Introduction to nematology
Wood-inhabiting nematodes
Quick Start lecture for beginners.

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Nematode Collection of ZIN RAS was used to prepare LM illustrations

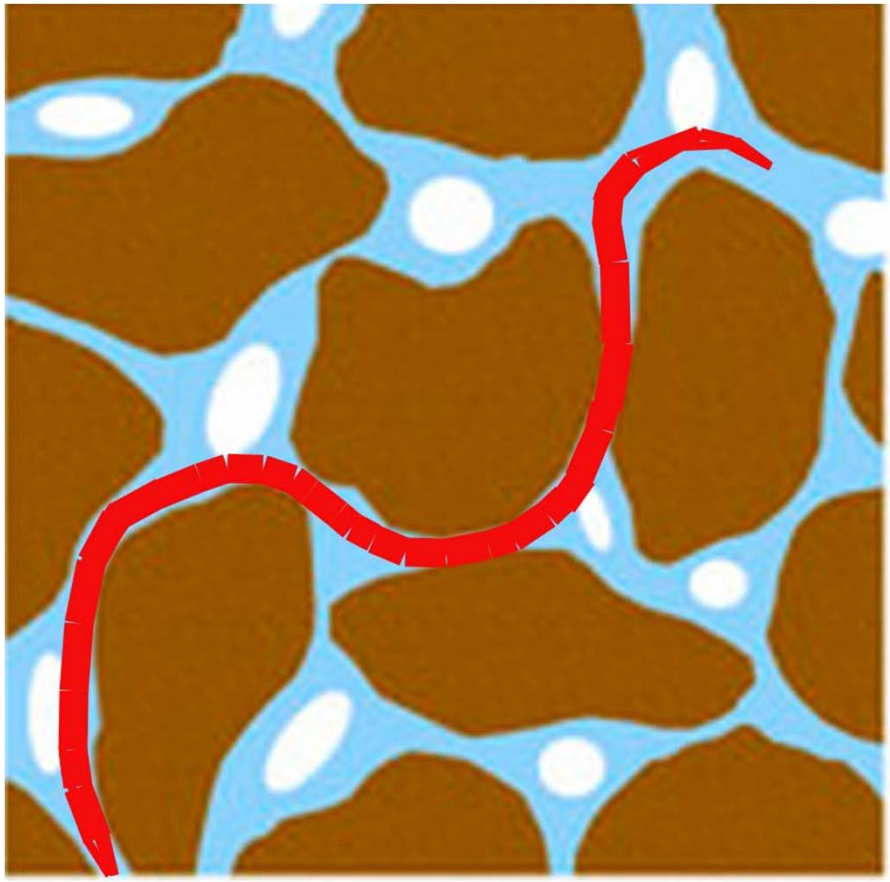
In frames of the Projects:

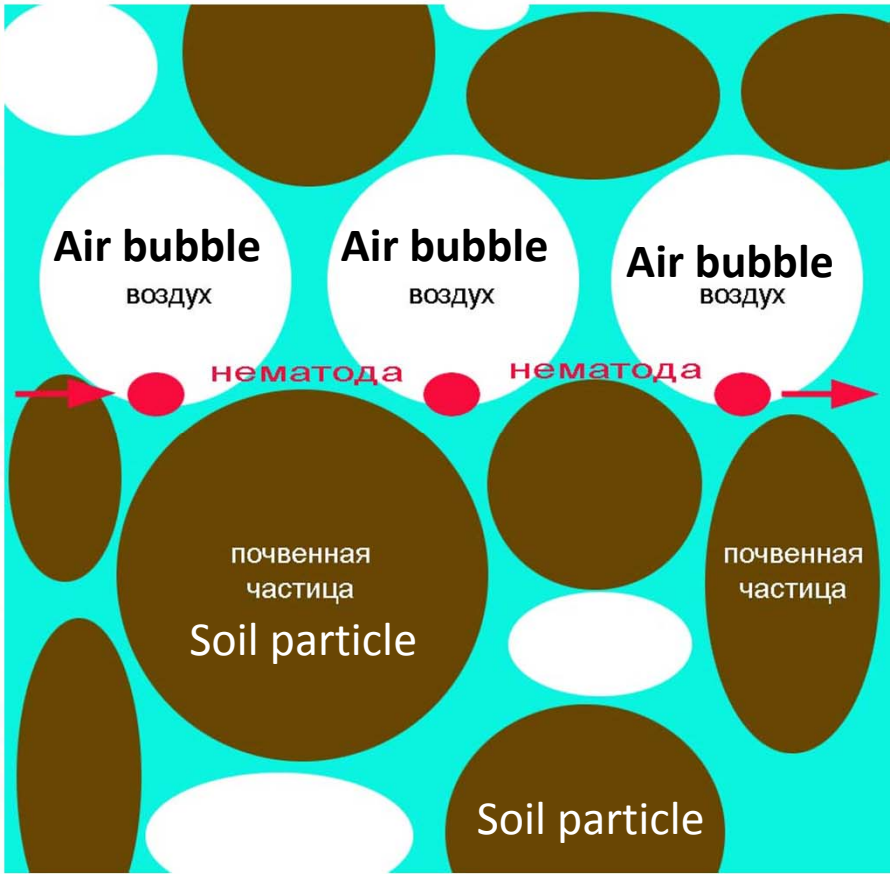
- ForBio and UiB Dead Wood Course 2019

- ZIN RAS Collection Fund
- State Academic Programs FSR:
- AAAA-A19-119020690109-2;
- AAAA-A17-117030310322-3;
- AAAA-A17-117080110040-3;

- RFBR 17-04-01397

Ryss, 2016





Upper view **Horizontal view**

NEMATODES ARE WORM-LIKE ORGANISMS INHABITING POROUS SUBSTRATES
They move wave-like in WATER FILM, but on LATERAL body surface (in horizontal plane)

What we know about the nematodes?

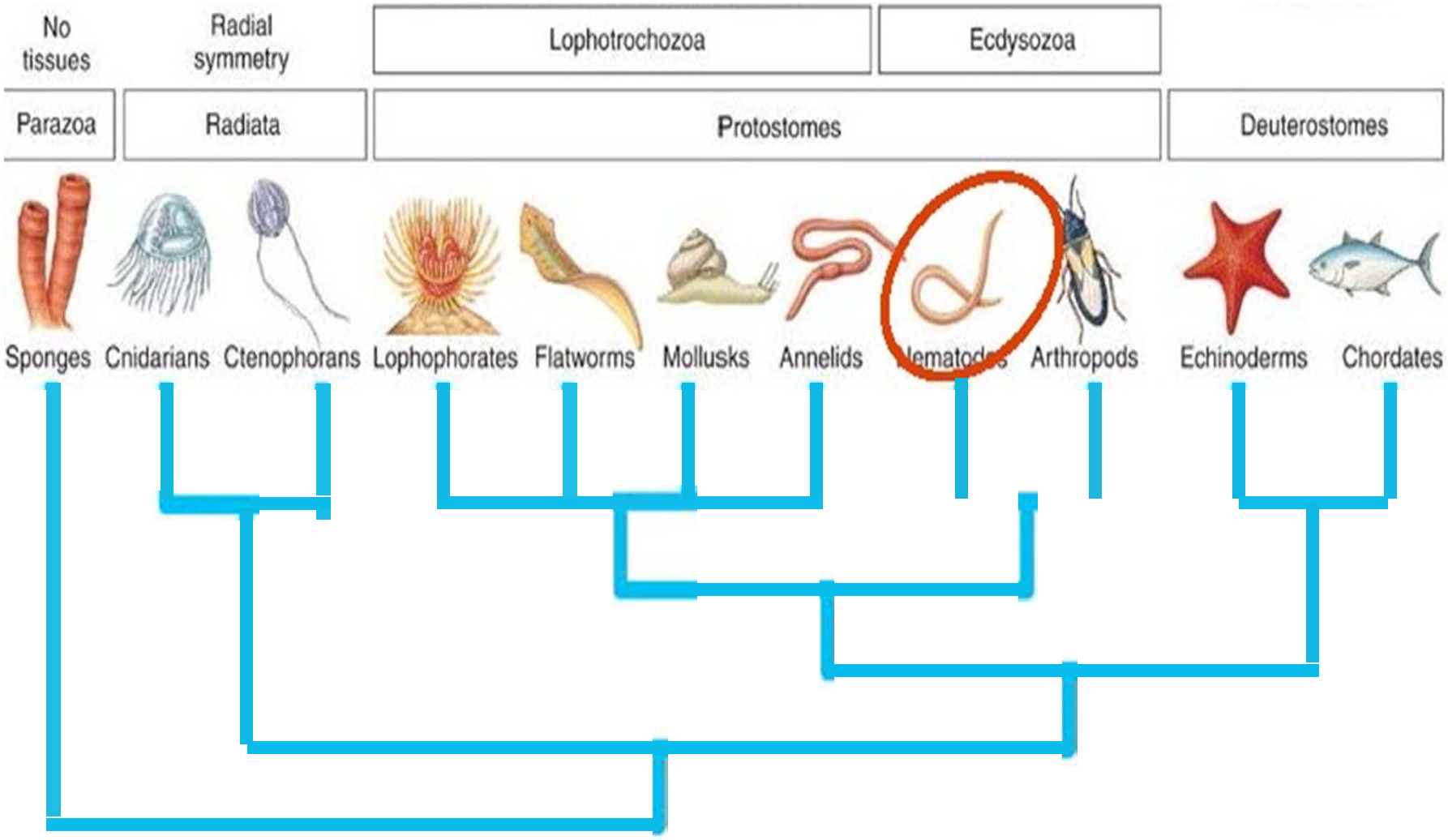
- - Biological model for the Developmental Biology
- - Dangerous parasites of humans and domestic animals

- Nematodes are the most popular highest animals for the molecular and genetics’ studies

- Goal and TASK:

- to demonstrate the Nematode Contribution to DeadWood successions process

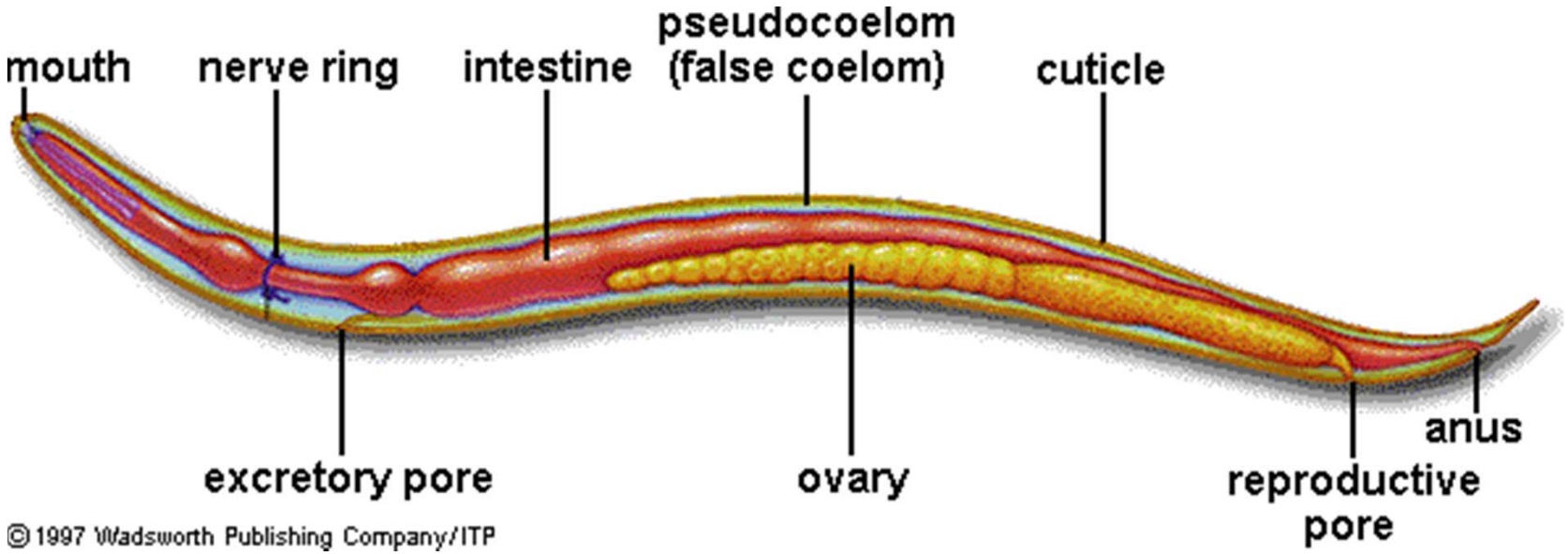
- GENERAL NEMATOLOGY:
- STRUCTURE AND EVOLUTION



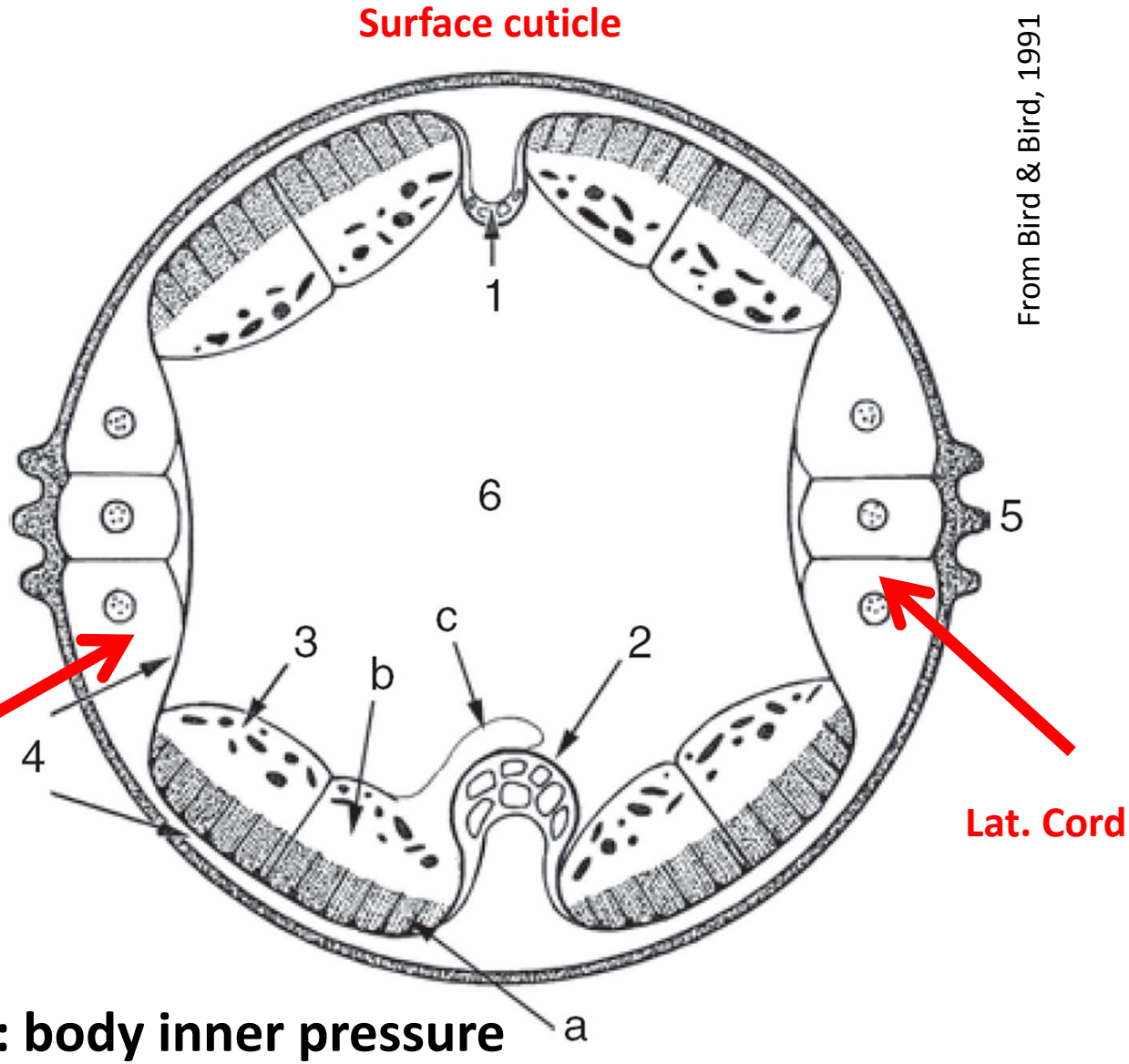
Main structural characteristics

- Growth with a series of molts
- Body surface isa covered by multi-layered cuticle, epithelium submerged (hypoderma) assembled in chords
- Mostly amphimictic, ovivivіparous
- Body cavity: no coelom, only shizocoel
- No blood or respiratory systems
- Muscles: only longitidinal, connection processus from muscle cell to the nerve cord
- Nerve cord: four cords and central neck ring with ganglia
- Sensory structures are integrative amphids and groups of head and genital papillae
- Excretory system: canals in hypodermic chords; excretory pore is supplied with excretory and secretory gland cells.

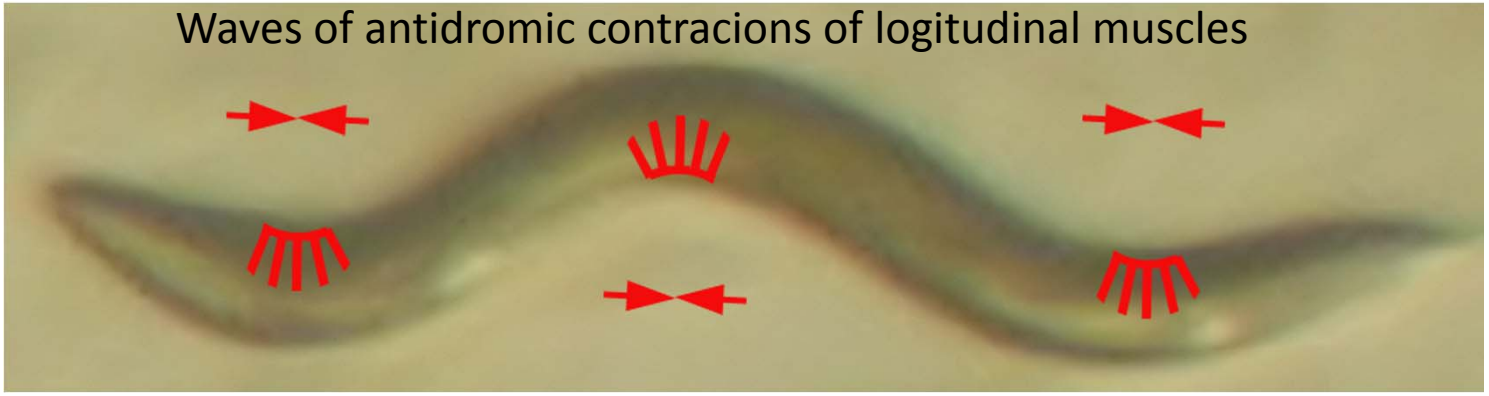
GENERAL ANATOMY

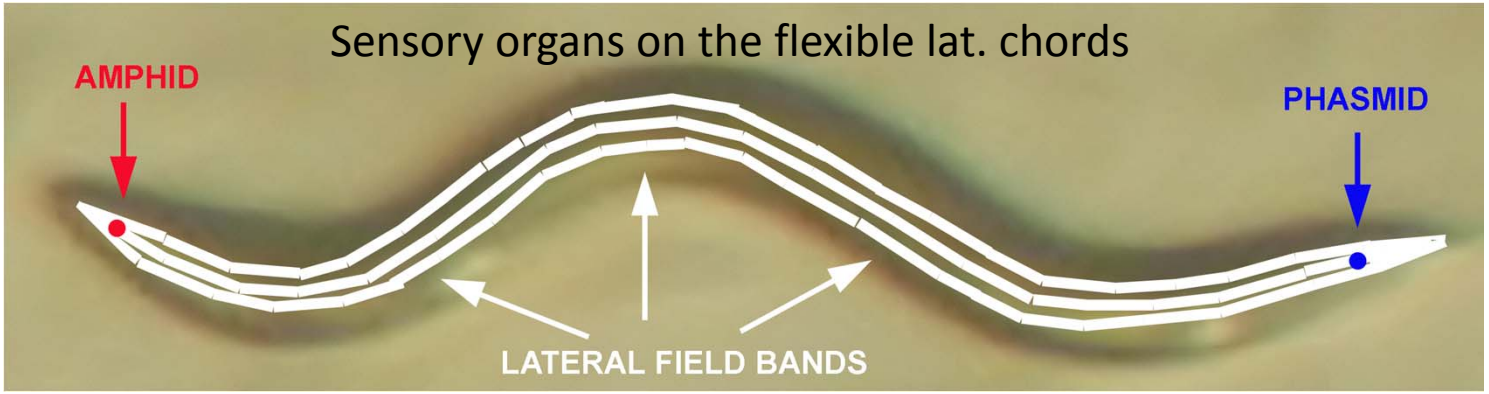


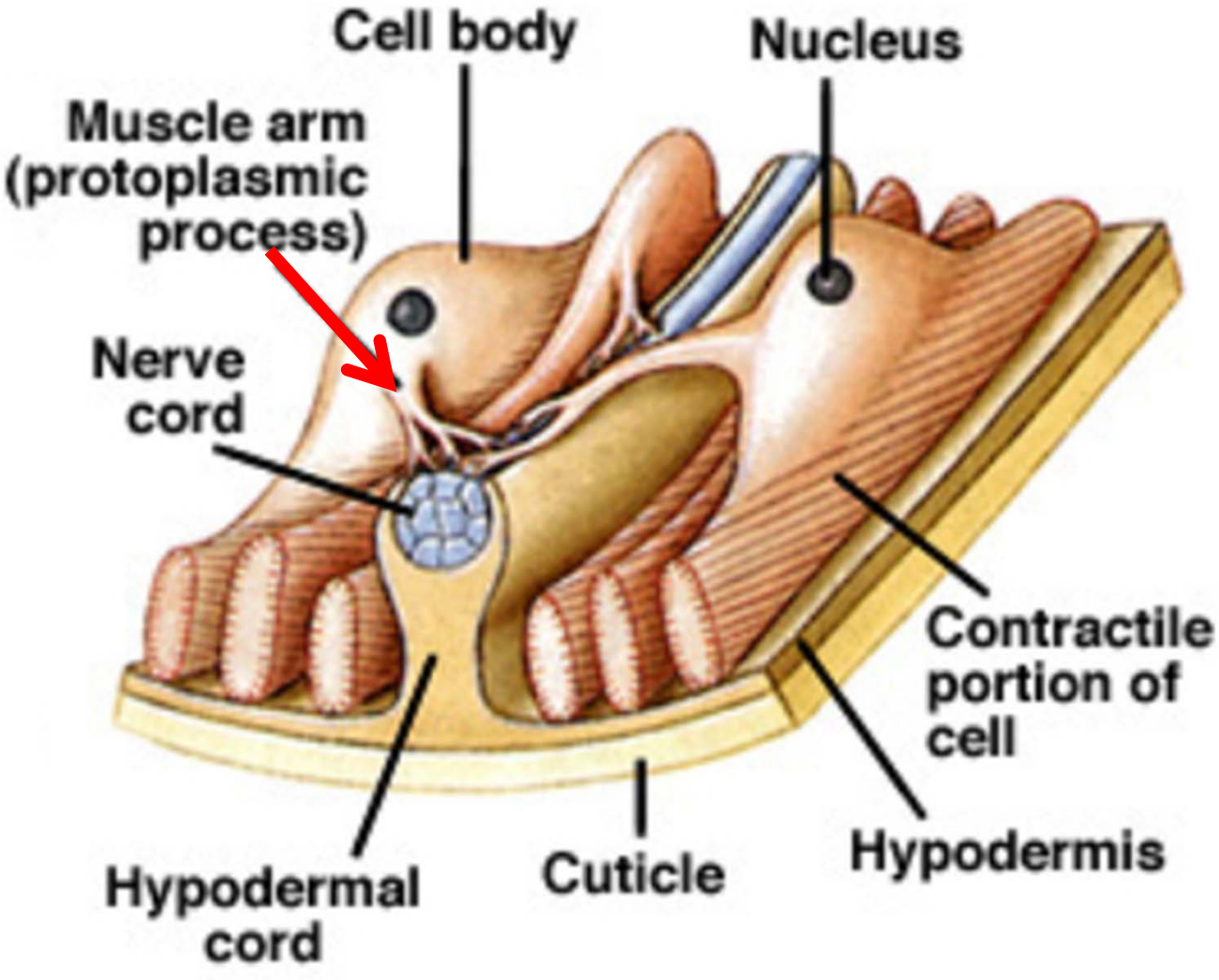
- The body is highly flexible because of **ANNULATED SURFACE CUTICLE,** **facilitated by the flexible LATERAL CHORDS** of **HYPODERMIS**



Support of undulation: body inner pressure
2.5-3 atm + lateral chords; muscles only longitudinal


Waves of antidromic contracions of logitudinal muscles


Sensory organs on the flexible lat. chords
AMPHID
PHASMID
LATERAL FIELD BANDS

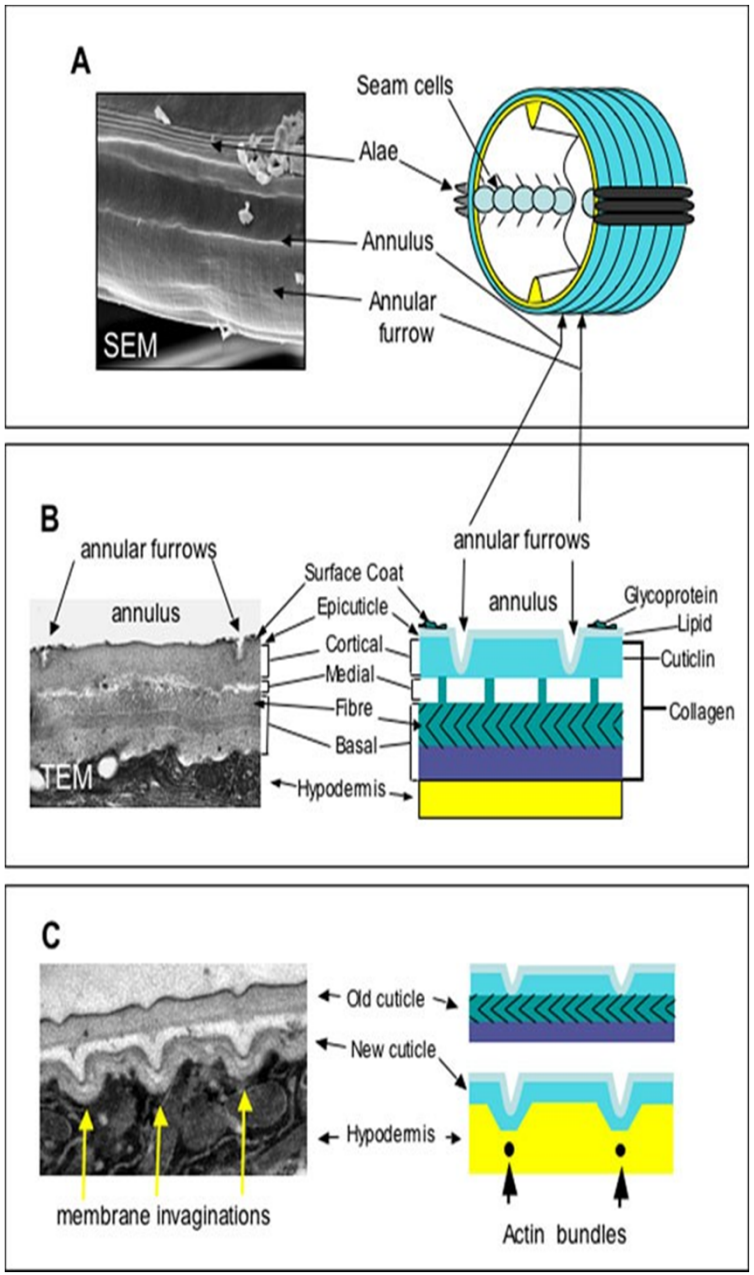


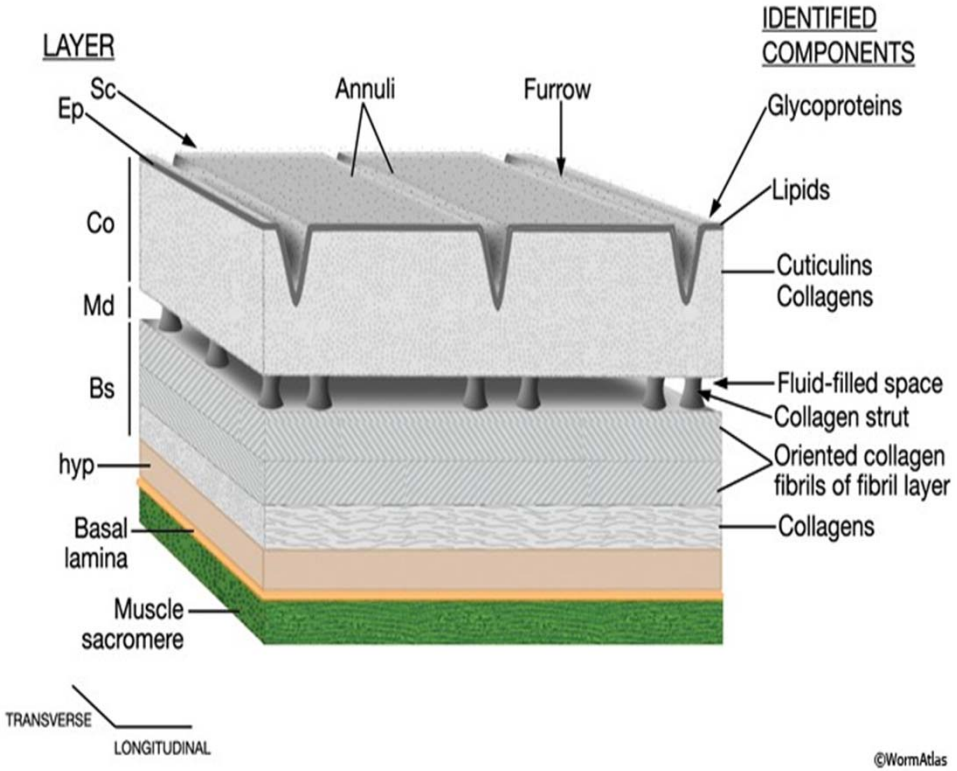
!!! Reverse connection:

The muscle cell sends its processus to nerve cord:

Nematode Adult Anatomy
https://www.slideshare.net/Fogellh/nematode-notes

Cuticle Structure



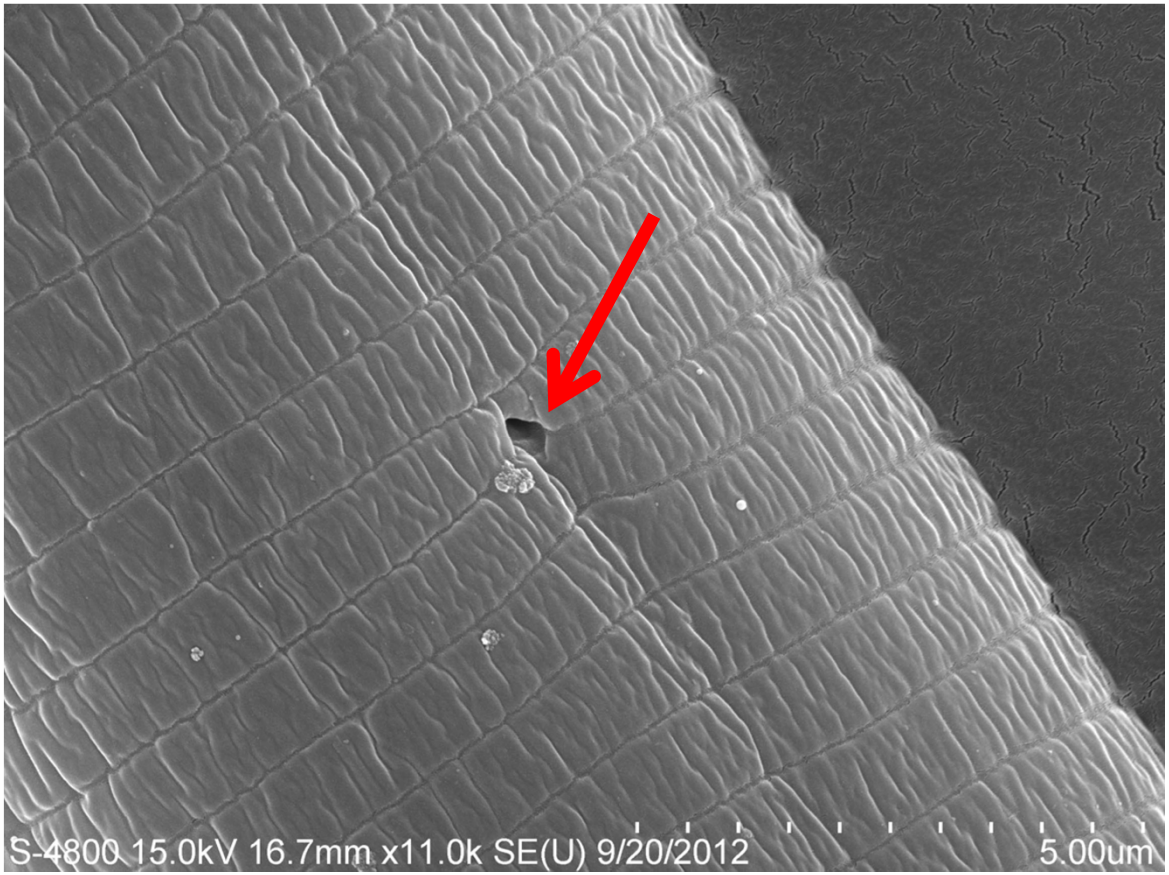


from WormAtlas

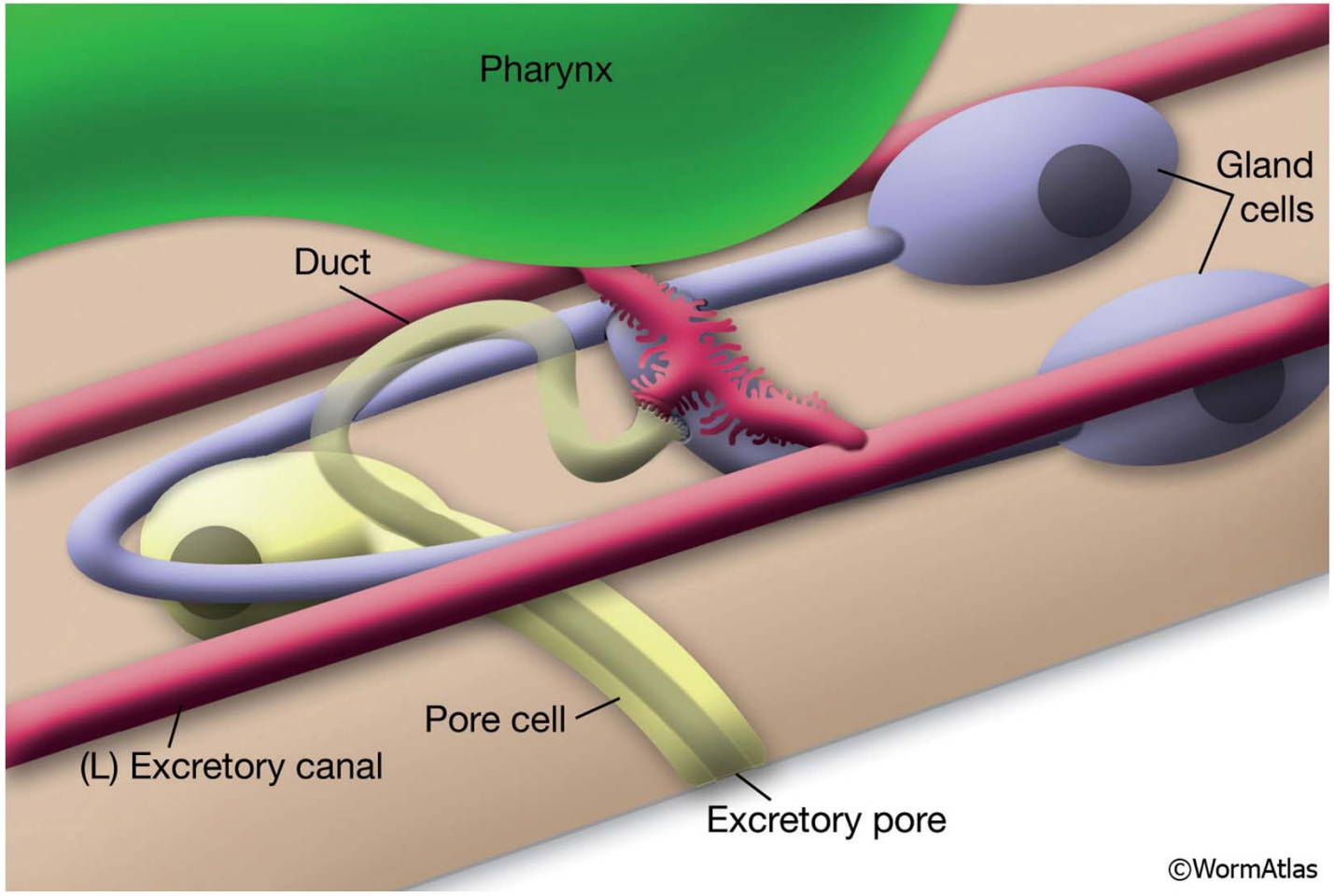
Ryss, 2002; 2013

Excretory Pore

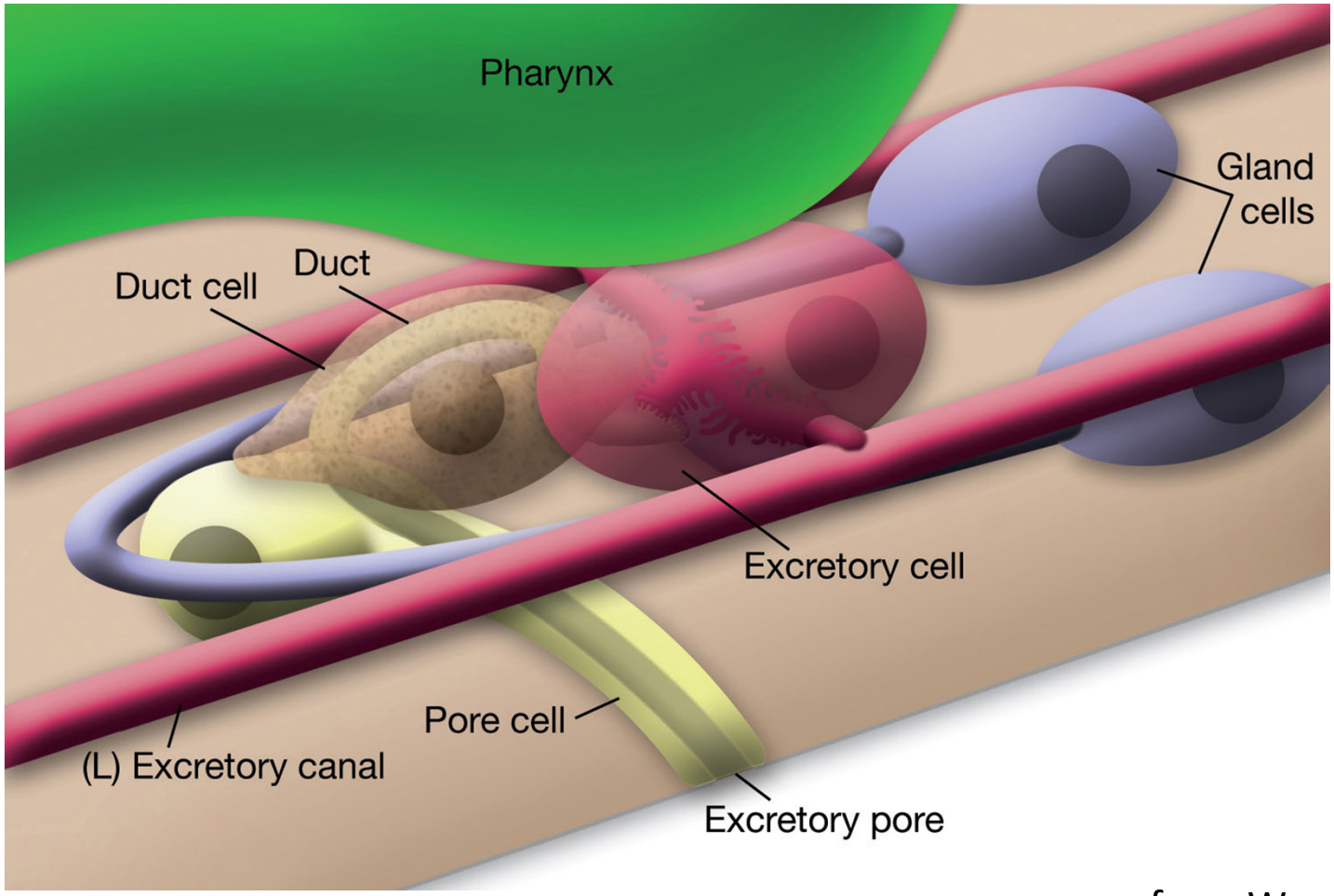
The outlet of the canal system located in hypodermic cords. It opens ventrally at the level of nerve ring and mid-pharynx



Excretory system – inter-cellular secretory and excretory space connections

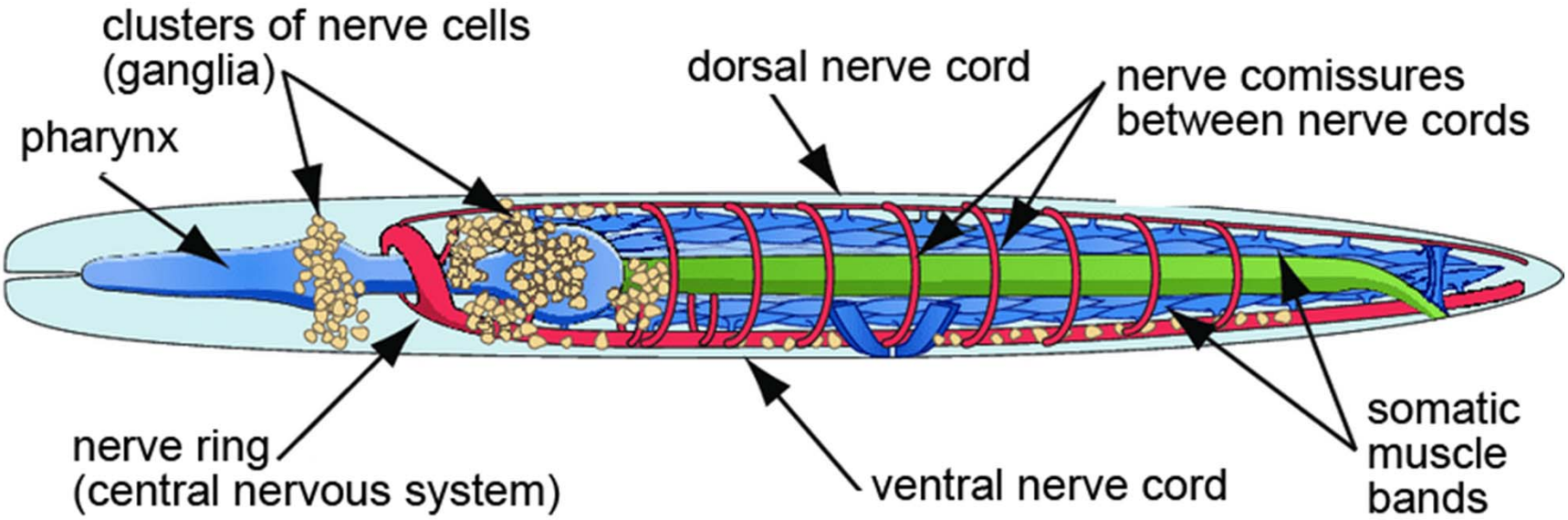


Excretory system – the duct cell and excretory cell



from WormAtlas

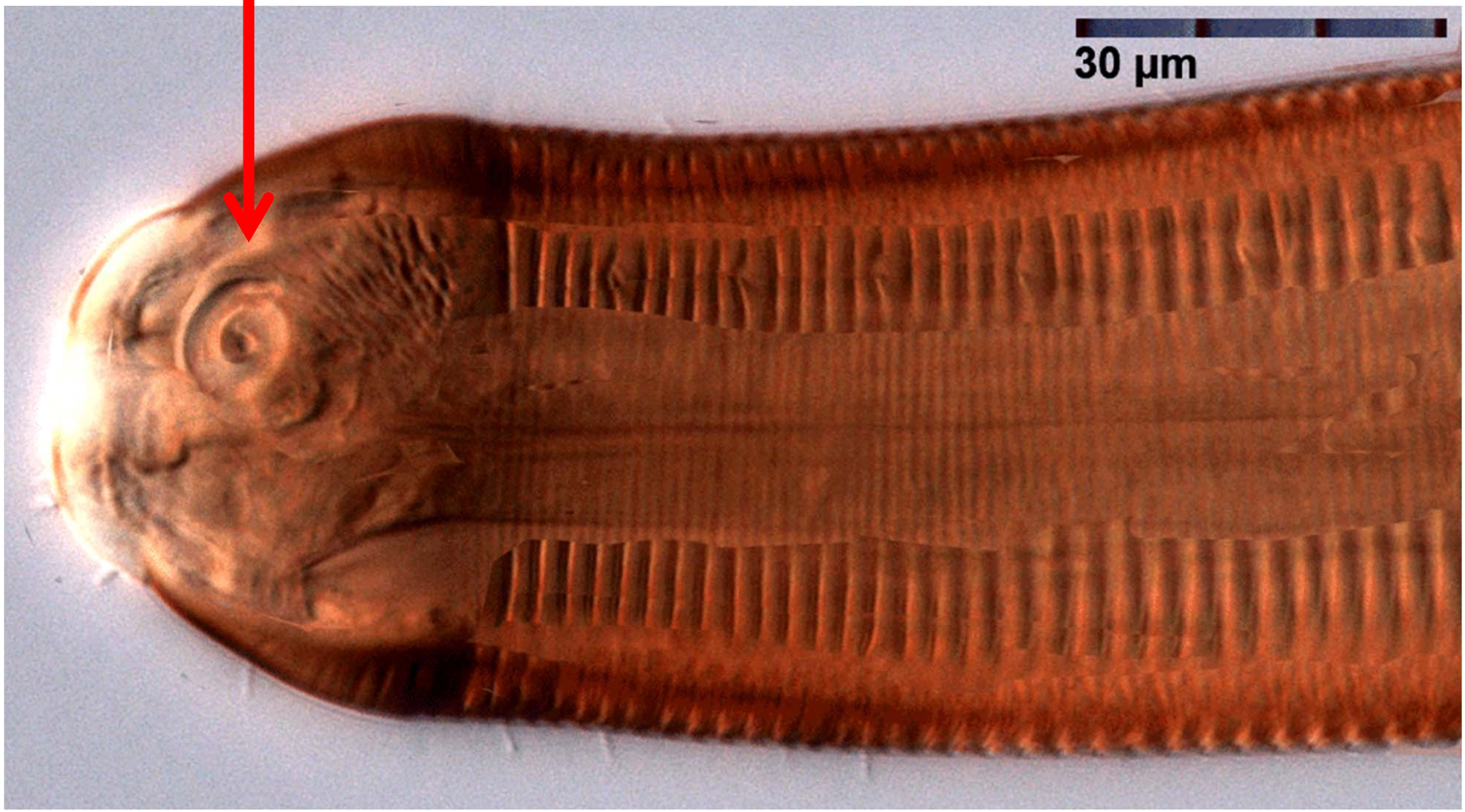
Nervous system



William R Schafer, https://www.researchgate.net/publication/309454437_Nematode_nervous_systems/

SENSORY STRUCTURES

amphid



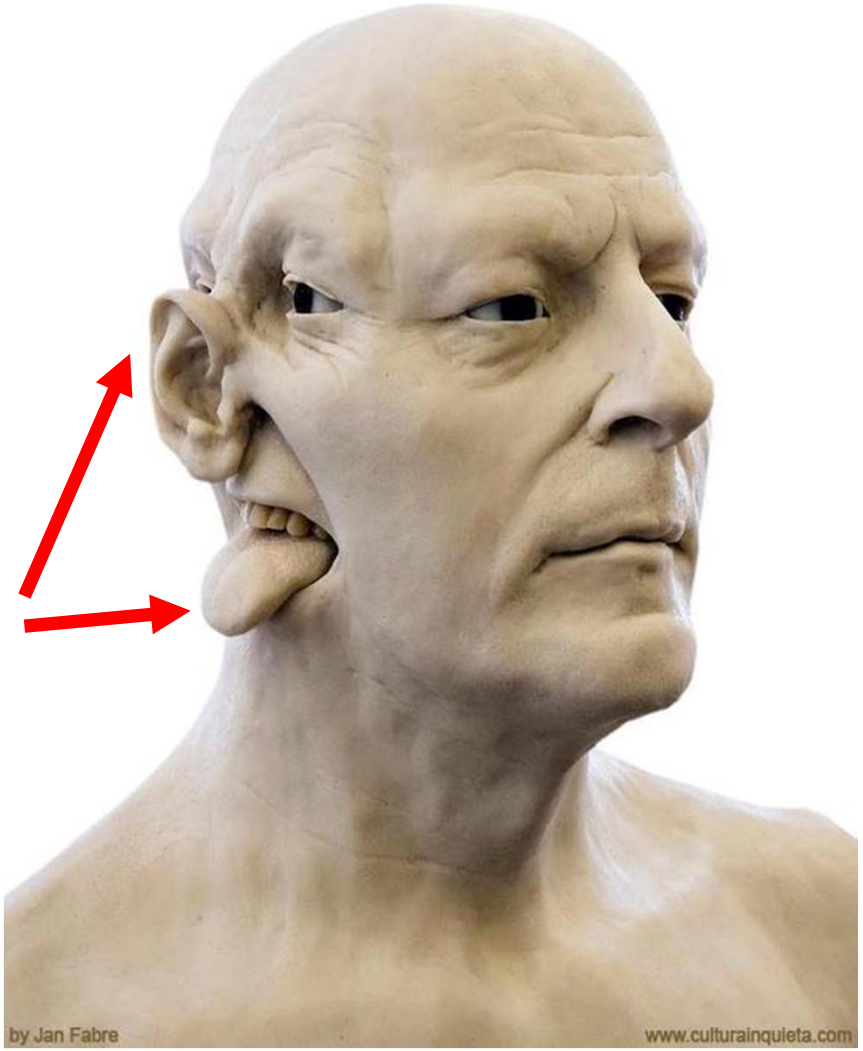
***Laxus septentrionalis* Cobb, 1914**

Ryss, 2013

i39.beon.ru/../Summer__by_MellyBaldin.2013

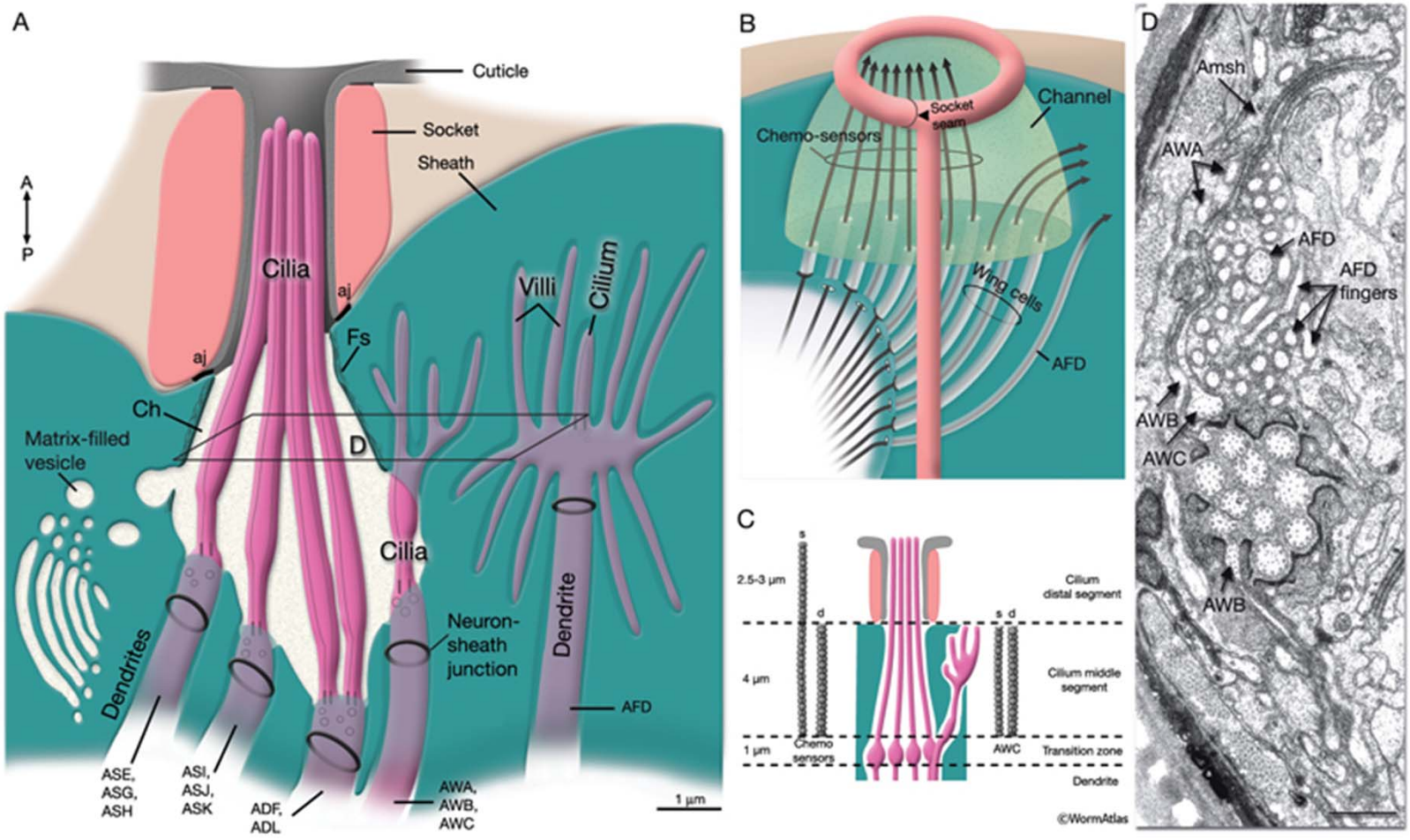
AMPHIDS: paired sensory organs combined feeling of chemical sense, temperature and vibration, including water film tension. They include sensory cells, sheaths, glands and framework structures

Analogies with human basic feelings are demonstrated



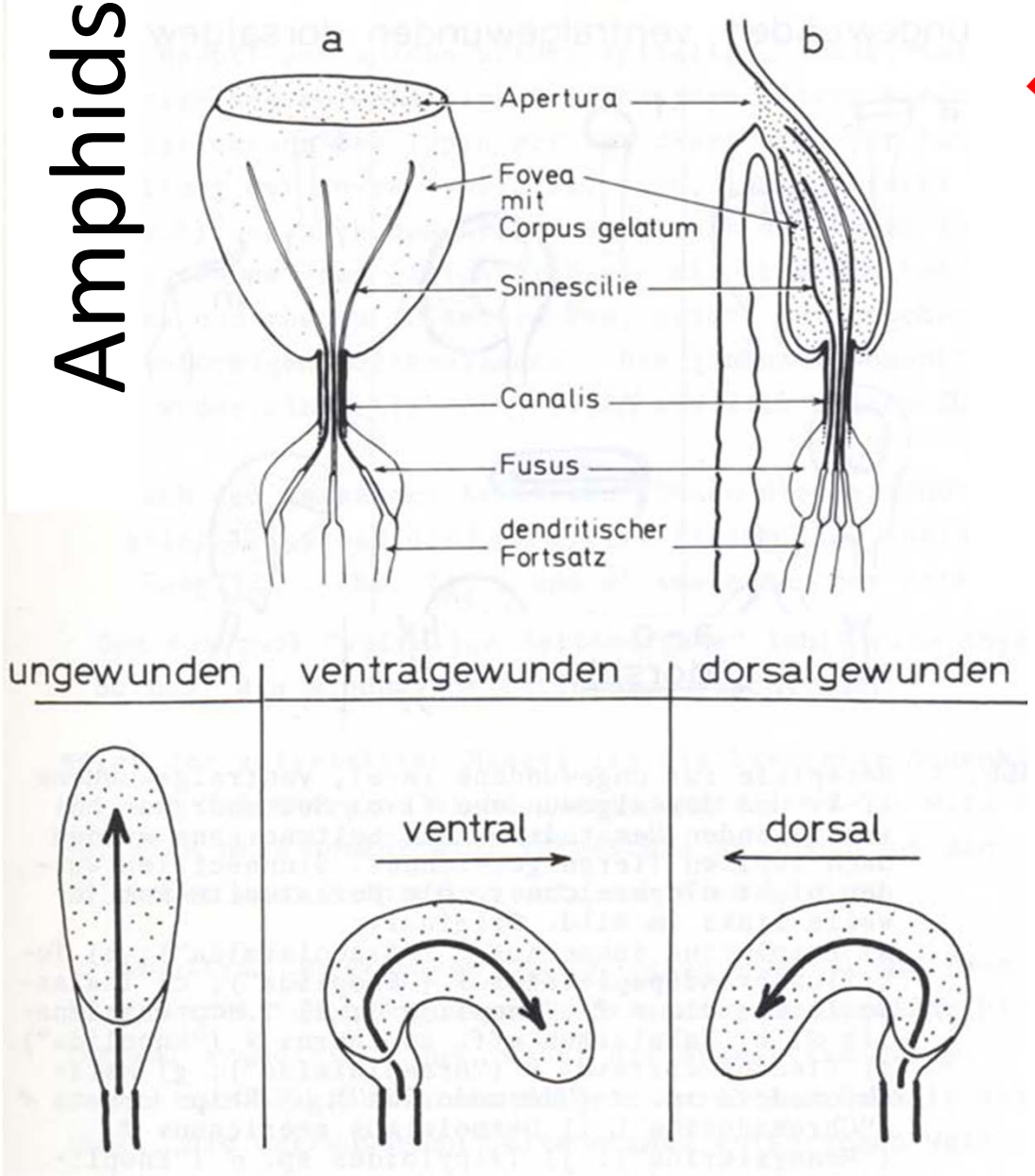
Jean Fabre, Cultura Inquieta 2013

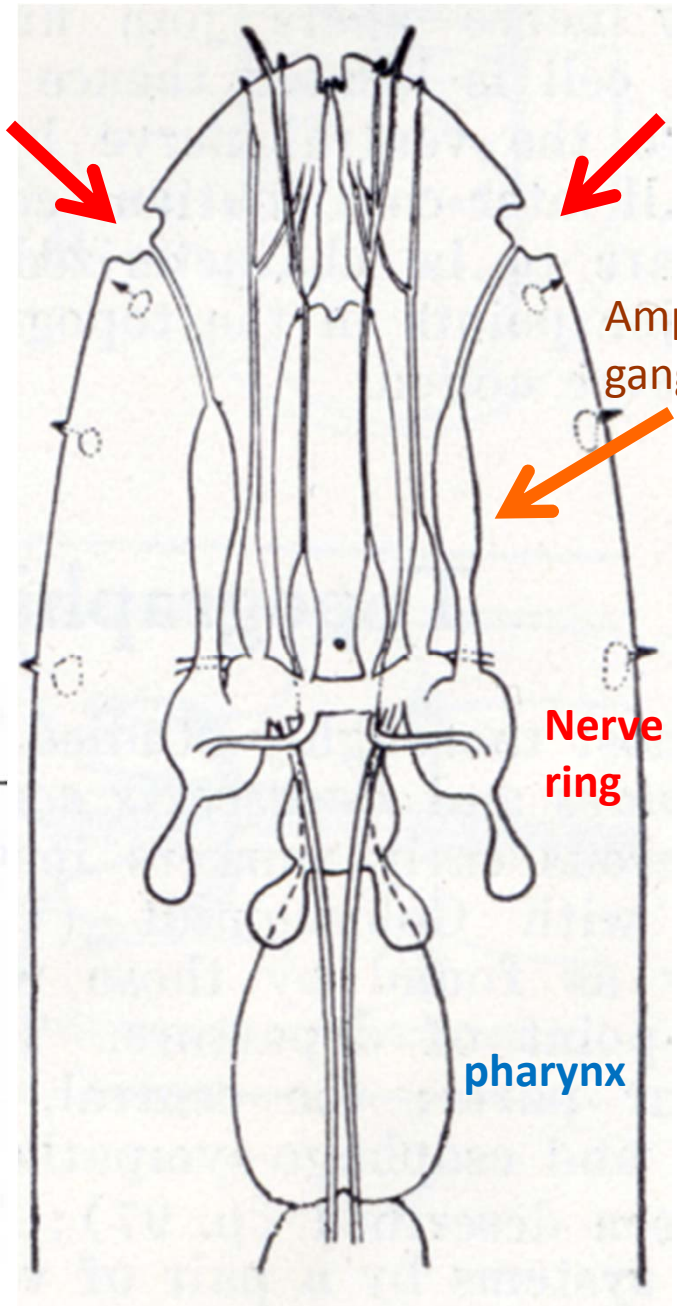
AMPHID, Ultrastructure



WormAtlas 2013: *Caenorhabditis elegans*

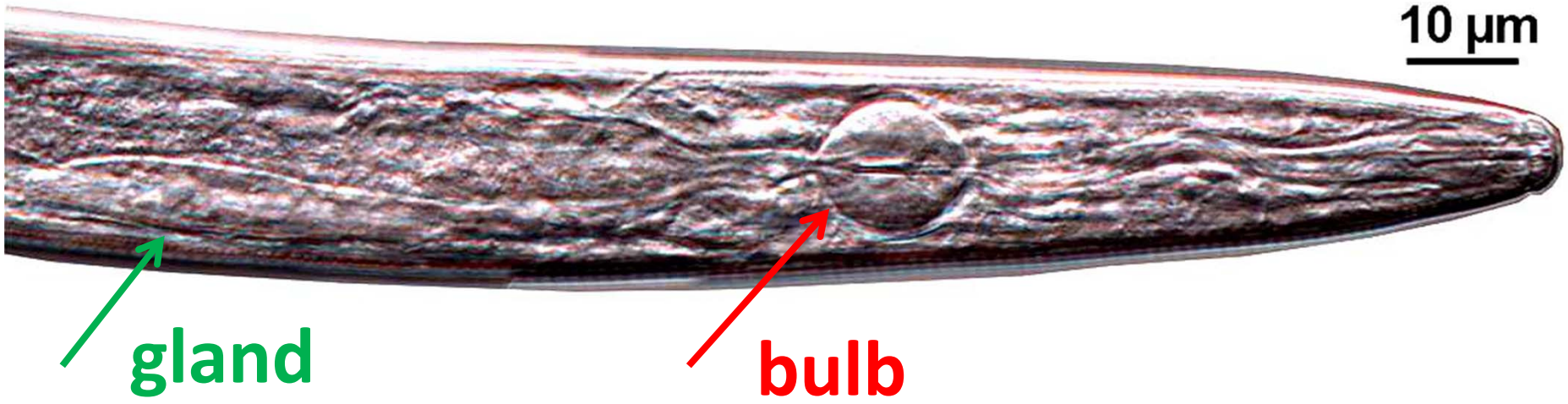
Amphids





Lorenzen, 1981; Chitwood, 1950

Bulb (pump) in pharynx of stylet-bearing nematodes: mycotrophic & plant parasites



Bulb (pump) facilitate a) the **gland secretion** output and b) the following suction of dissolved host cell content

Trophic groups

- Bacteriotrophic
- Fungivores
- Plant parasites
- Predators
- Parasites of animals (insects and vertebrates)

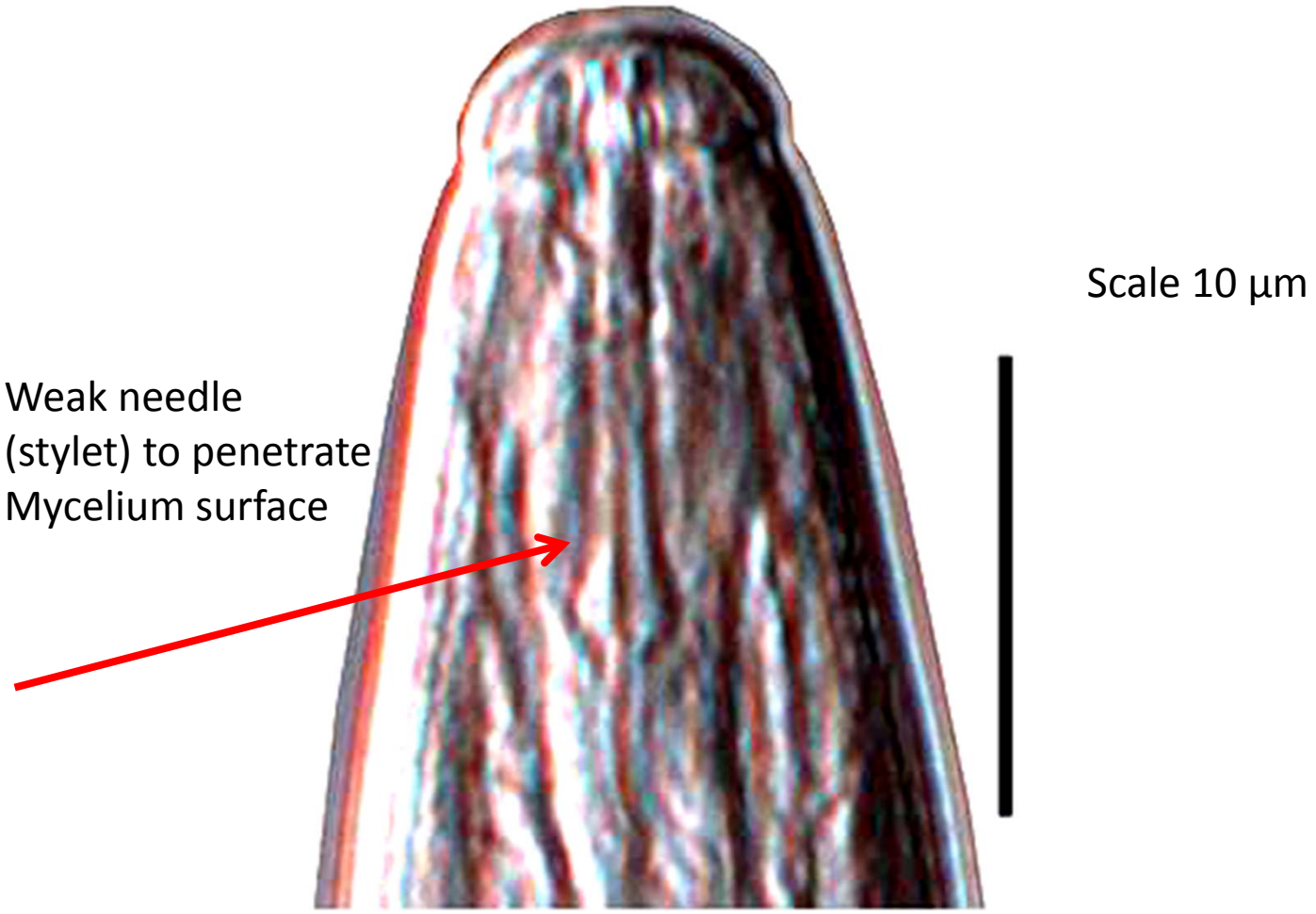
- *Different trophism = >> Different structure of stoma!!*

Stoma of BACTERIOTROPHIC NEMATODE: : funnel-like, with biting cuticular lips (photo)

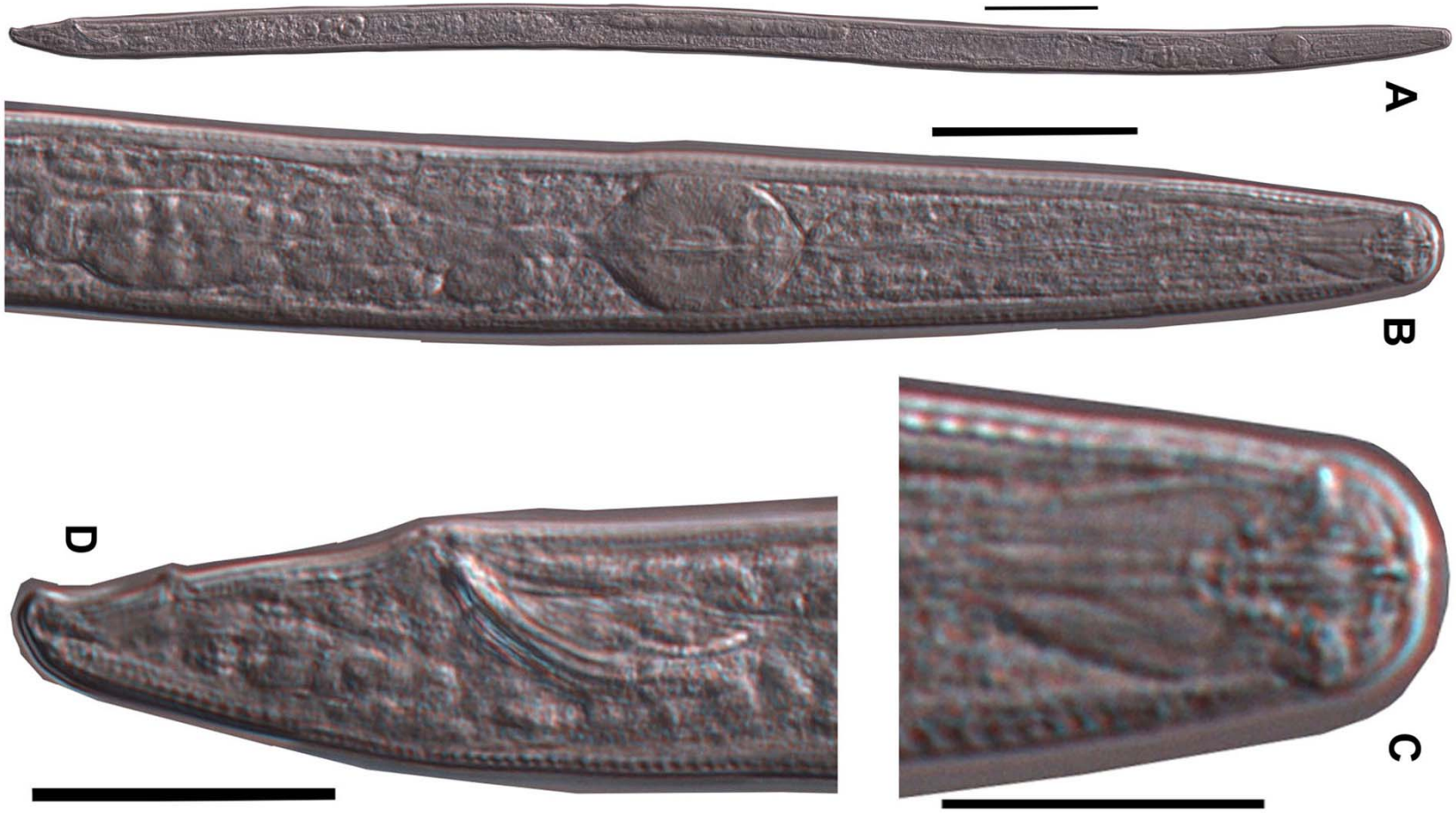
Scale 10 µm

BACTERIOTROPHIC, with tubular stoma and a crone of sticky lip appendages

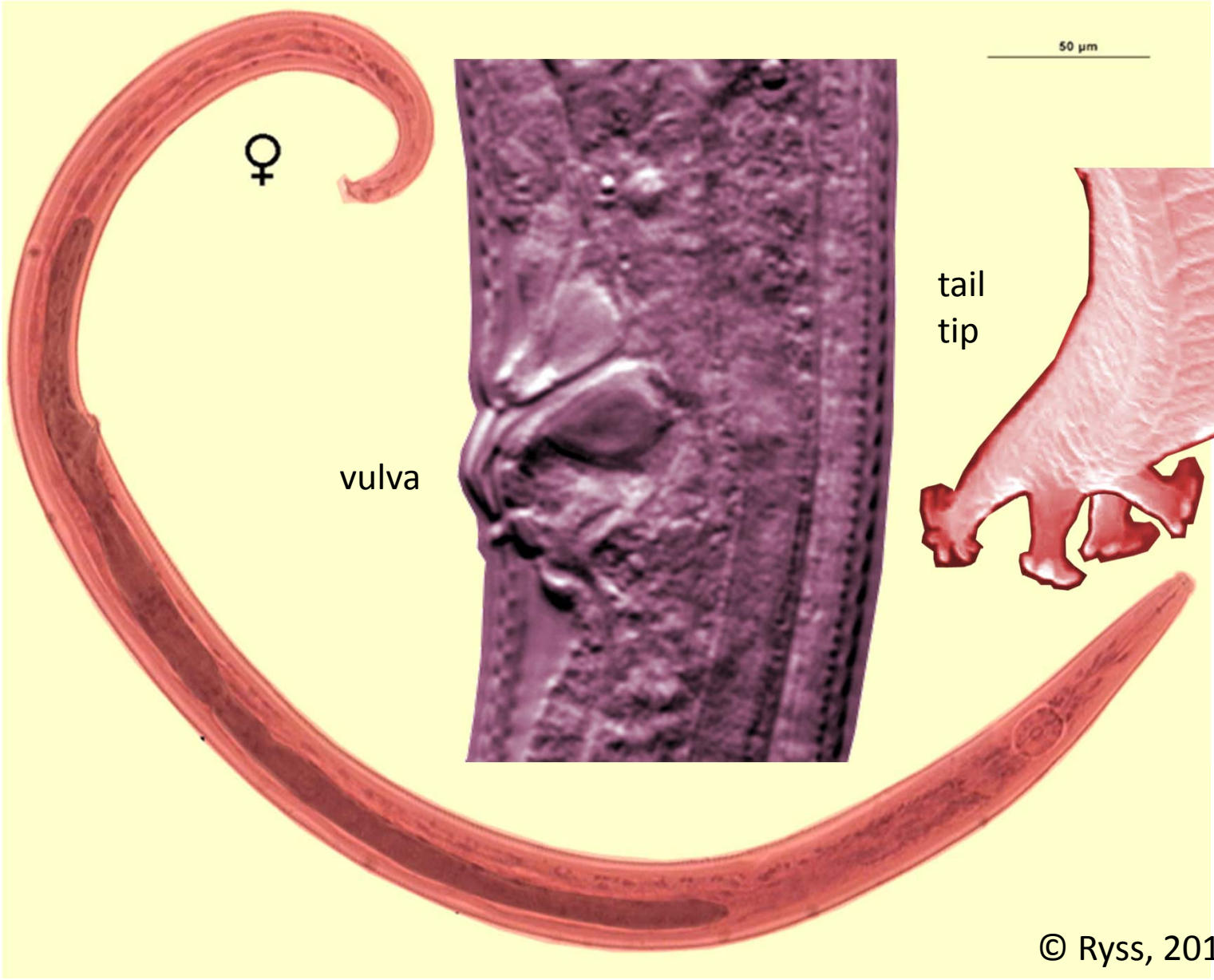
Fungivore nematode



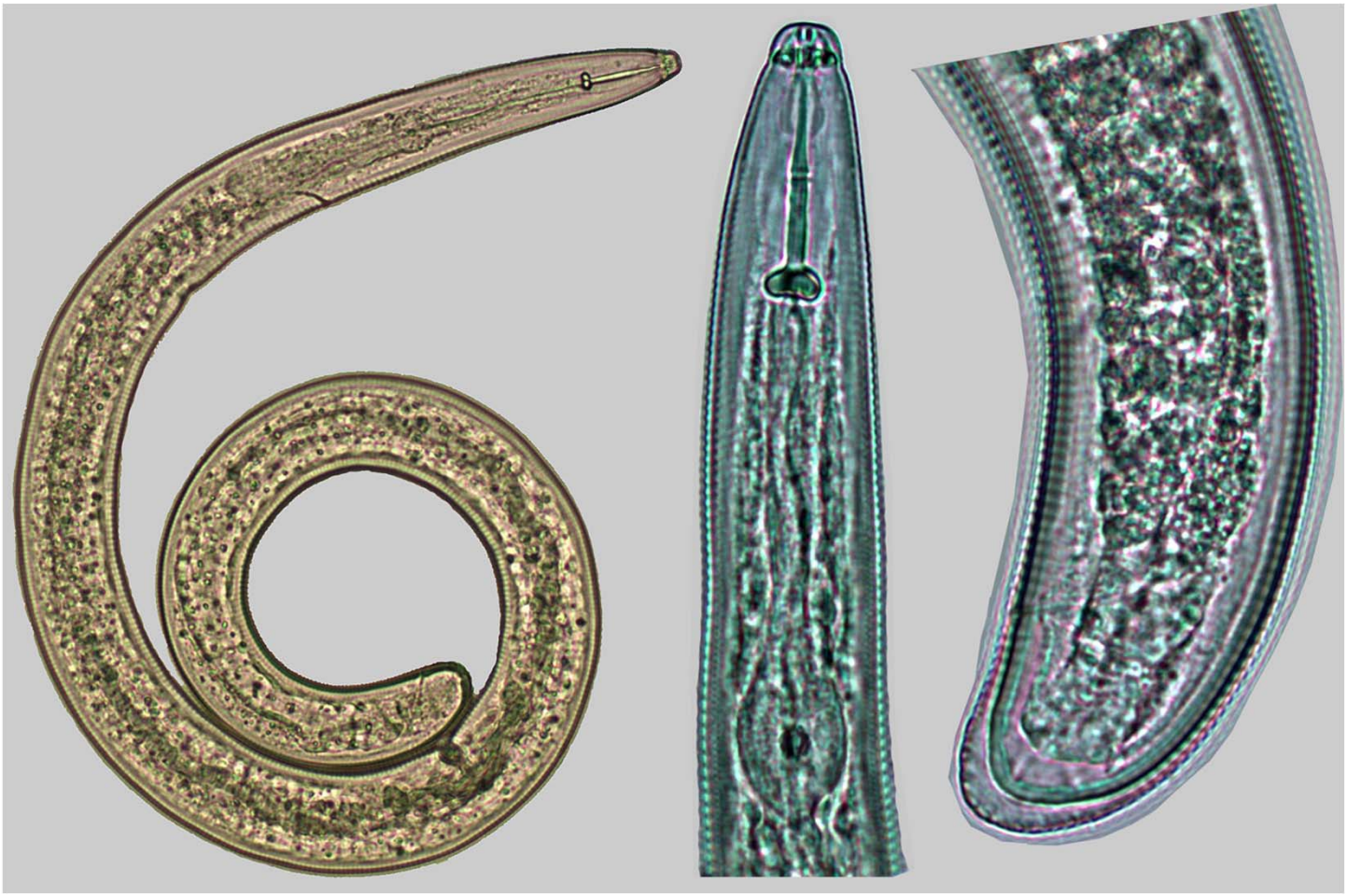
Fungivore : *Paraphelenchus* MALE



Fungivore: *Laimaphelenchus.* FEMALE



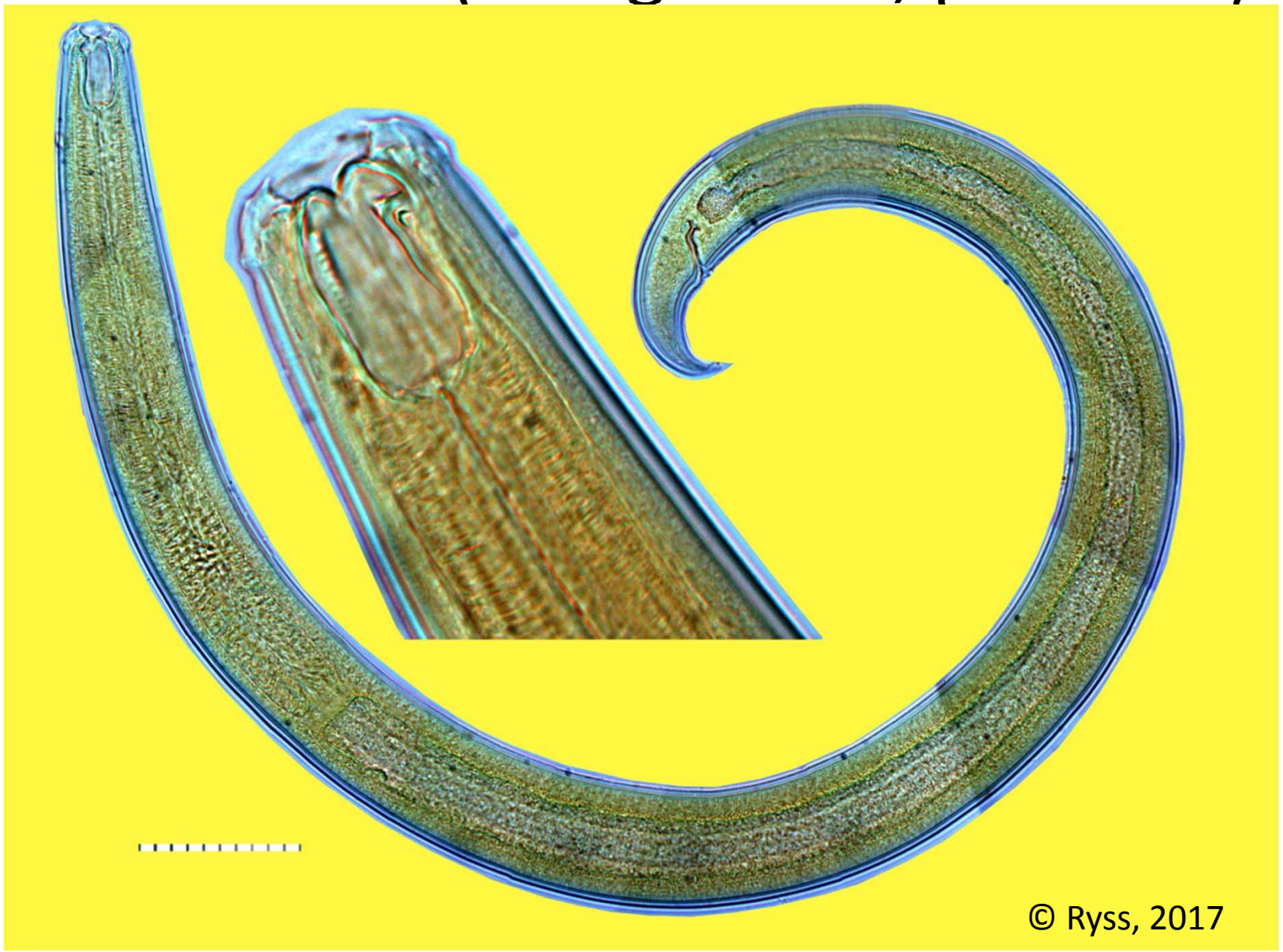
Plant parasite: *Rotylenchus*



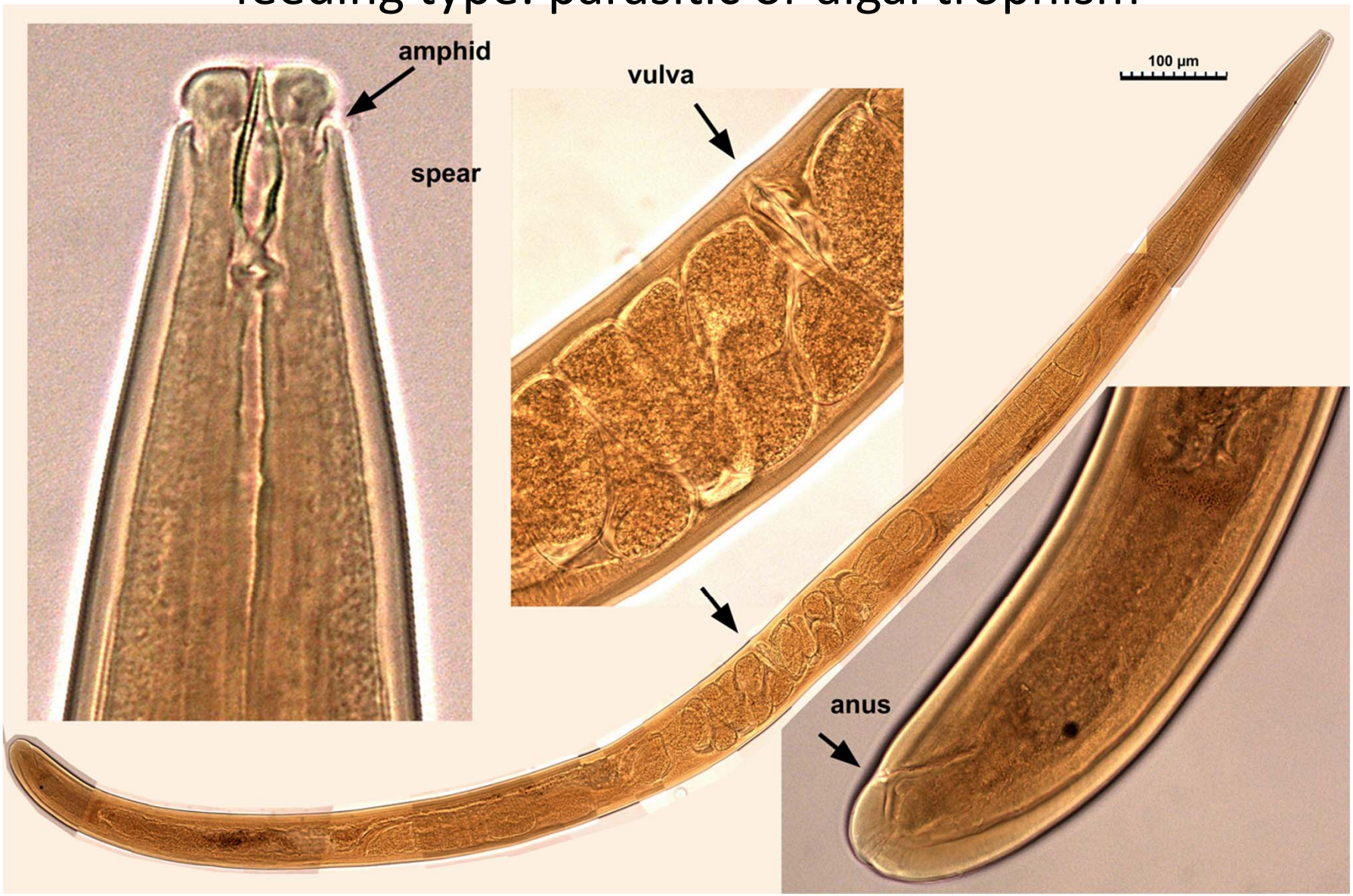
PLANT-PARASITIC NEMATODE WITH STRONG STYLET

Ryss, 2003

Anatonchus (biting stoma, predator)



Aporcelaimus: omnivore comb. predation and other feeding type: parasitic or algal trophism

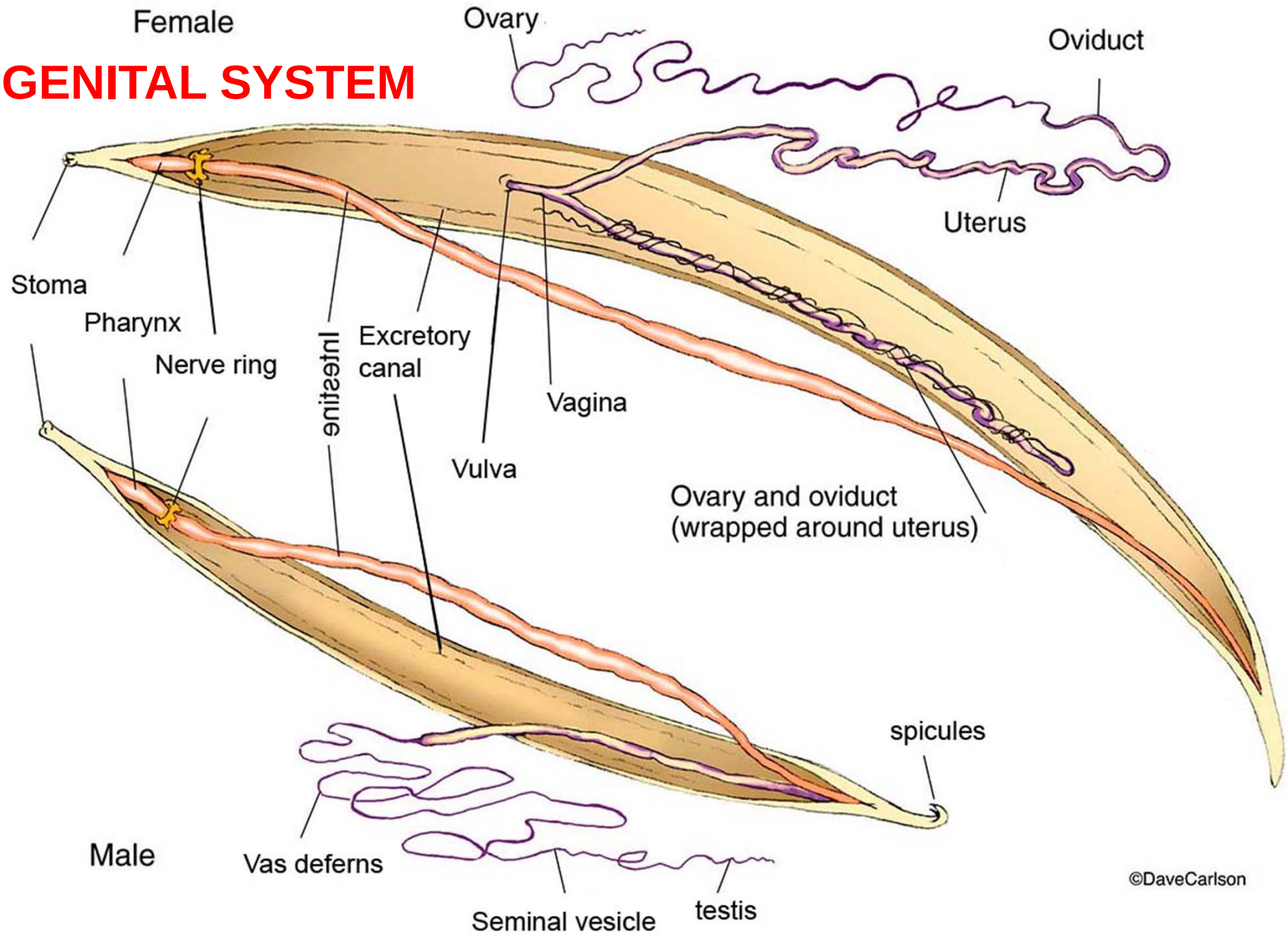


Nematodes are AMPHIMICTIC animals.

Male has hook-like tail with cuticular copulatory SPICULES.

Females are usually longer than males.

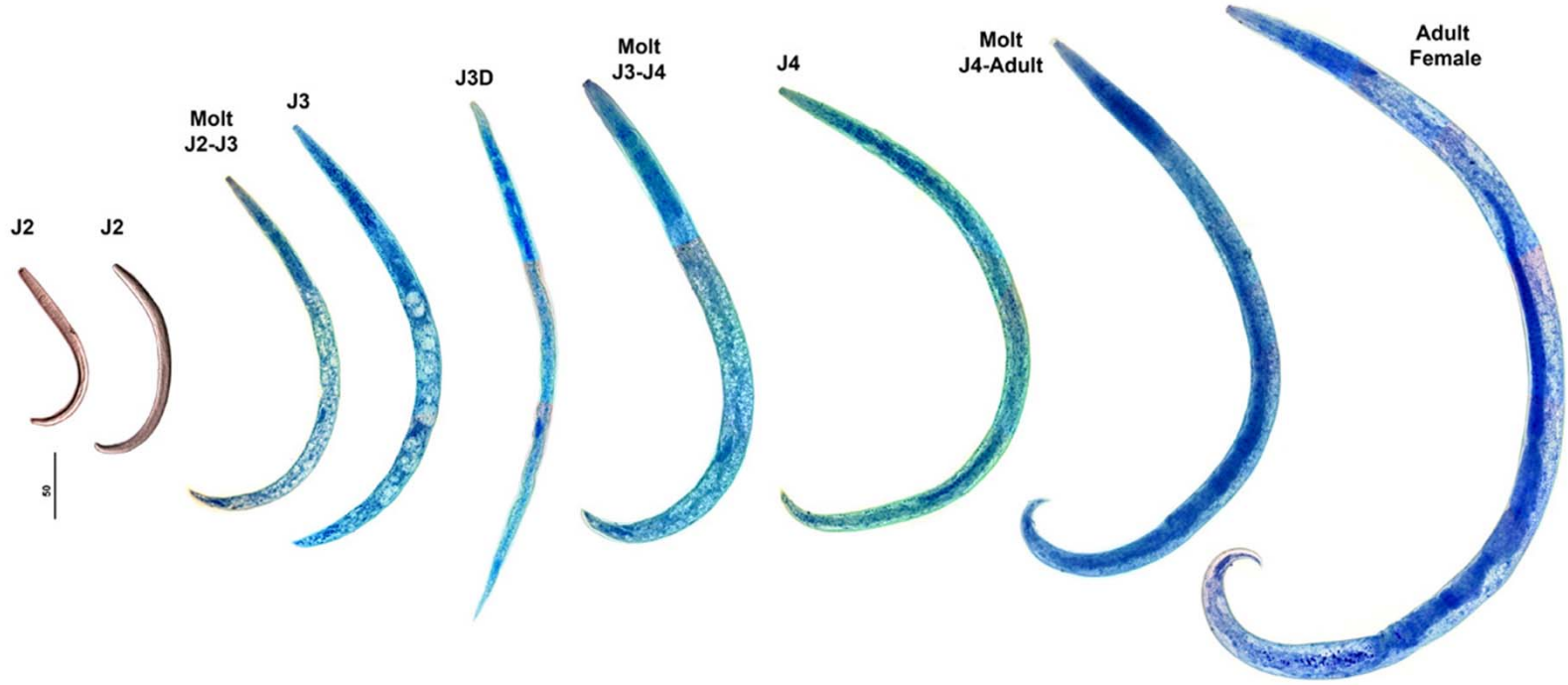
GENITAL SYSTEM



DEVELOPMENT: first molt in egg shell

Bursaphelenchus crenati Ryss & Polyanina 2018 © Journal of Nematology

Four molts, five stages of development



Bursaphelenchus crenati Ryss & Polyanina 2018 © Journal of Nematology

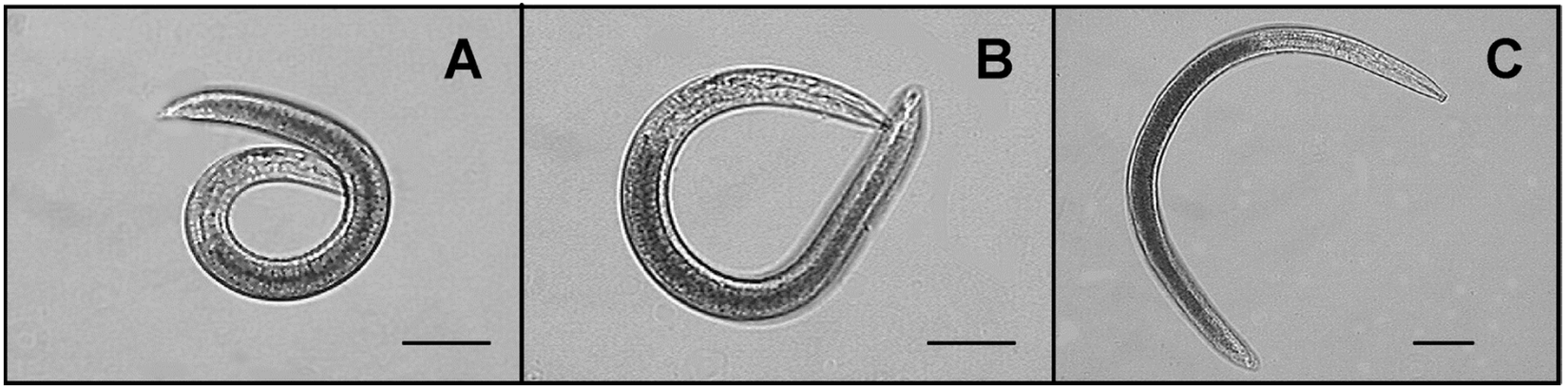
- **Stress resistance:**
- **dormant stages and survival**

SURVIVAL TYPES

- **QUIESCENCE*:*** (ОЦЕПЕНЕНИЕ) – a **response to rapid change** of environment, e.g. **sudden** freezing
- **DIAPAUSE:** obligatory (seasonal) **dormancy stage in the normal life cycle** stimulated by the specific stimuli, e.g. a cyst stage of cyst-forming nematode
- **DAUER**– a reserve **transmissive** stage in **ALTERNATIVE GENERATION** in a life-cycle as a response to specific stimuli from a host

Time series micrograph of uncoiling nematode.

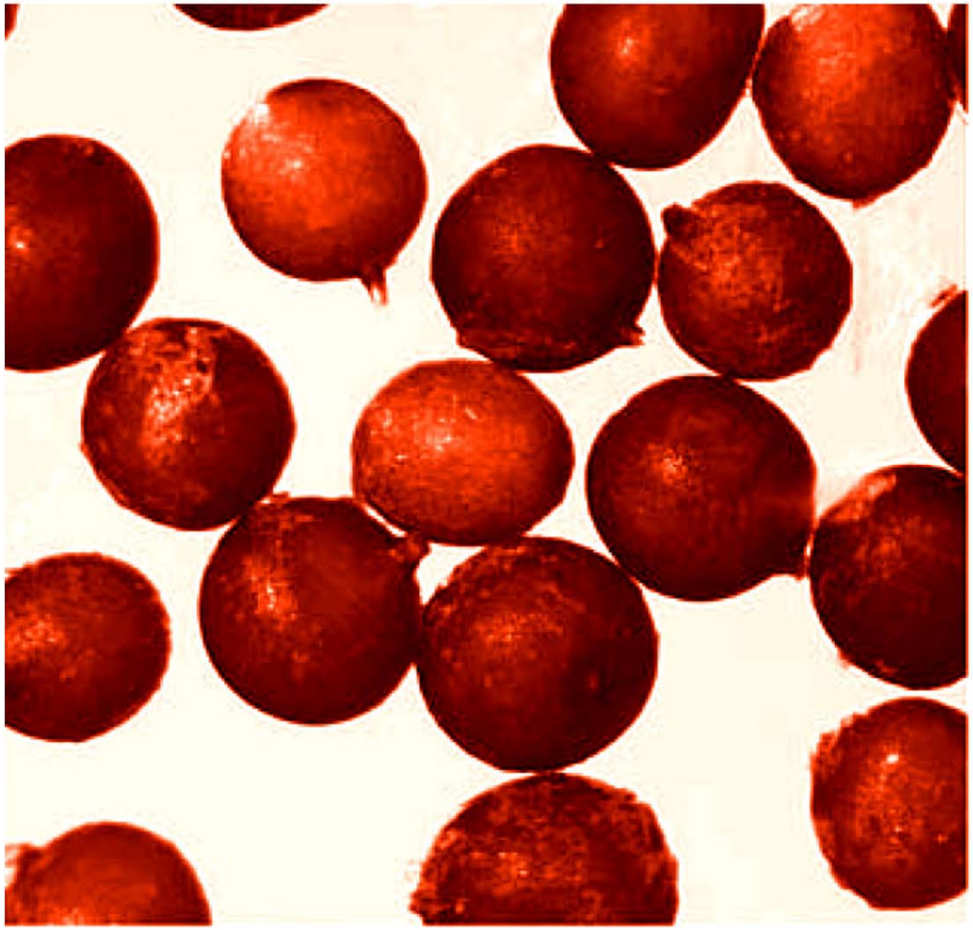
A reverse from coiling quiescence (A) to active moving stage (C)? “uncoiling response”

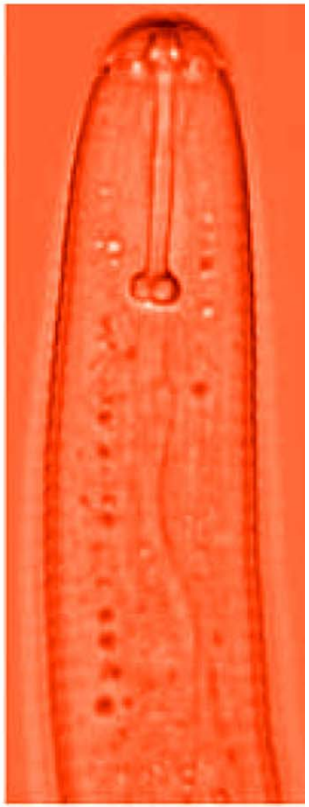


Quiescence is reversible! After watering nematodes wake up to active moving

Treonis A M , and Wall D H Integr. Comp. Biol. 2005;45:741-750

DIAPAUSE in cyst forming potato nematodes (*Globodera rostochiensis*)
Cyst is the mature dead female with 150-200 eggs inside; each egg contain an alive infective juvenile

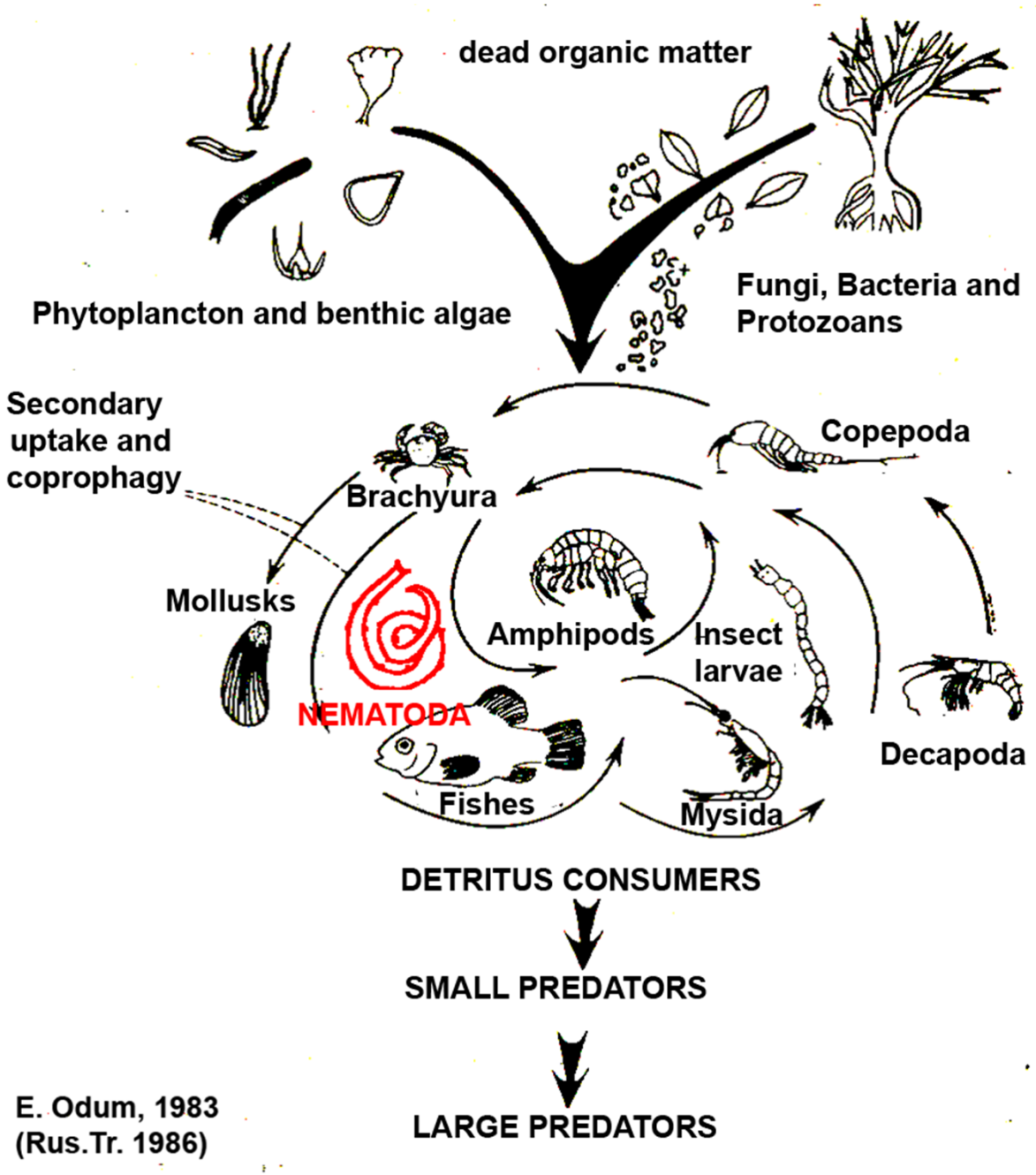




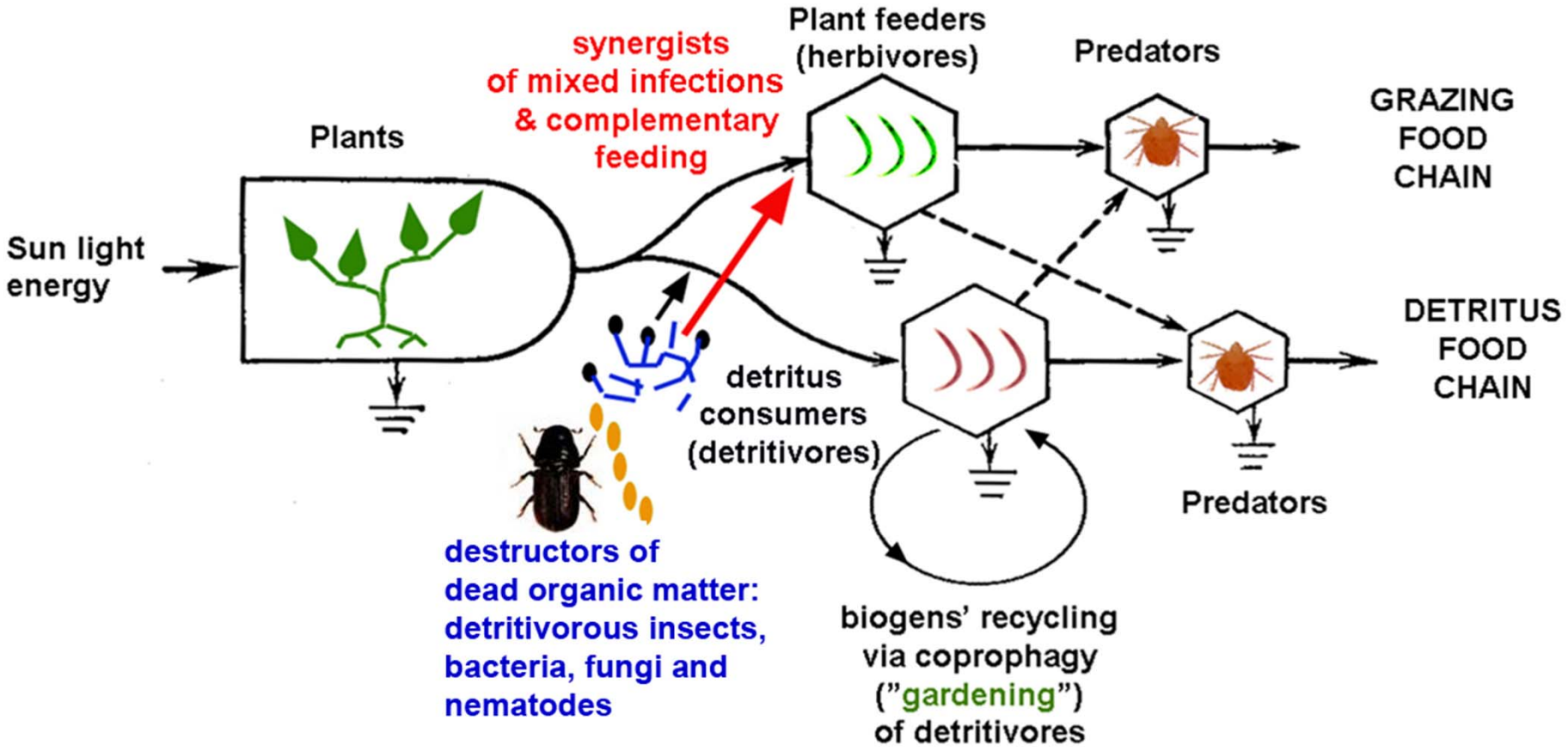
Turner & Subbotin, 2013

Nematodes in Ecosystem

The position of the free-living nematodes in the food web (from E. Odum)



Mycotrophic nematodes – a part of multi-pathogens' association



Odum, 1986: modified

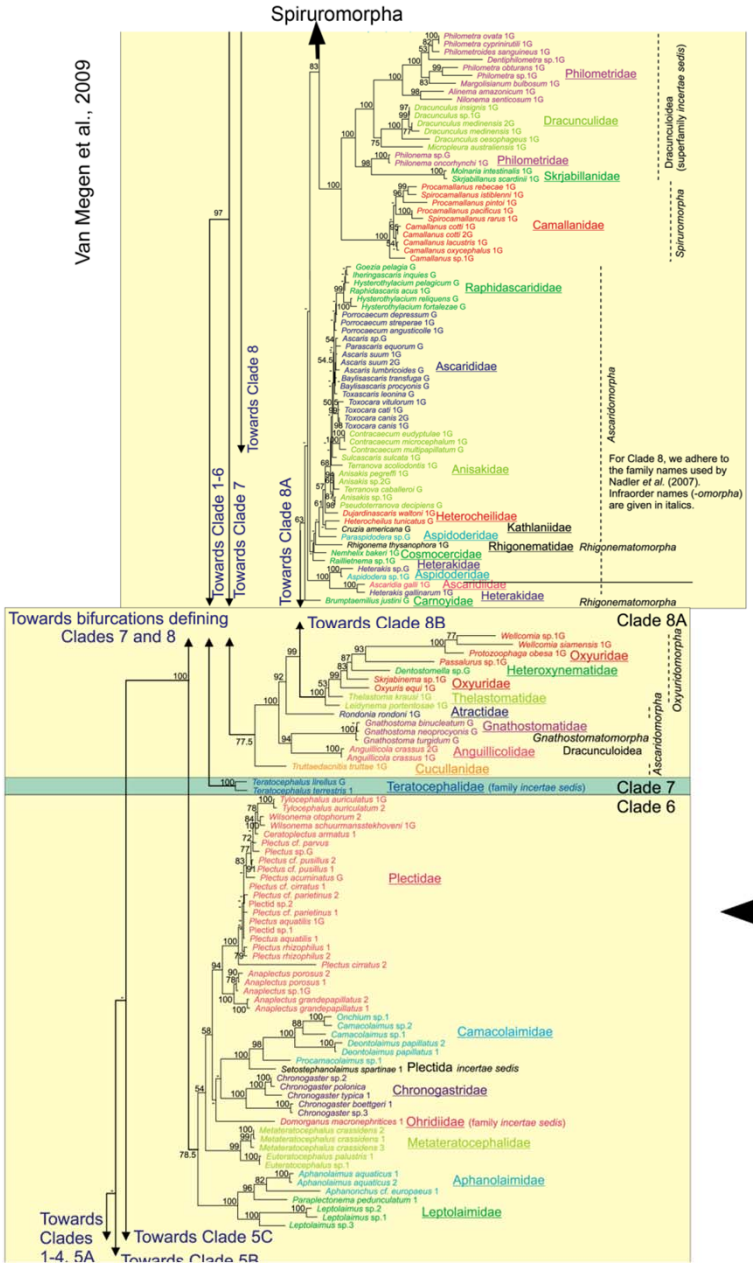
Historically, **nematodes, bacteria** & **fungi** are partners in processes of bio-utilization, i.e. they are actors of the **DETRITUS FOOD CHAIN**

Decomposition processes for dead plants and animal cadavers are different

- In plant decomposition the basic compound is **cellulose** and similar chemicals (the main destructors are **fungi**!)
- For animal cadavers the basic compounds are **proteins** (processed by **bacteria**).

- From the combined Molecular and Morphotaxonomic Phylogram, the helminths ancestors are taxa - colonizers of the dead organic matter substrats :
- **Fungivores for plant parasitic nematodes,**
- **Bacteriophages for the parasites of animals**.

- Fungivores – are members of microbiotic communities which decompose the dead matter with the high content of celluloses which are processed by fungi



CLADES 6,7,8

Advanced taxa are Parasites of vertebrates and Insects (Ascarididae, Anisakidae, Rhigonematidae)

Basal Taxa: Plectidae and Teratocephalidae, the bacterivores

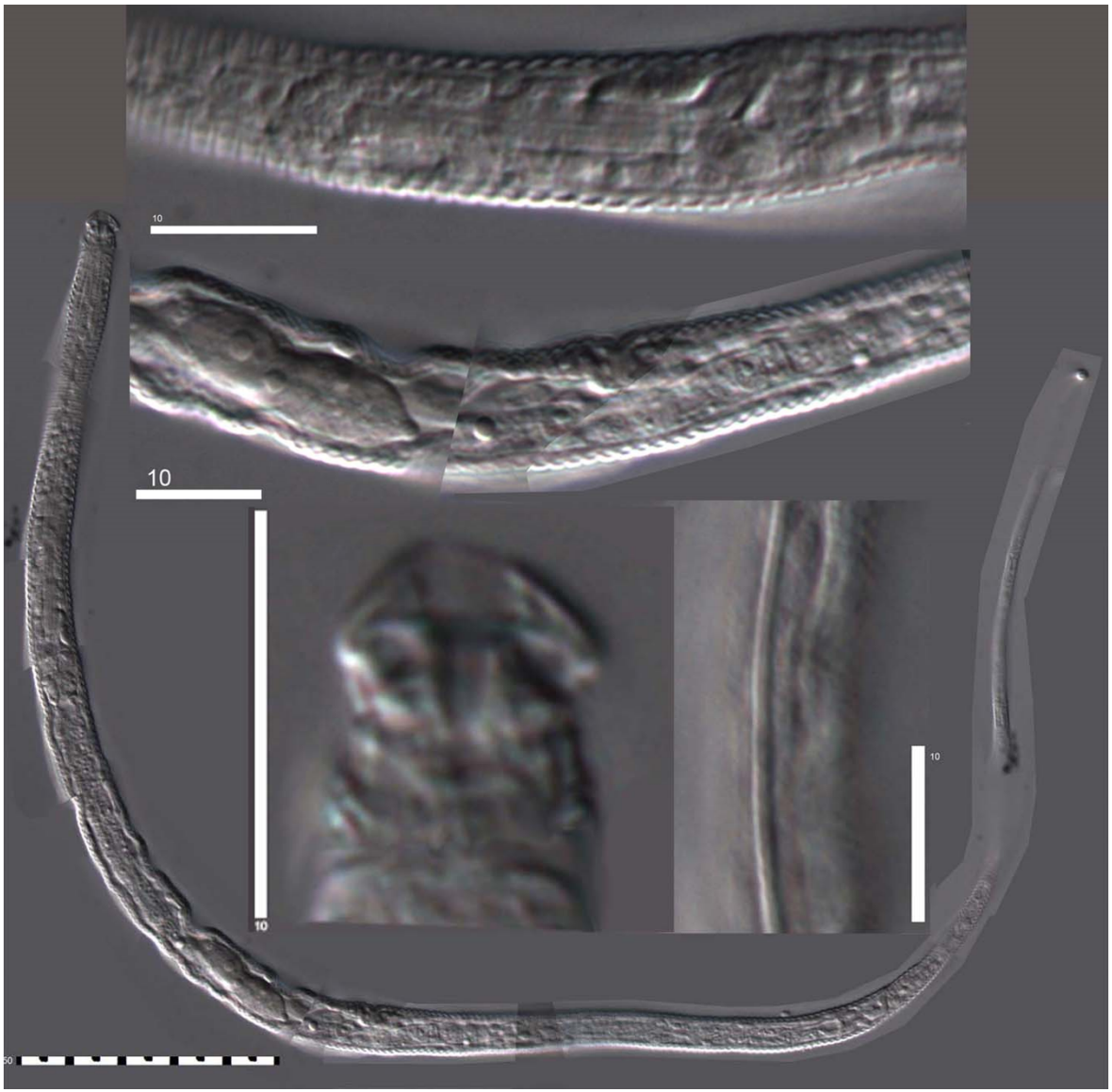
Origin of ascaridids and anisakids from bacterivorous nematodes

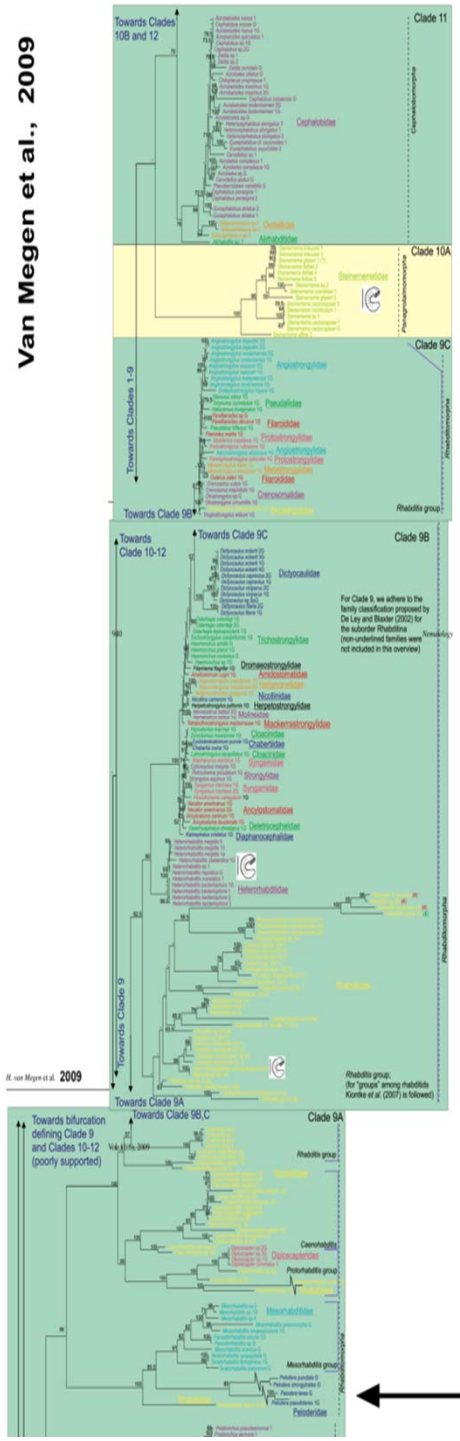
Plectus elongatus

Bacterivorous nematode from basal Clade

Teratocephalus terrestris

Bacterivorous nematode from basal Clade





Clade 10
Advanced taxa
Insect Parasites

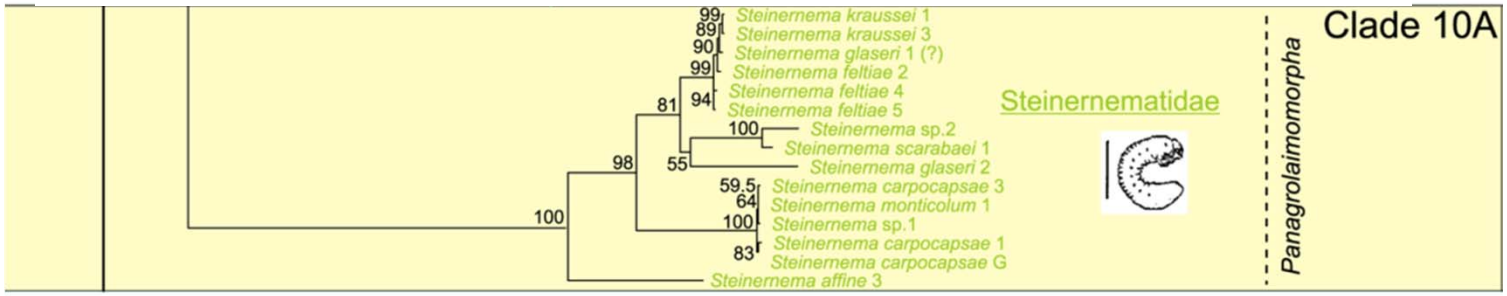
Clade 9
Advanced taxa
Insect Parasites

Clade 9
Basal taxa are
Bacterivores

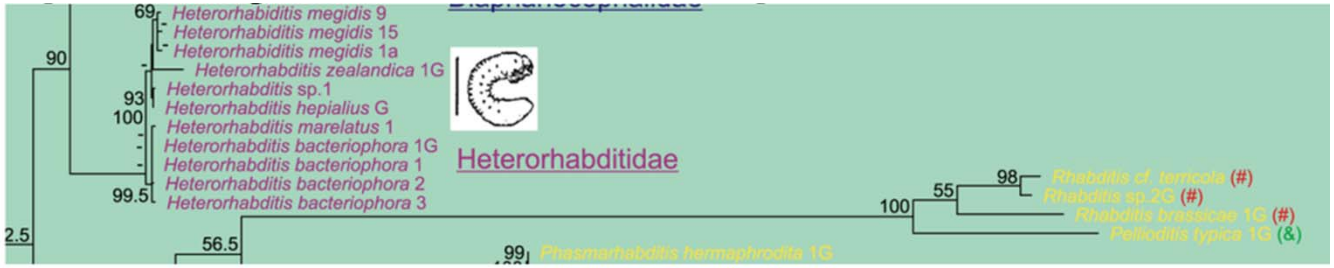
Origin of the insect parasites from bacterivorous nematodes

Macro Clades 9 and 10

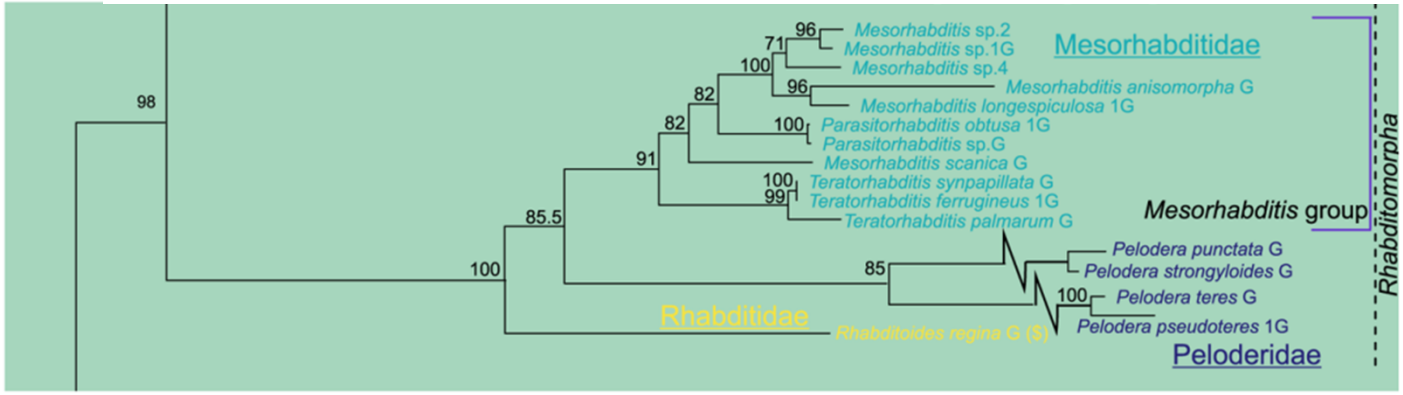
Advanced taxa: Parasites of Insects



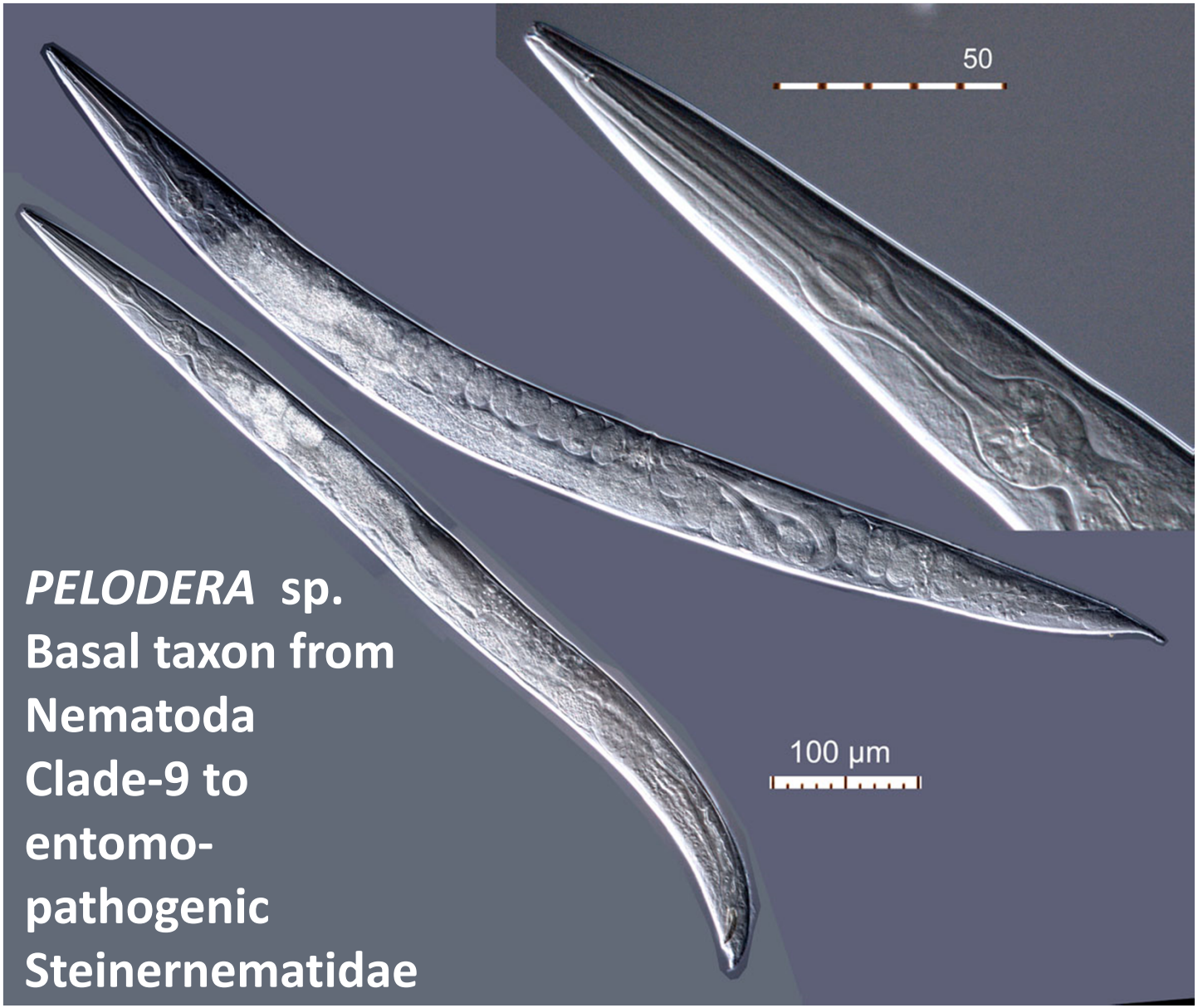
Advanced taxa: Parasites of Insects



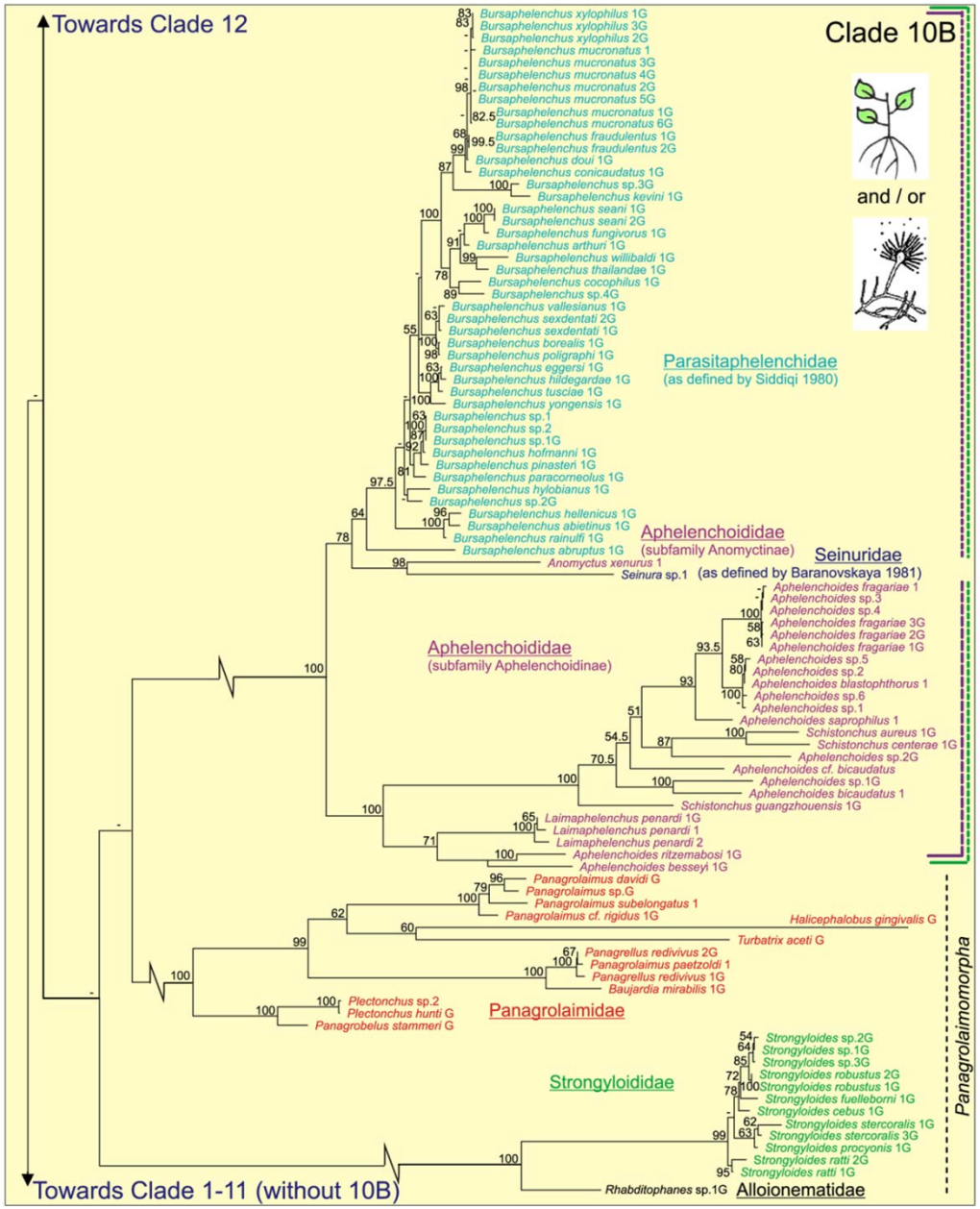
Basal taxa: Bacterivorous nematodes



Fragments from Van Megen et al., 2009

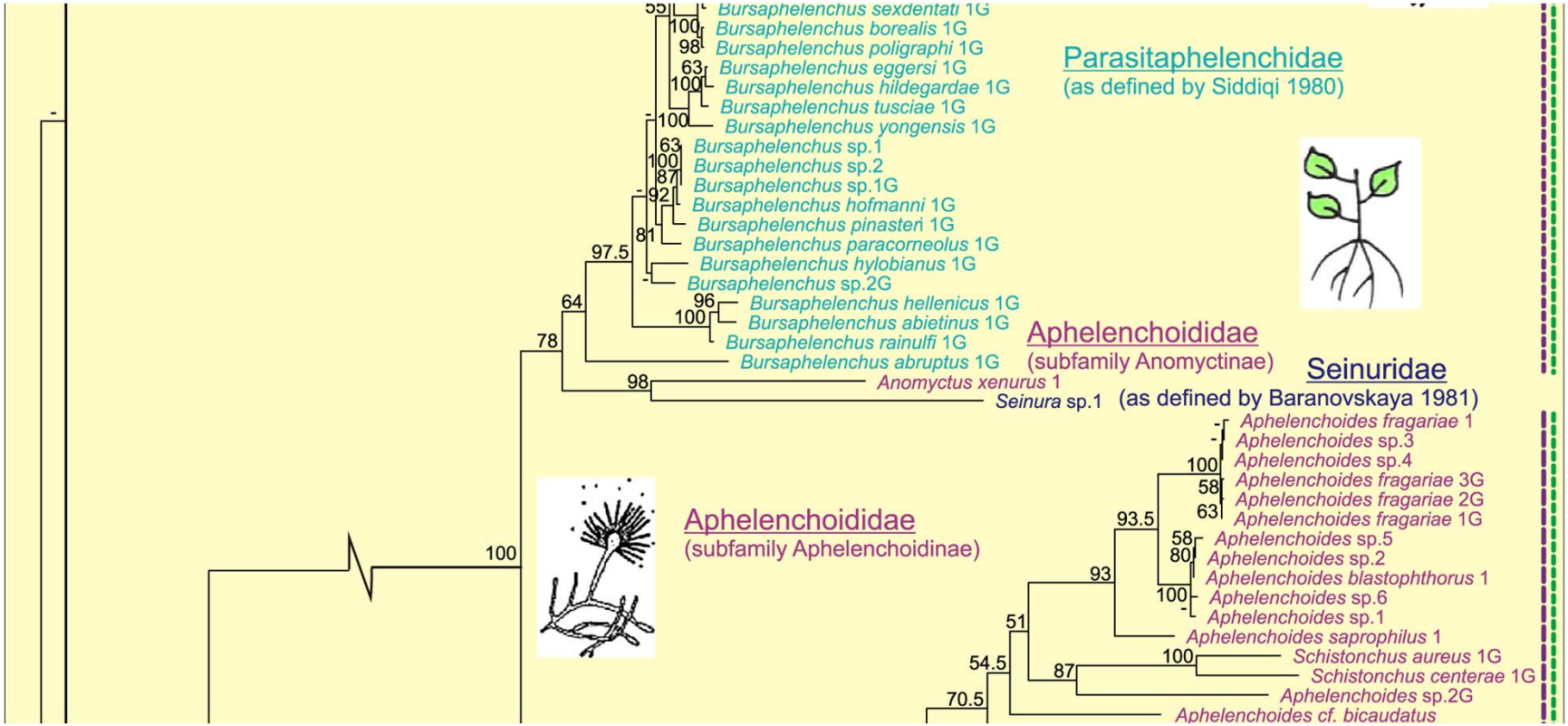


PELODERA **sp.**
Basal taxon from Nematoda Clade-9 to entomo-pathogenic Steinernematidae



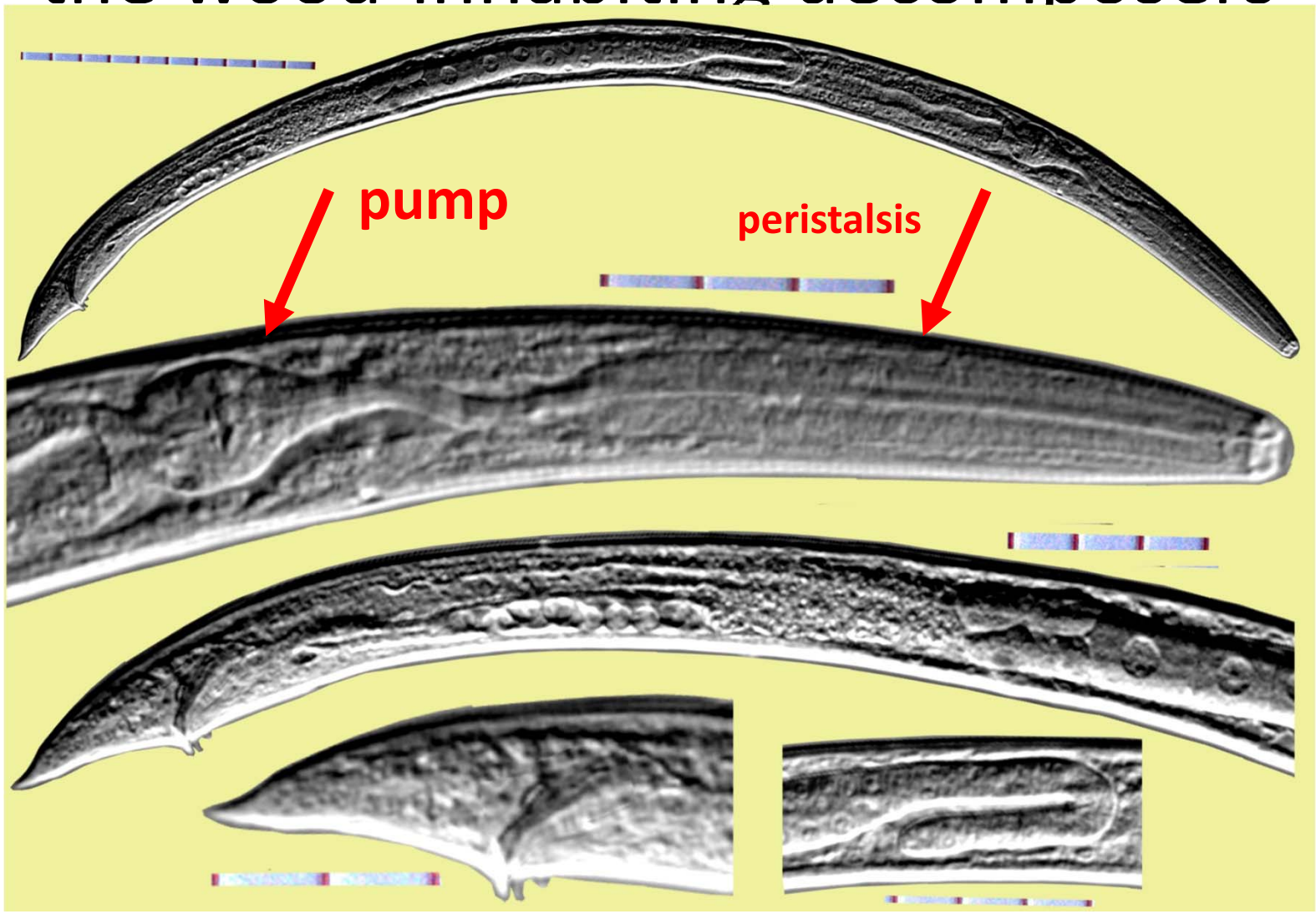
Cladoram from
Van Megen e.a., 2009

MacroClade 12,
basal Taxa are mycophages,
Advanced Taxa –
plant parasites combining
parasitic feeding on
fungal mycelium and
live plant cells of
dying woody
plants



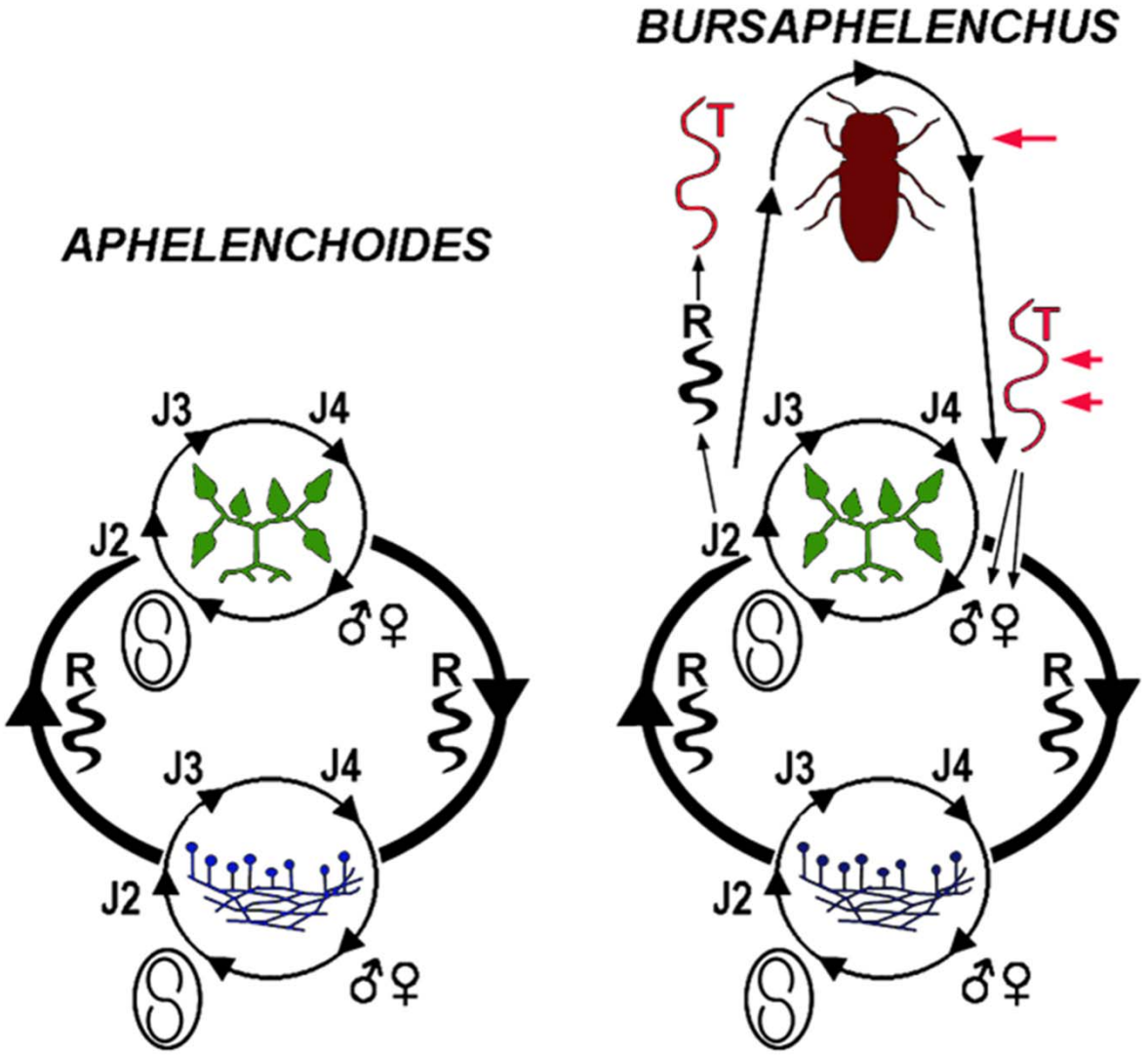
Clade 12 from Van Megen et al., 2009
Splitting of clades of plant parasites and mycophages (look at icons)

Basal clade taxon *Panagrolaimus* for the wood-inhabiting decomposers



LIFE CYCLES OF WOOD-DECOMPOSER NEMATODES WITH INSECT VECTOR (BURSAPHELENCHUS)

JJ –juveniles
R – resistant (DIAPAUSE) juveniles



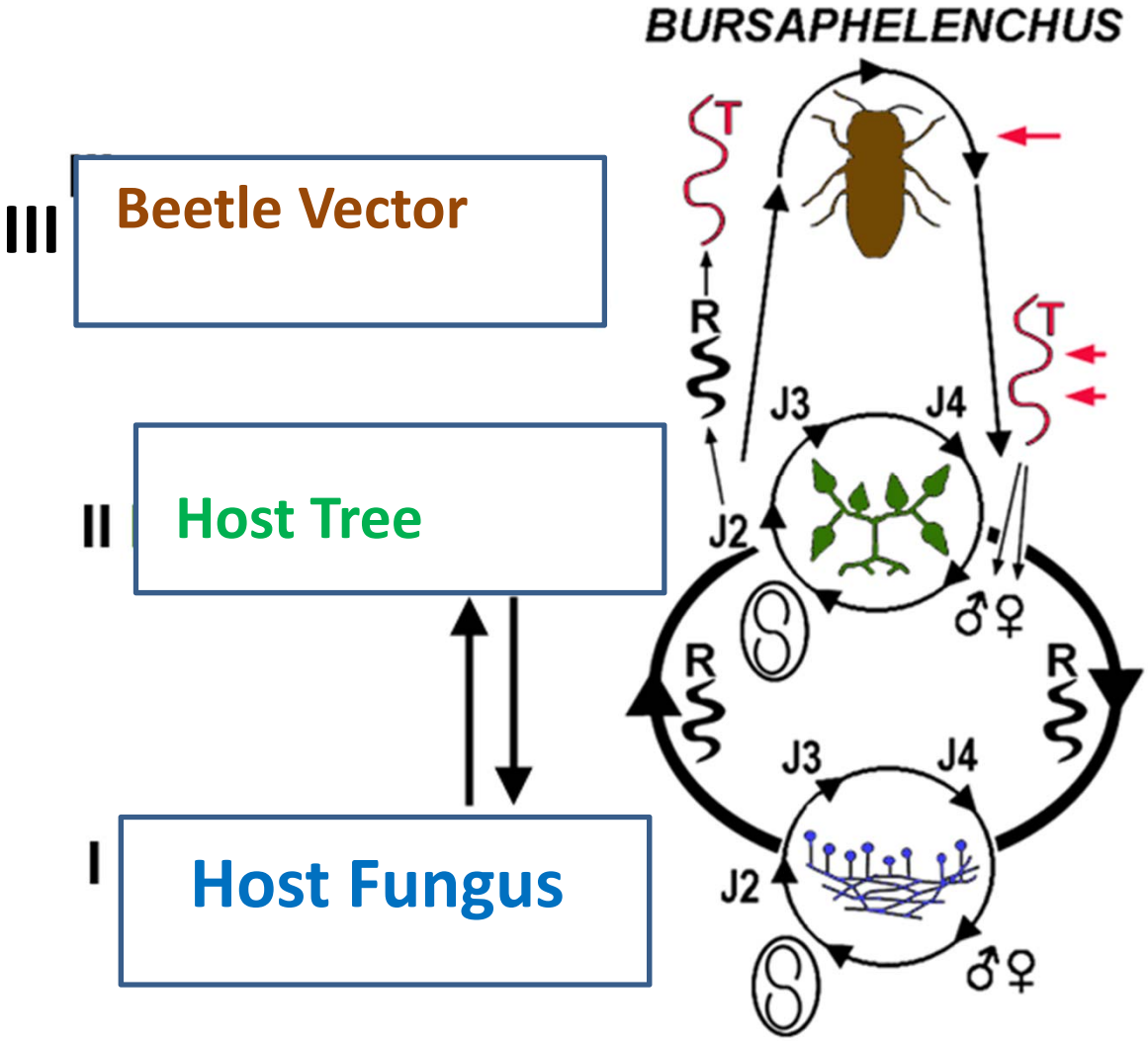
JJ –juveniles
T – DAUERS = transmissive juveniles

Life cycle without insect vector

Life cycle with insect vectoring DAUERS (T)

Ryss, 2009

Hosts in the Life cycle of wood- inhabiting nematodes



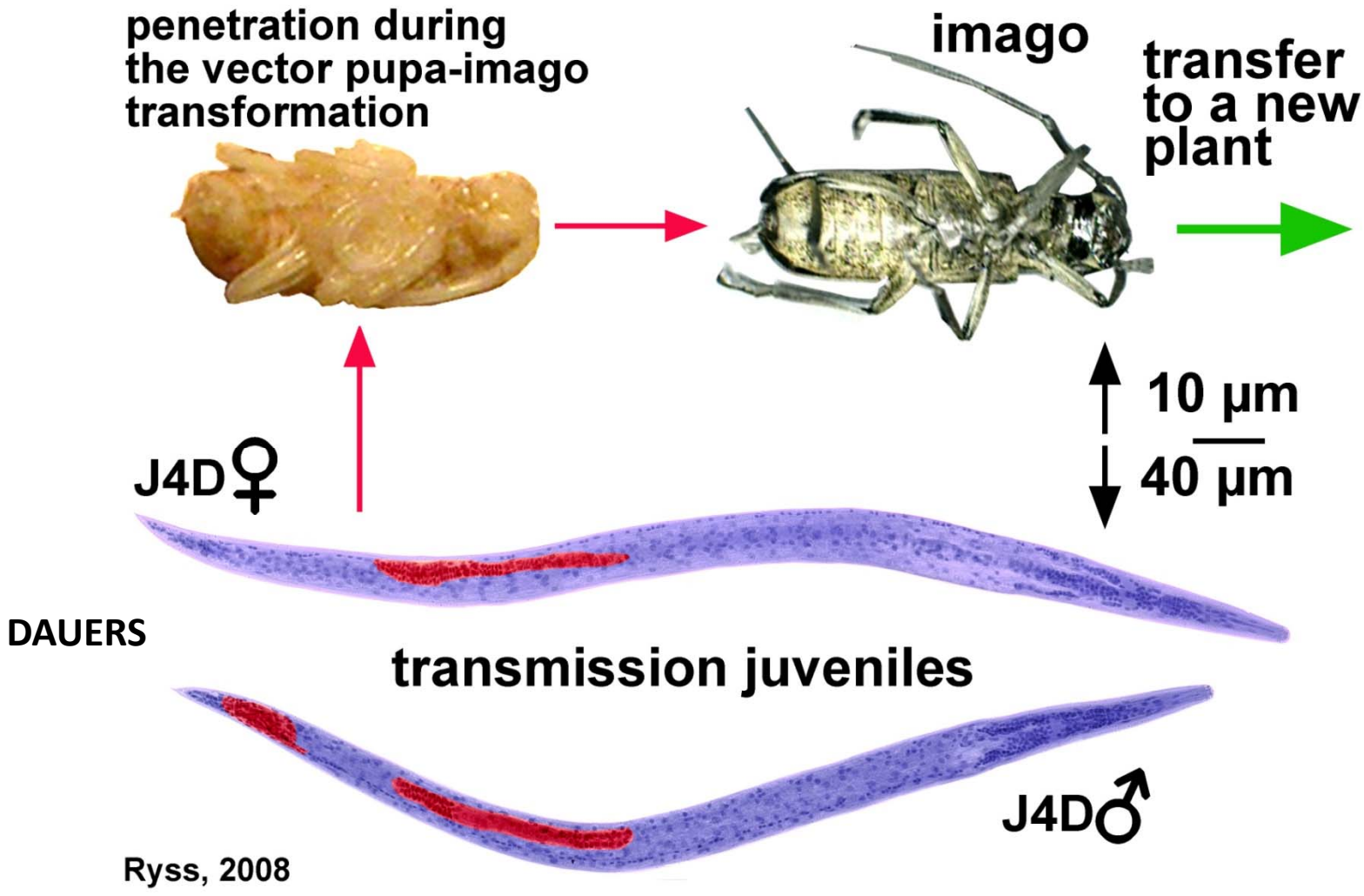
JJ –Juveniles;
R – resistant Juveniles;
T –DAUERS (= transmissive juveniles).

Ryss, 2009

Dauer larvae of free living nematodes

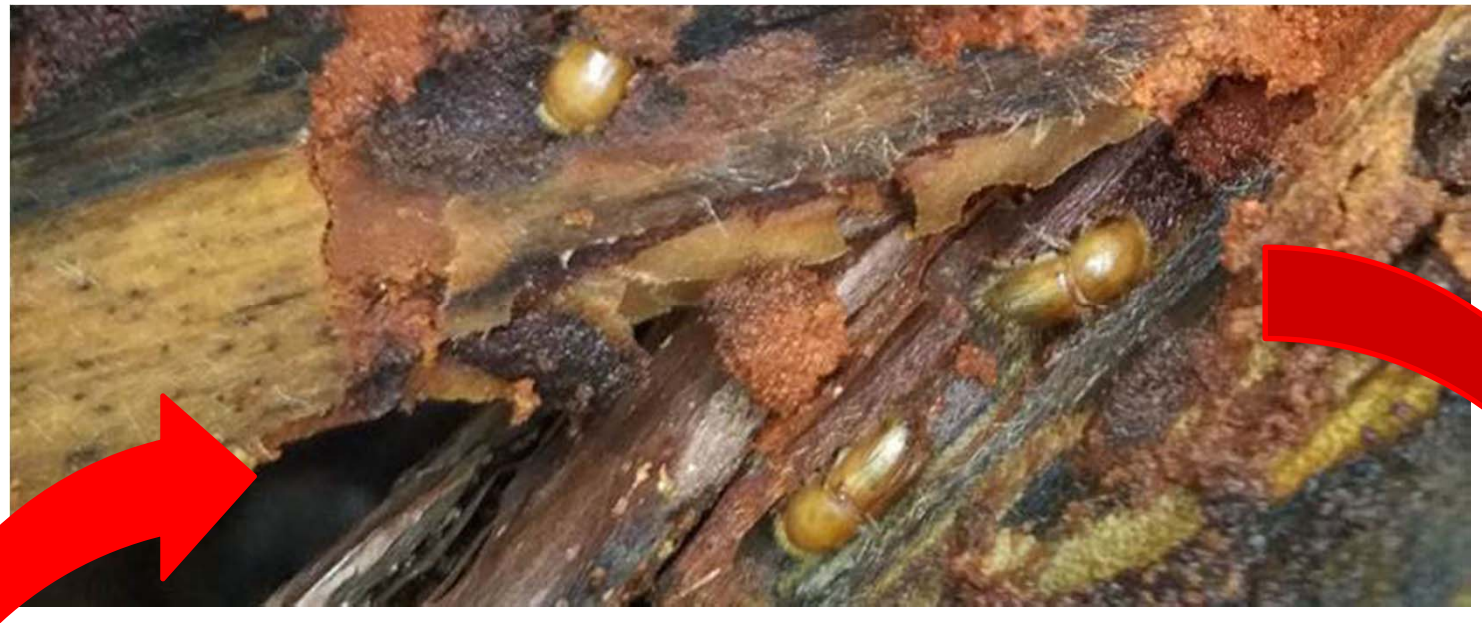
- Both bacterivorous and mycofagous nematodes have the juveniles of long term diapause (“DAUER larvae”); as an adaptation to the start stages of succession in the detritus food web.

Dauers in the life cycle of wood parasitic *Bursaphelenchus*

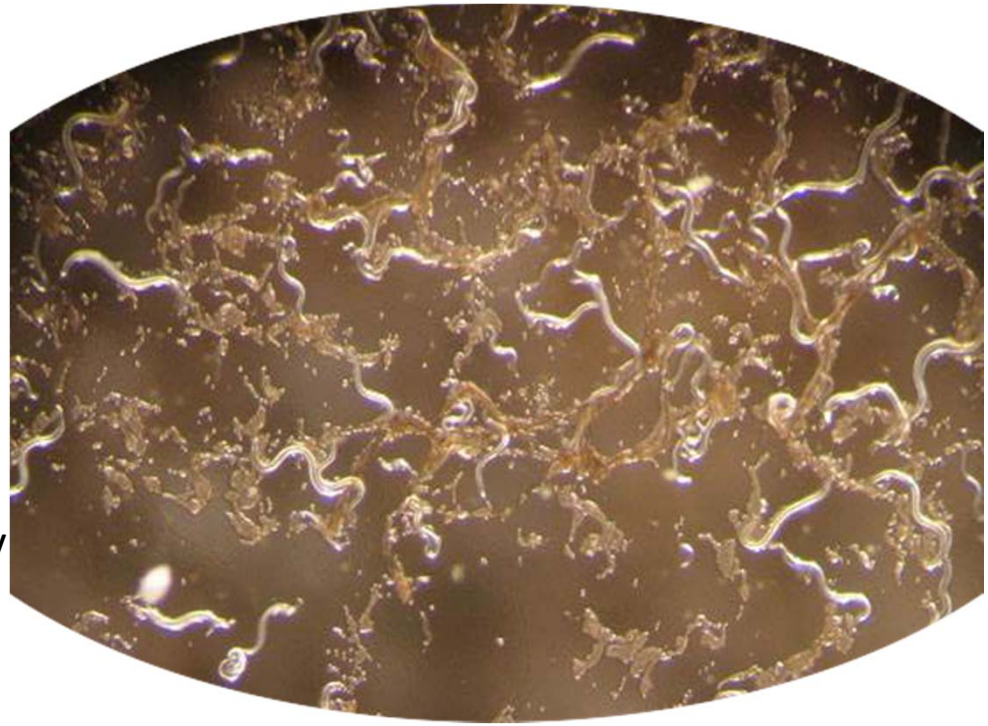


Why nematodes are so important in the DeadWood succession?

- Detritus food chain – **repeated cycling of the dead matter through the intestine** of the detritivorous arthropod or annelid with enrichment of bacterial and fungi communities – nematodes help to infiltrate the porous substrate with bacteria and fungi acting as the "grower of the bacterial garden" ("*Mikrobiologische Gärtnerei"*).

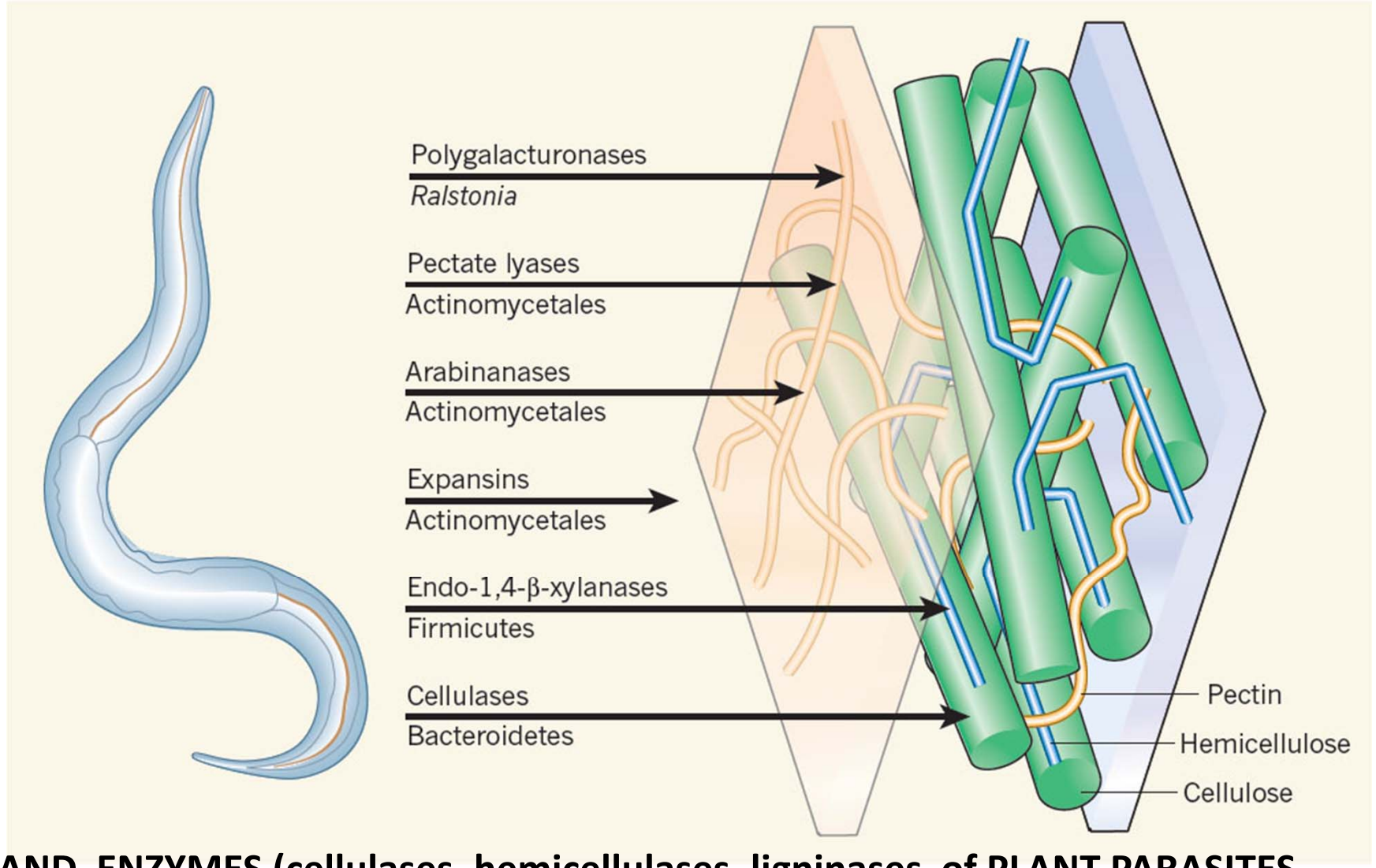


Bark beetles in decaying deadwood



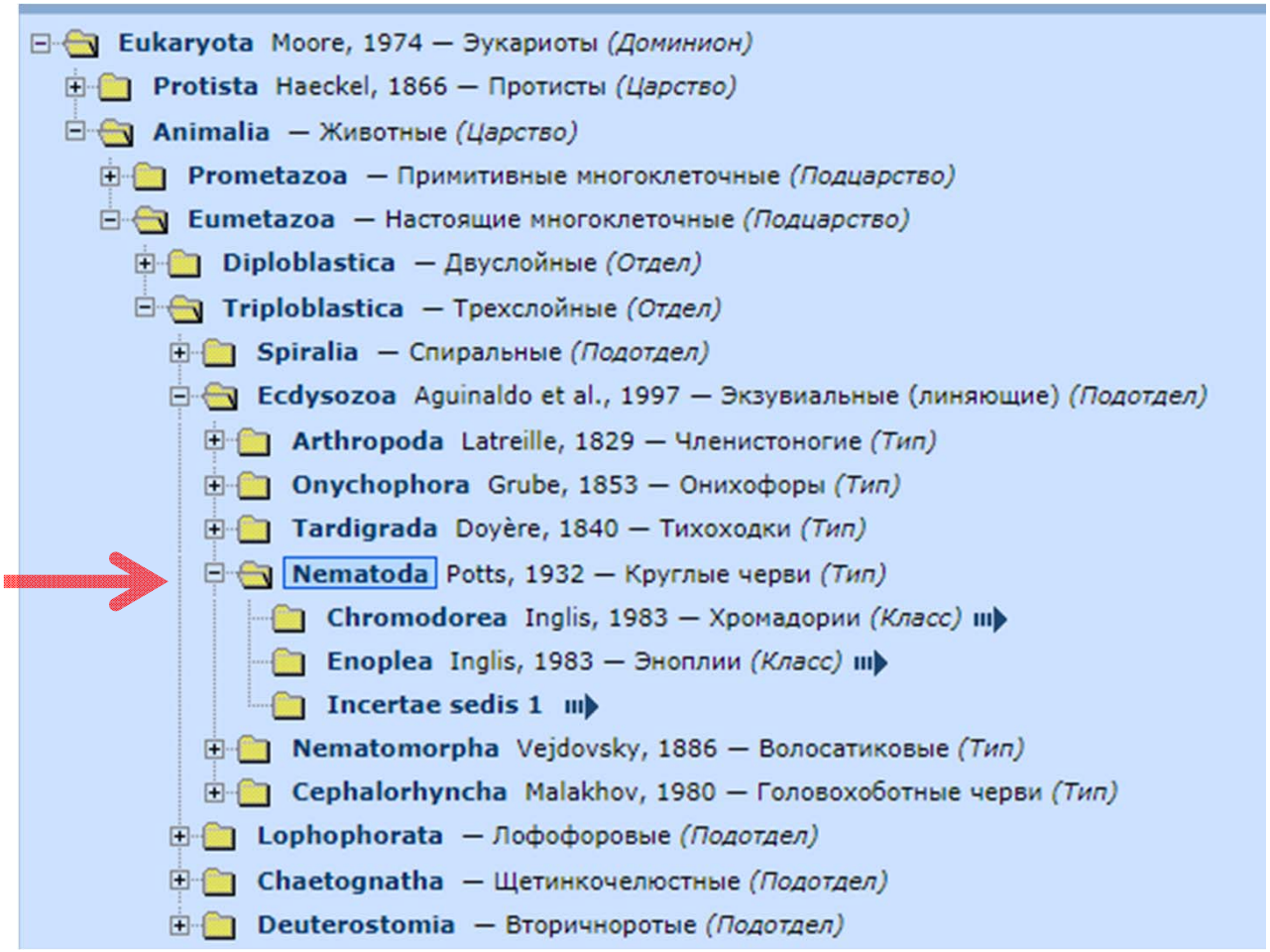
Nematodes and bacteria in beetles' faeces In decaying sapwood

After fermentation passed through beetle intestine and enrichment with bacterial colonies, the decaying matter is ingested repeatedly in the cycle of decomposition



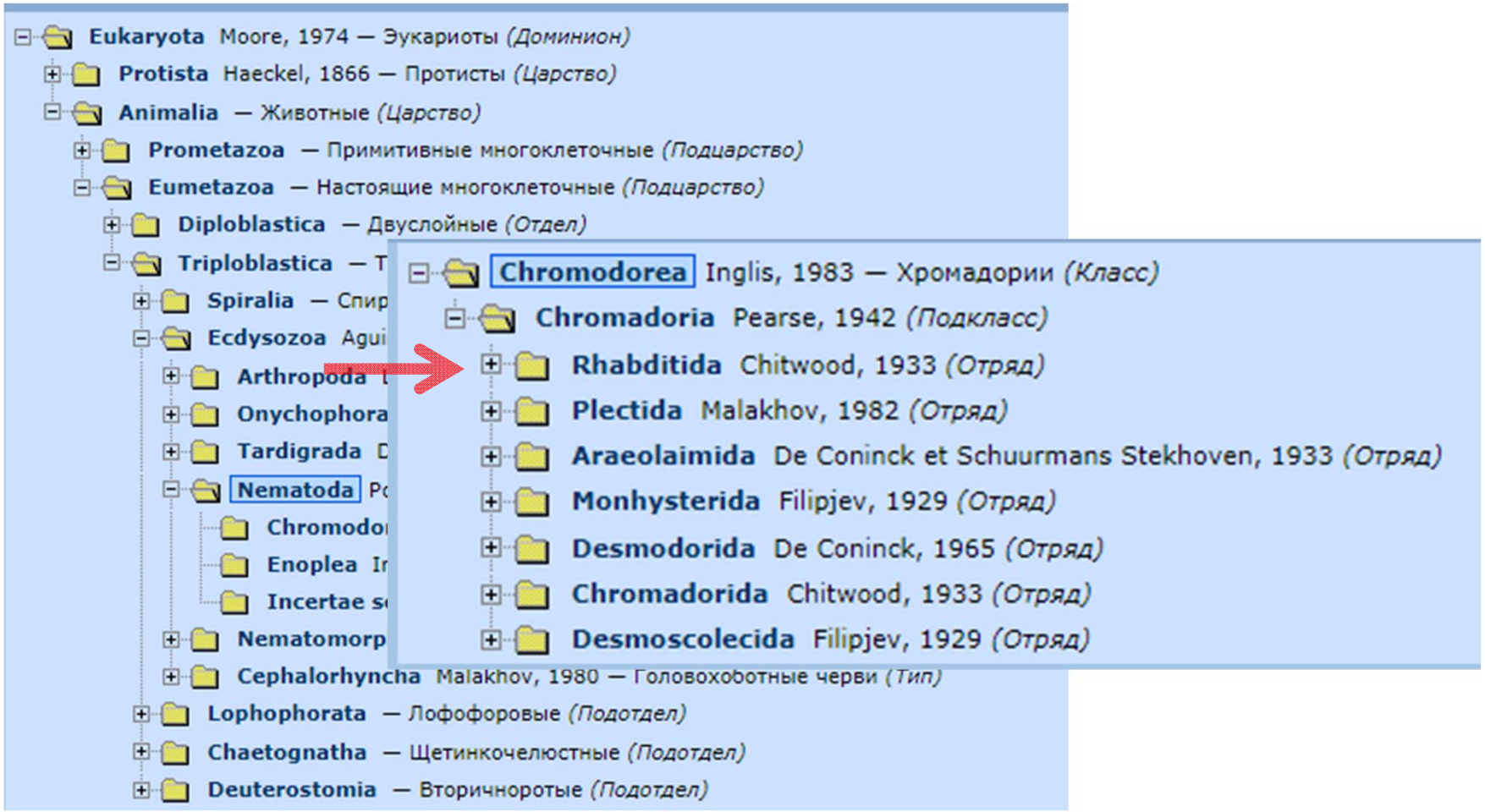
GLAND ENZYMES (cellulases, hemicellulases, ligninases of PLANT PARASITES (*Meloidogyne*) are controlled by genes inherited from ancestral bacteria (during LGT< lateral gene transfer) (Whiteman, Gloss, 2010; Danchin et al., 2010; Vieira et al., 2011).

Modern CLASSIFICATION: De Ley & Blaxter, 2002 with later emendations

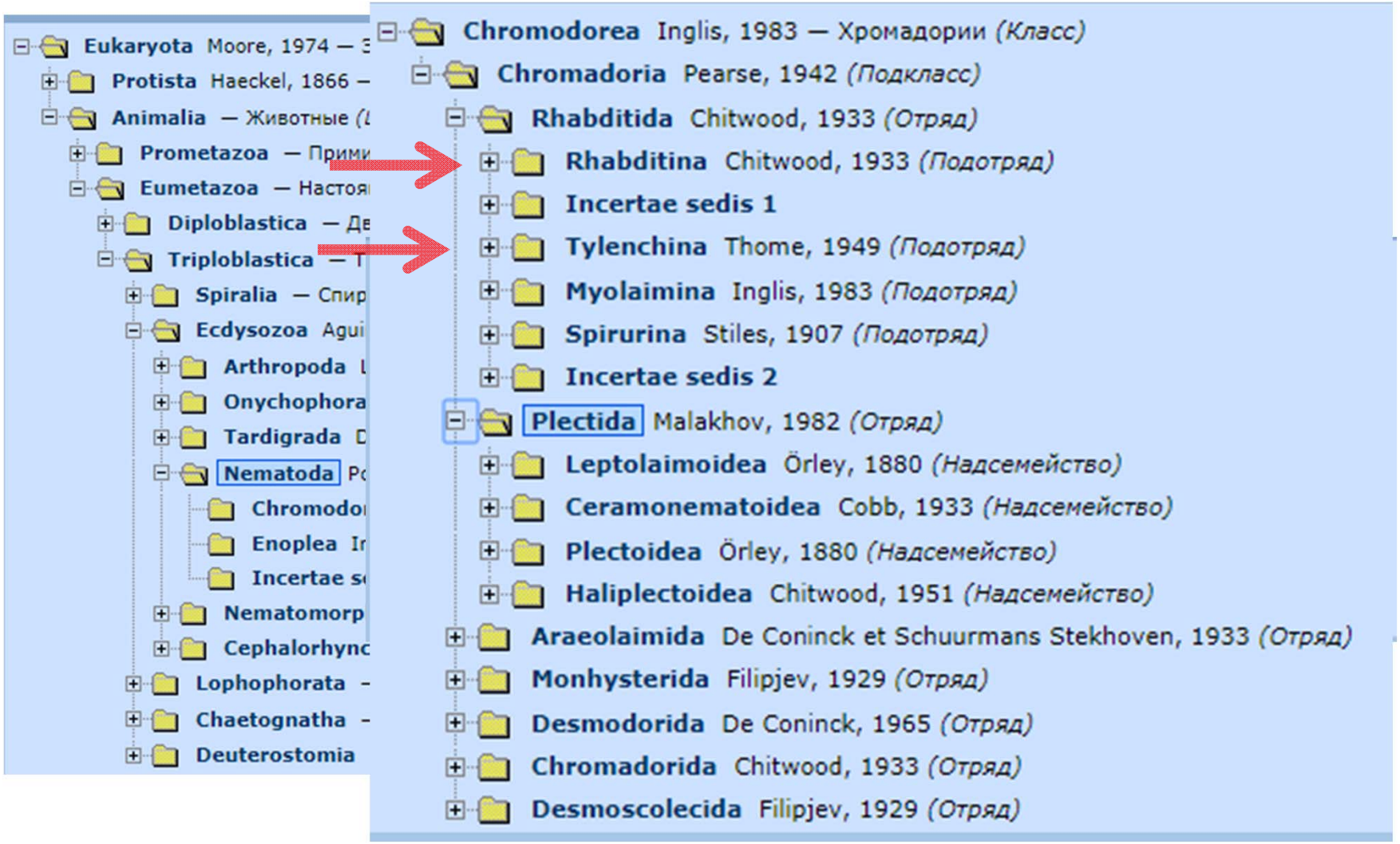


https://www.zin.ru/ZooDiv/animals

Modern CLASSIFICATION: De Ley & Blaxter, 2002 with later emendations



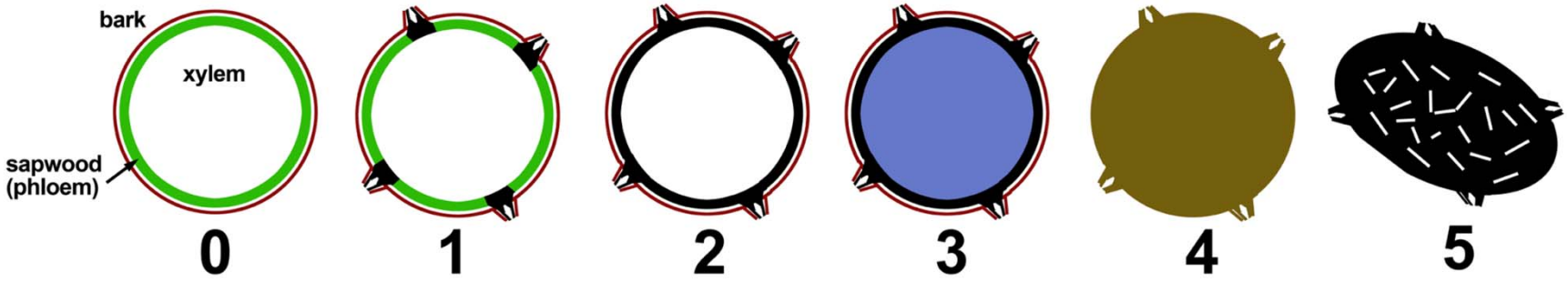
Modern CLASSIFICATION: De Ley & Blaxter, 2002 with later emendations



Nematode succession in wood destruction stages (*Betula, Ulmus, Fraxinus, Quercus*)

Stage N	wood tissue in destruction	Insect vector	Nematode dominants	Leading trophic groups
0	healthy tissues		Laimaphelenchus , Plectus	algotrophic
1	destruction in phloem	no	Aphelenchoides, Anguinidae Panagrolaimidae, Rhabditidae	mycotrophis and bacteriotrophic
2	Complete phloem destruction expanded to surface of softwood	Scolytinae	Bursaphelenchus, Spaerulariidae	mycotrophic
3	Xylem destruction	Cerambycidae	Bursaphelenchus, Cryptaphelenchus, Ektaphelenchus, Sychnotylenchus, Parasitotylenchus	mycotrophic
4	Complete browining of wood with the dissolving of filamentous structure but the structure is tough Woody and not soft	Nitidulidae	Neotylenchidae	mycotrophic
5	wood softening and viscous gradually transforming to soil	no	Monhysteridae, Quitsinematiidae, Mononchidae, Rhabditida, Tardigrada	Predators and bacteriotrophic

Nematode succession in wood destruction stages (*Betula, Ulmus, Fraxinus, Quercus, Malus*)



0: healthy tissues
1: destruction in phloem (sapwood)
2: Complete phloem destruction expanded to surface of softwood, **Scolytinae** involved
3: Xylem destruction, blue stain fungi, **Cerambycidae** involvement
4: Complete browining of wood with the dissolving of filaments, **Nitidulidae** involved
5: wood softening to complete destruction

Decomposition of
The dead old pines:
(Insect vector/Nematode/Fungus)
Ips sexdentatus
-Bursaphelenchus sylvestris
-Ophiostoma abietinum.

Entomo-Transmissive
Nematode-Fungal infection:
Hylastes ater
-Bursaphelenchus chitwoodi
- Leptographium truncatum

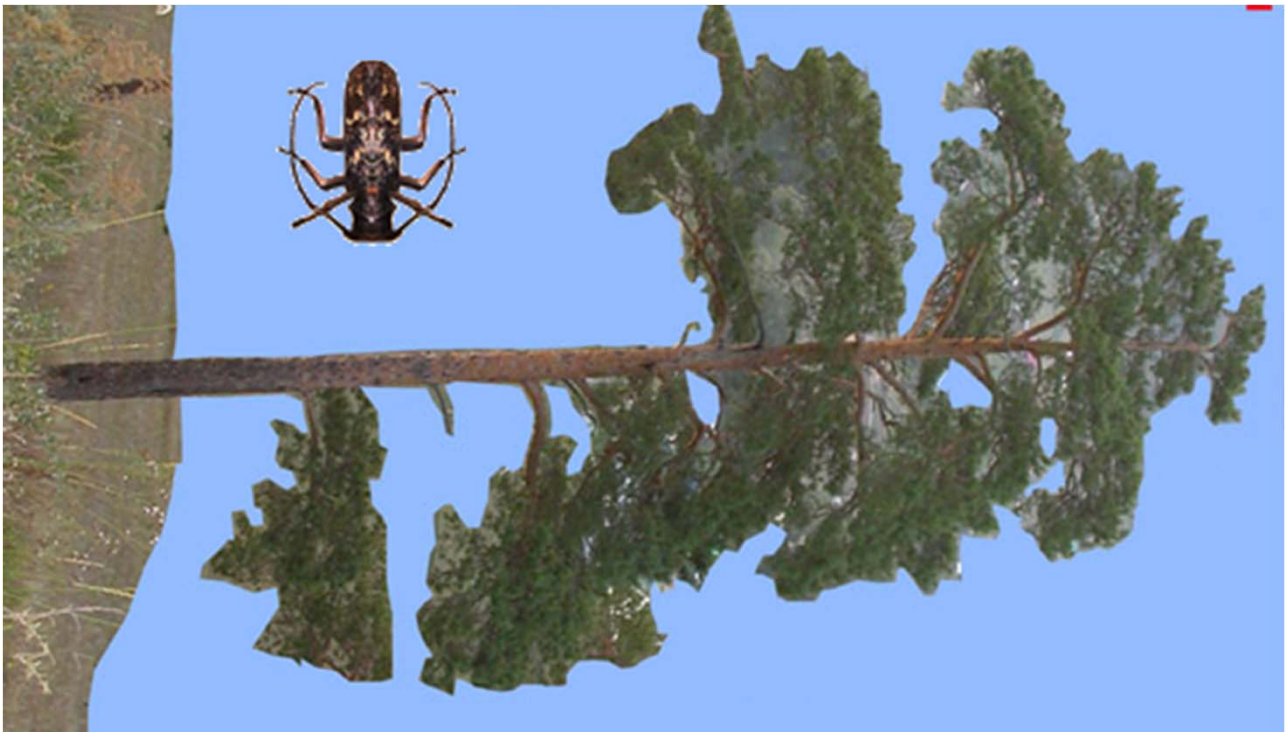
Wilt of young pine

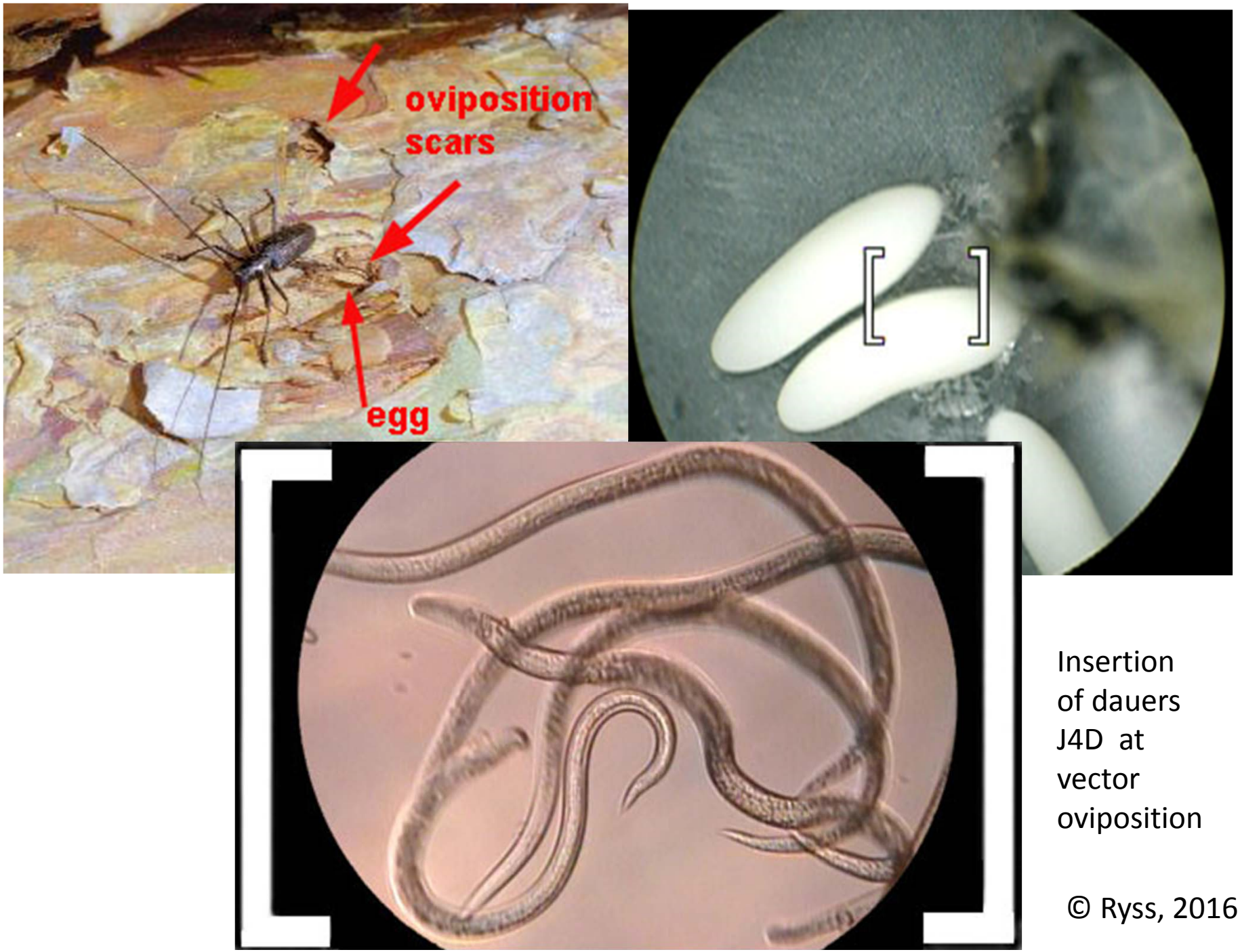
Wilt of 50-y.old pine forest:




Forest diseases caused by nematode associations

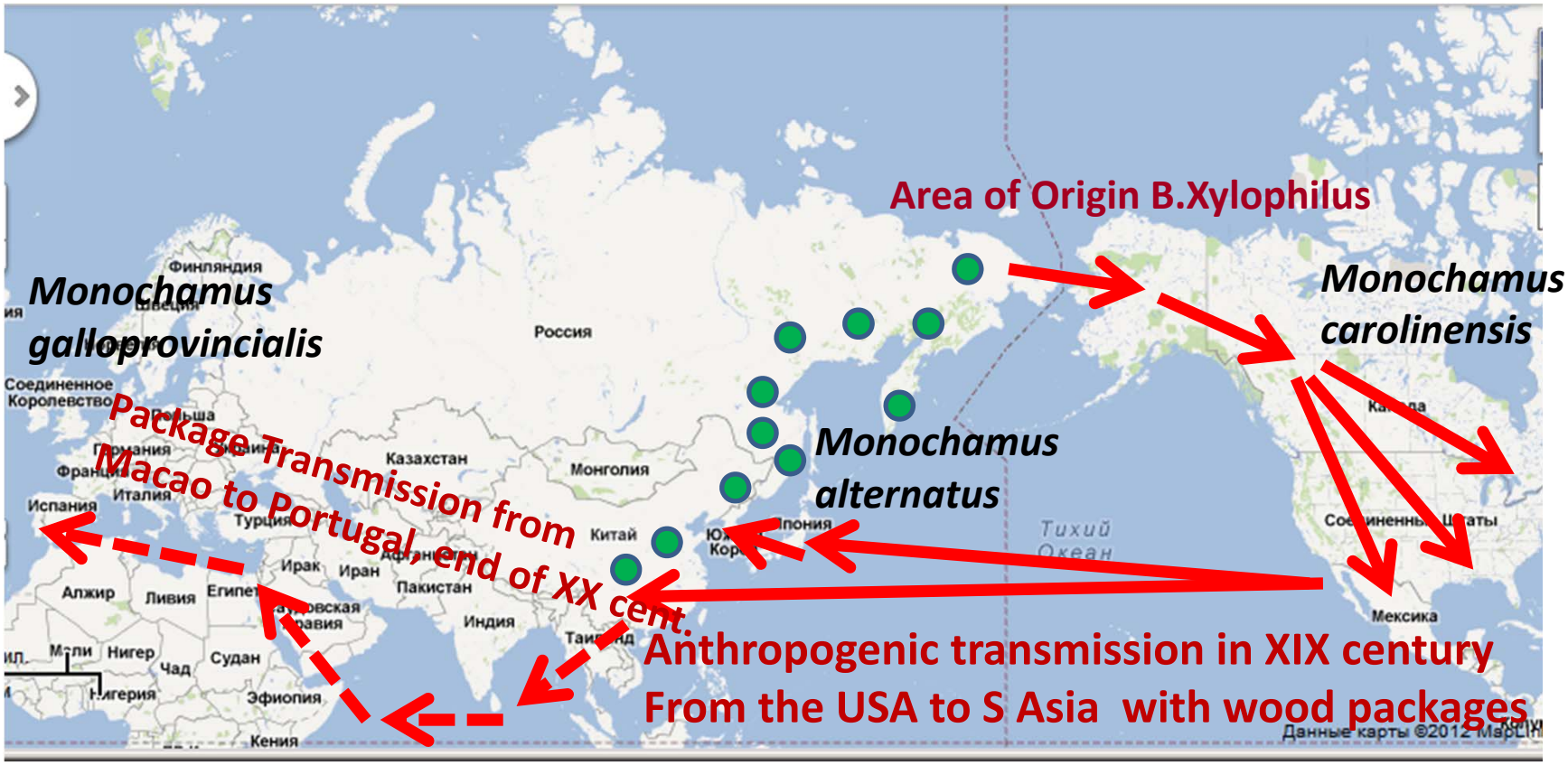
Pine wilt caused by the *nematode+Ophiostoma* fungus





Insertion of dauers J4D at vector oviposition

Invasive species : *Bursaphelenchus. xylophilus, causative agent of the Pine Wilt Disease*



Native areals of other *Bursaphelenchus* spp.

Dutch Elm Disease in Saint Petersburg, Russia. City Parks.

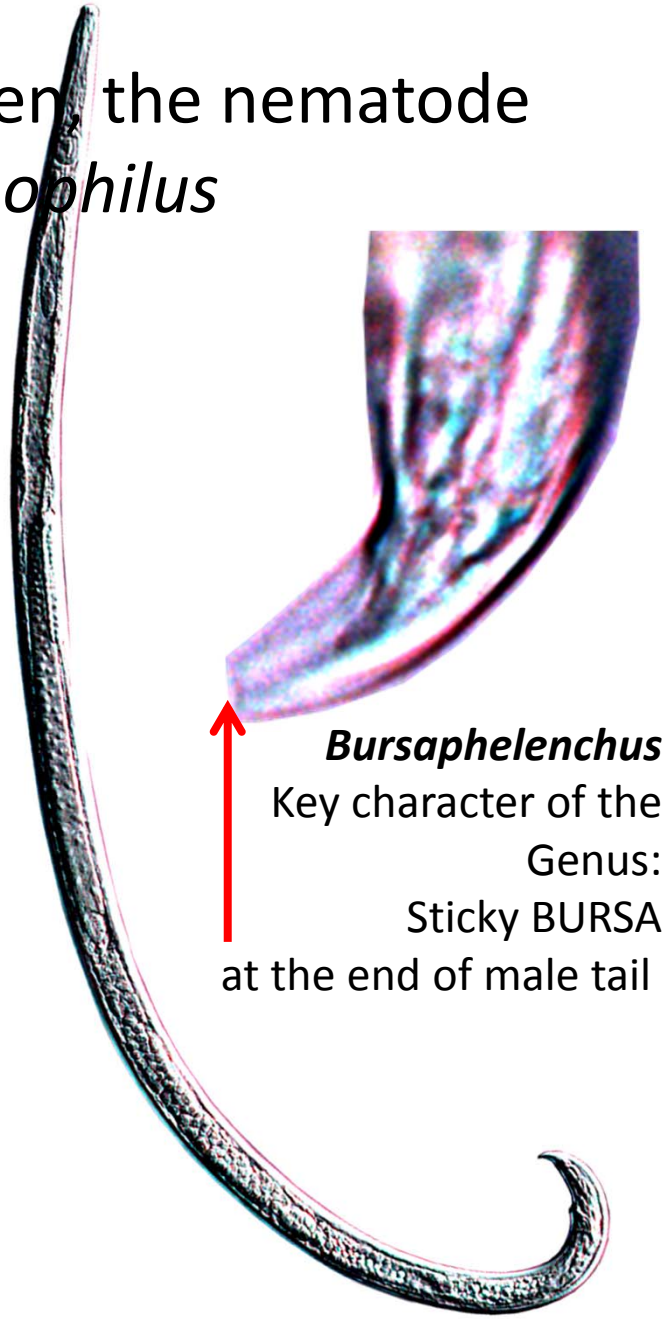
Dutch Elm Disease (DED). Symptoms: Red-Brown Rings of SapWood(Phloem)

Dutch elm disease of *Ulmus* spp. in parks of St. Petersburg

Pathogen: fungus
Ophiostoma novo-ulmi

Vectors = beetles:
Scolytus scolytus
S. multistriatus

In DED association: as a pathogen, the nematode *Bursaphelenchus ulmophilus*



Bursaphelenchus
Key character of the Genus:
Sticky BURSA
at the end of male tail

Palm weevil
Rhynchophorus palmarum

Palm nematode
Bursaphelenchus cocophilus + fungus *Blastocysis* sp.

SOCHI, Palms, Material from Elena Zhuravlyova ,
Weevil *Rhynchophorus ferrugeneus*
Nematodes *Aphelenchoides bicaudatus*
and fungus *Blastocysis* sp.