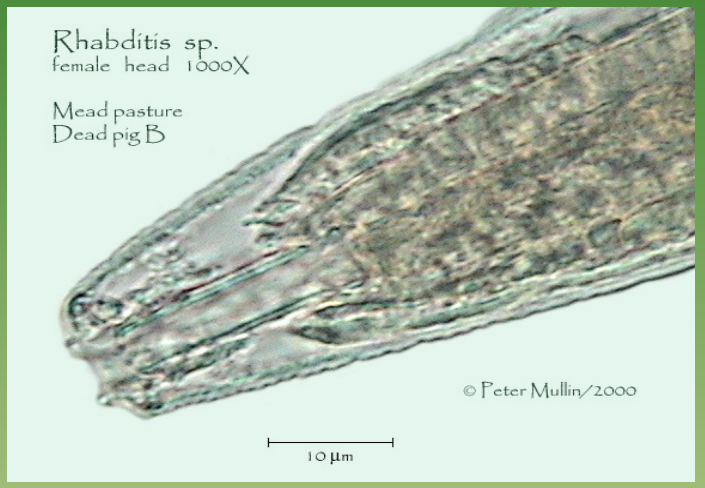
Bursa of H2 (40x)

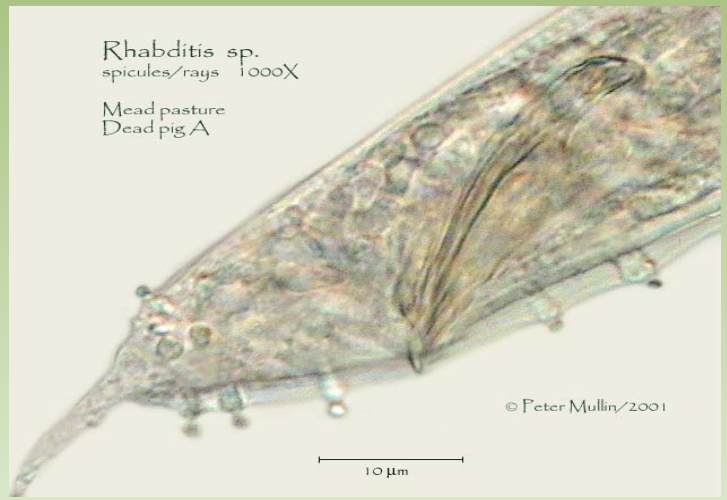
Referent characters of Rhabditis

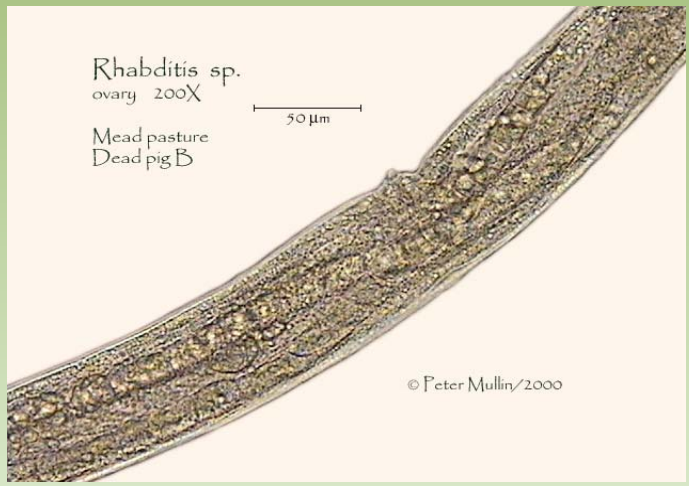


Referent characters of Rhabditis









Conclusion

- The 4 isolates are EPNs (more data support)
- 4 isolates have no morphological difference
- RFLP patterns of Molecular ID are different
- 4 isolates belong to insectivora group in subgenus *Oscheius* of *Rhabditis*

Walter Sudhaus,
Journal of Nematology, 33(1):7,31,33,60 (2001)

Discussion

- Two groups in subgenus *Oscheius*
  Insectivora group: 5 species
  Dolichura group: 7 species

- Spicules distally shaped like a crochet needle (I)
- Tail longer than bursa (I)

- Bursa peloderan (D)
- Female rectum proximally expandable (D)

- Need further observation in SEM

Acknowledgement

cost

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Thank you!