

Trichodoridae parasitos de plantas: principais características

- Ectoparasitos migradores
- Forma do odontostilete



50 μ m 150 μ m

Paratrichodorus minor: entire female



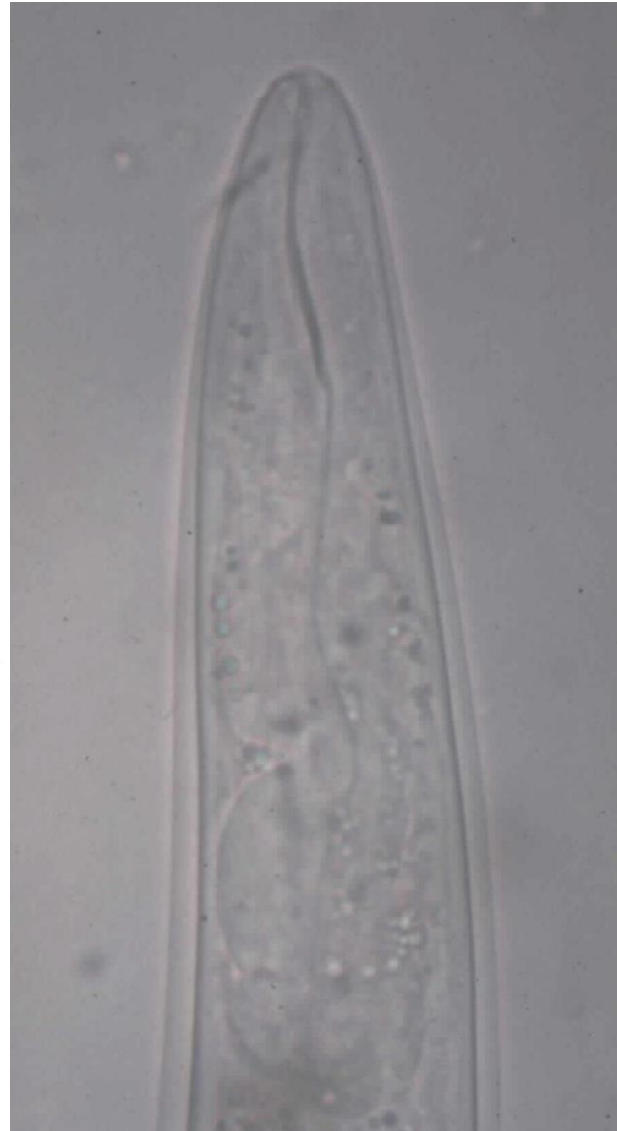
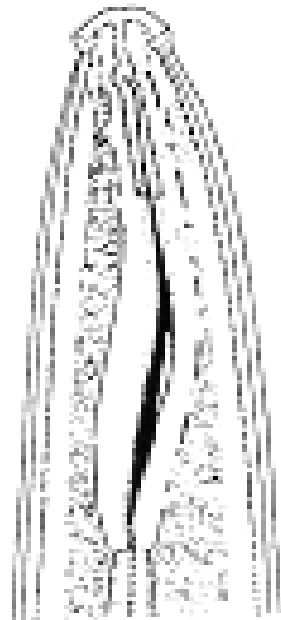
UCR vCinema (c) 2001



ODONTOESTILETE

Trichodoridae

O onquioestilete é curto, curvo e sólido, não canaliculado, sendo pois usado apenas para perfurar as paredes celulares vegetais

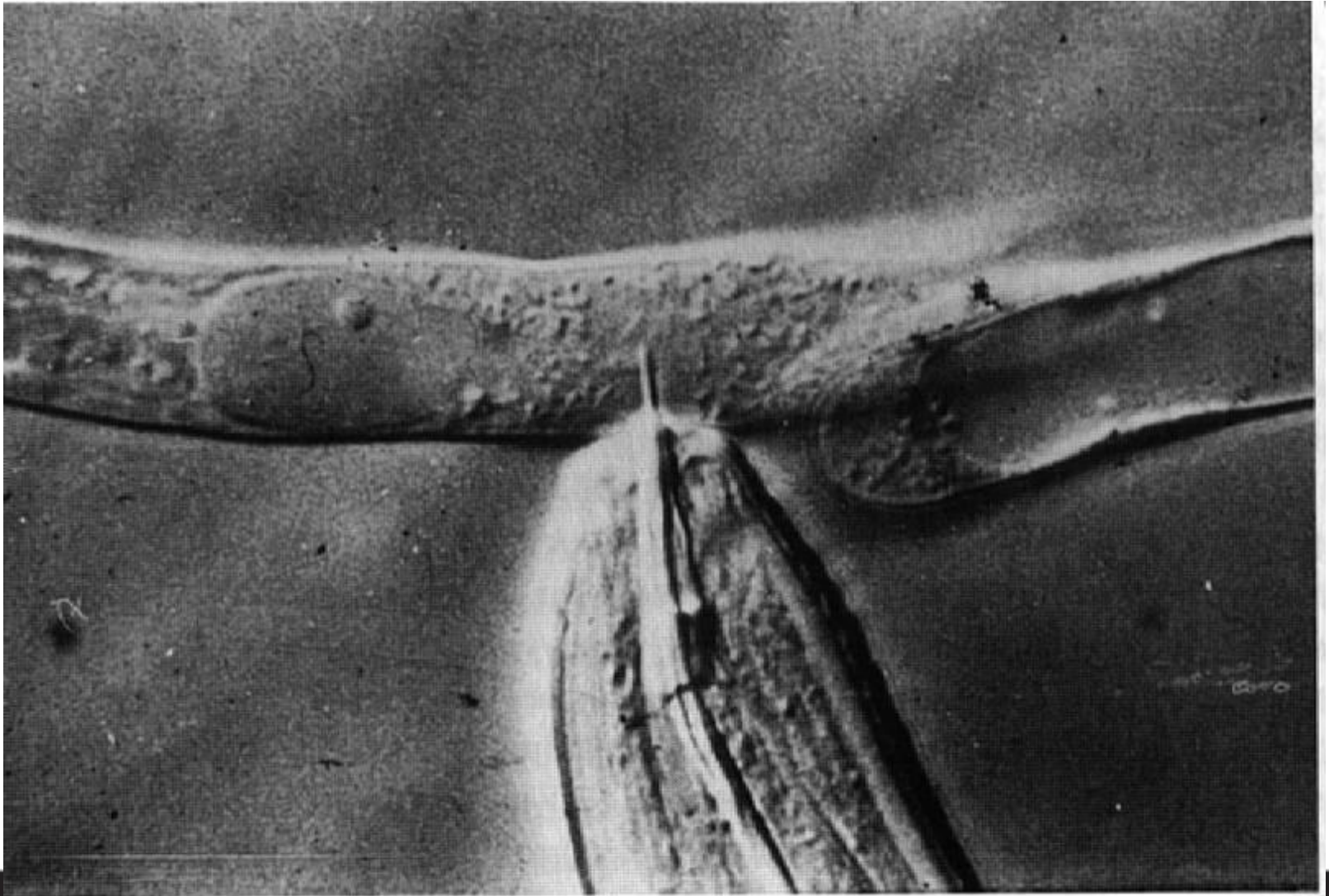


Importância da família Trichodoridae

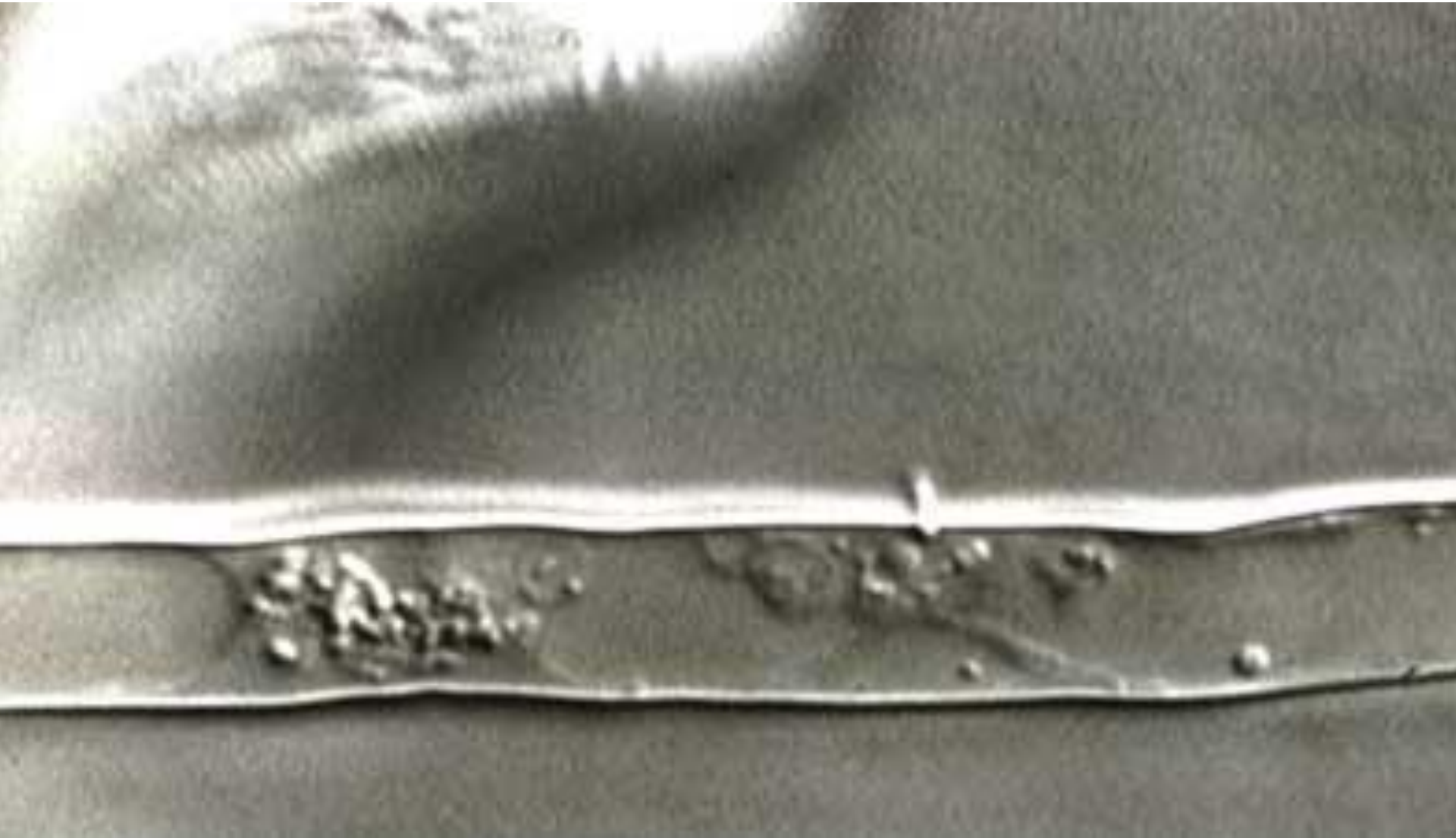
- Danos diretos
 - ex.: trigo e milho
- Transmissão de viroses (Tobravirose)
 - Ex.: batata



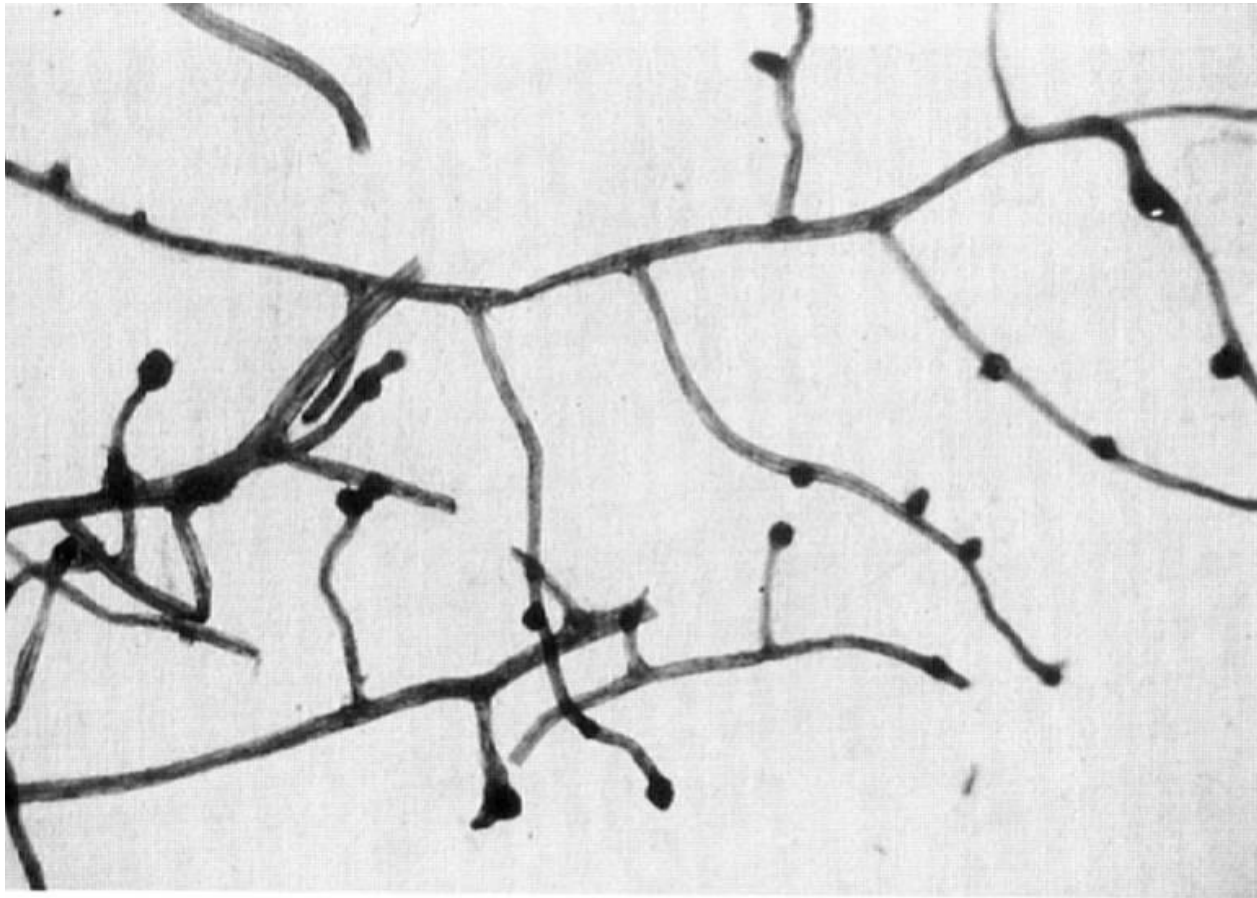
Trichodorus similis



Tubo de alimentação



Sintomas causados por *Paratrichodorus* spp. em raízes de trigo.

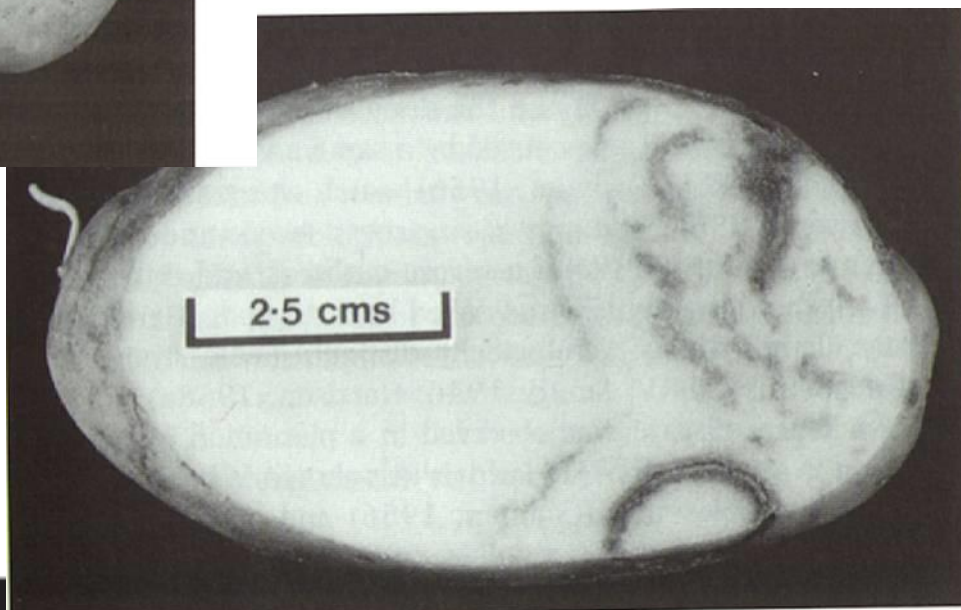
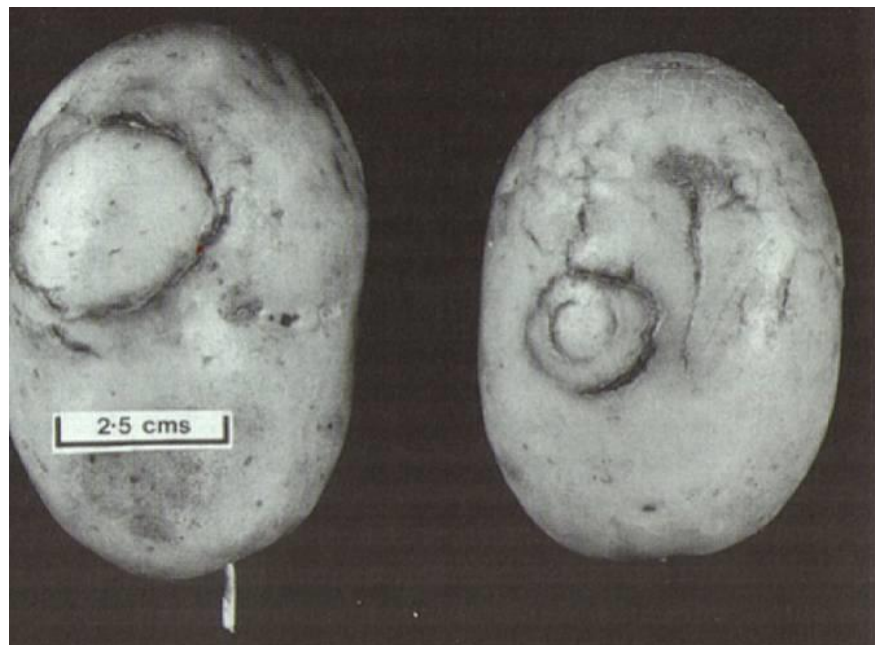


Raízes de milho parasitadas com *Paratrichodorus minor*



VETOR	VÍRUS (Tobravírus)
<i>Paratrichodorus hyspanus</i>	tobacco rattle (TRV)
<i>Trichodorus tunisiensis</i>	tobacco rattle (TRV)
<i>Paratrichodorus allius</i>	tobacco rattle (TRV)
<i>P. anemones</i>	tobacco rattle (TRV)
	pea early-browning (PEBV)
<i>P. minor</i>	pepper ringspot (PRV)
	tobacco rattle (TRV)
<i>P. nanus</i>	tobacco rattle (TRV)
<i>P. pachydermus</i>	pea early-browning (PEBV)
<i>P. porosus</i>	tobacco rattle (TRV)
<i>P. teres</i>	pea early-browning (PEBV)
<i>Trichodorus cylindricus</i>	tobacco rattle (TRV)
	pea early-browning (PEBV)
<i>T. primitivus</i>	tobacco rattle (TRV)
	pea early-browning (PEBV)
<i>T. similis</i>	tobacco rattle (TRV)
<i>T. viruliferus</i>	tobacco rattle (TRV)
	pea early-browning (PEBV)

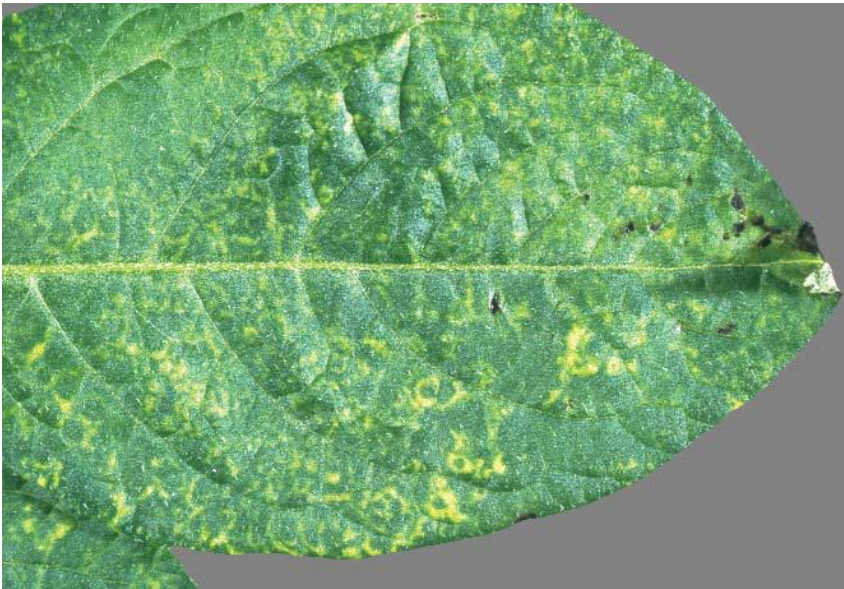
Batatas exibindo anéis necróticos causados por tobacco
ratle virus transmitidos por *Trichodoridae*.



Tuberculos de batata exibindo anéis necróticos causados por *Tobacco rattle virus* transmitidos por Trichodoridae



Folhas de batata com sintomas causados por *Tobacco rattle virus* transmitidos por Trichodoridae

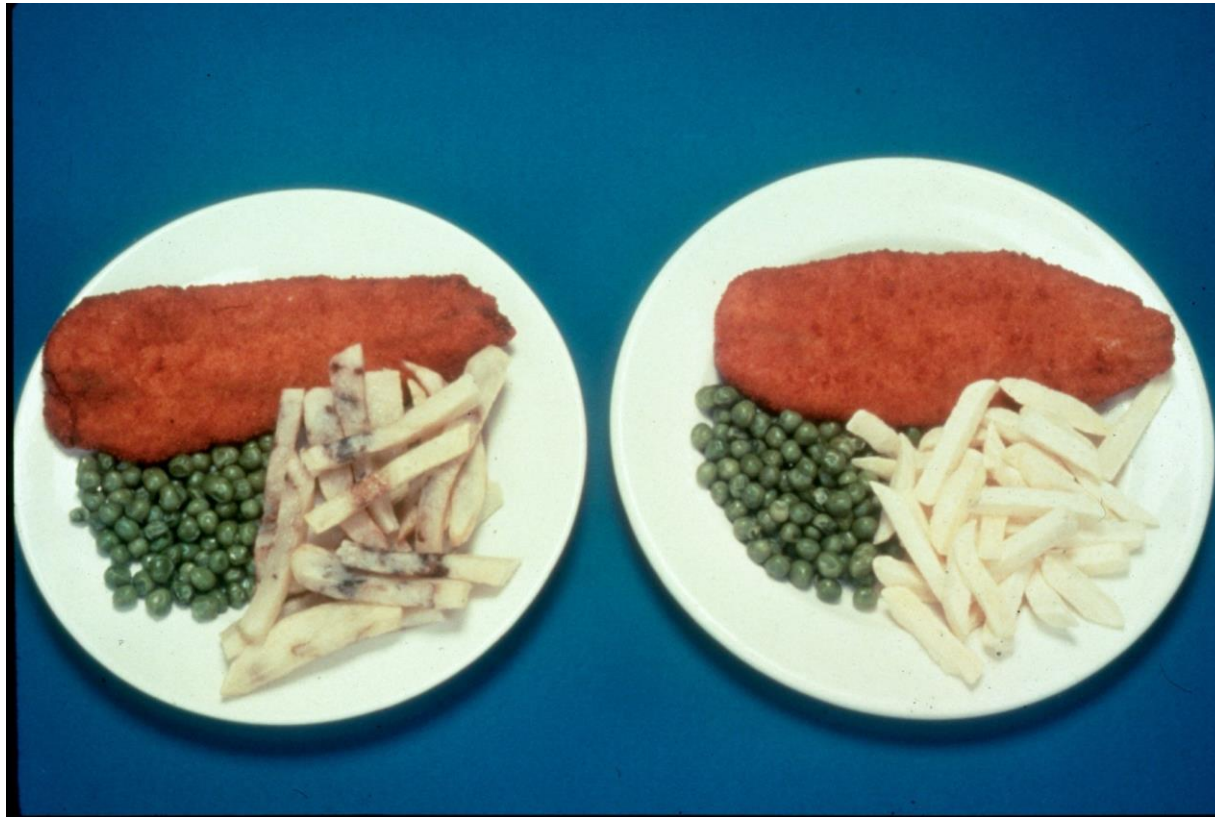


Anéis amarelos



Folhas retorcidas e necrose

O prato tradicional da cozinha britânica (*fish and chips*), preparado com batatas infectadas com tobacco rattle virus (TRV).

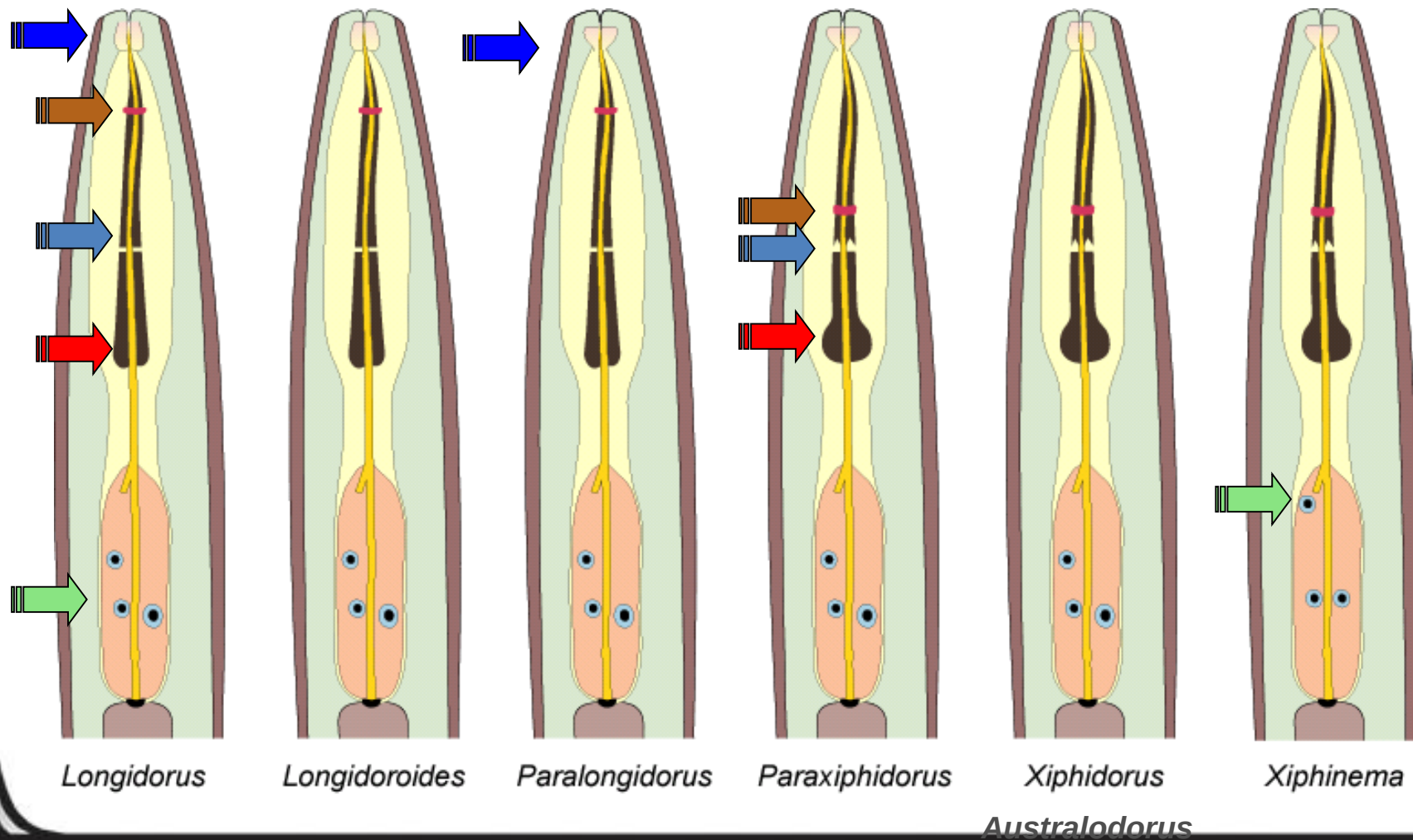


Diagnose das viroses transmitidas por nematoídes

- Identificação do vírus
 - Sintomatologia
 - Plantas indicadoras
 - Teste ELISA
 - Primers específicos
- Identificação do nematoíde vetor
 - Morfologia e morfometria
 - Primers específicos



Caracteres usados para diferenciar os gêneros da família Longidoridae

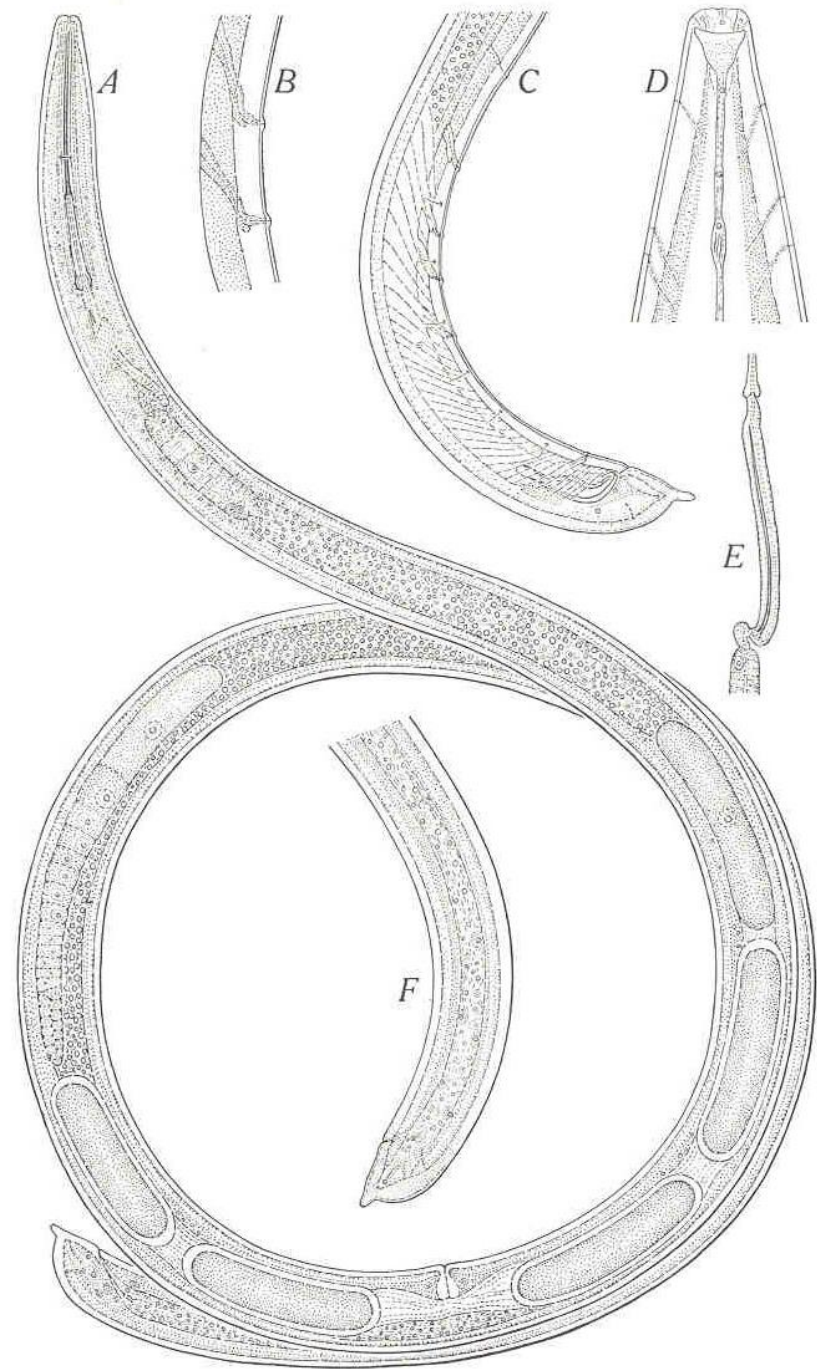


Identificação de espécies de *Xiphinema*

Morfologia

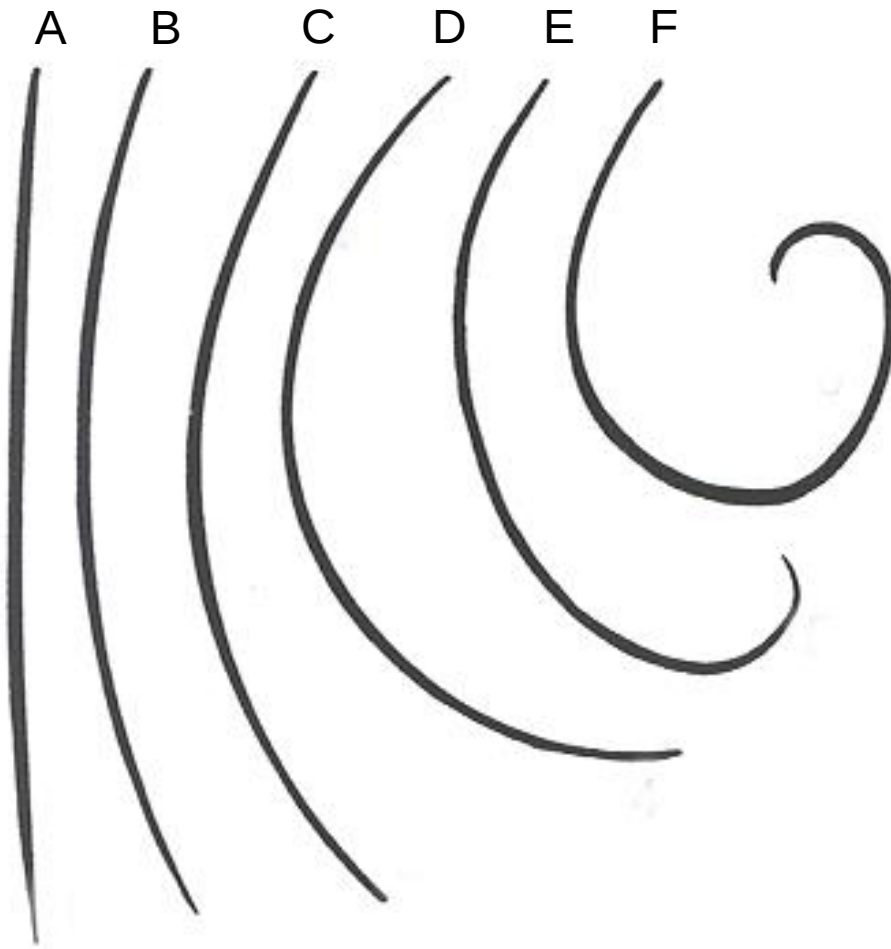
+

Morfometria
das fêmeas



Xiphinema index

Hábito: forma do corpo



A: retilíneo

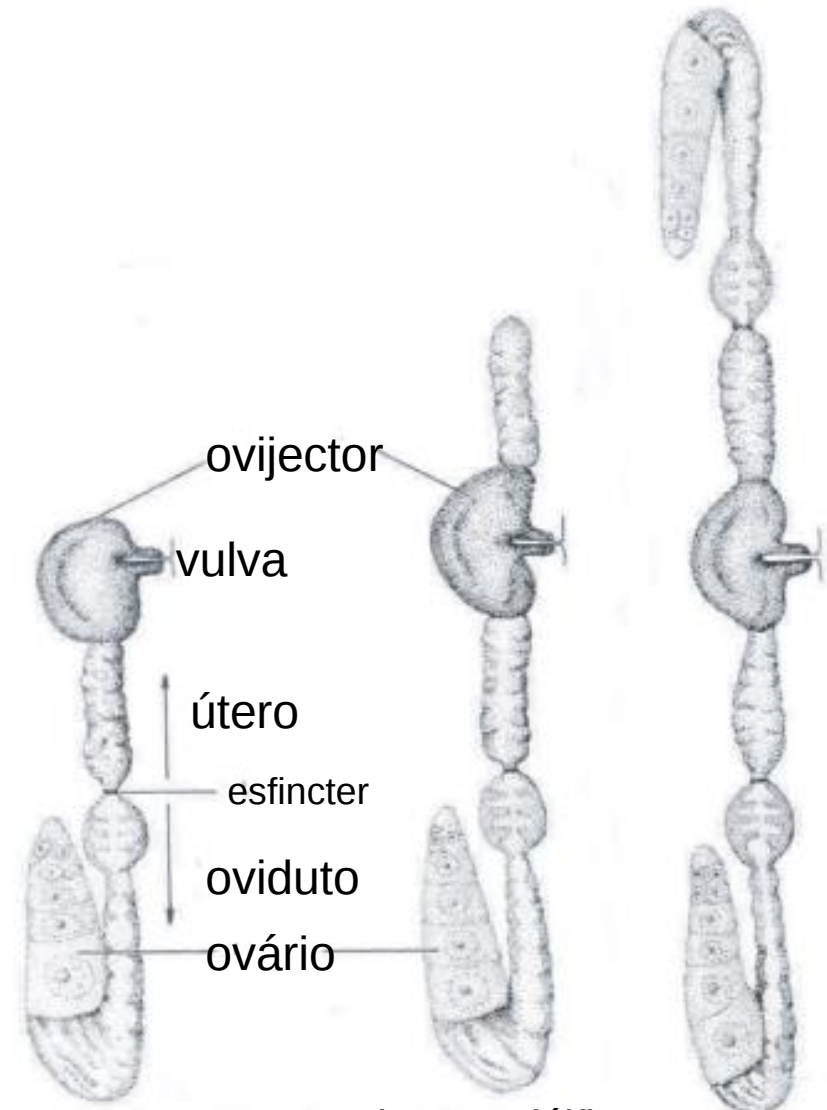
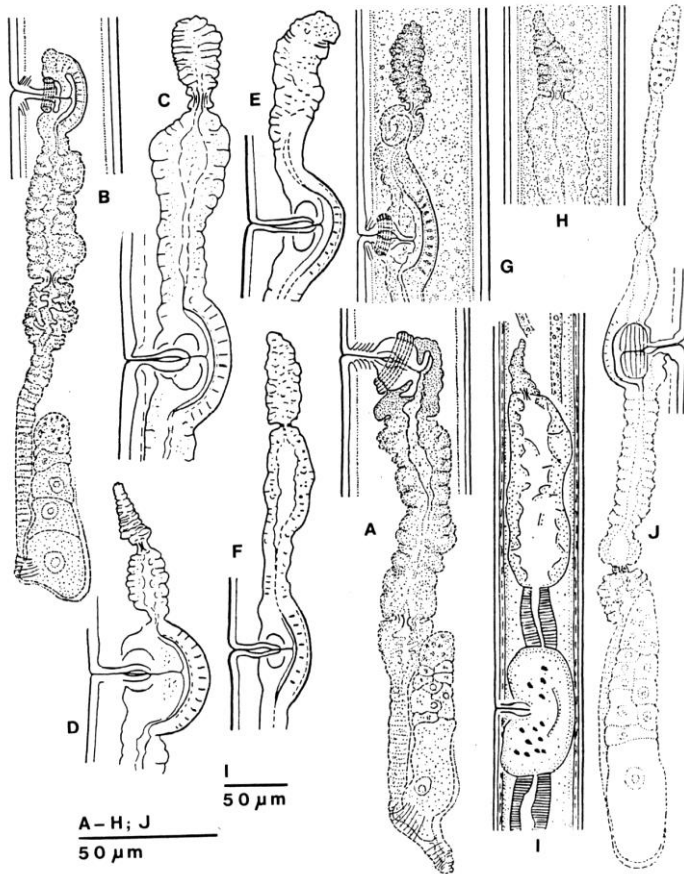
B: ligeiramente retilíneo

B-D: ligeiramente recurvado

E: recurvado, assumindo o formato de um C ou J ou gancho

F: espiralado

Organização do aparelho reprodutor feminino



Monodélfico (A-B) Pseudomonodélfico anfidélfico

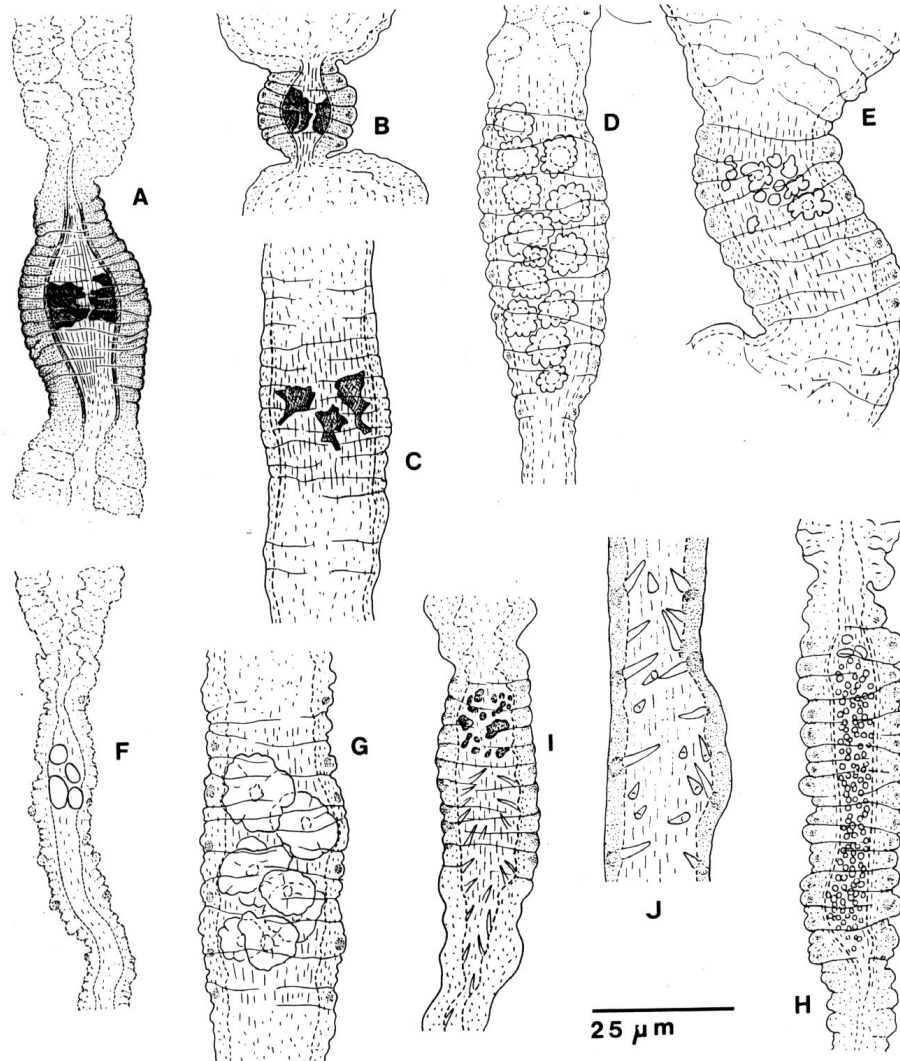
Tipos de diferenciação uterina

A e B: Órgão Z

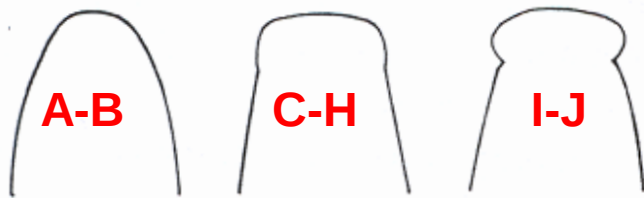
C-H: Pseudo-órgão Z

I: Pseudo-órgão Z +
Espinhos uterinos

J: Espinhos uterinos



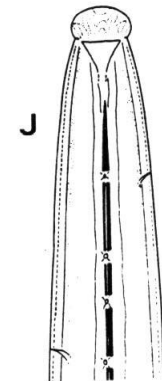
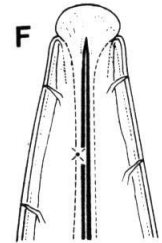
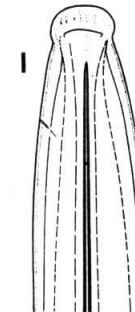
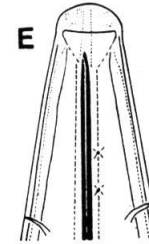
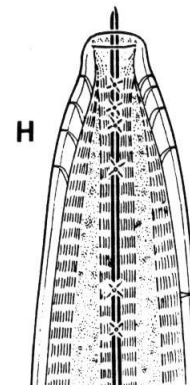
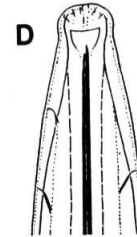
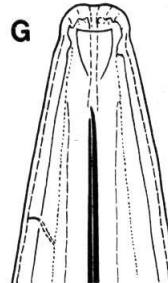
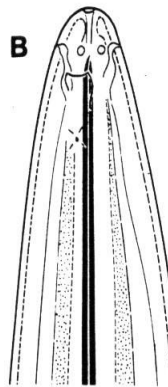
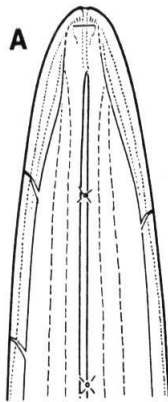
Região labial



A-B: Sem
constricção,
contínua com o
resto do corpo

C-H: Separada por
fraca constricção

I-J: Separada por
evidente constricção



C-F 50 μ m

A, H, I 50 μ m

B, G 25 μ m

J 25 μ m

A: *X. hygrophyllum*; B: *X. stenocephalum*; C-D: *X. elongatum*; E-F: *X. coxi europaeum*; G: *X. insigne*; H: *X. ingens*; I: *X. conurum*; J: *X. pachydermum*
(Loof and Luc, 1990)

Forma da cauda

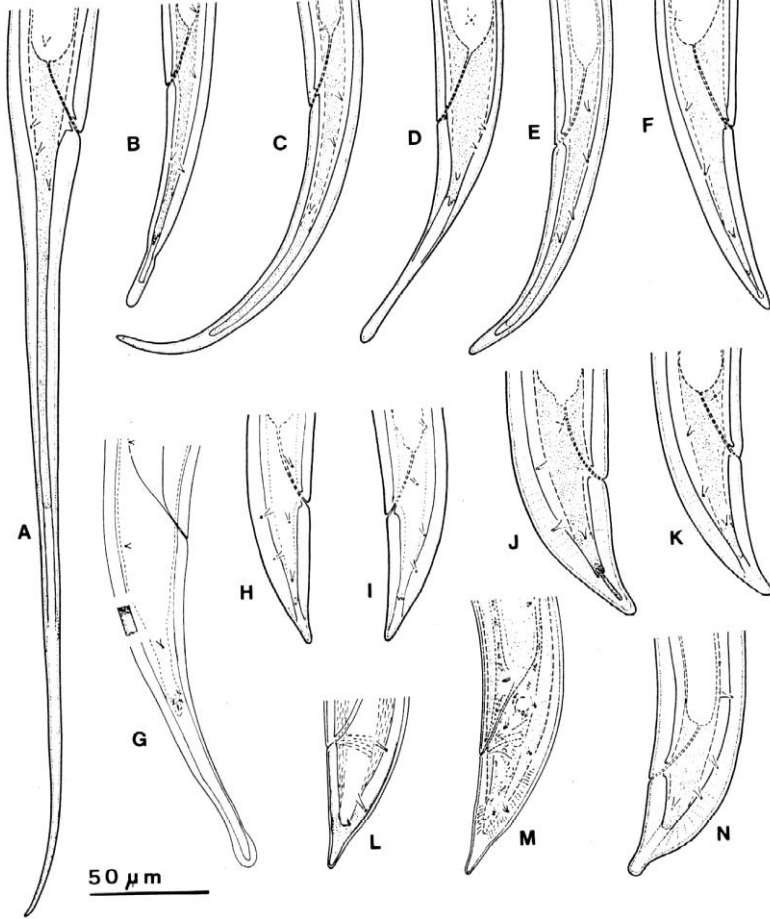


Fig 1

A: longa ($C' > 7,5$), atenuada ou filiforme

B-G: longa ($2,5 < C' < 7,5$), cônica ou clavada

H-K: curta ($C' \pm 2,5$), cônica ou levemente subdigitada

N (L-M): curta, cônica e digitada

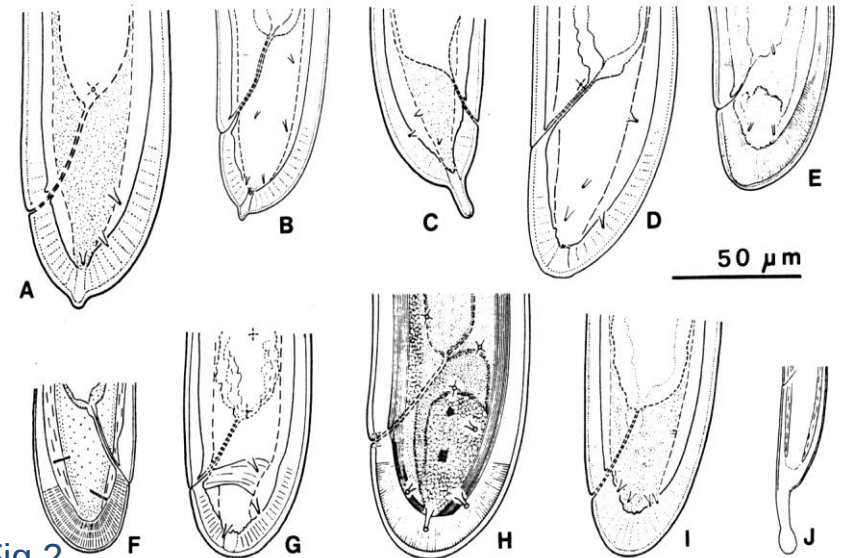


Fig 2

A-C: cônica-hemisférica com término na forma de dedo ou mamilo (mucro)

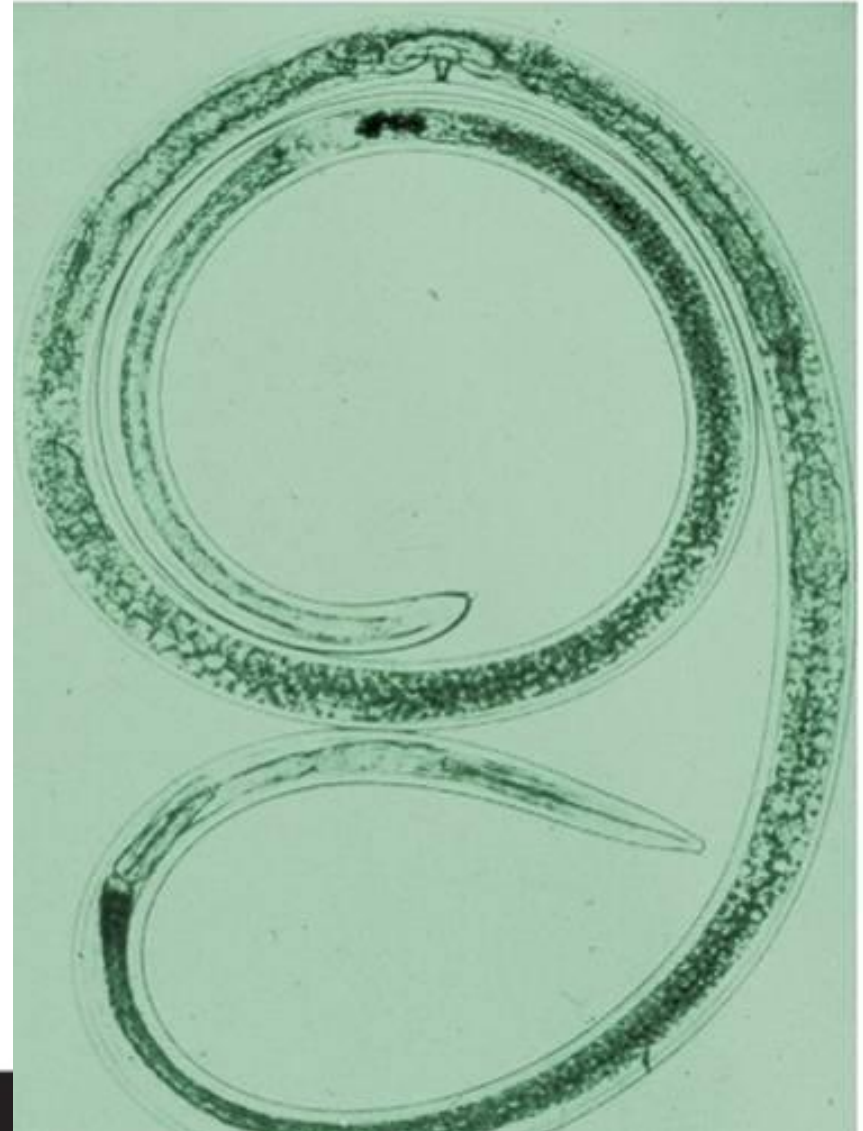
D-F: cônica-arredondada

G-I: hemisférica

J: arredondada com longo término na forma de mucro

Morfometria

- Comprimento do corpo (L)
- odontostíleo
- odontóforo
- estilete
- Comprimento da cauda (TL)
- diâmetro do corpo ao nível do anus (abw)
- diâmetro maior do corpo (bw)
- V% (posição da vulva)
- a (L/bw)
- c (L/TL)
- c' (TL/abw)



grupo *Xiphinema americanum*

- Pequena variação morfológica/morfométrica: mesmo código na chave politômica
- Controvérsias!!!!!!
- Atualmente: 50 espécies
- Brasil: 8 espécies:
 - *X. brevicolle*, *X. californicum*, *X. georgianum*, *X. pachtaicum*,
 - *X. diffusum*, *X. oxycaudatum* e *X. peruvianum*
 - *X. luci*



Características do grupo *Xiphinema americanum*

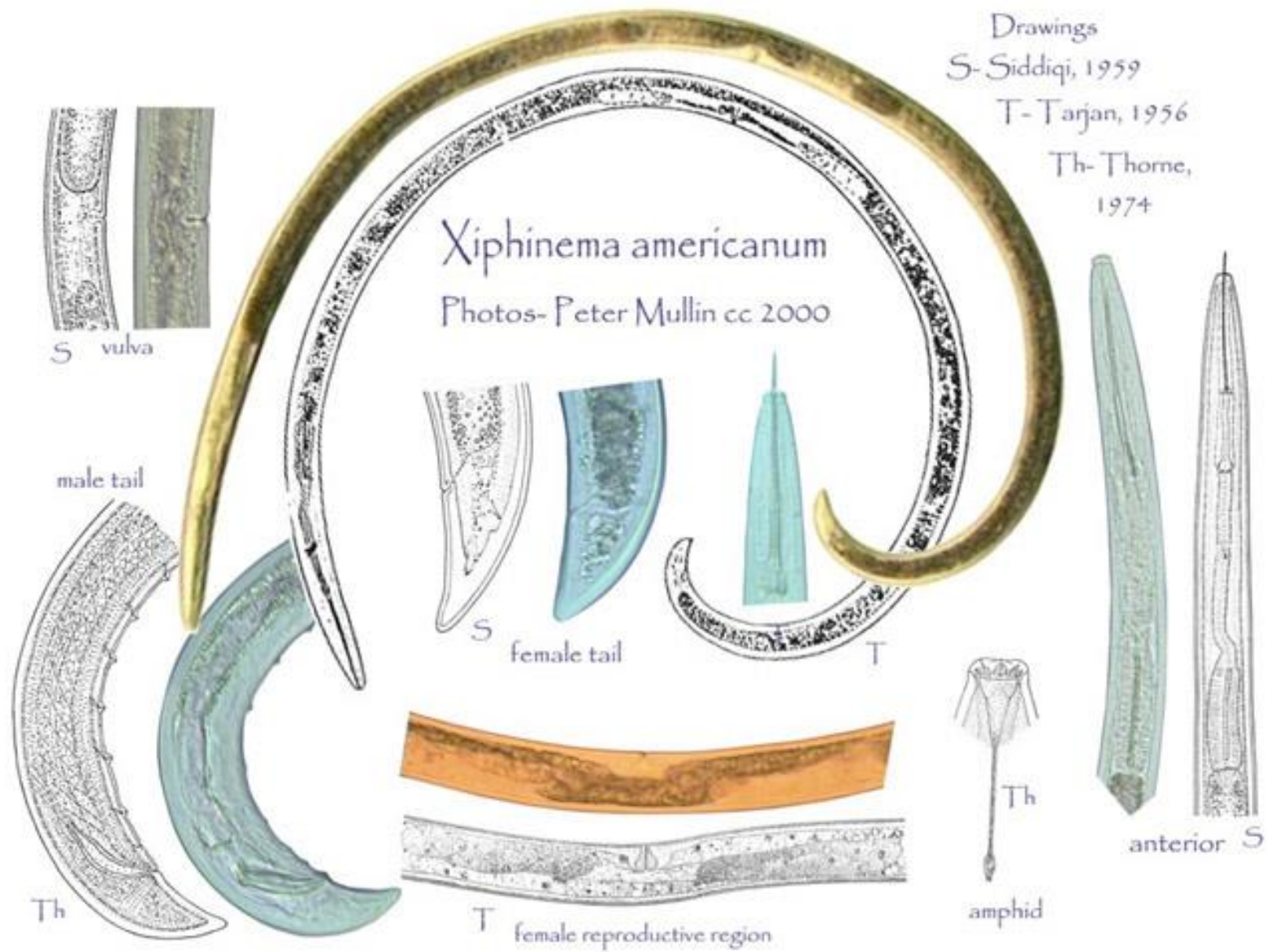
- $L < 2,3 \text{ mm}$
- Espiralado ou forma de C
- Estilete $< 150 \text{ um}$
- Fêmeas anfidélficas
- $V > 50\%$
- Útero curto, sem diferenciação
- Presença de bactérias endossimbiontes no ovário
- Cauda curta: conóide-arredondada a levemente digitada
- Machos raros: reprodução por partenogênese

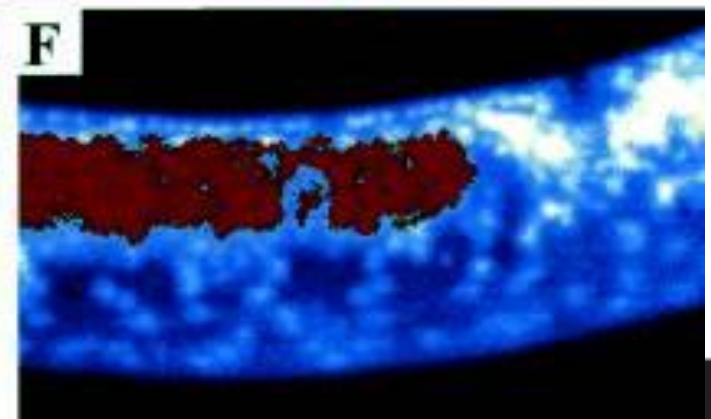
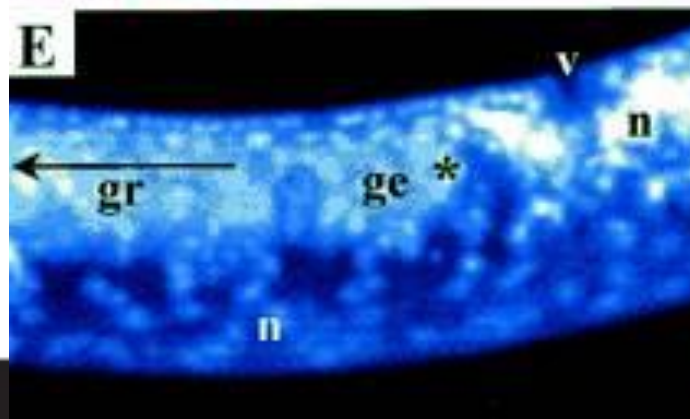
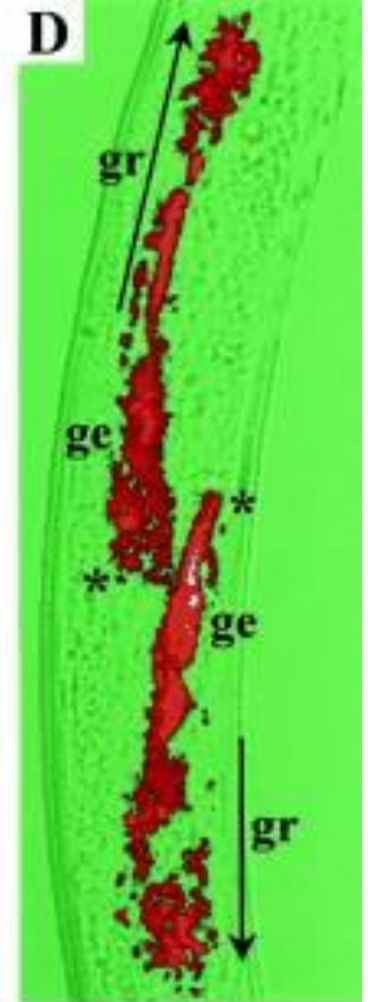
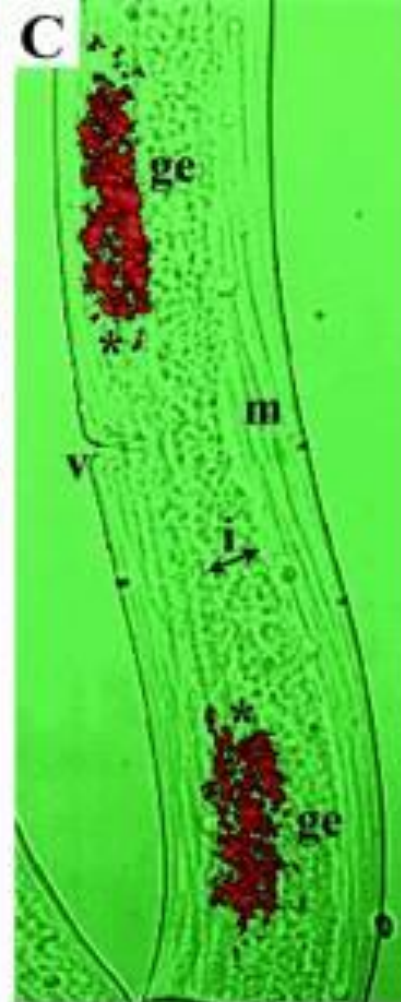
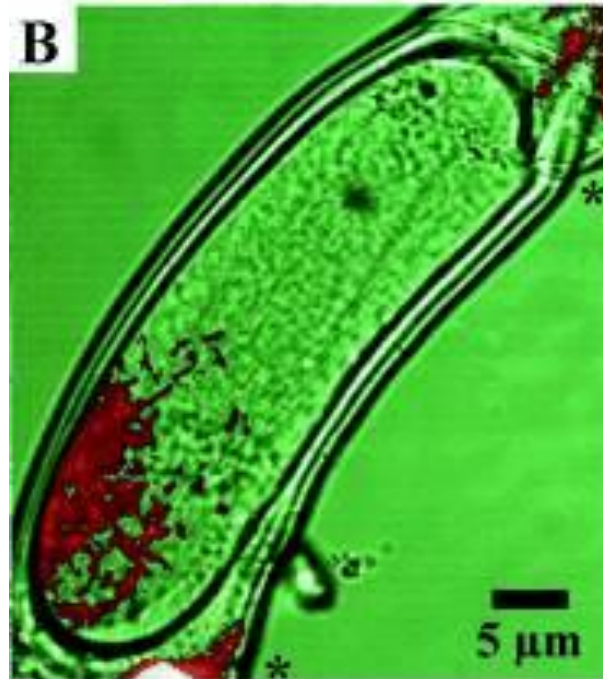
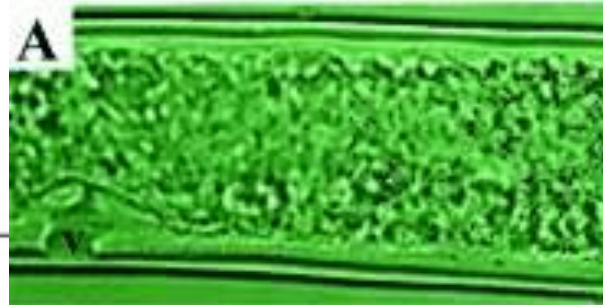


Drawings
S- Siddiqi, 1959
T- Tarjan, 1956
Th- Thorne,
1974

Xiphinema americanum

Photos- Peter Mullin cc 2000





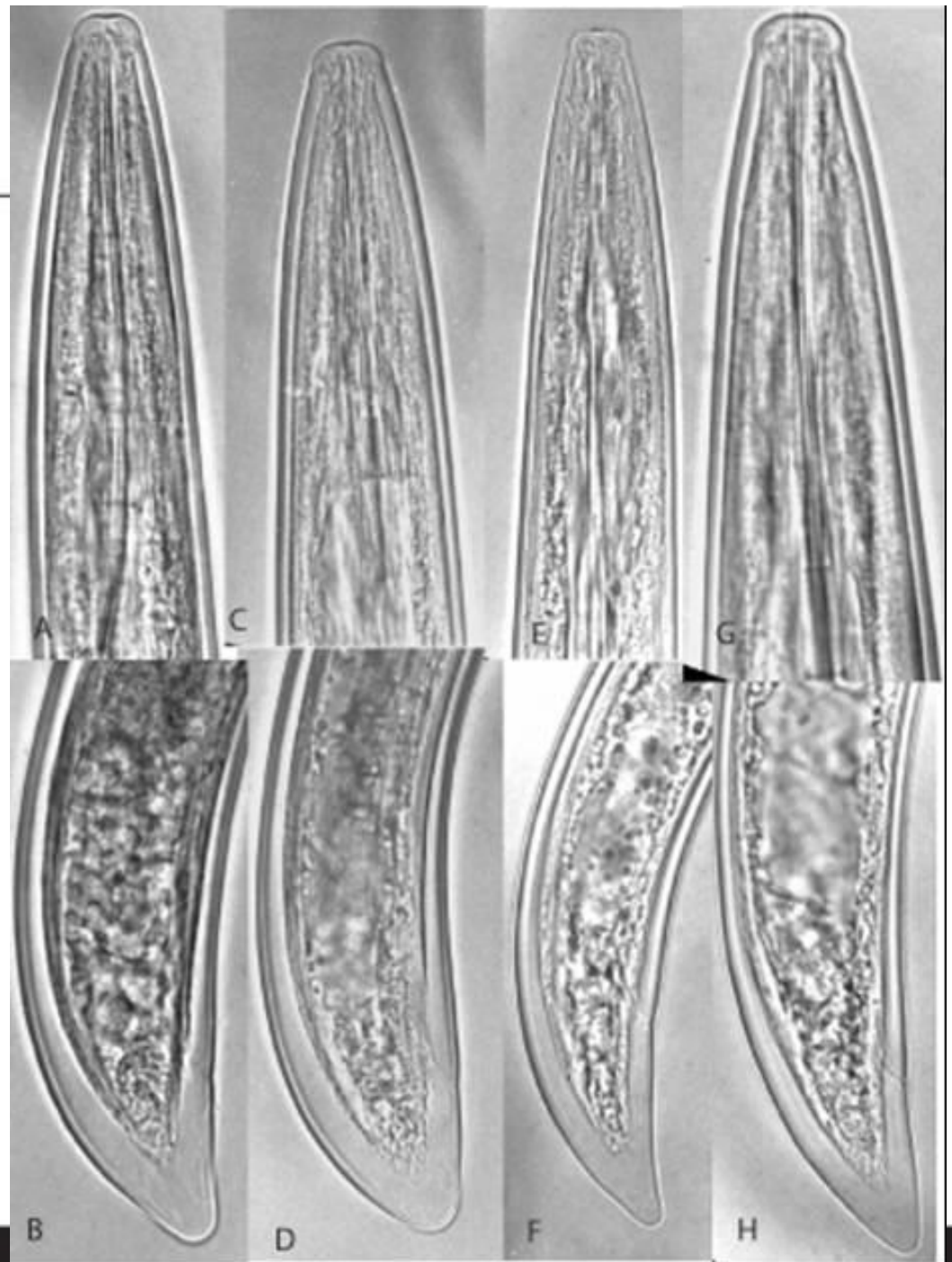
Região anterior e posterior de fêmeas do grupo *X. americanum*

A-B, *X. brevicolle*;

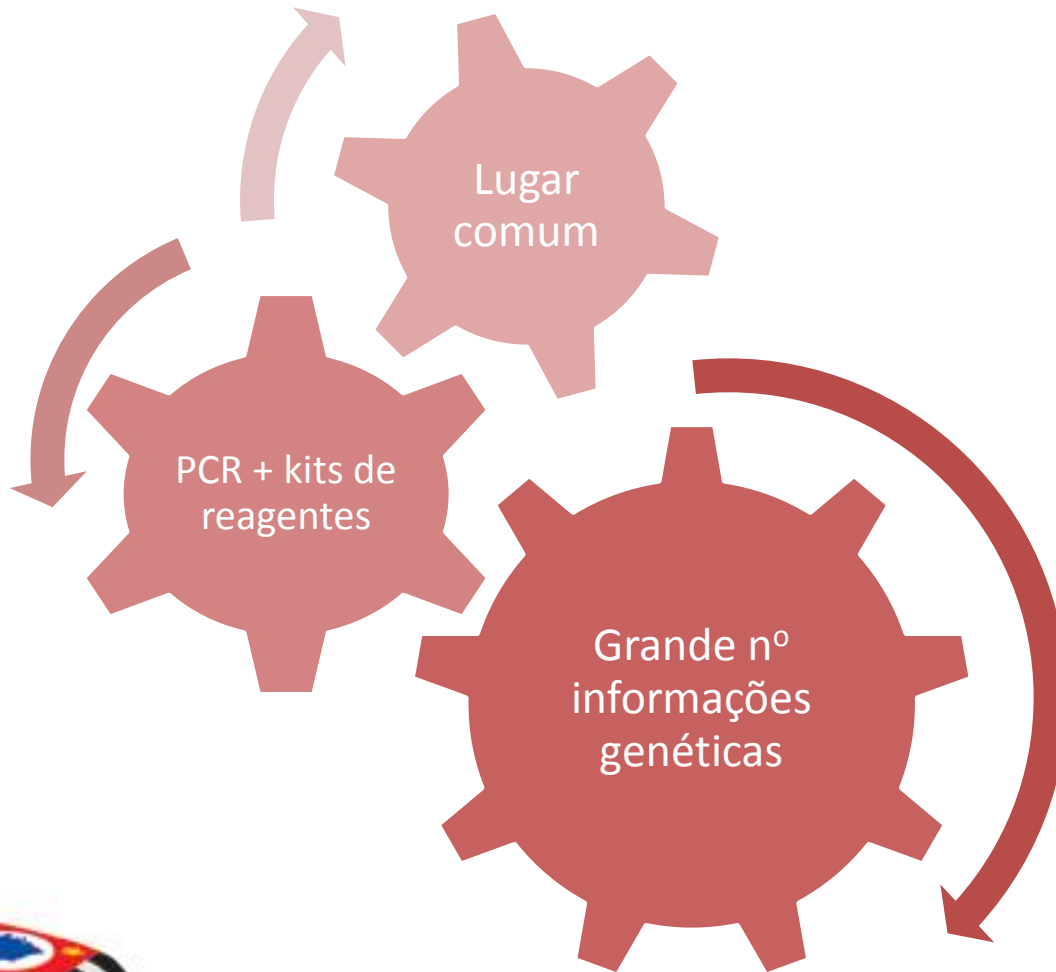
C-D, *X. diffusum*;

E-F, *X. oxycaudatum*;

G-H, *X. peruvianum*



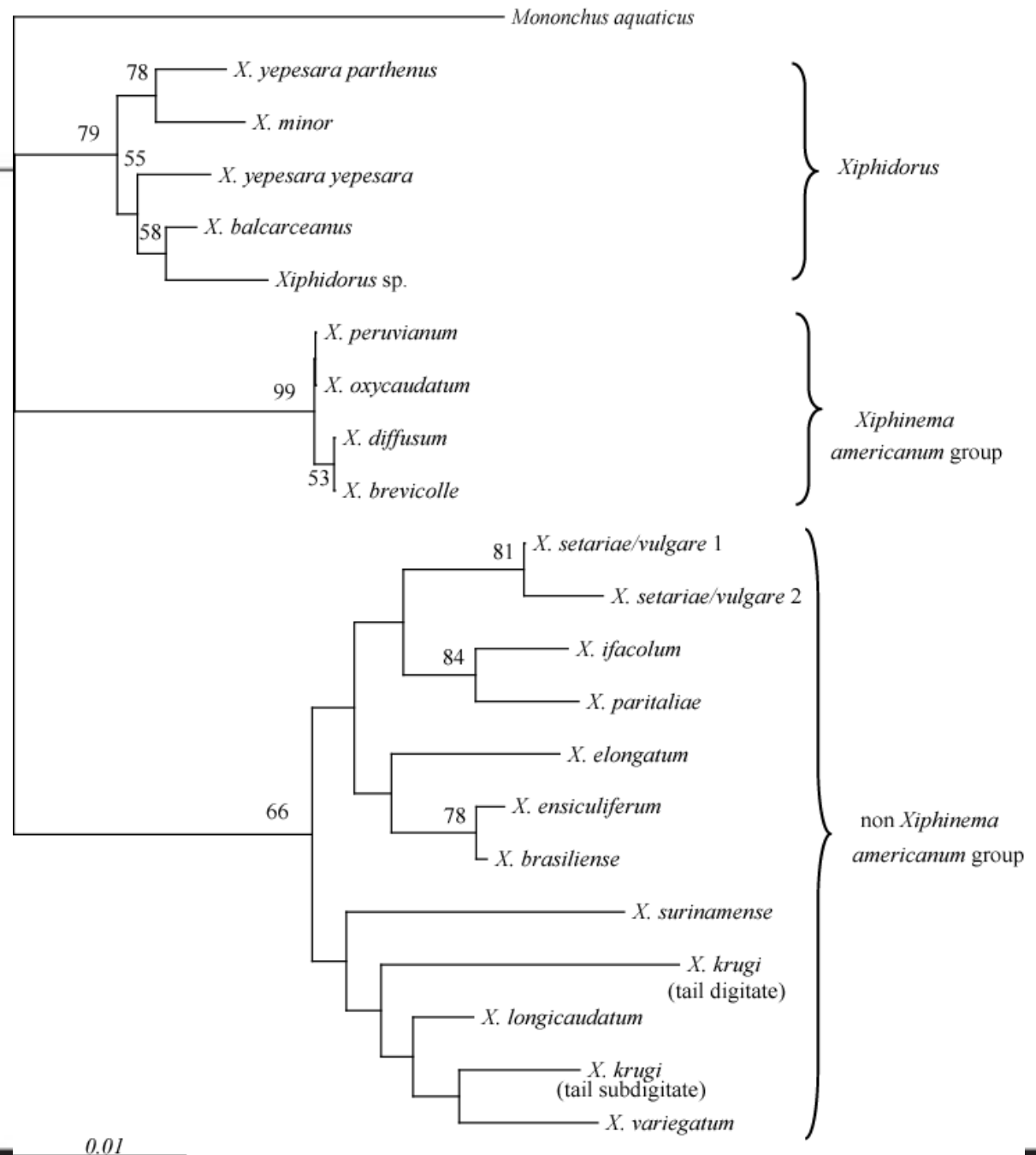
Técnicas moleculares



GenBank: www.ncbi.nlm.nih.gov

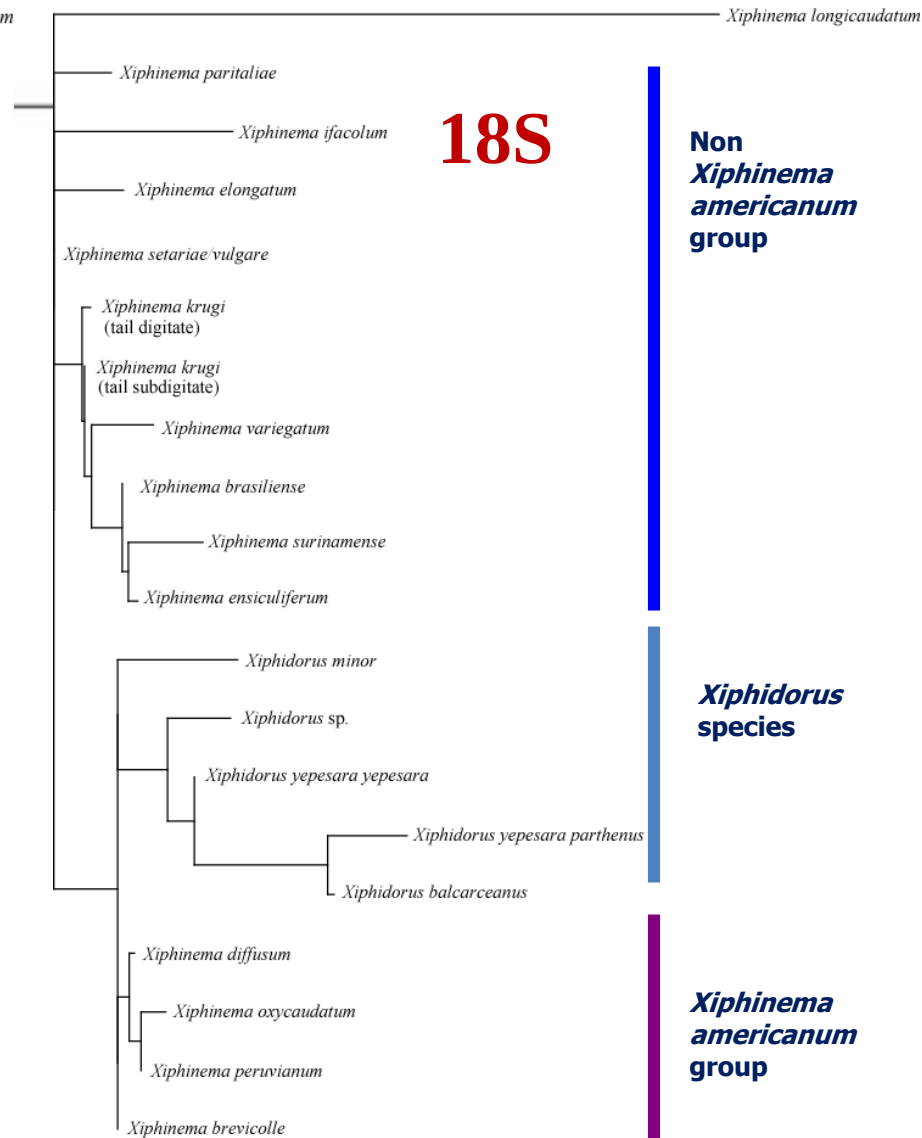
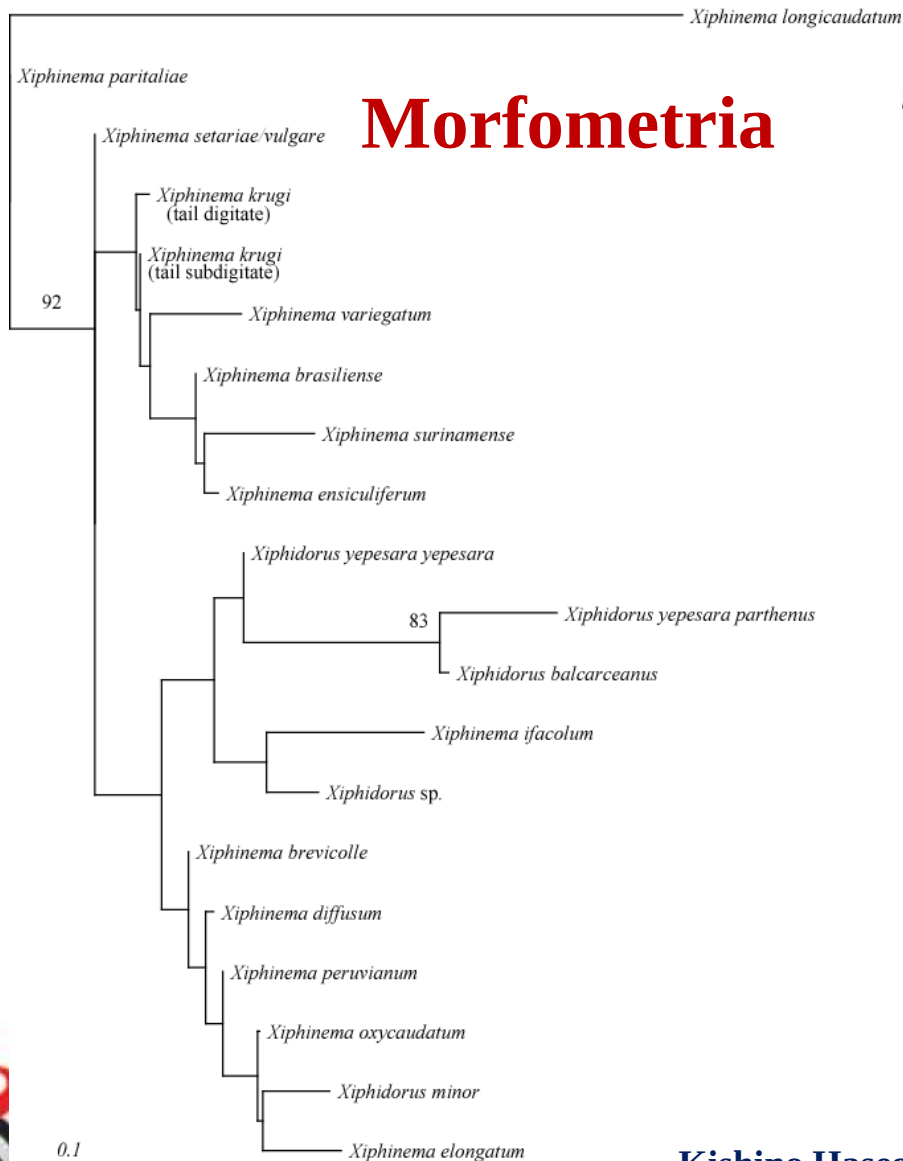


Árvore filogenética
mostrando as relações
entre as espécies de
Xiphinema e *Xiphidorus*
baseando-se nas
seqüências da região 18S
rDNA



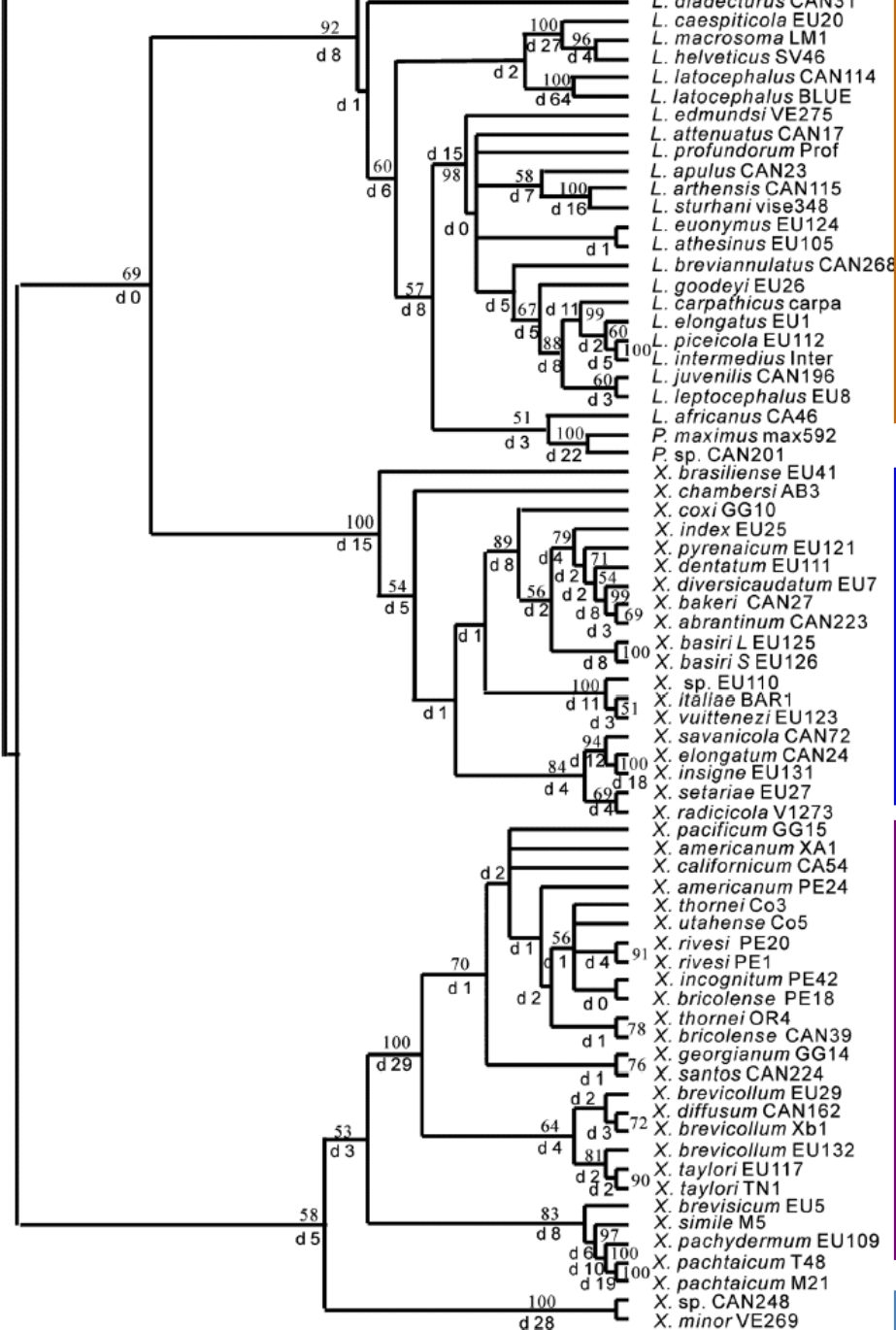
-
- Árvore filogenética
 - Maximum Likelihood trees → programa PHYLIP CONTML
 - Teste Kishino Hasegawa (KH)
 - Comparação das árvores 18S rDNA e morfometria





Kishino Hasegawa (KH)

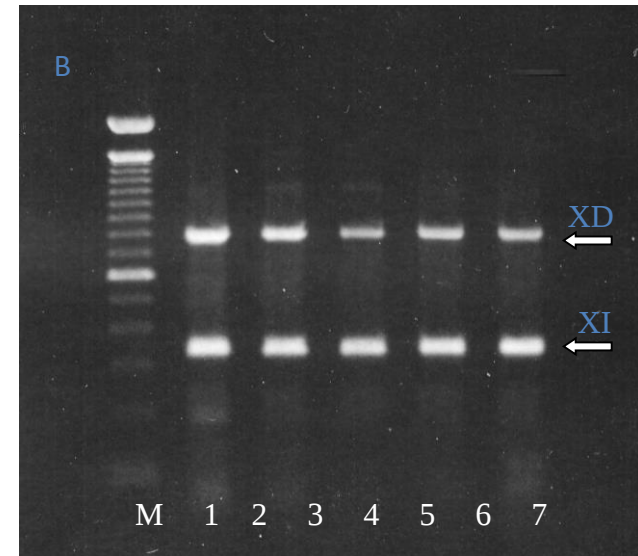
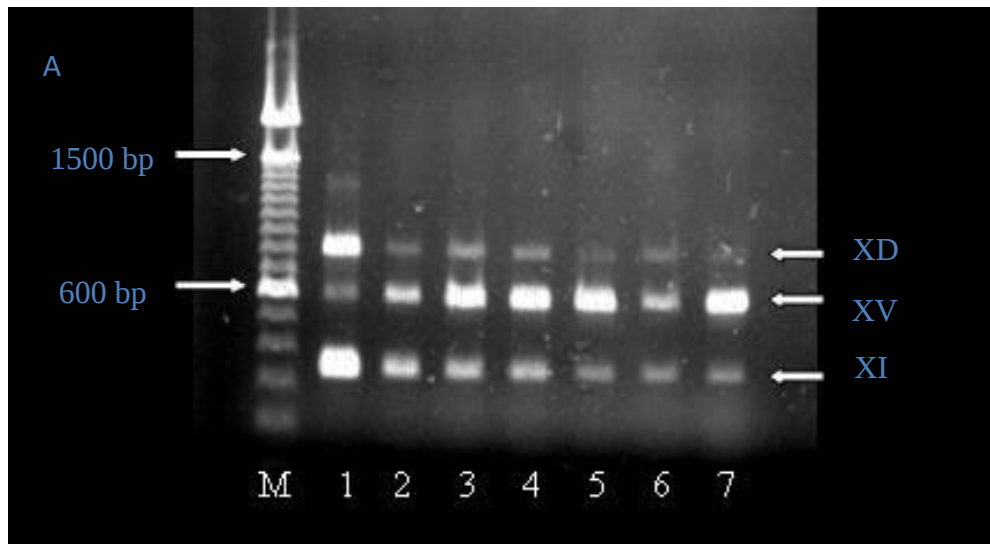
Filogenia da família Longidoridae baseada
no LSU rRNA gene (28S)



Primers específicos disponíveis para diagnose de longidorídeos vetores de viroses

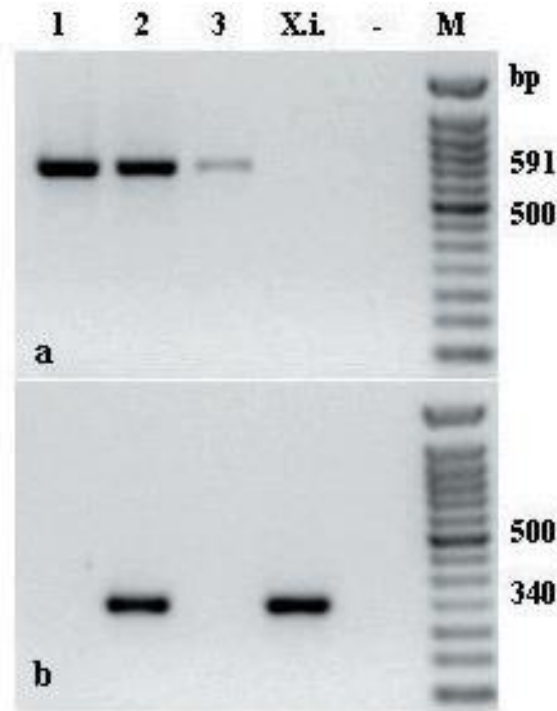
Oligo primer	Espécie de nematoide	Nº pb	Sequência do Primer (5'-3')	Referencia
GenF	Universal	23	TTG ATT ACG TCC CTG CCC TTT GT	Hübschen et al. (2004a)
Latten3	<i>Longidorus attenuatus</i>	30	TTC CCT TTT CCC TGA TTA TAA TTT TCT ATC	Hübschen et al. (2004a)
Lelong1	<i>Longidorus elongatus</i>	23	TTA TCG TAC GTA TTC CCA GTT CT	Hübschen et al. (2004a)
Lmacro2	<i>Longidorus macrosoma</i>	21	GTT CCC GAC GAT TAT TTT TGT	Hübschen et al. (2004a)
Pmax1	<i>Paralongidorus. maximus</i>	22	TGC ATT TCA CCA CTT CTC ACT C	Hübschen et al. (2004a)
D24	<i>Xiphinema diversicaudatum</i>	24	GAG ATA TAA AGC GAA AAC CGC GAG	Wang et al. (2003)
I27	<i>Xiphinema index</i>	27	GAG TCG TAA CGT TTC TCG TCT ATC AGG	Wang et al. (2003)
ITA26	<i>Xiphinema italiae</i>	26	GAA ATA AGA ACC CTG AAA AAG ATA GG	Wang et al. (2003)

Primers específicos para diagnose de
X. diversicaudatum (XD) ; *X. index* (XI) e *X. vuittenezi* (XV)



Hübschen et al. (2004)

Identification of *Xiphinema index* in an Austrian vineyard

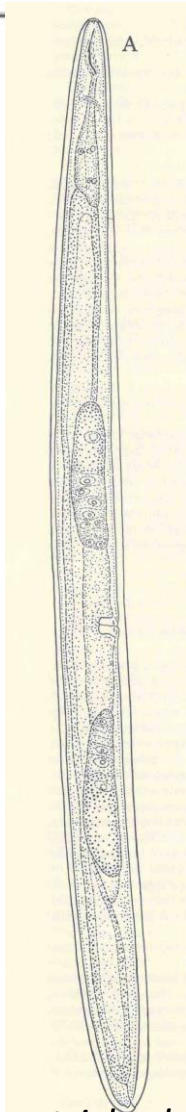


Molecular analysis of nematode populations (1-3) from the rhizosphere of three GFLV-infected grapevines using species specific primers for a) *X. vuittenezi* and b) *X. index*. *X. index* was identified in a mixture with *X. vuittenezi* in sample No. 2. X.i. = *X. index*, - = negative control, M = molecular size marker, bp = basepairs.

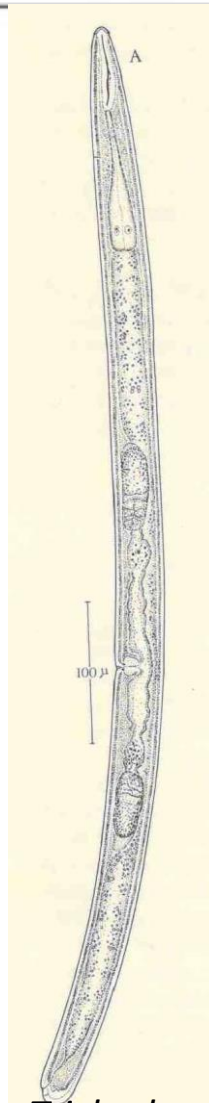
Caracteres usados para diferenciar os gêneros da família
Trichodoridae

característica	gênero			
	Trichodorus	Paratrichodorus	Monotrichodorus	Allotrichodorus
fêmeas	didelfas	didelfas	monodelfas	monodelfas
poros laterais advulvares	presente	raro	presente (1 par)	ausente
cutícula após fixação	normal	expandida	normal	normal/expandida
machos				
bursa	ausente	presente	ausente	presente
espículos	curvo	reto	curvo	longo

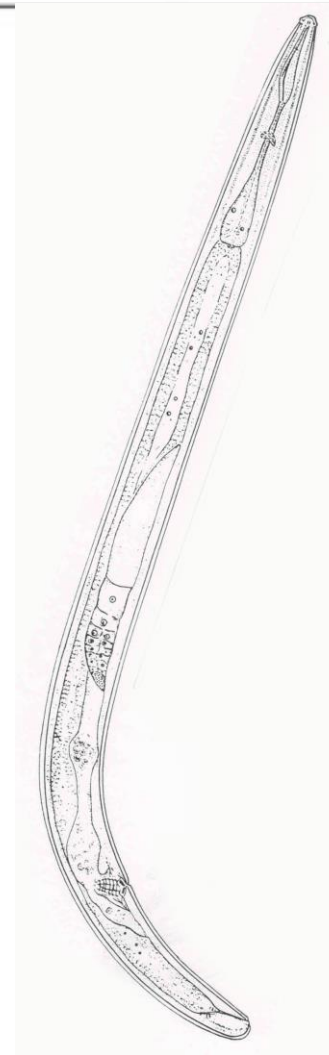
Morfologia das fêmeas após fixação



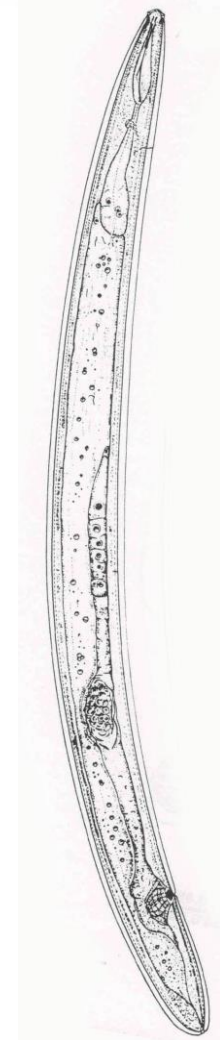
Paratrichodorus



Trichodorus



Monotrichodorus

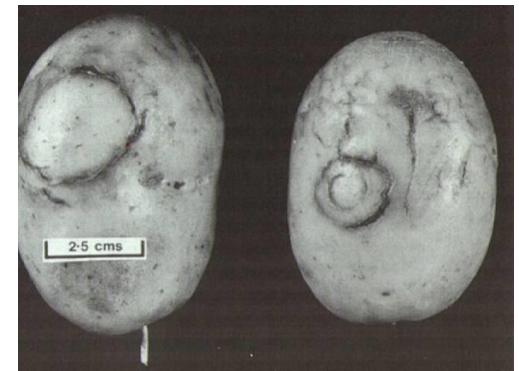
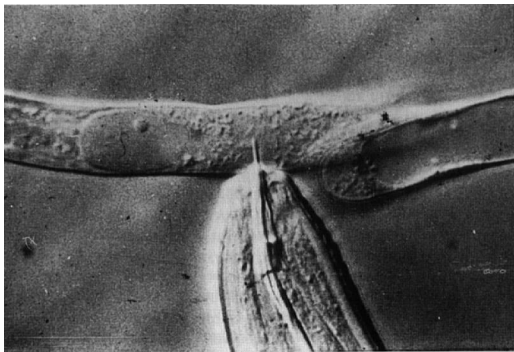


Allotrichodorus



Identificação Molecular de duas espécies de *Paratrichodorus*

- E. Riga, E. Karanastasi, C. M. G. Oliveira and R. Neilson (2007). Molecular Identification of Two Stubby Root Nematode Species. *Amer J. of Potato Res* 84:161-167.
- *Paratrichodorus allius* and *P. teres* são similares morfológicamente
- Danos diretos
 - ex.: trigo e milho
 - Transmissão de viroses (*Tobacco rattle virus*, Tobravirose)
 - Ex.: batata
- Desenvolvimento de primers específicos

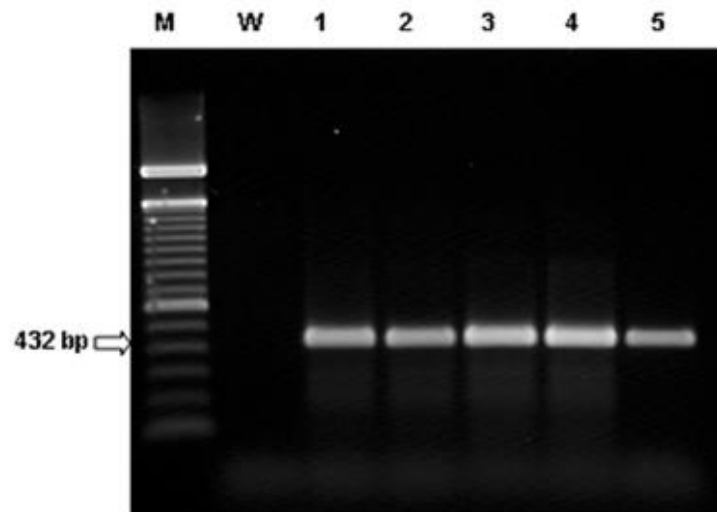


Populações utilizadas para determinar a especificidade e confiabilidade dos primers específicos PAR2 (*P. allius*) e PTR4 (*P. teres*)

Nematode species and GenBank Accession numbers	Plant Hosts	Locality
<i>P. allius</i> (Pa 1-4)	<i>Solanum tuberosum</i>	Columbia Basin, WA, USA
<i>P. allius</i> (Pa NE)	<i>Solanum tuberosum</i>	Columbia Basin, WA, USA
<i>P. allius</i> (Pa al)	<i>Medicago sativa</i>	Columbia Basin, WA, USA
<i>P. allius</i> (Pa kp)	<i>Solanum tuberosum</i>	Columbia Basin, WA, USA
<i>P. allius</i> (Pa m)	<i>Mentha X piperita</i>	Columbia Basin, WA, USA
¹ <i>P. macrostylus</i> (AJ439508)	<i>Quercus cerris</i>	Kralovsky Chlmec, Slovakia
<i>P. minor</i> (Pm 11a)	Fallow	Amaliada, Greece
¹ <i>P. pachydermus</i> (AJ439513)	<i>Solanum tuberosum</i>	Carnoustie, Scotland
<i>P. teres</i> (Pt 6a)	<i>Cynara scolymus</i>	Candia, Argolis, Greece
<i>P. teres</i> (Pt 7a)	<i>Cynara scolymus</i>	Candia, Argolis, Greece
<i>P. teres</i> (Pt cb)	Potato Field	Columbia Basin, WA, USA
¹ <i>T. primitivus</i> (AJ439518)	Pasture	Kinshaldy, Scotland
¹ <i>T. similis</i> (AJ439523)	<i>Solanum tuberosum</i>	Tentsmuir, Scotland
<i>Paratylenchus</i> spp.	Potato field	Columbia Basin, WA, USA
<i>Tylenchorhynchus</i> spp.	Potato field	Columbia Basin, WA, USA
<i>Criconebella</i> spp.	Potato field	Columbia Basin, WA, USA
<i>Meloidogyme hapla</i>	Potato field	Columbia Basin, WA, USA
<i>Meloidogyme chitwoodi</i>	Potato field	Columbia Basin, WA, USA
<i>Ditylenchus dipsaci</i>	Alfalfa field	Columbia Basin, WA, USA
<i>Caenorhabditis elegans</i>	Laboratory culture	Cold Spring Harbor, USA

¹Data from Boutsika 2002.

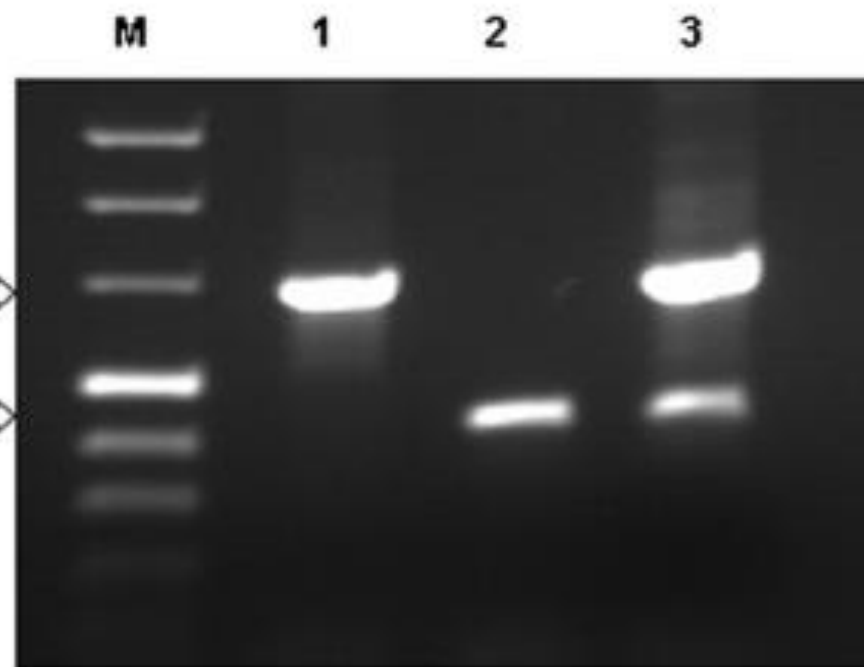




Confiabilidade do primer PAR2 (*Paratrichodorus allius*)

PTR4 (*P. teres*) 677 bp ⇨

PAR2 (*P. allius*) 432 bp ⇨



Uso em multiplex dos primers PAR2 (*P. allius*) e PTR4 (*P. teres*)

Controle

- Nematicidas
 - Fumigantes
 - Não fumigantes
- Rotação de culturas
 - Polífagos
 - GFLV/*X. index*: uva
- Cultivares resistentes
 - *Vitis rotundifolia* não foi infectada por GFLV
 - *Vitis candicans* cv. Dog Ridge resistência a *X. index*
 - Batata Bintje: imune a TRV

Legislação

Plantas livres de vírus e nematoides
Serviço quarentenário



Raspberry Ringspot Virus on Raspberry

Vetor = *Longidorus* sp.



Sem tratamento



Controle: brometo de metila

OBRIGADO

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