



Základy humánní parazitologie

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oddělení parazitologie
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Základy humánní parazitologie

Základní údaje o předmětu:

přednáška: čtvrtek, od 9 do 11.50 hodin,

budova A11, místnost 306

zkouška (ústní) se bude konat v kampusu MU
Bohunicích v pavilonu Ústavu botaniky a zoologie
A31 v místnosti 332 – bude info mailem

studijní materiály na IS

Základy humánní parazitologie

- Struktura přednášky:
- Úvodní část přednášky, základní parazitologické pojmy
- Protozoologie
- Helmintologie
- Arachnoentomologie
- Kombinace orgánového přístupu a systematiky

Z historie parazitologie I

Egypt: (1250 – 1000)

Schistosoma hematobium
Taeniarhynchus saginatus
Ascaris lumbricoides
Dracunculus medinensis

Řecko a Řím:

Plinius starší
Aristoteles
Galén
Hippocrates

Z historie parazitologie II

- Čína
Ascaris lumbricoides
- Amerika
helminti
Tunga penetrans
Pediculus humanus
Malárie (Plasmodium)
Leishmanióza (Leishmania)

Z historie parazitologie III

- Evropa (1200 – 1650)
 - 1379: Fasciola hepatica
 - 1592: Diphyllbothrium latum
 - 1674: Eimeria
 - 1681: Giardia intestinalis

 - 2. pol. 17.stol – Francesco Redi – „otec parazitologie“ - redie

Z historie parazitologie IV

- Středověk – mnoho falešných představ o cizopasnících
- Rudolphi:
 - Nematoidea
 - Acanthocephala
 - Nematoda
 - Cestoda
 - Cystica
- 1773: cerkarie (Muller)
- 1816: cercárie – motolice (Nitzsch)
- 1842: životní cyklus motolice (Thomas, Leucard)
- 19. století – parazitologie jako věda (Zeder, Rudolphi, Frolich, Butschli, Dolfein, Dujarden, von Sielbold, Schaudin, Loos aj.)

Z historie parazitologie V

- Rozvoj parazitologie u nás:
- Do 1. světové války: Praha – Dušan Lambi, Stanislaw Prowazek
- Mezi válkami: Praha: Briendl, Komárek, Jírovec – otec naší parazitologie
Brno: Rašín
- Po 2. světové válce: Akademie věd - ČSAV, SAV, AV ČR,
Parazitologický ústav AV ČR v Českých Budějovicích
Univerzity (UK, ČZU, JčU, MU, MENDELU, VFU)
Veterinární a hygienická služba
Armáda, nemocnice, referenční laboratoře
Soukromé firmy a diagnostické laboratoře

Z historie parazitologie VI

- 20. století – parazitologie vyhraněná vědní disciplína
- Fauna cizopasníků
- Morfologie, taxonomie a systematika
- Životní a vývojové cykly
- Biologie a ekologie
- Fyziologie, biochemie, imunologie
- Epidemiologie a matematické modelování
- Genetika a molekulární biologie
- Evoluční biologie a fylogenetika
- Genomika a transkriptomika

- Histologie, histochemie, imunohistochemie
- Ultrastruktura a anatomie
- SCAN, TEM, CLSM

Přehled základních mikroskopických technik



Small drop has a nearly perfect spherical shape, and so it can serve as a lens.

17th century - Stephen Gray used a water drop as a lens for a microscope he built

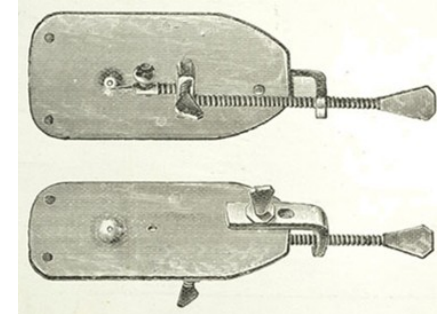


Fig: 4.

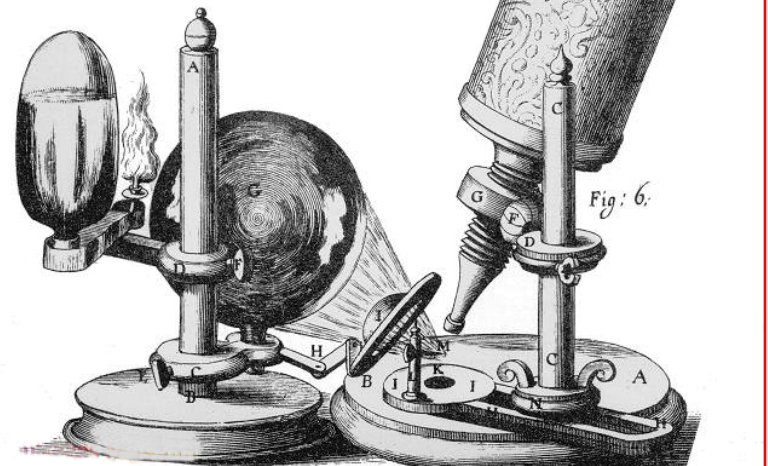
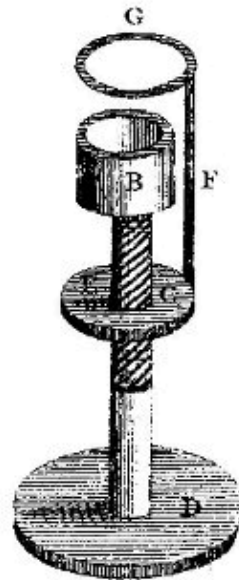
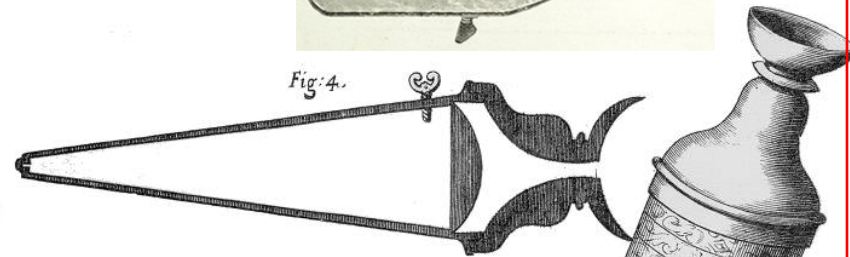
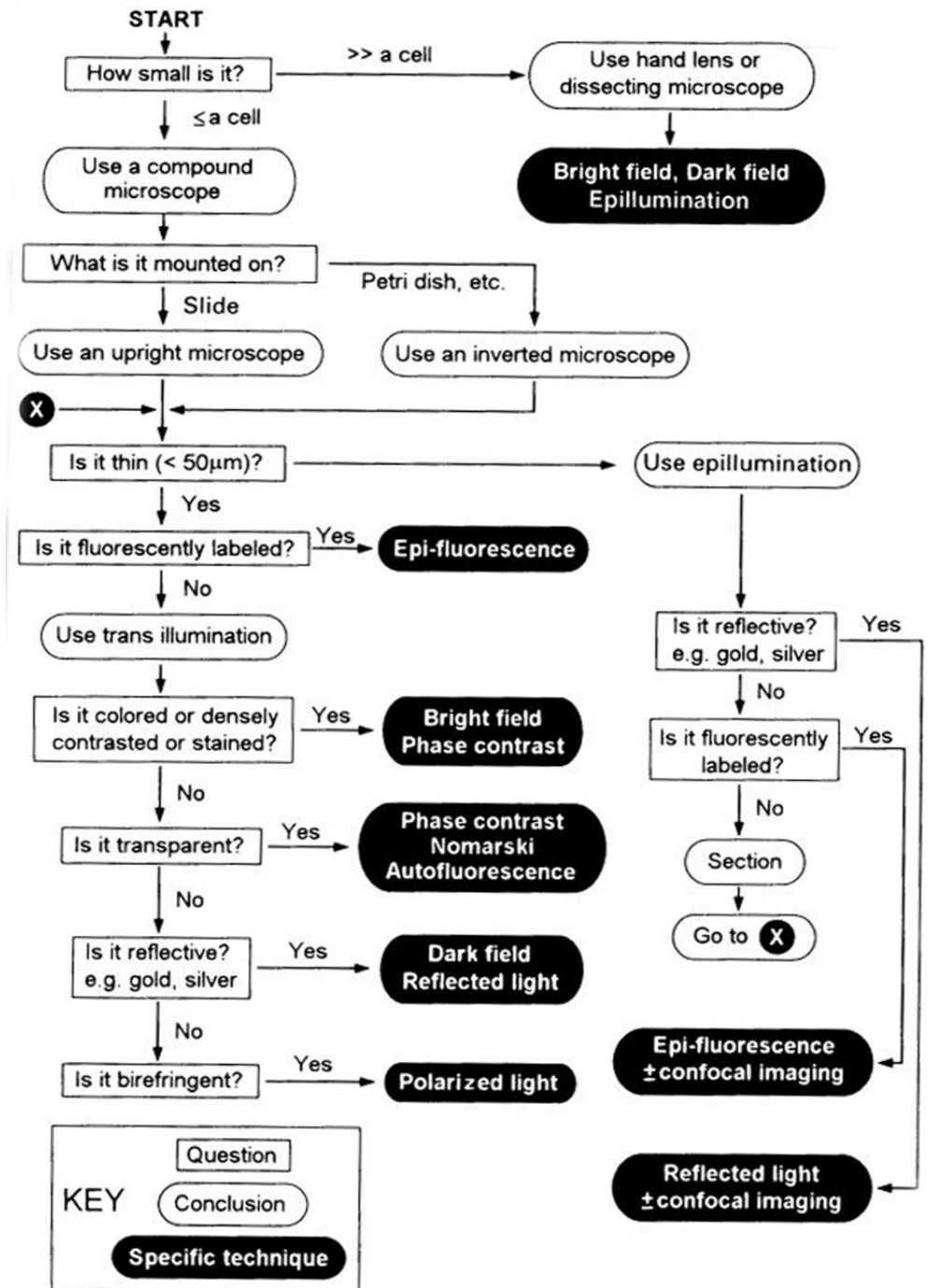


Fig: 6.

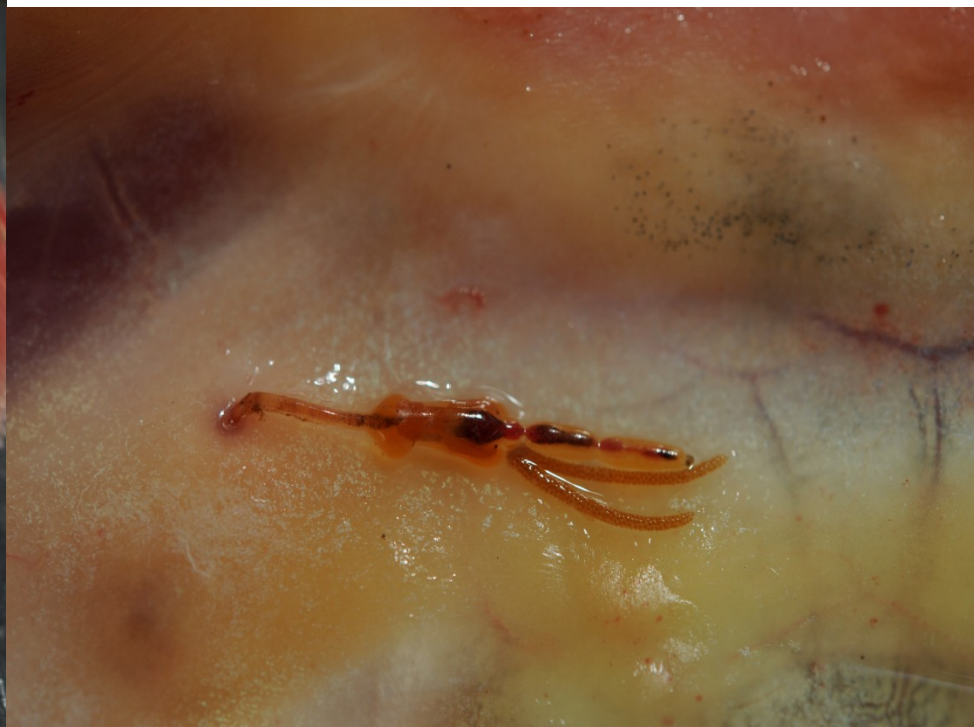
Jak vybrat správnou mikroskopickou techniku ?



Parasitic crustacea



ECTOPARASITES
Parasitic crustaceans,
leeches - found
externally:
usage of hand lens or
dissecting microscope



Oddělení parazitologie - infrastruktura

Laboratoř speciální mikroskopie



Mikroskop
Olympus BX51 s fázovým
kontrastem a analýzou obrazu

Bright field

Eudiplozoon nipponicum



- Acetylcholine visualised with 5-bromo-chloro-indolyl acetate

(Zurawski T.H. et al., 2001)

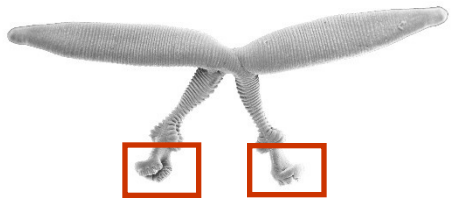
Gomori Trichrom Staining

E. nipponicum

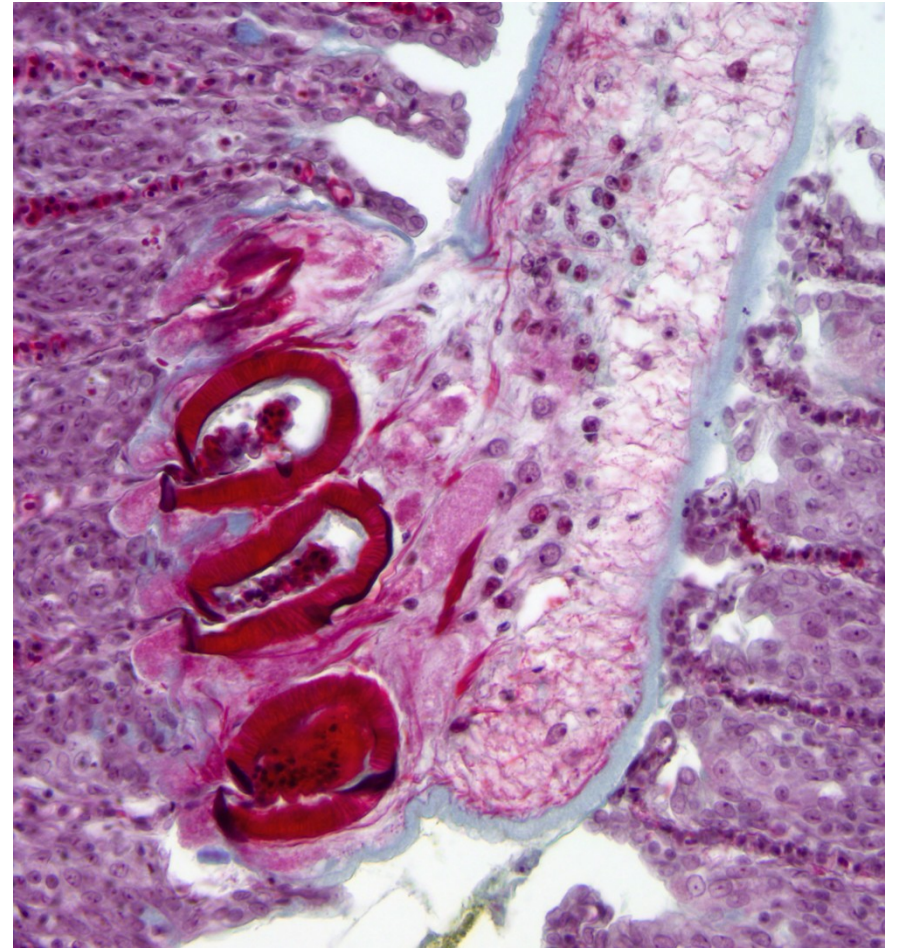


P. homoion

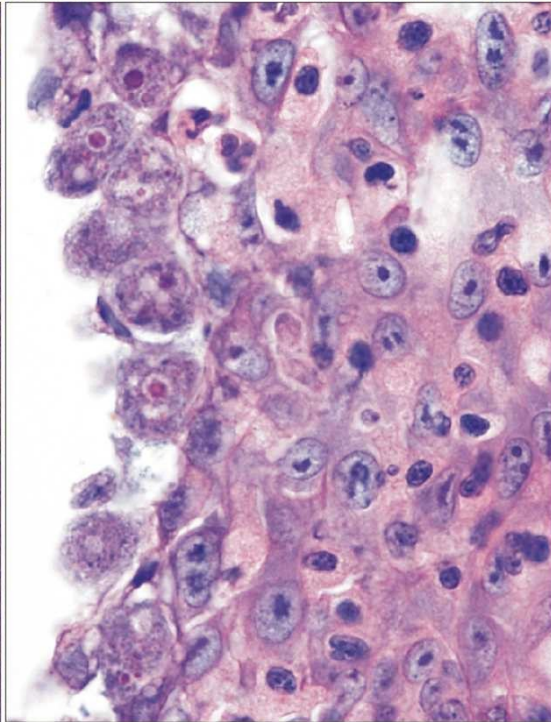
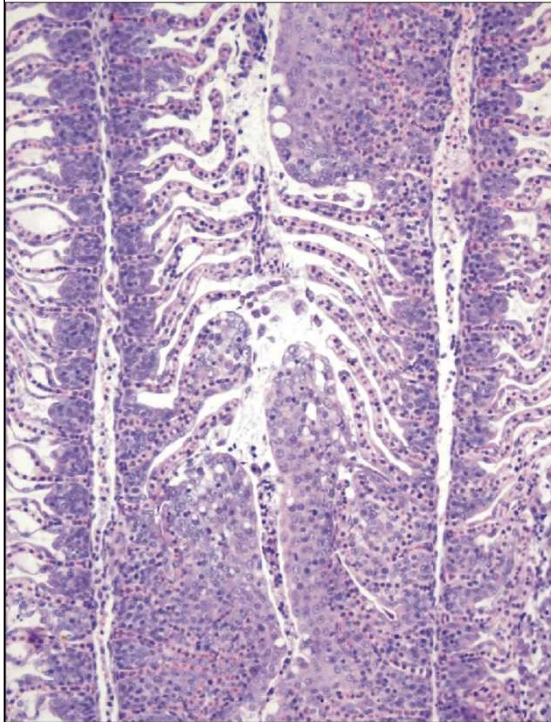
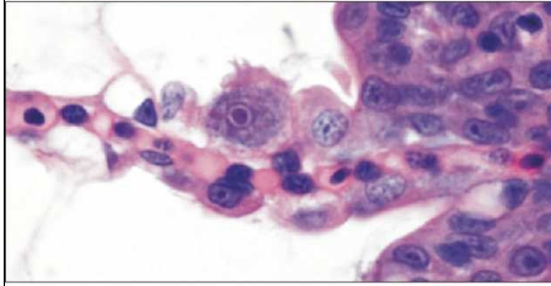
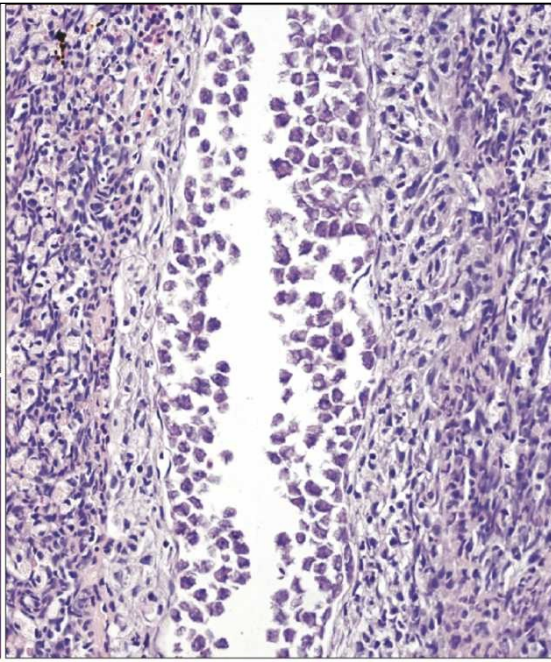




E. nipponicum - histology

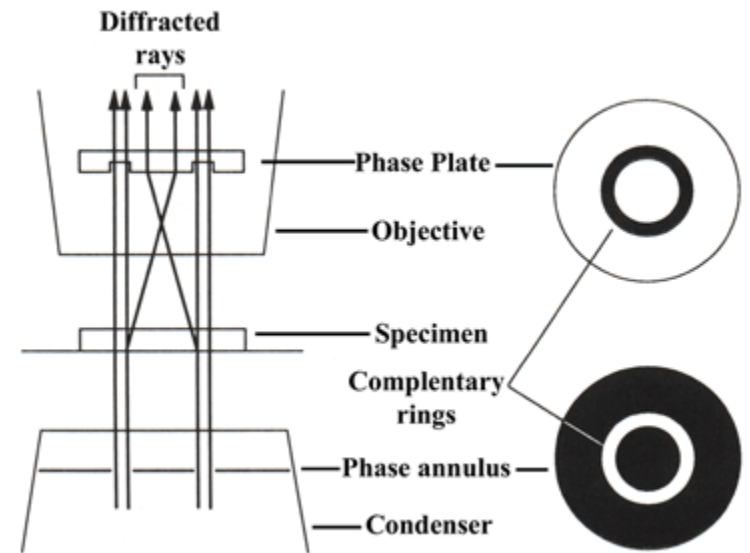


LM (Histological sections, Masson's trichrome)

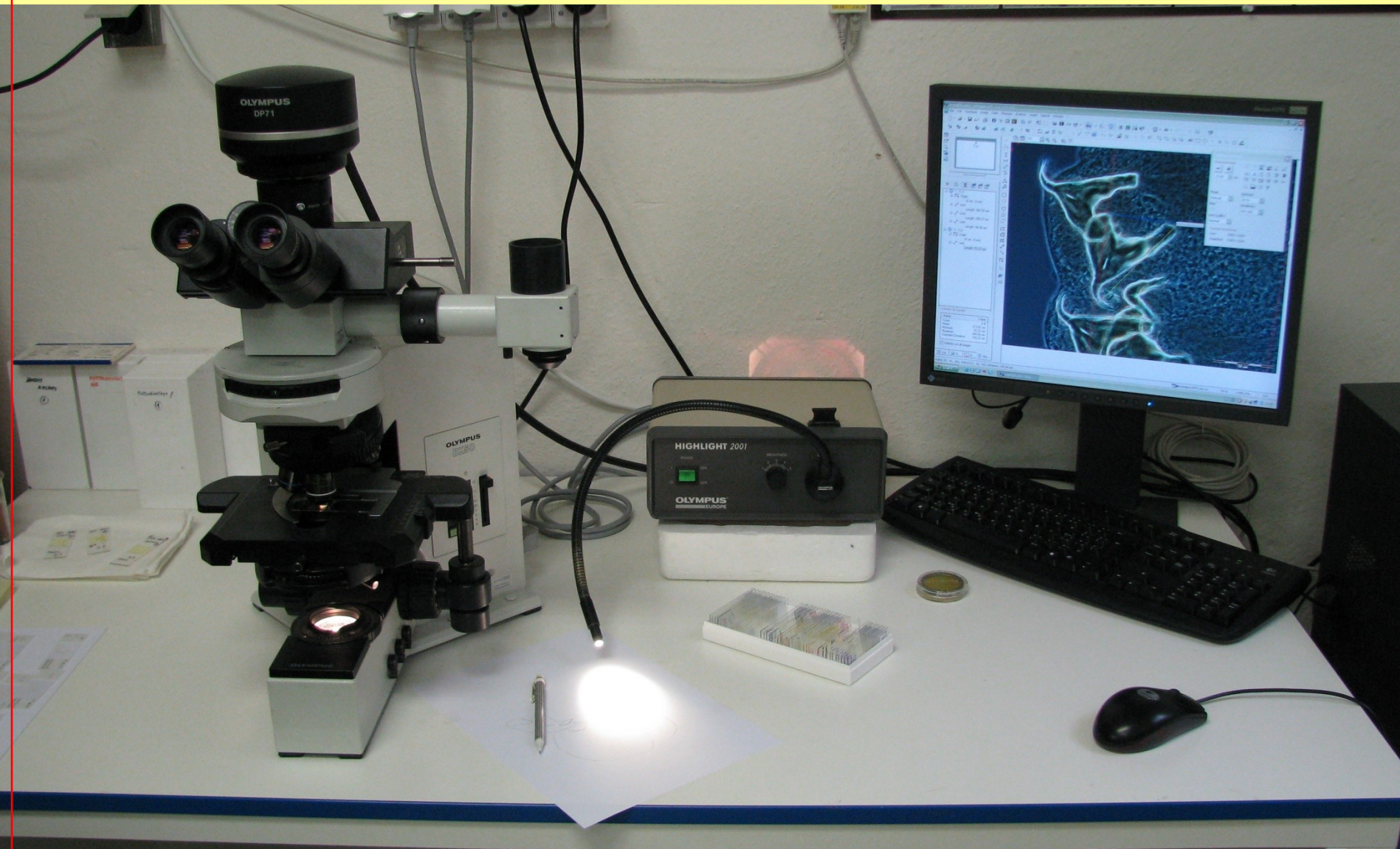


Phase contrast (PC)

- PC enhances contrasts of transparent and colorless objects by influencing the optical path of light
- Light passing through a transparent part of the specimen travels slower and, due to this is shifted compared to the uninfluenced light
- Difference in phase is not visible to the human eye → change in phase can be increased to half a wavelength by a transparent phase-plate in the microscope and thereby causing a difference in brightness
- Transparent object becomes shining out in contrast to its surroundings

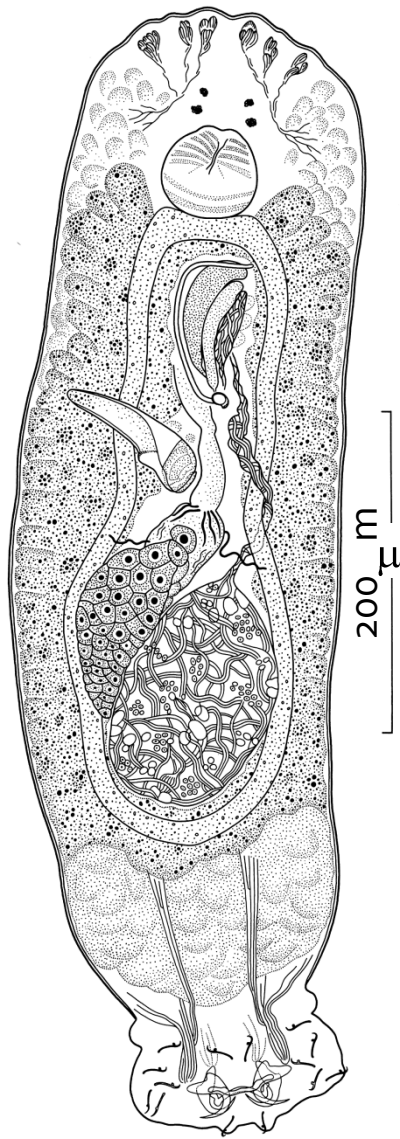


I. Organismal and structural diversity - species identification

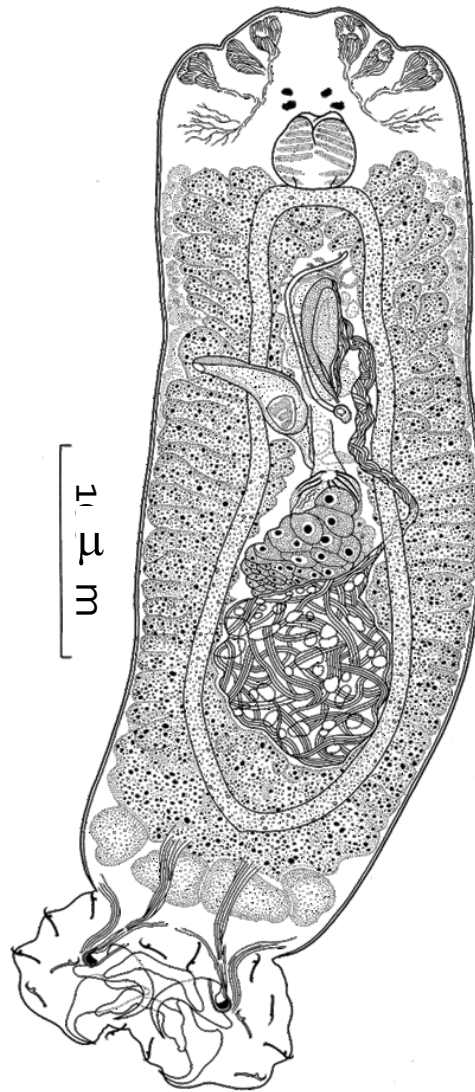


Drawings: microscope Olympus BX 50 equipped with a phase contrast optics and drawing tube; **Measurements:** Digital Image Analysis

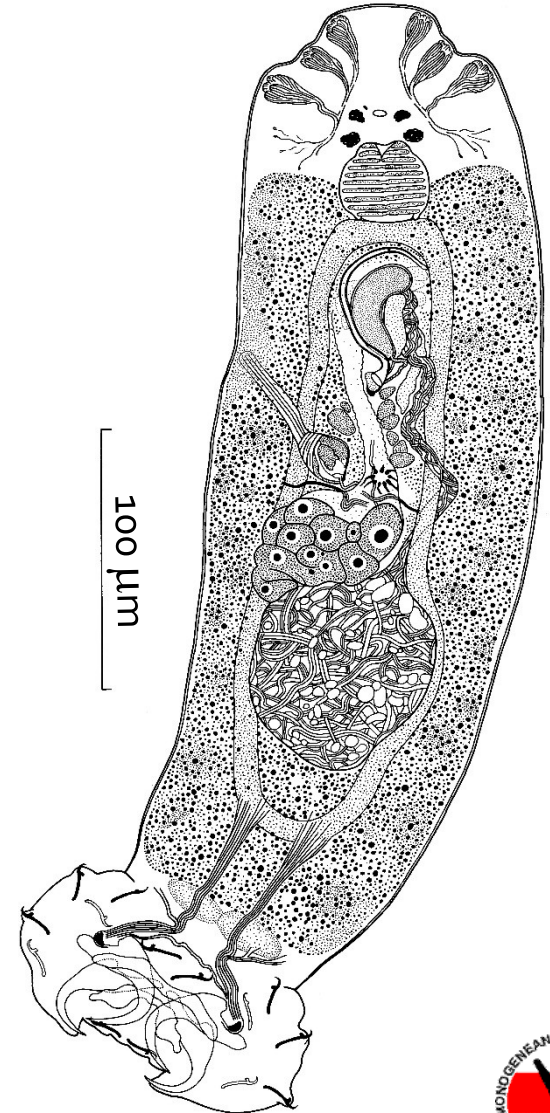
Thylacicleidus serendipitus



Thylacicleidus
sp. 1



Thylacicleidus
sp. 2

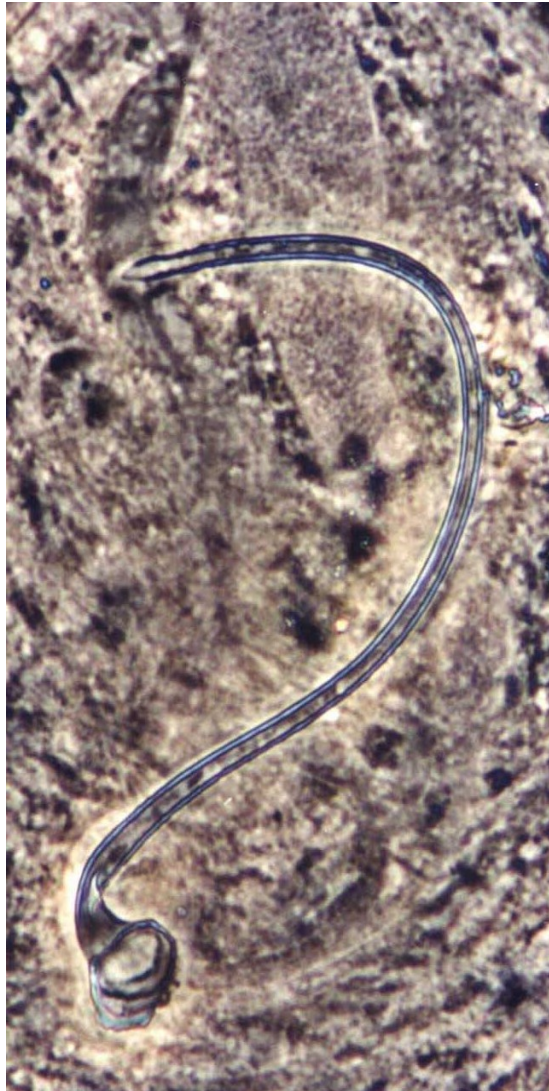


Laboratoř speciální mikroskopie

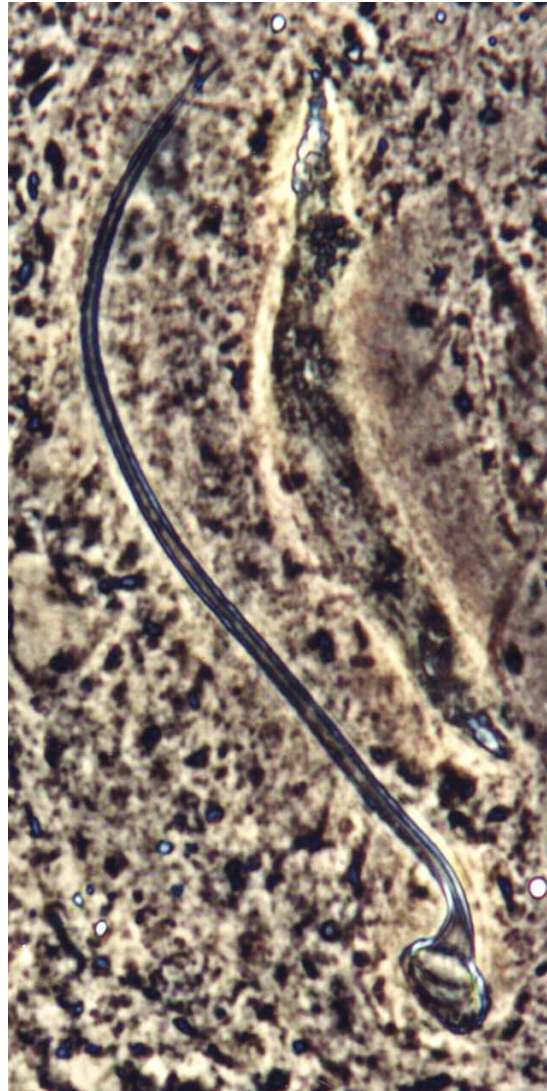
Fázová mikroskopie



**Thylacicleidus
serendipitus**



**Thylacicleidus
sp. 1**



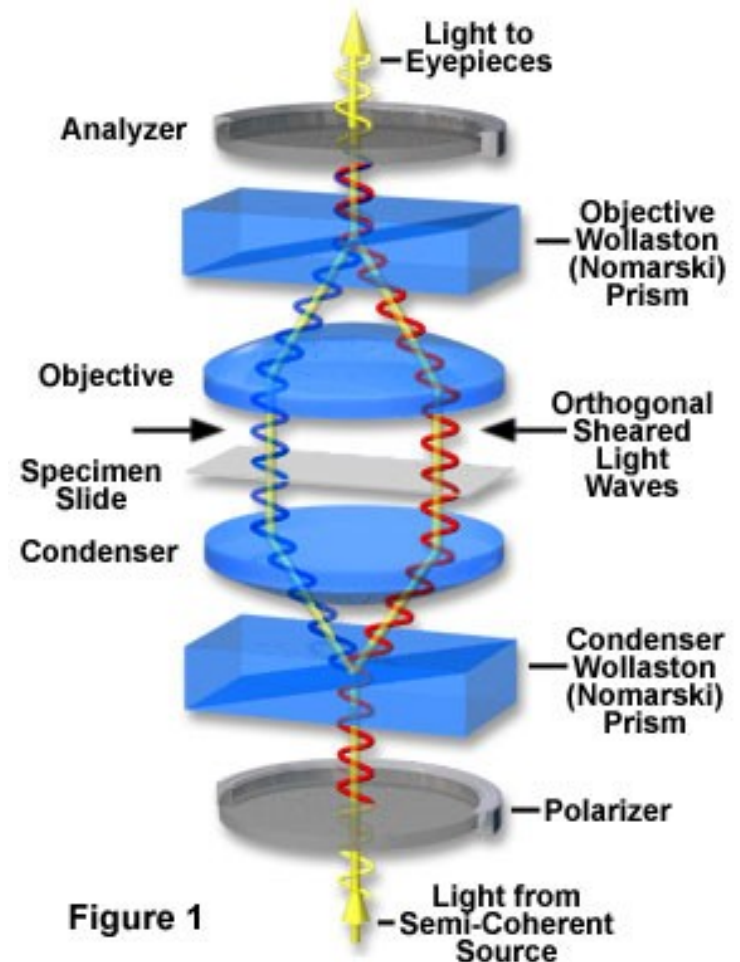
**Thylacicleidus
sp. 2**



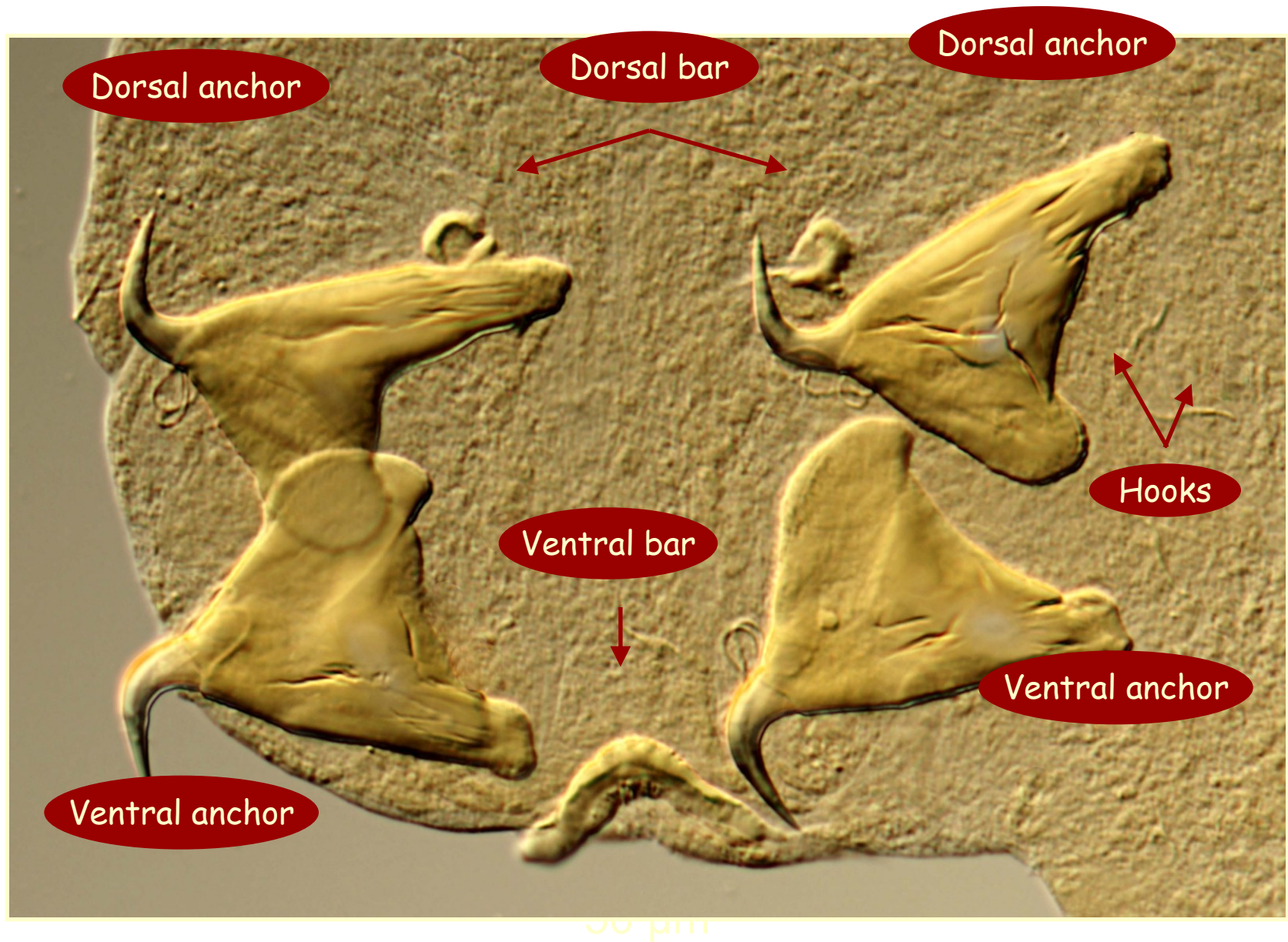
Nomarski differential interference contrast

- DIC Nomarski
- mid-1950s - Georges Nomarski modified the Wollaston prism
- Living or stained specimens, which often yield poor images when viewed in brightfield illumination, are made clearly visible by optical rather than chemical means

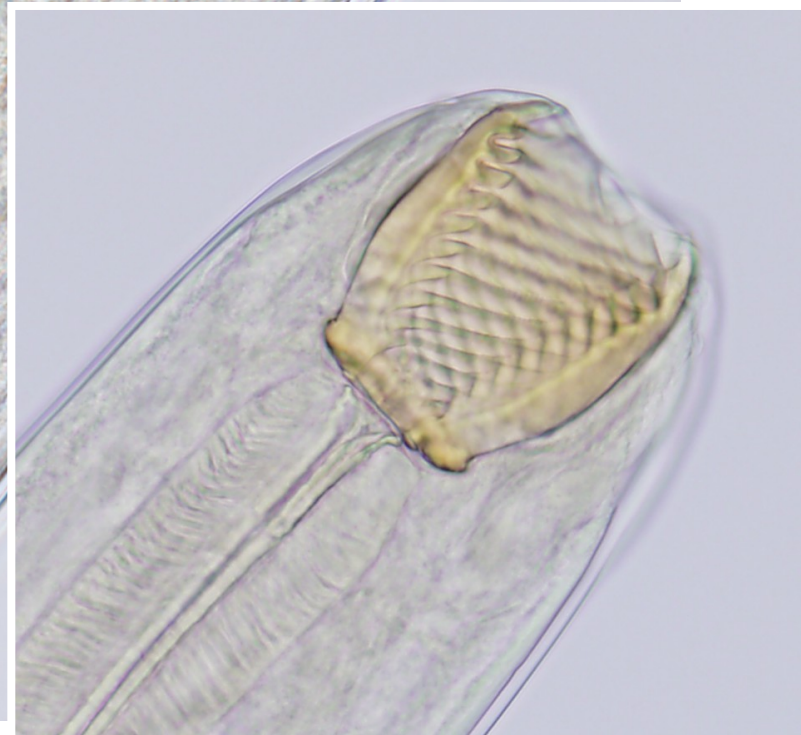
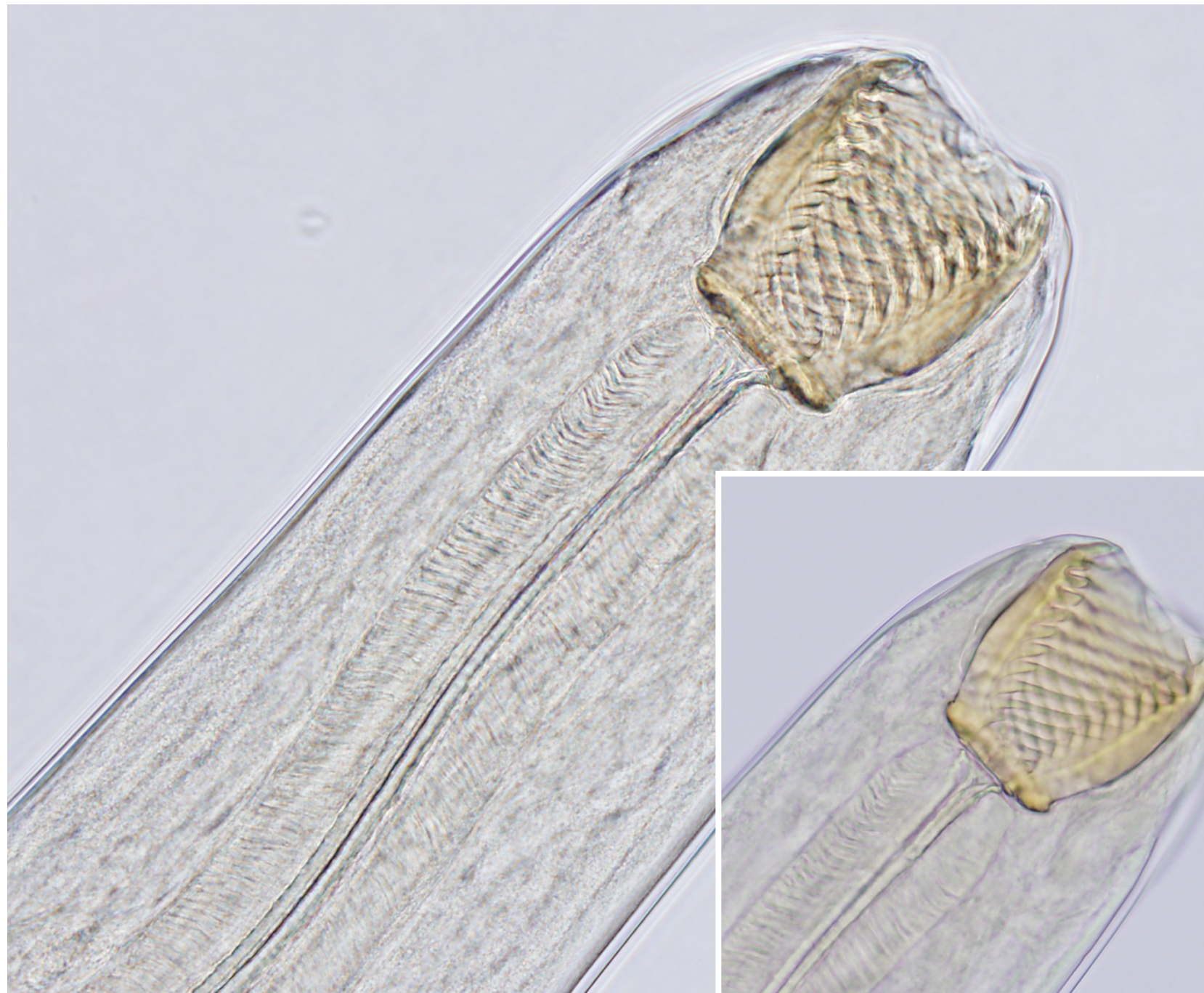
Differential Interference Contrast Schematic



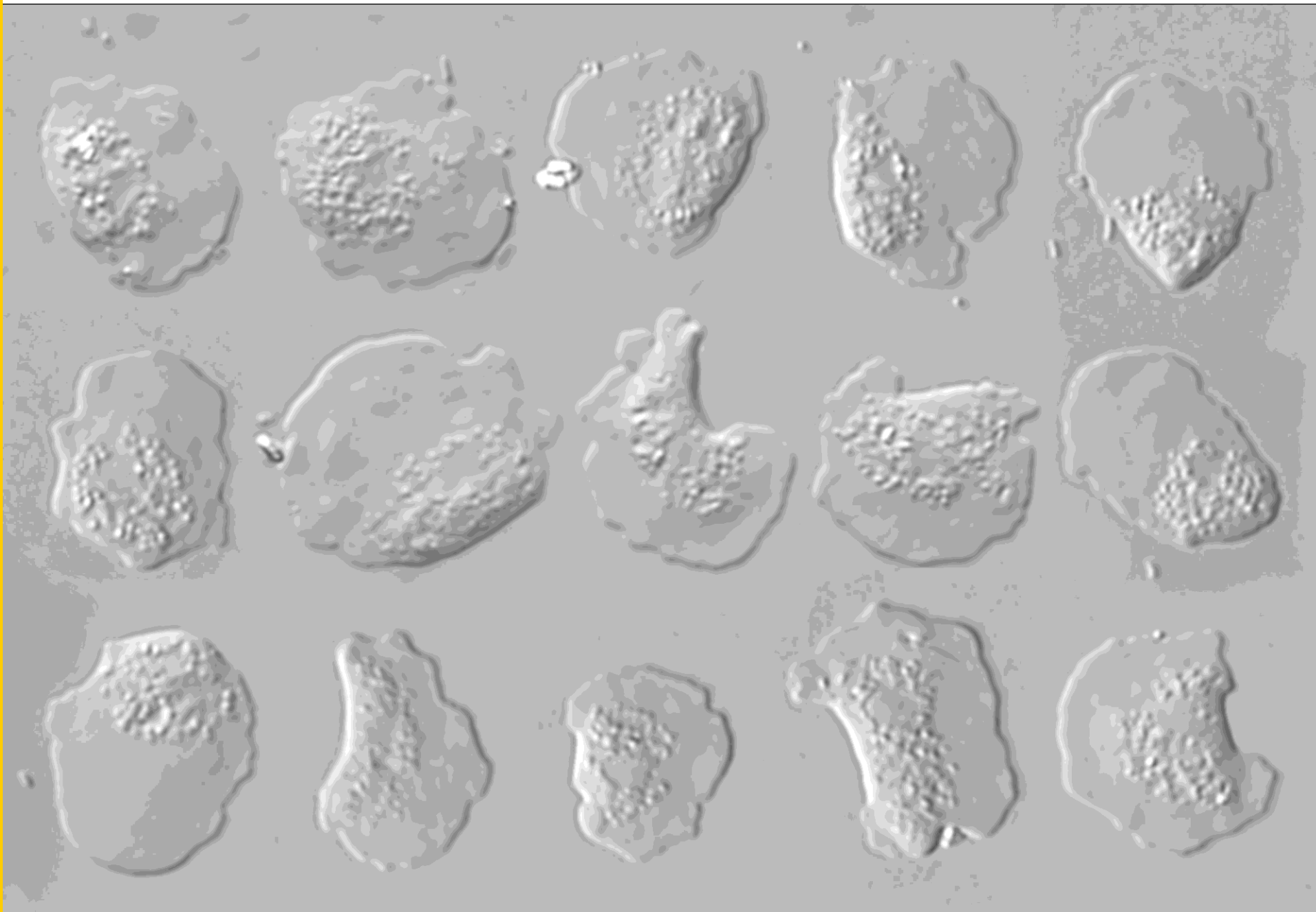
Laboratoř speciální mikroskopie (DIC Nomarski)





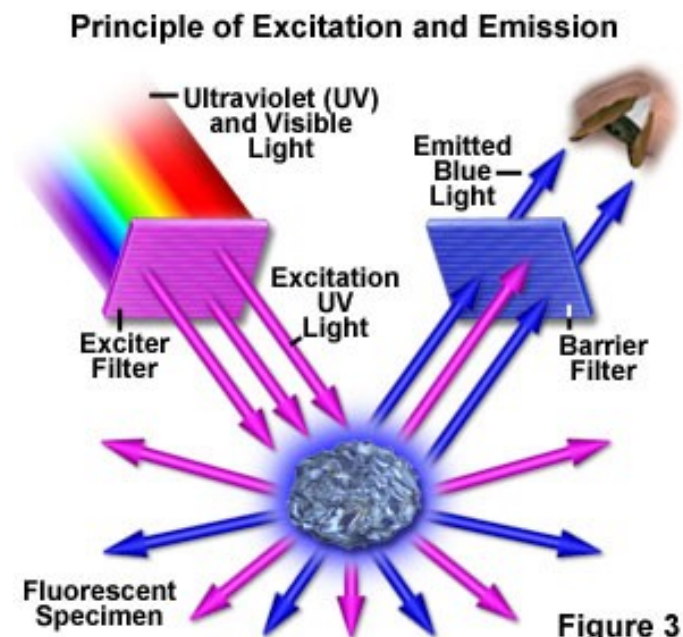


Model parasites group: free living amoebae

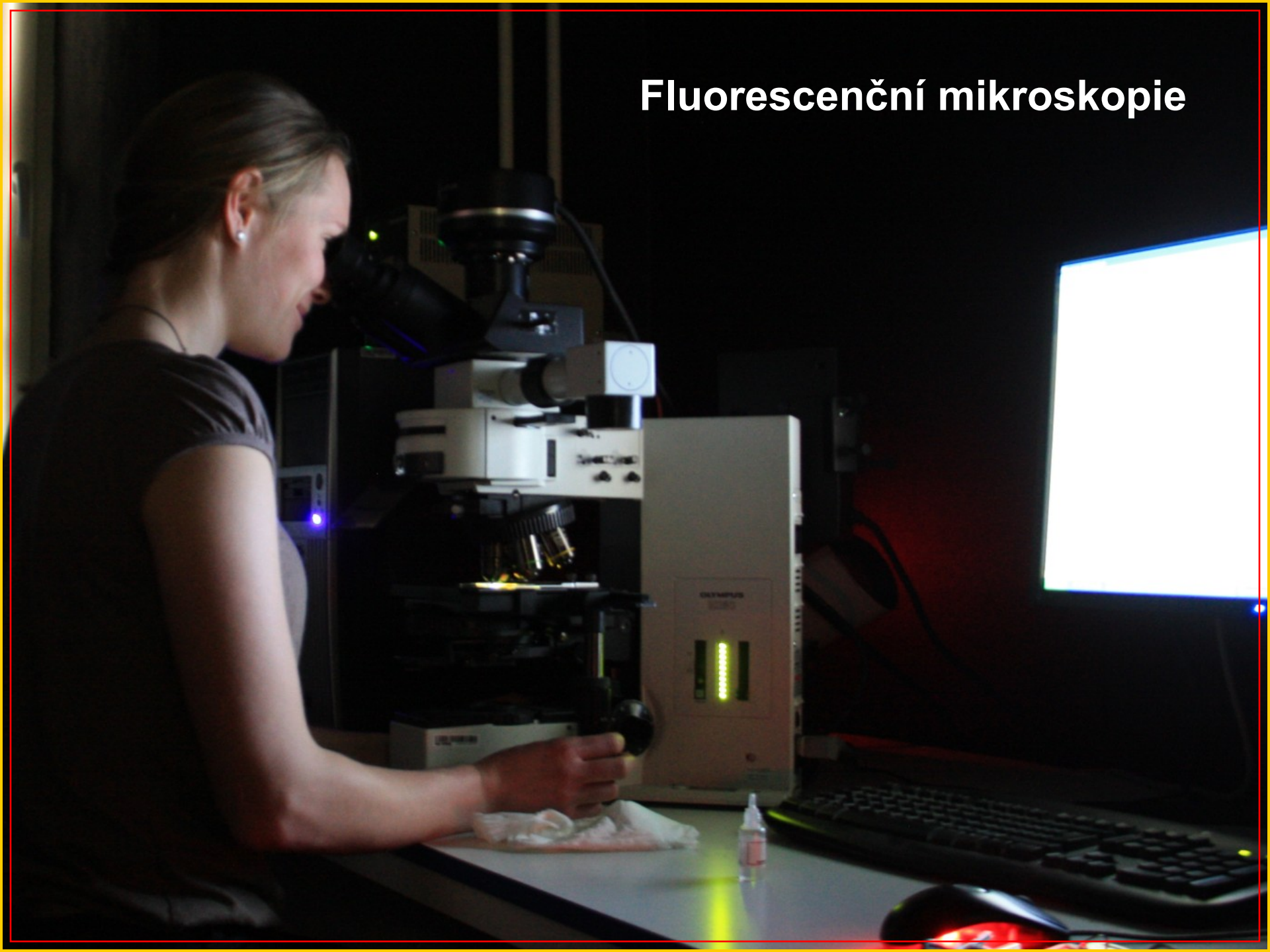


Fluorescent illumination

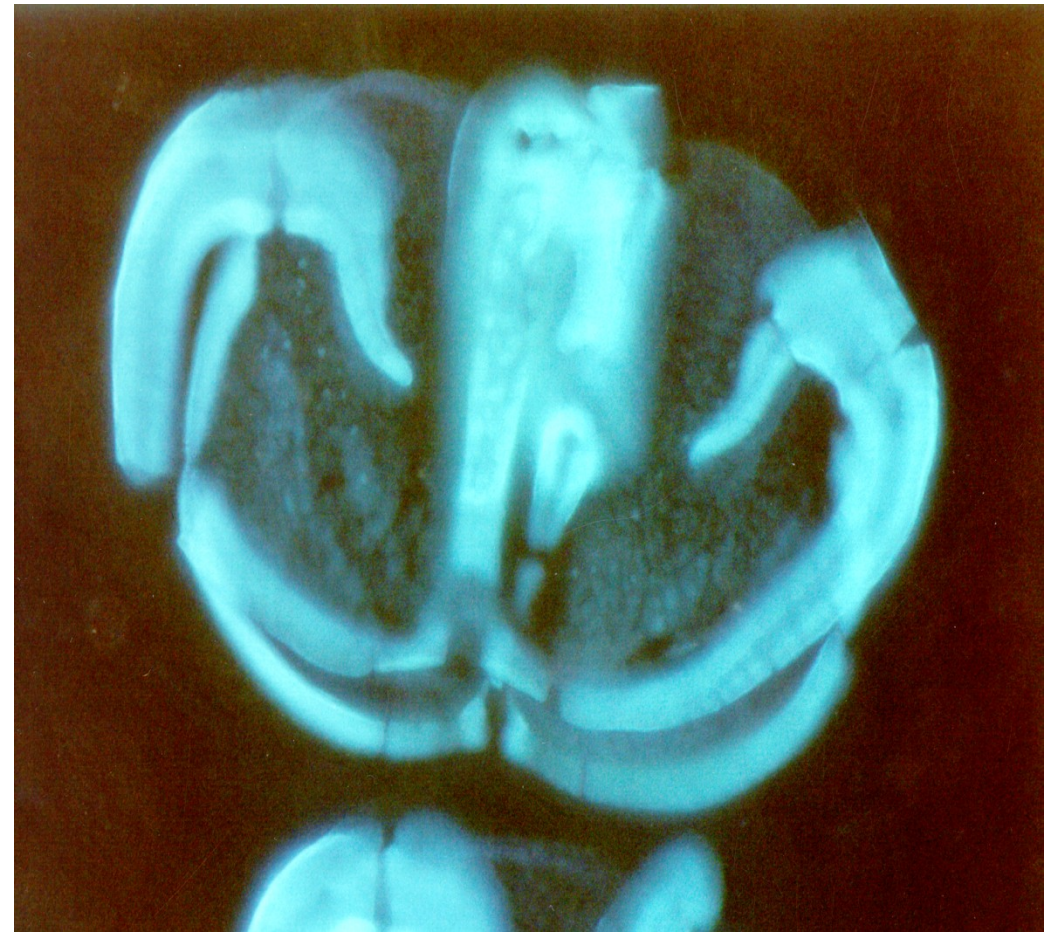
- emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength
- emitted light has:
 - longer wavelength
 - lower energy



Fluorescenční mikroskopie

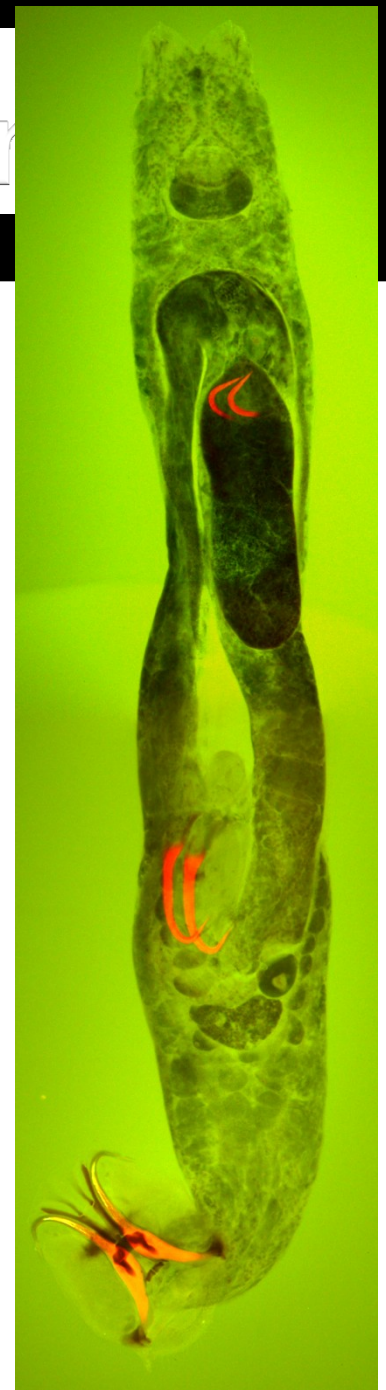


Fluorescent illumination



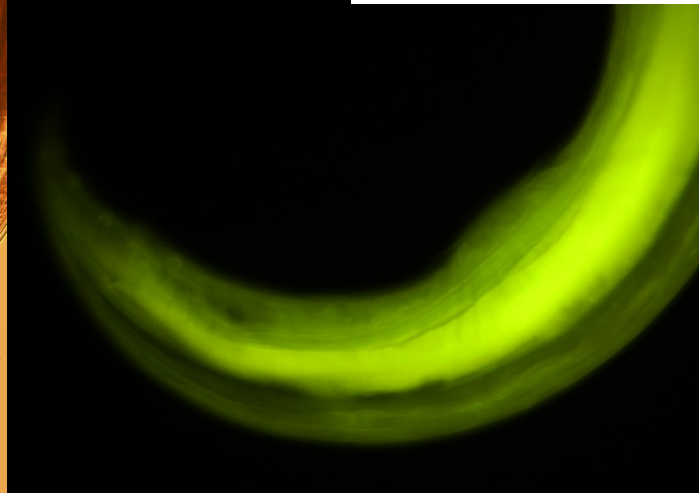
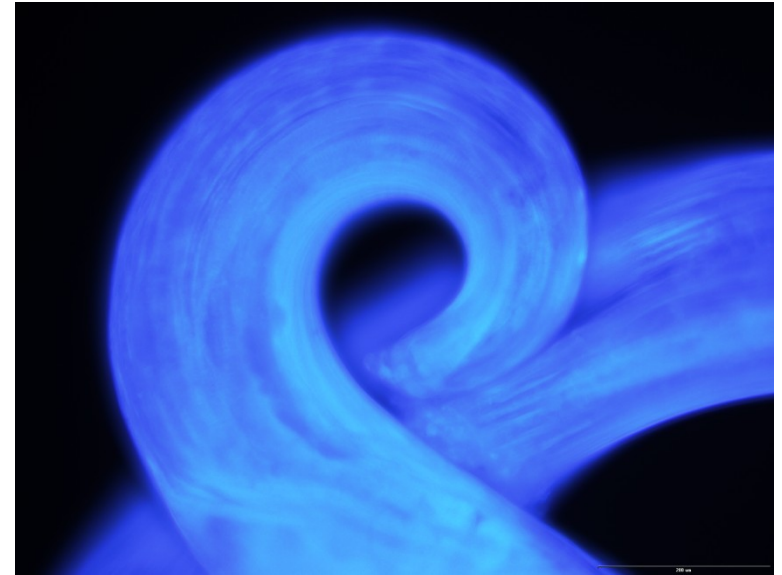
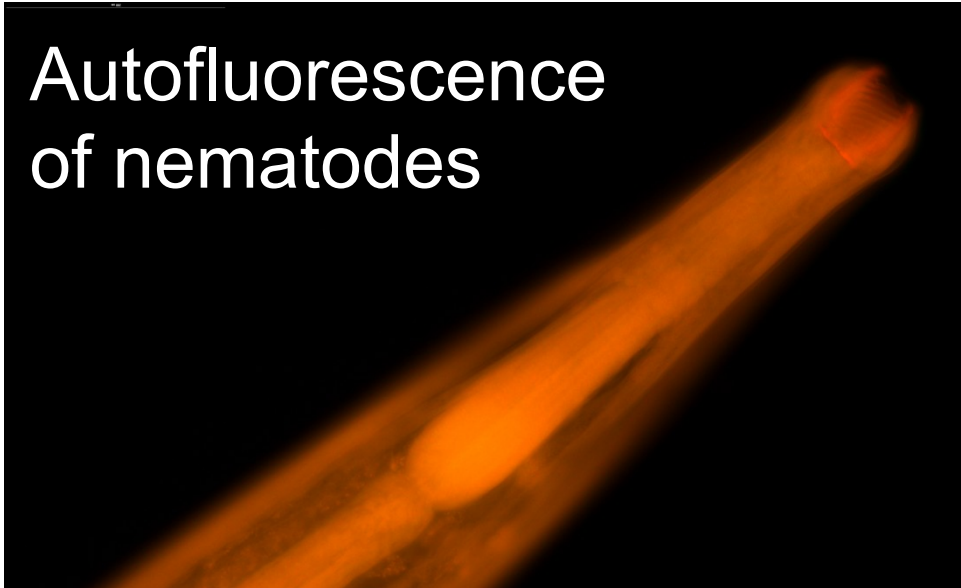
Fluorescent illumination

Gomori trichrom

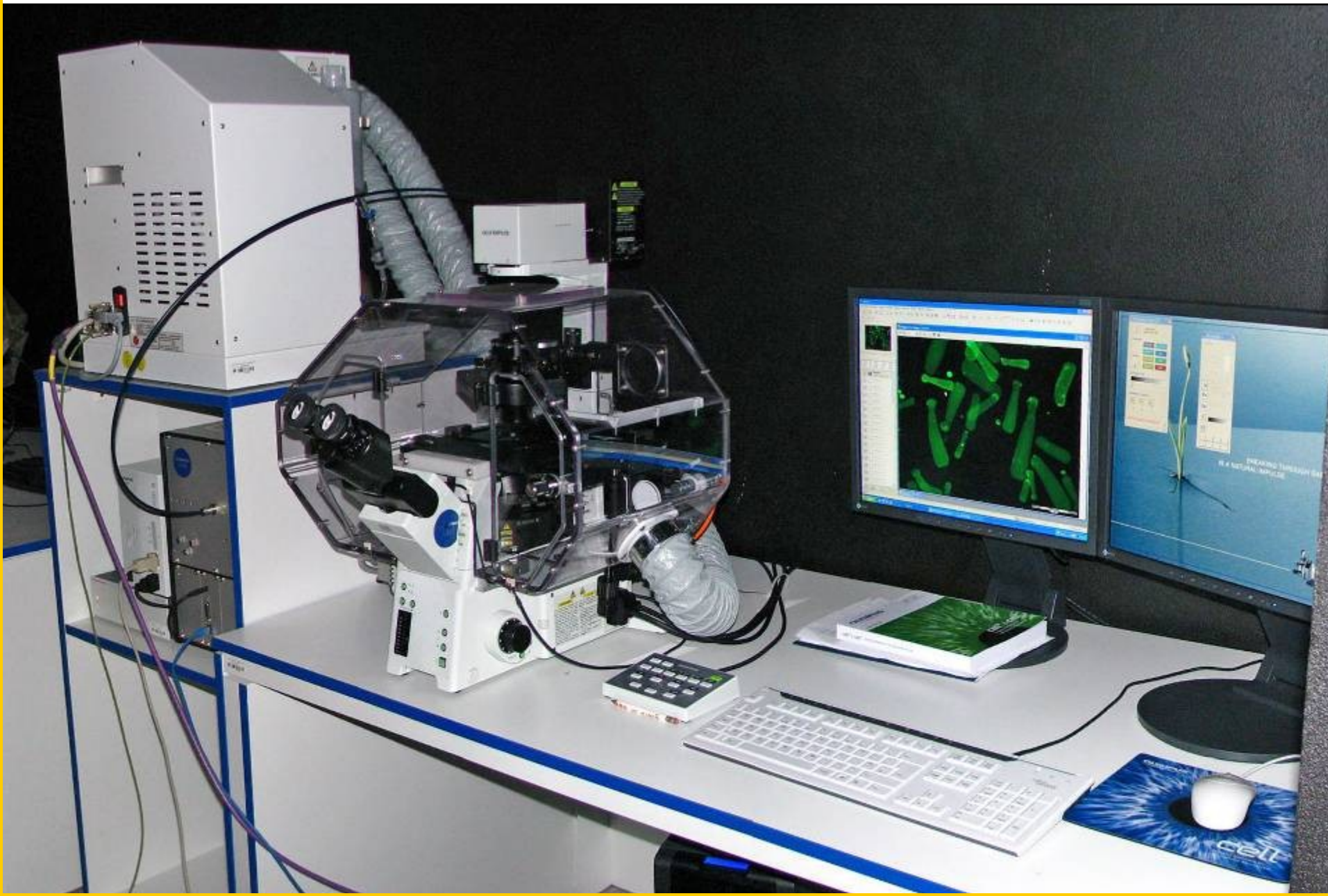


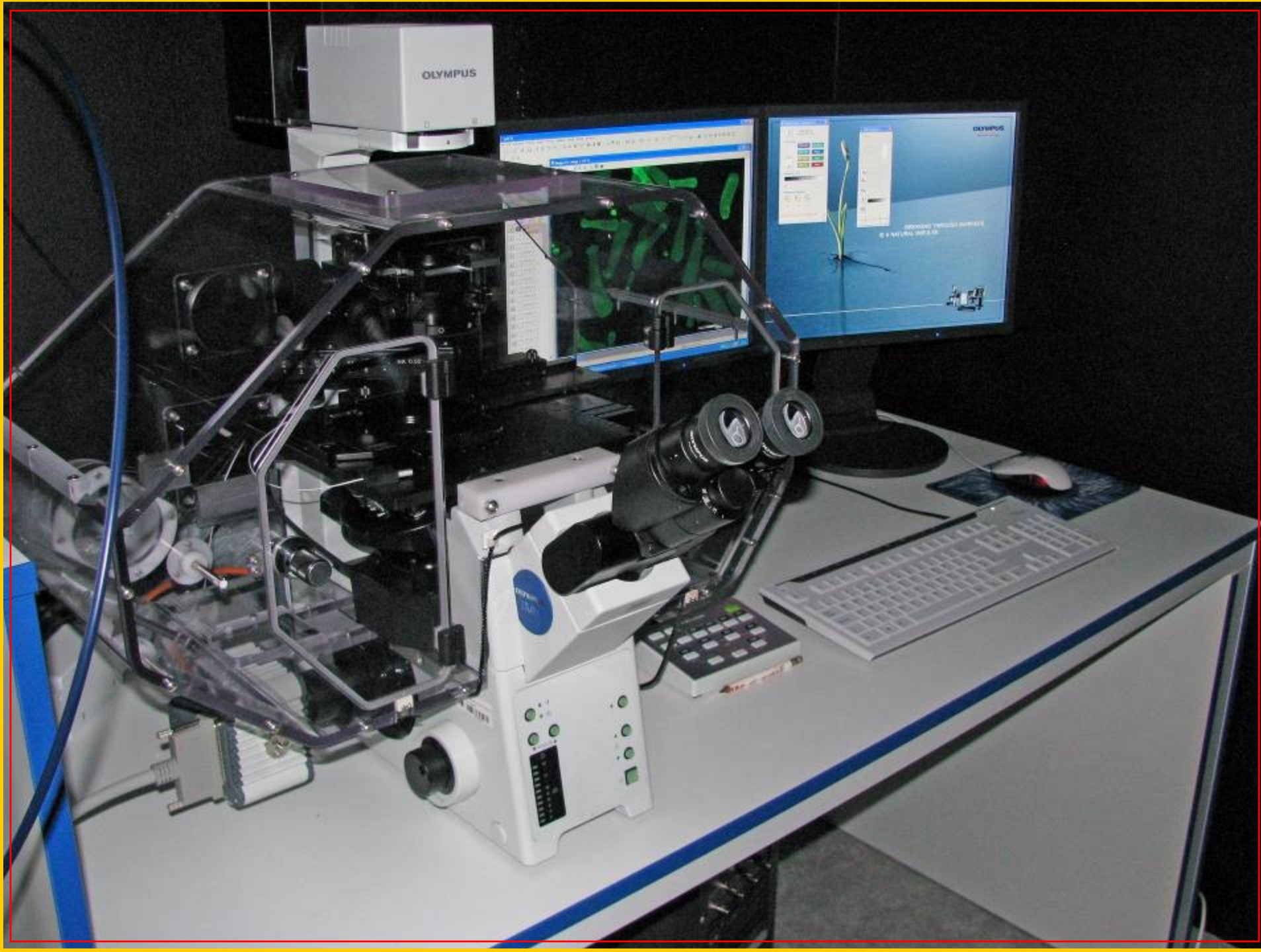
Fluorescent illumination

Autofluorescence
of nematodes

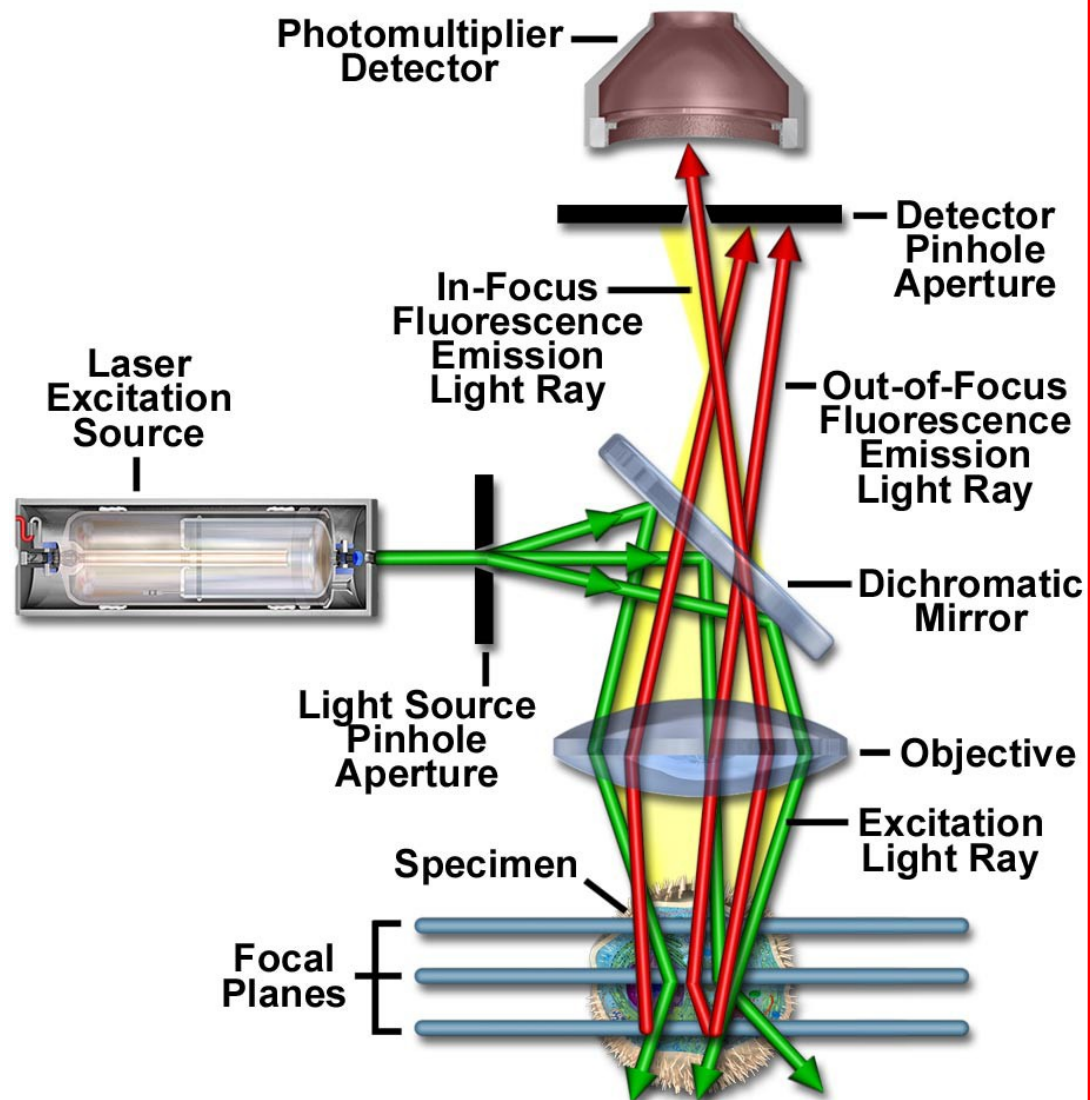
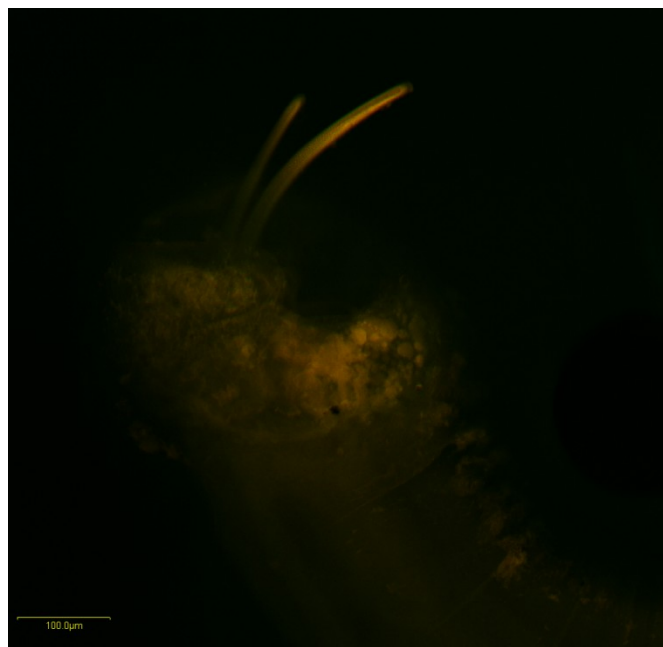


Olympus Cell^R - motorizovaný invertovaný mikroskop se systémem rychlé fluorescence pro sledování procesů v živých buňkách





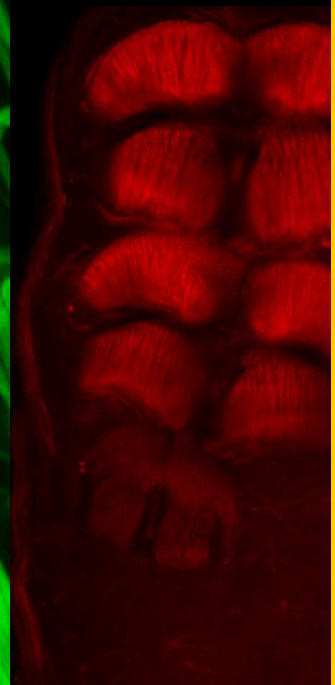
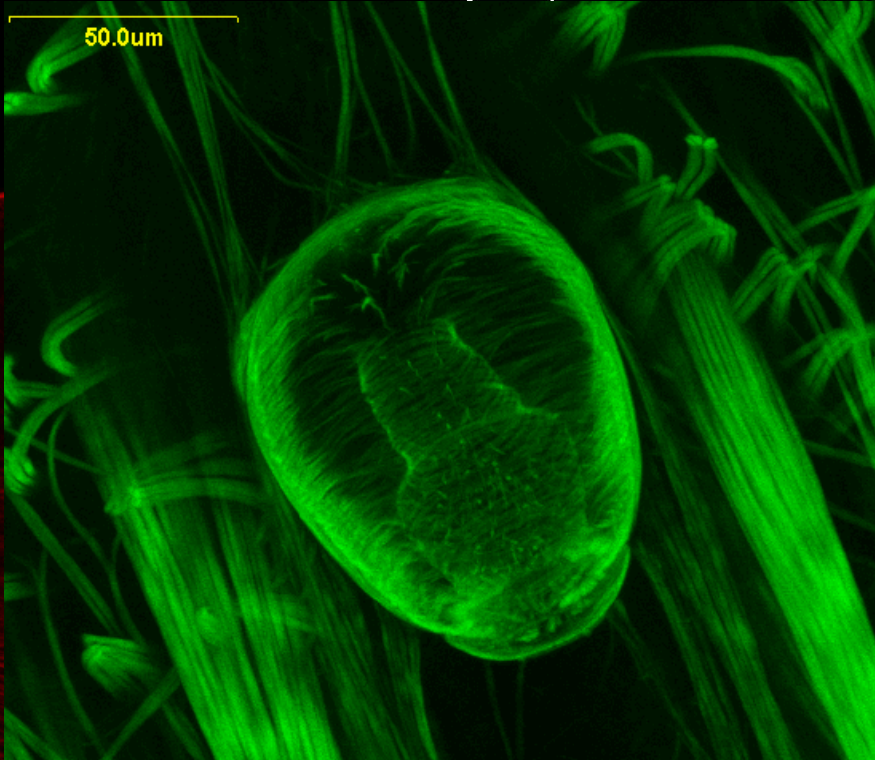
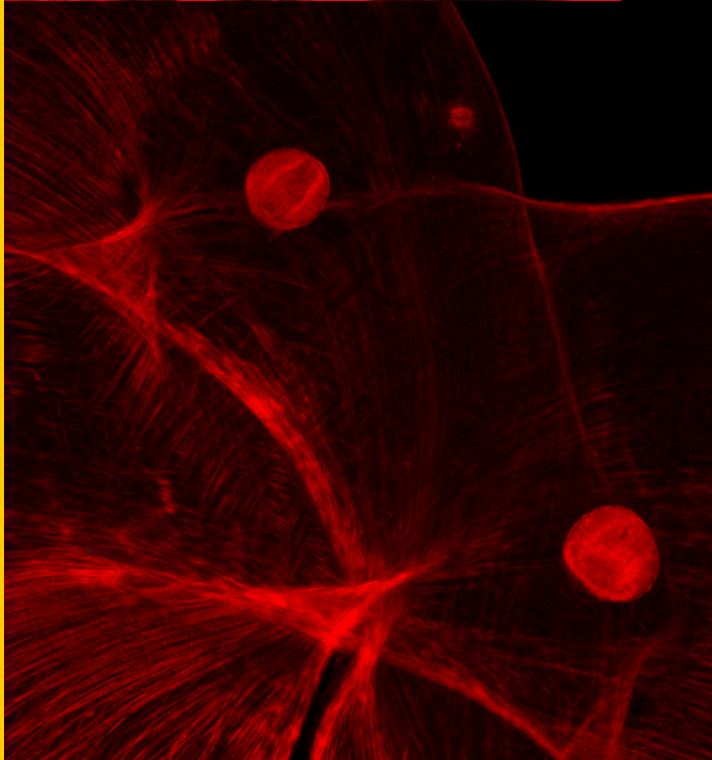
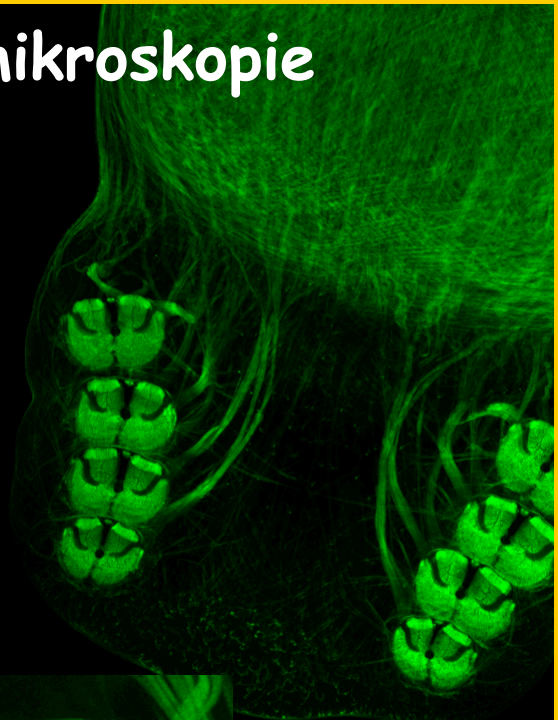
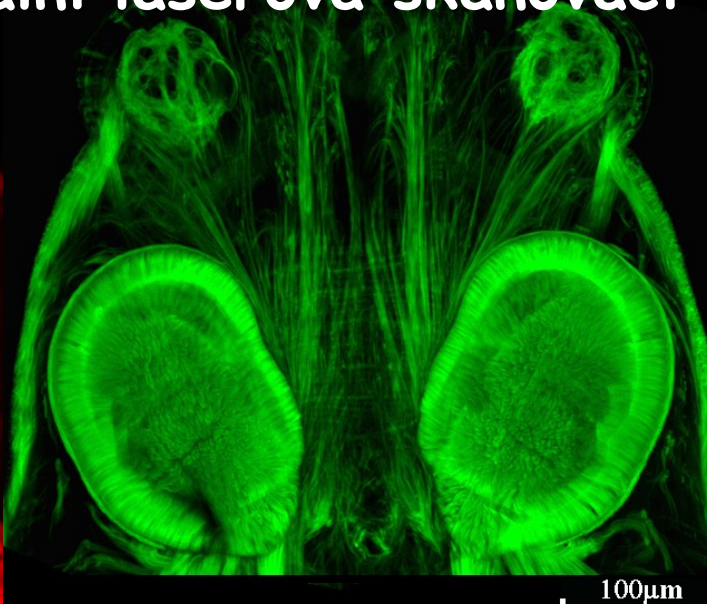
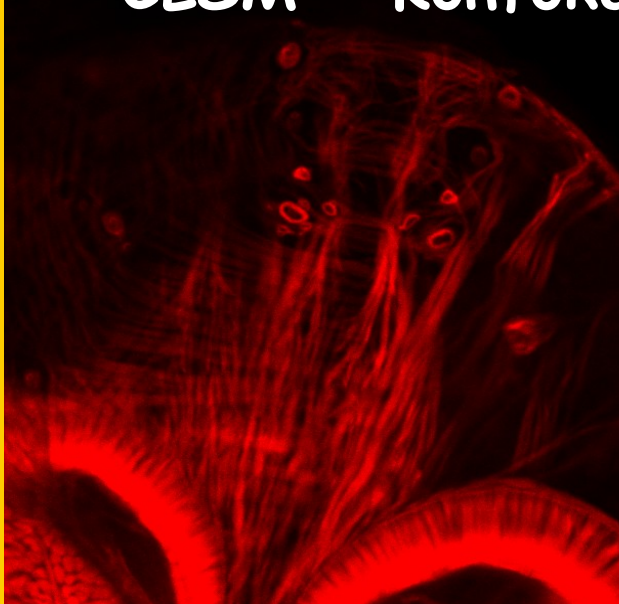
Confocal Laser Scanning Microscopy



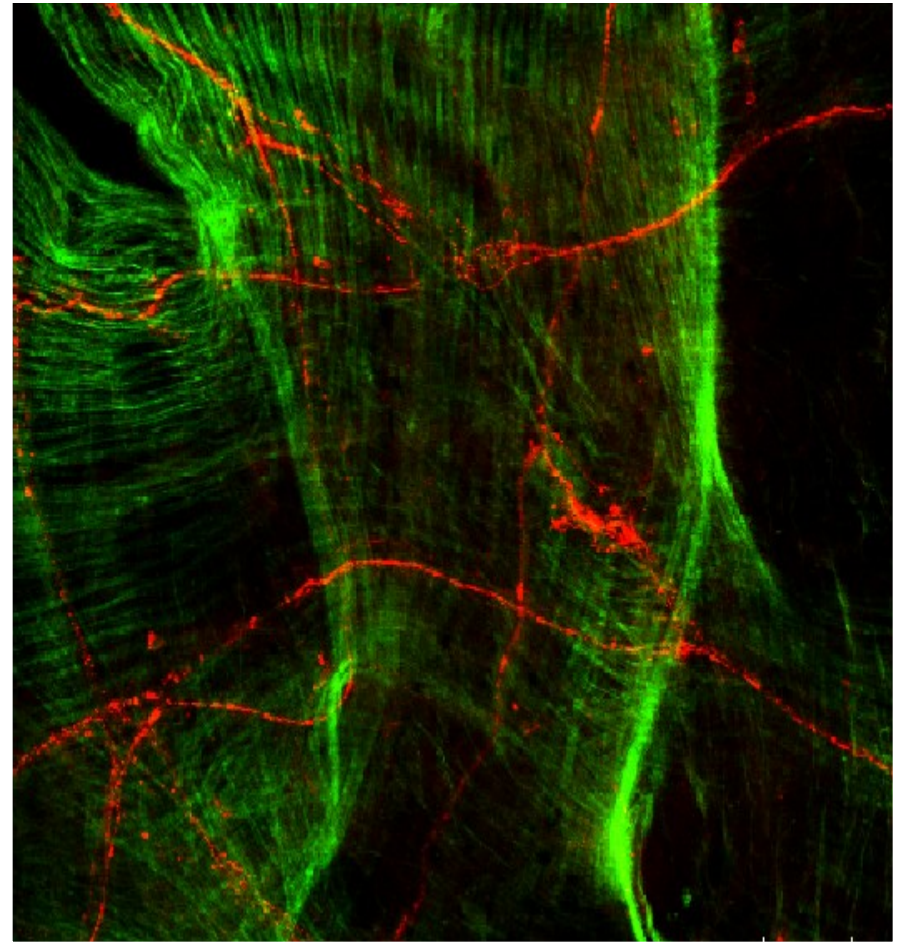
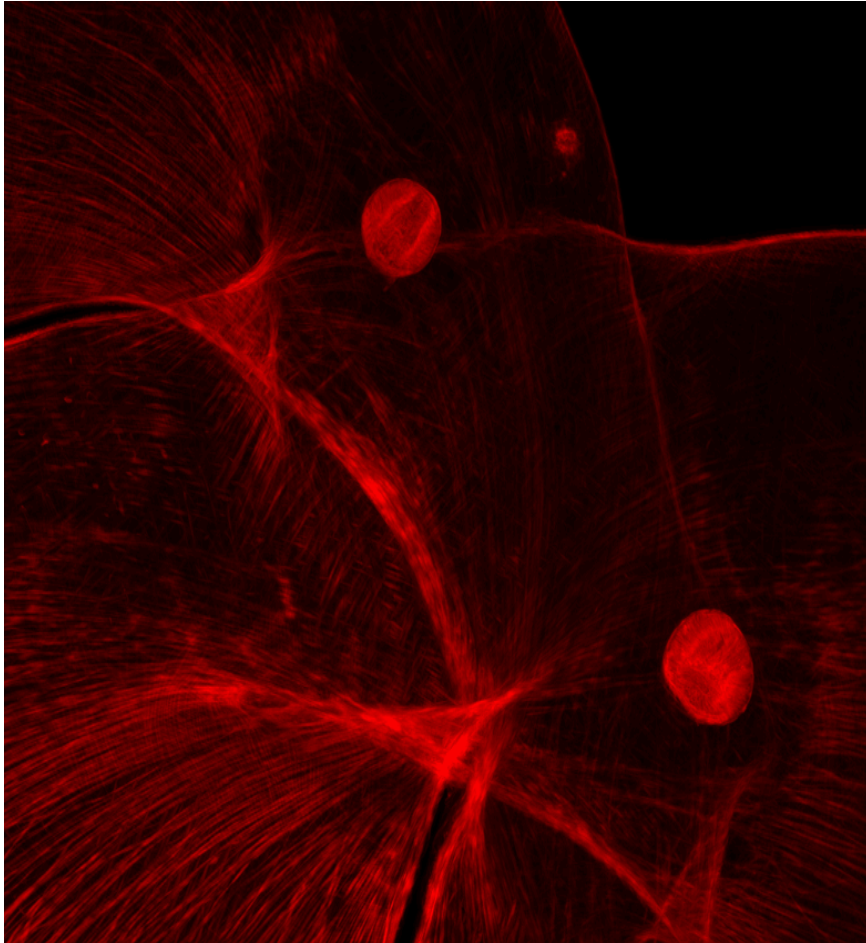
CLSM



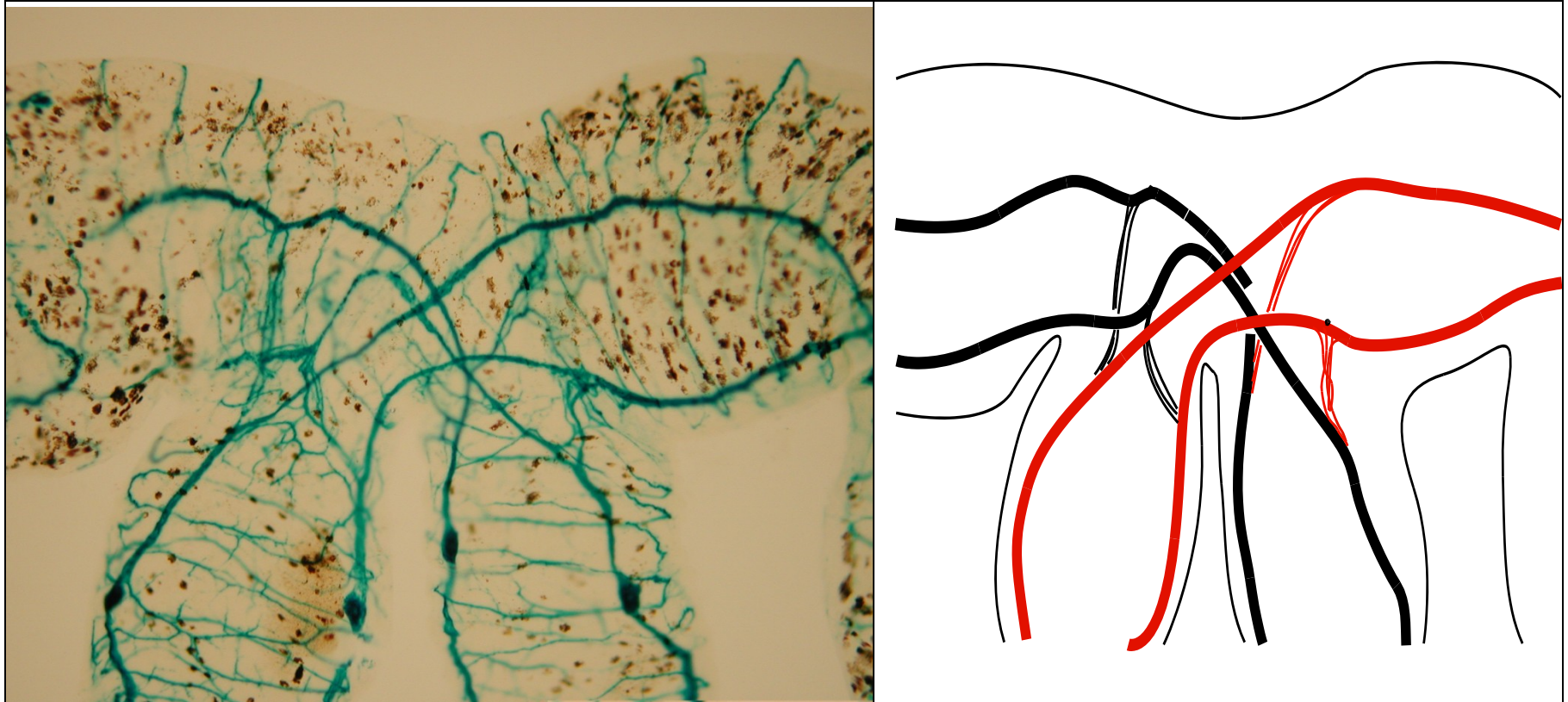
CLSM - Konfokální laserová skanovací mikroskopie



Možnost kombinace barvících technik



Schematic illustration of neuronal interspecific connectivity between 2 heterogenic CNS

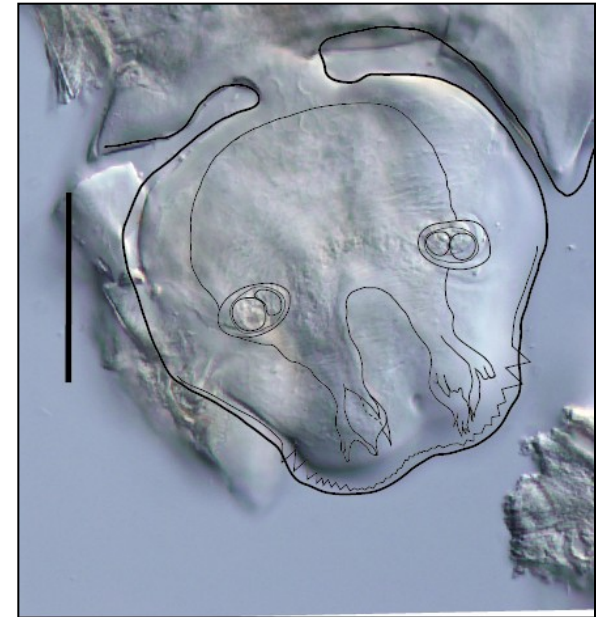
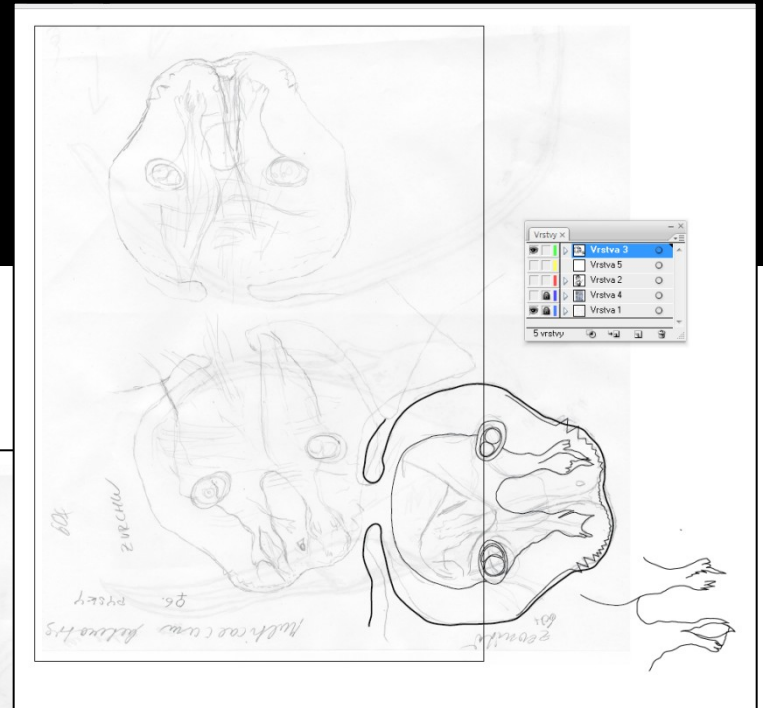
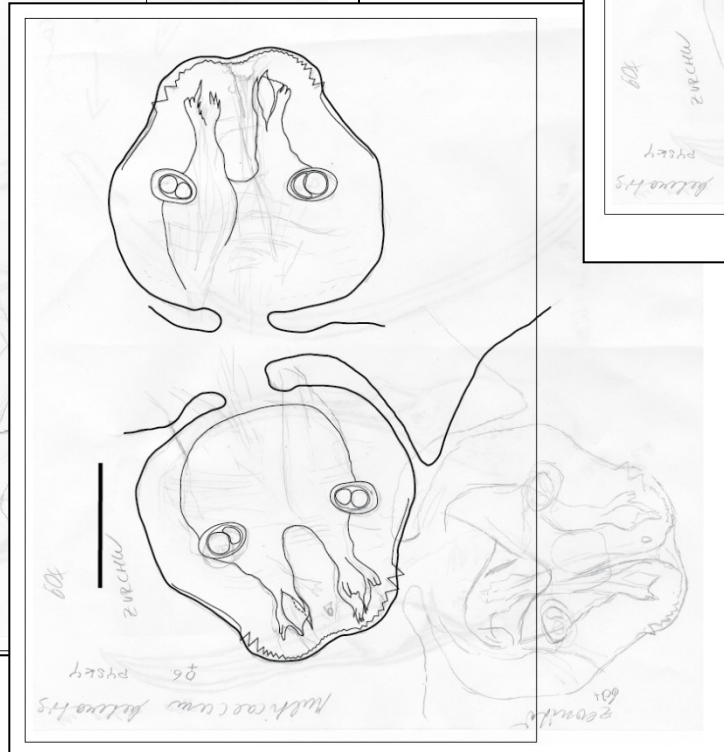


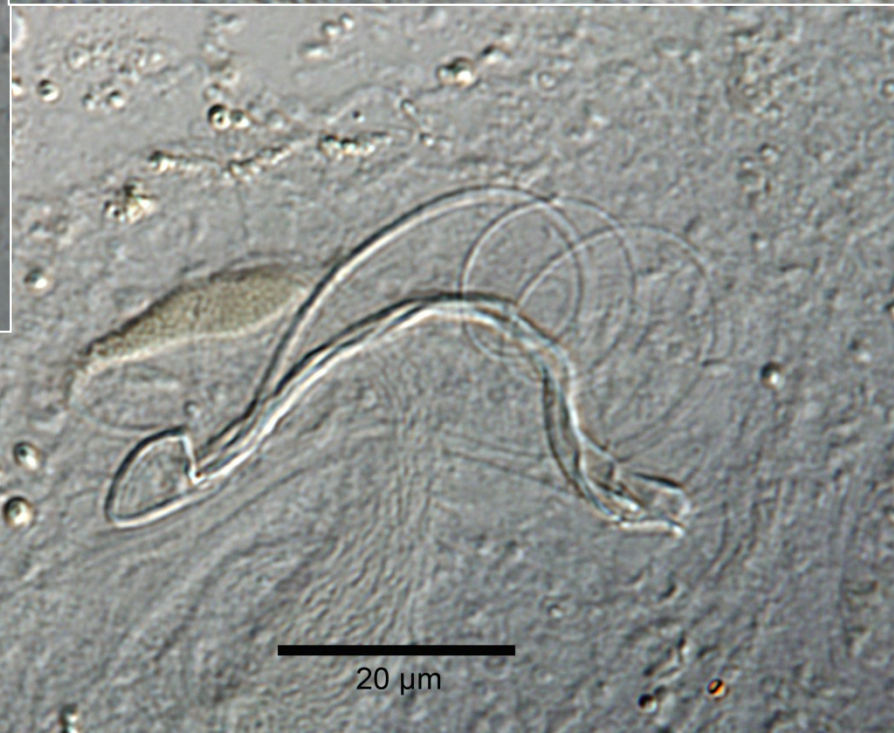
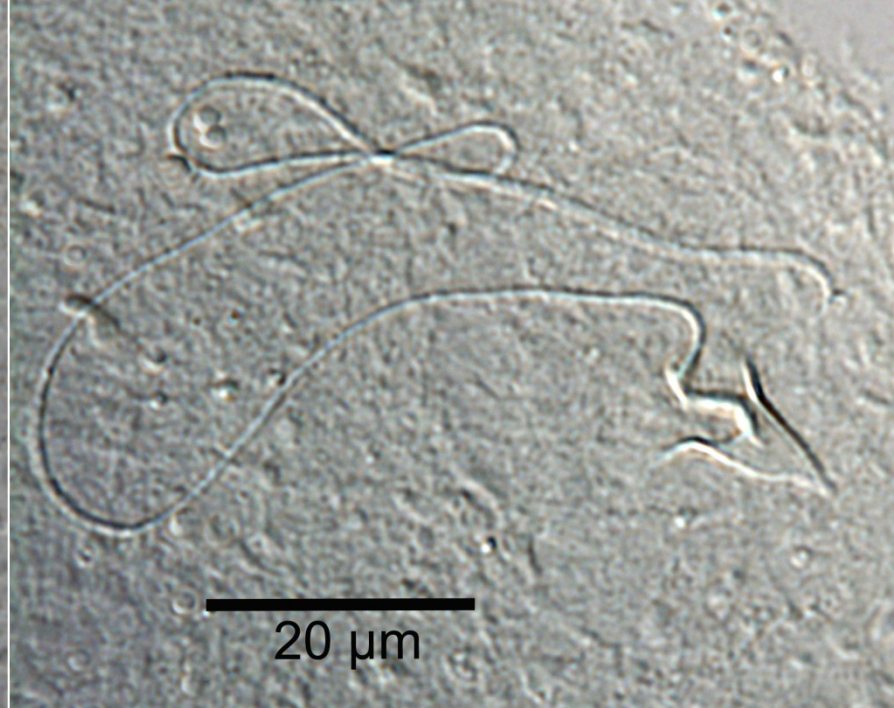
Zurawski T.H. et al., 2003: Microscopic evaluation of neural connectivity between paired stages of *Eudiplozoon nipponicum*. J. Parasitol 89:198-200

Line-drawings



Line-drawings

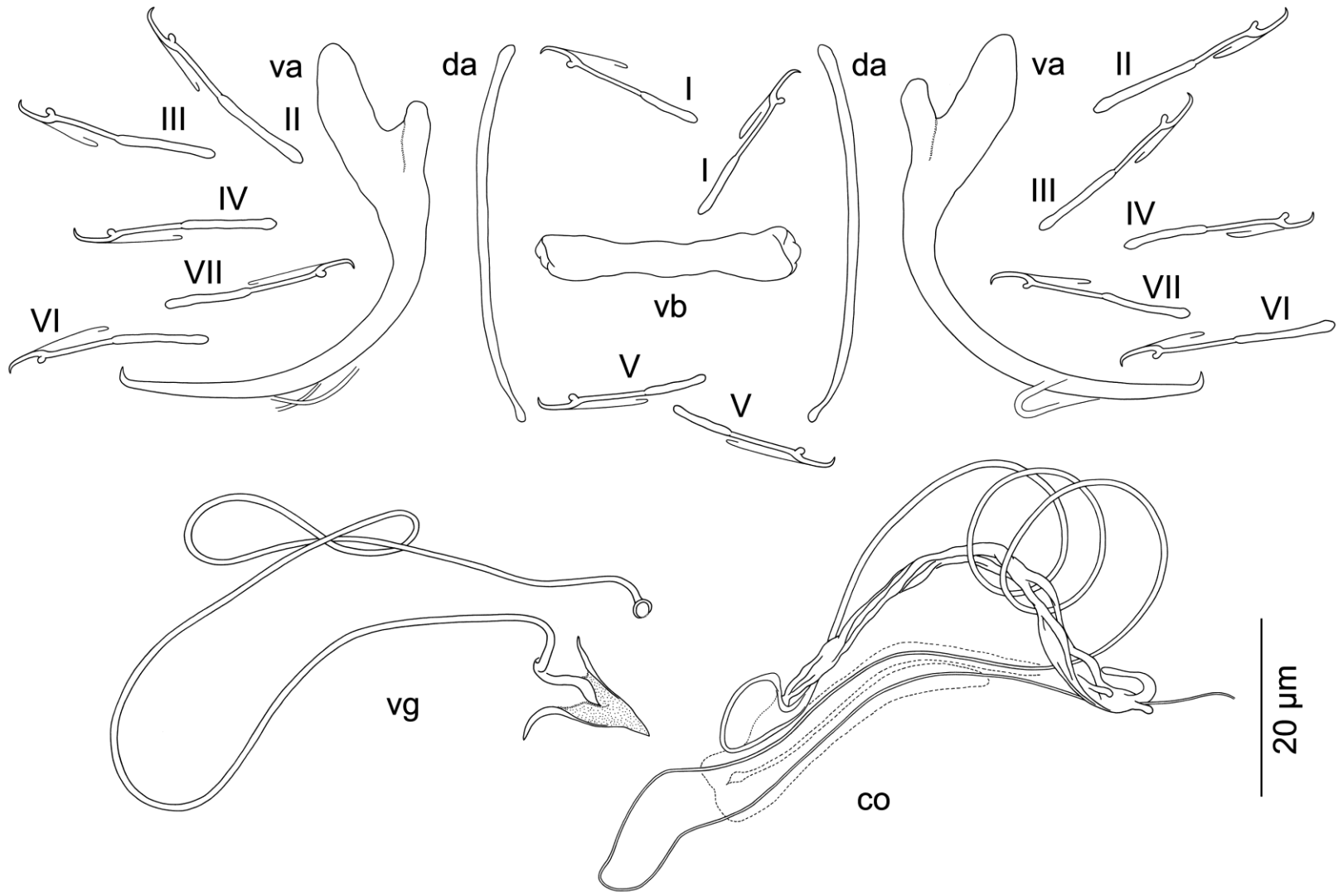




Nanotrema niokoloensis n.sp.

Host: *Citharinus citharus citharus*

Locality: Niokolo Koba River near Pont
Suspendu Niokolo-Koba National Park,
Senegal



Sclerotised structures of Nanotrema niokoloensis sp.nov.: va = ventral anchor, vb = ventral bar, da = dorsal anchor, I-VII = pairs of hooks, co = copulatory organ, vg = vagina

Sampling of parasite individual

Variety of monogenean body shapes and haptor morphology

Variety of types of scolexes of cestodes

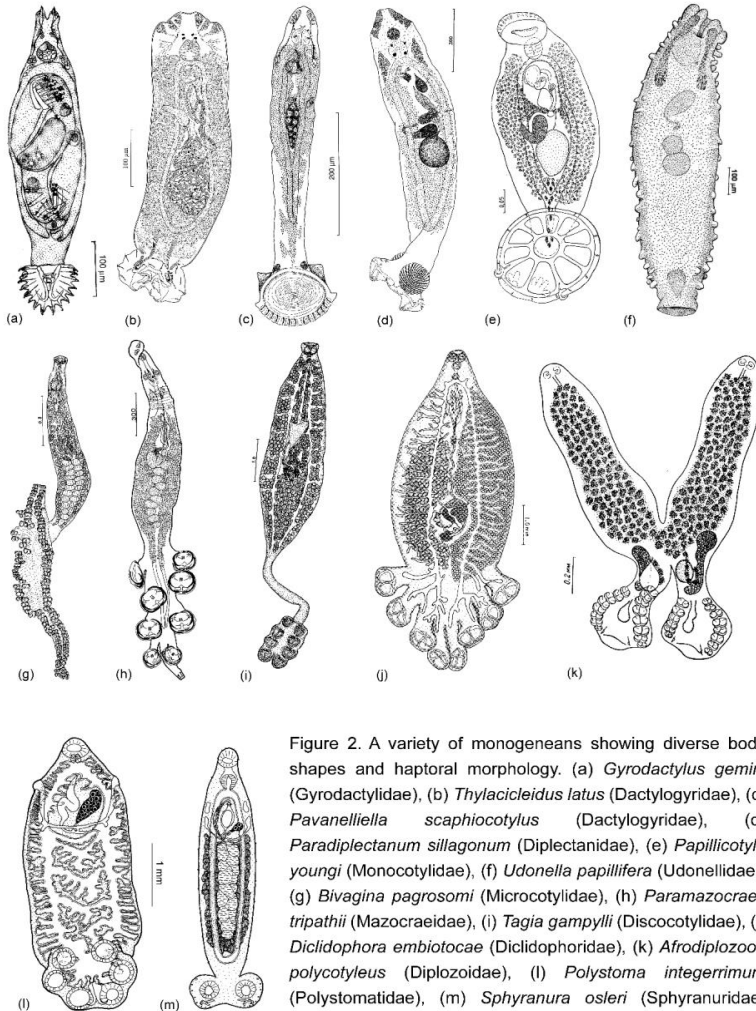
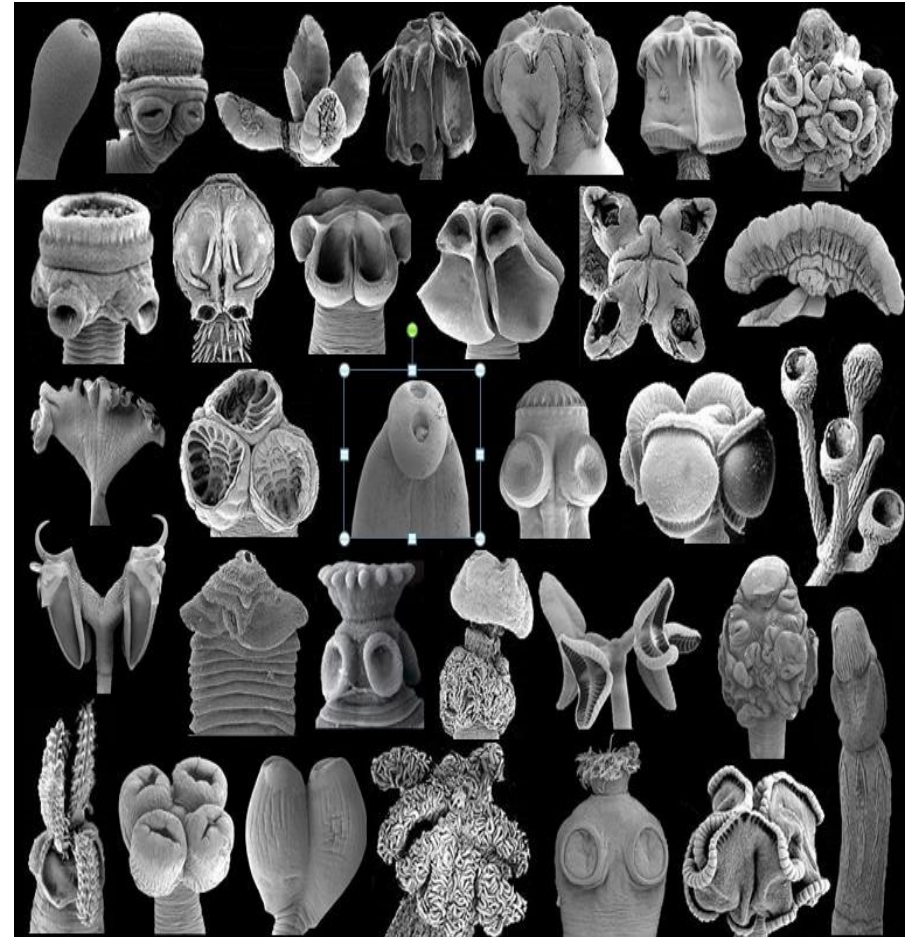
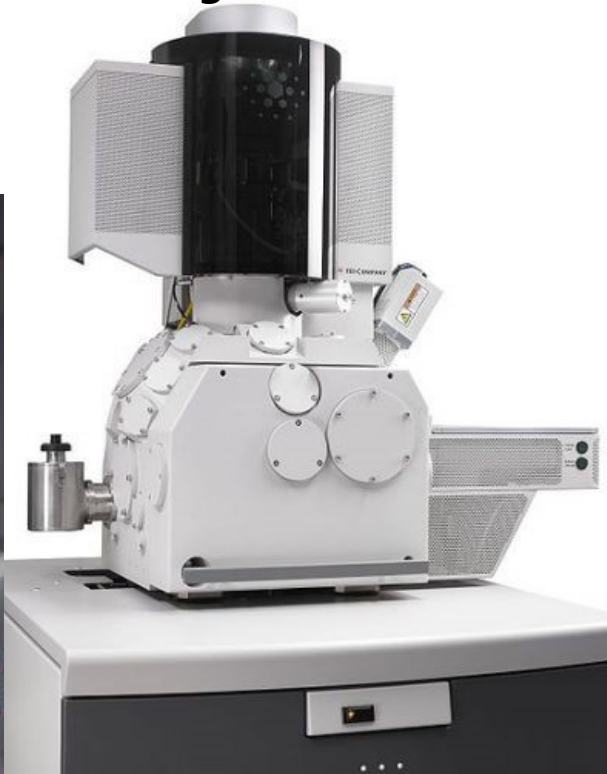


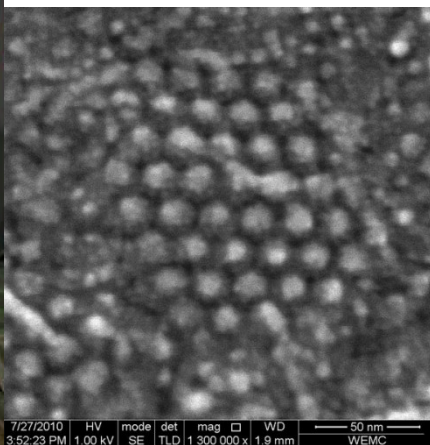
Figure 2. A variety of monogeneans showing diverse body shapes and haptor morphology. (a) *Gyrodactylus gemini* (Gyrodactylidae), (b) *Thylacicleidus latus* (Dactylogyridae), (c) *Pavanelliella scaphiocotylus* (Dactylogyridae), (d) *Paradiplectanum sillagonum* (Diplectanidae), (e) *Papillicotyle youngi* (Monocotylidae), (f) *Udonella papillifera* (Udonellidae), (g) *Bivagina pagrosomi* (Microcotylidae), (h) *Paramazocraes tripathii* (Mazocraeidae), (i) *Tagia gampylli* (Discocotylidae), (j) *Dicliphora embiotocae* (Diclidophoridae), (k) *Afrodiplozoon polycotyleus* (Diplozoidae), (l) *Polystoma integerrimum* (Polystomatidae), (m) *Sphyranura osleri* (Sphyranuridae)



Ultra High Resolution SEM



Magellan (FEI).

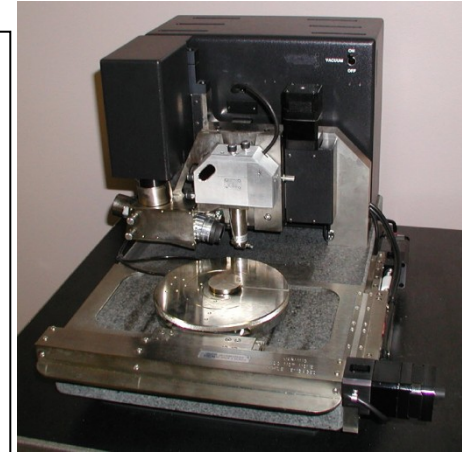


resolutions
below one
nanometer

7/27/2010 HV mode det mag □ WD 50 nm
3:52:23 PM 1.00 kV SE TLD 1 300 000 x 1.9 mm WEMC

www.fei.com

AFM



Example of AFM image is shown below where the shape of single DNA and protein molecules are seen. (<http://nano.uib.no/AFM.php>)

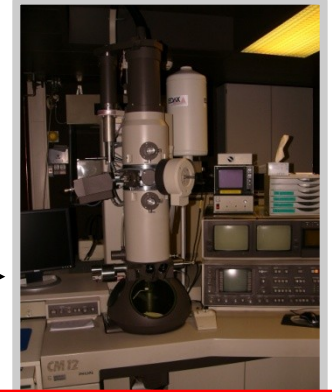
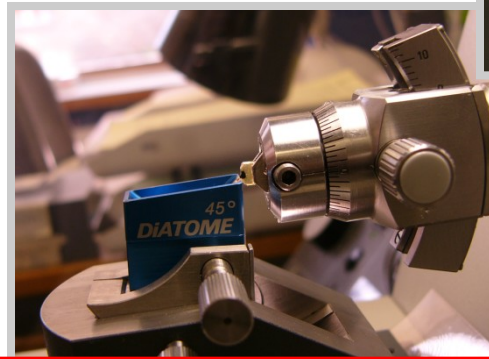
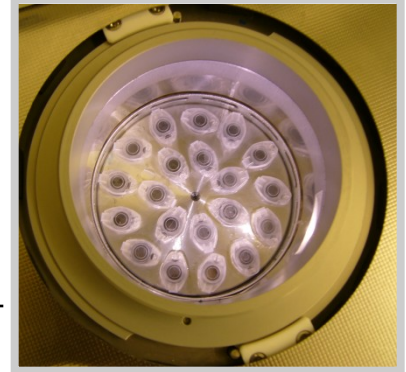
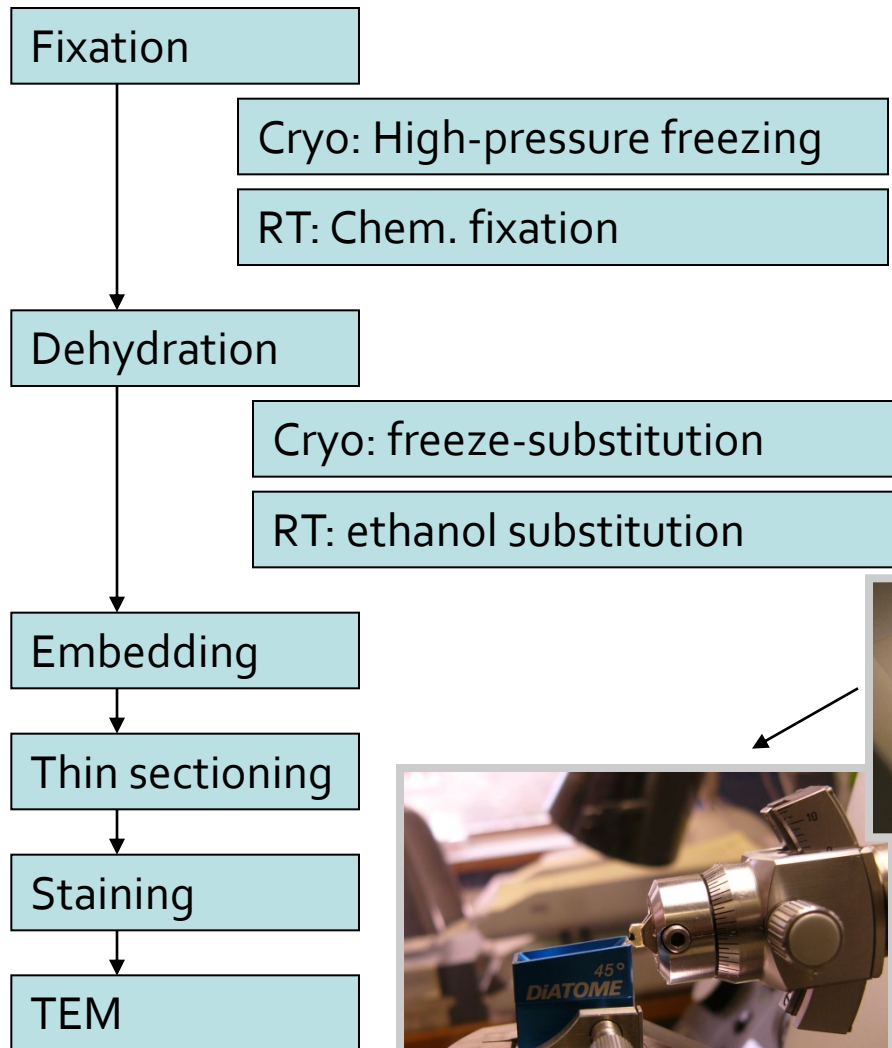


AFM determines the topology of a surface with a resolution down to 0.8 nm.

Electron microscopy

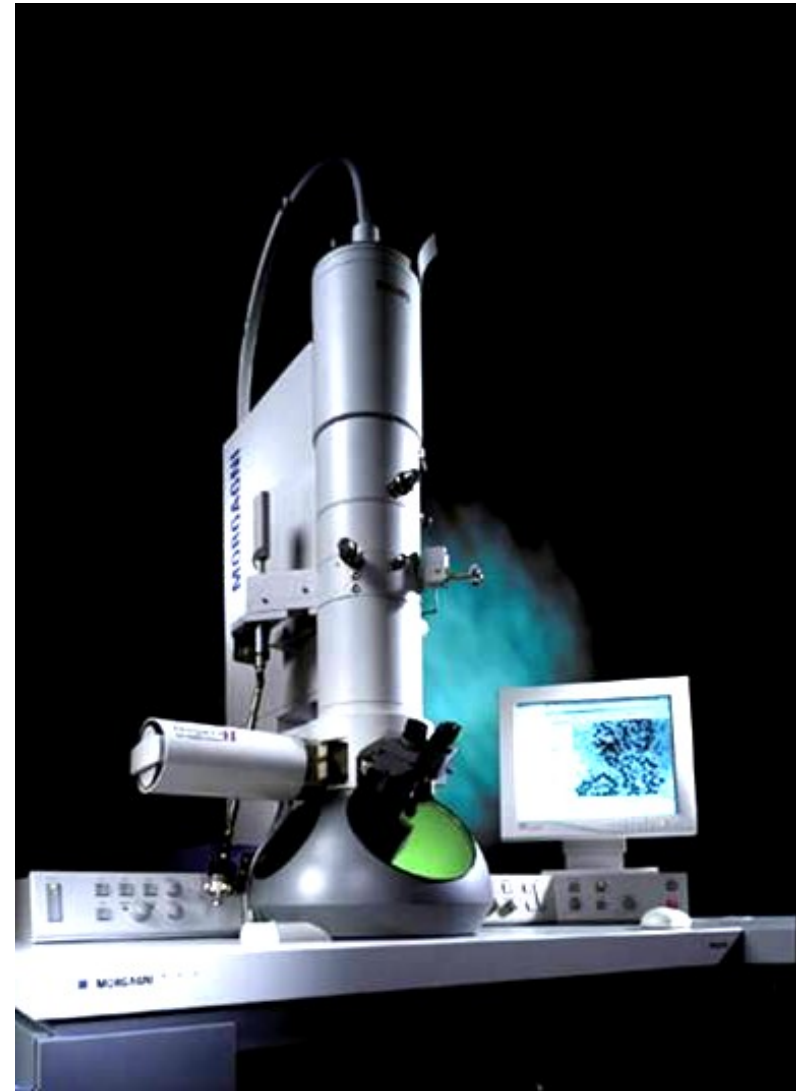
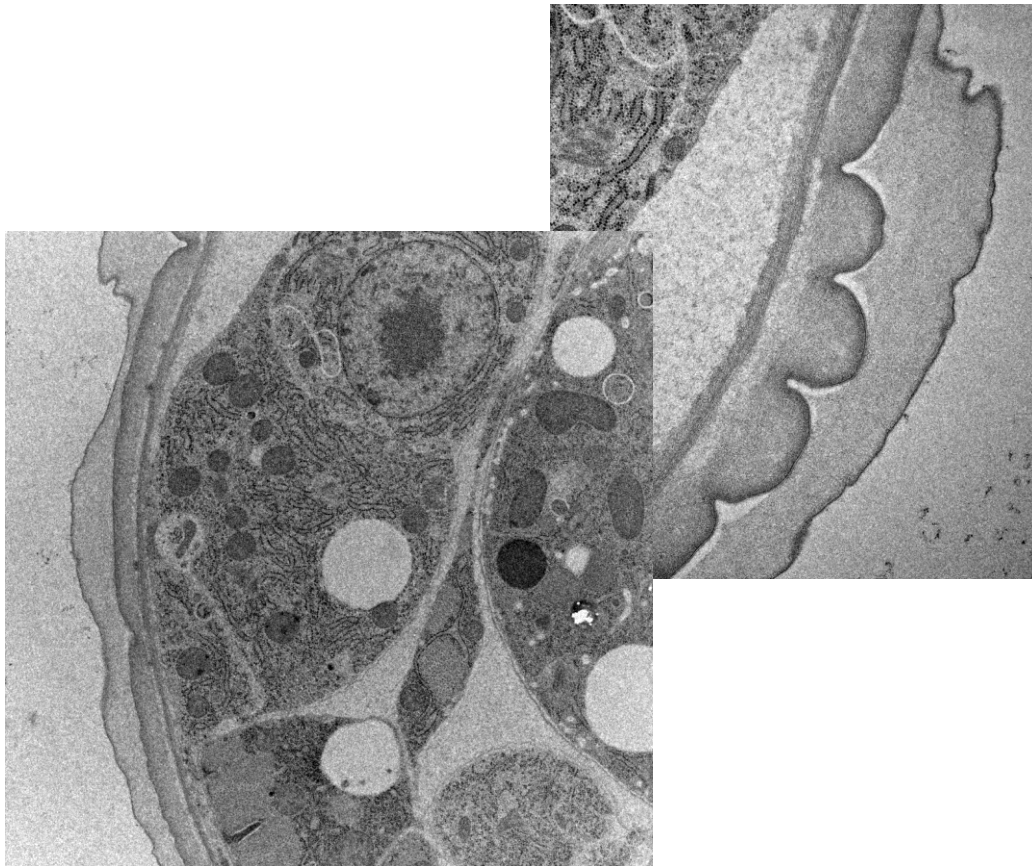
- Transmission electron microscopy (TEM)
- Scanning electron microscopy (SEM)
- Environmental scanning electron microscopy (ESEM)

TEM - Sample preparation

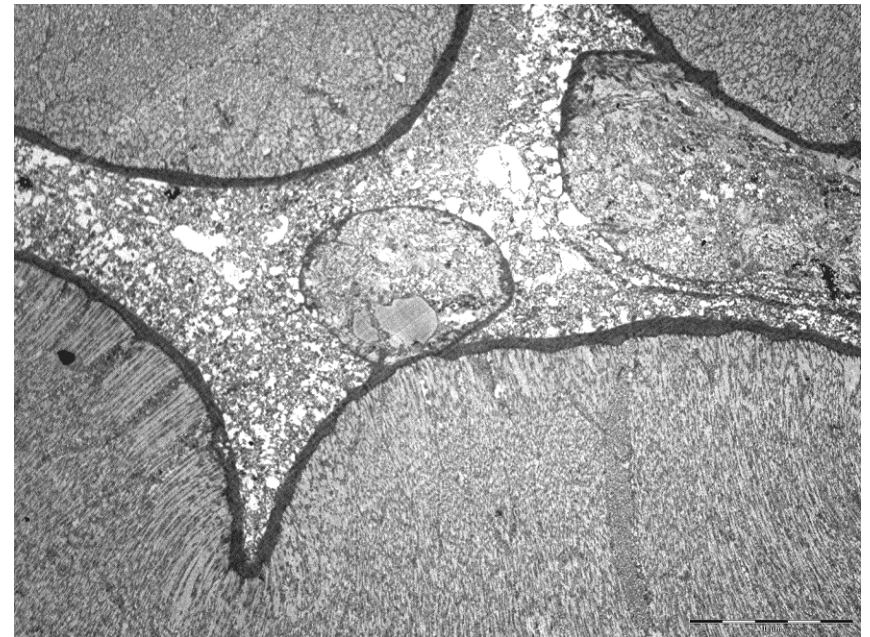
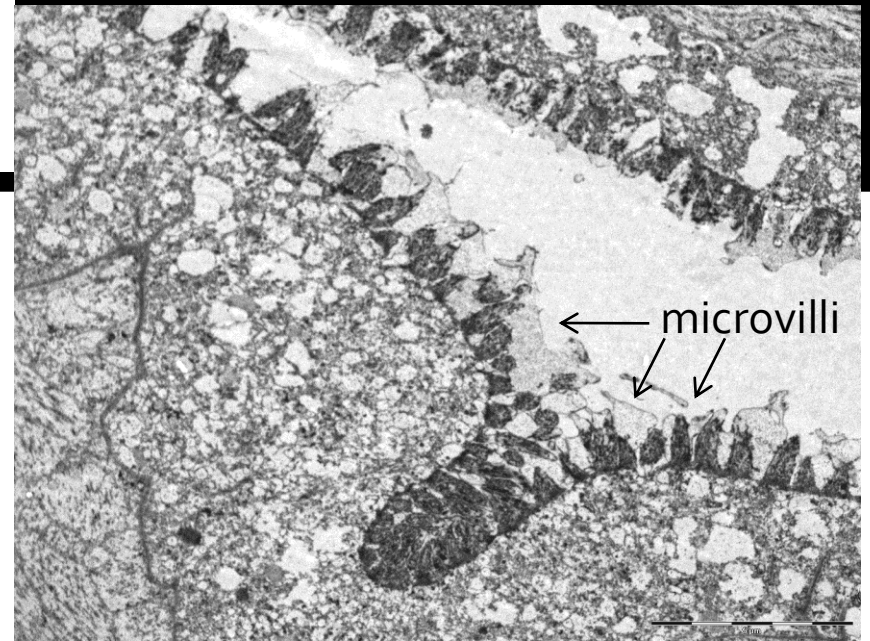
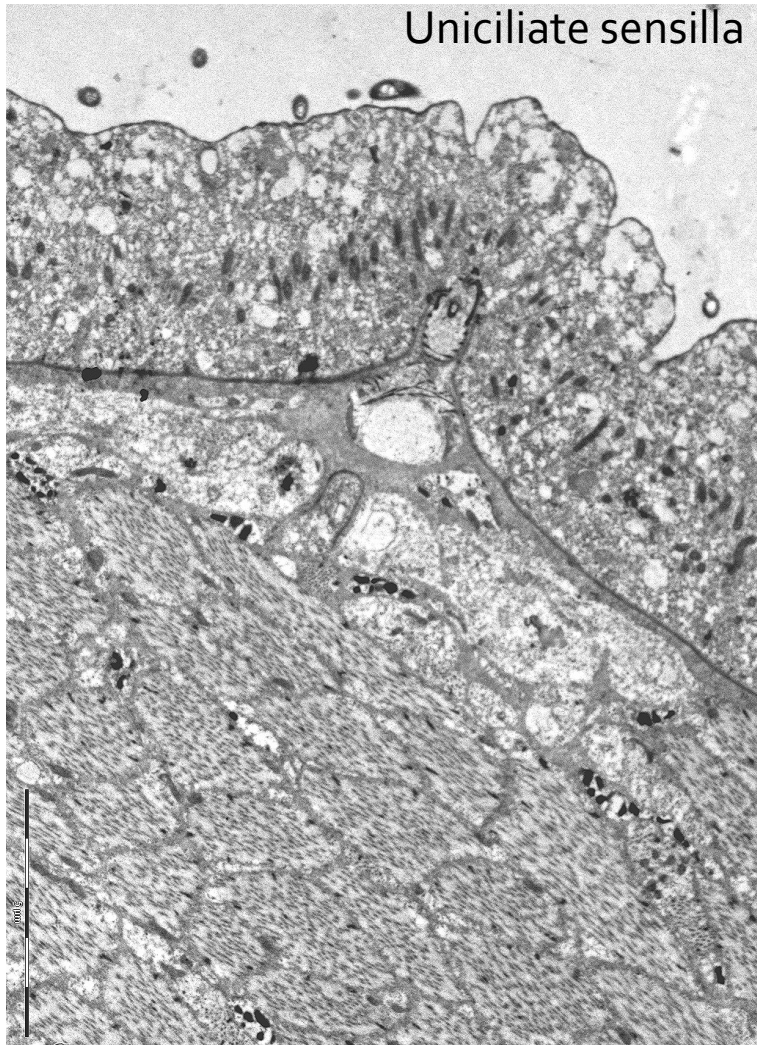


TEM

- Philips Morgagni



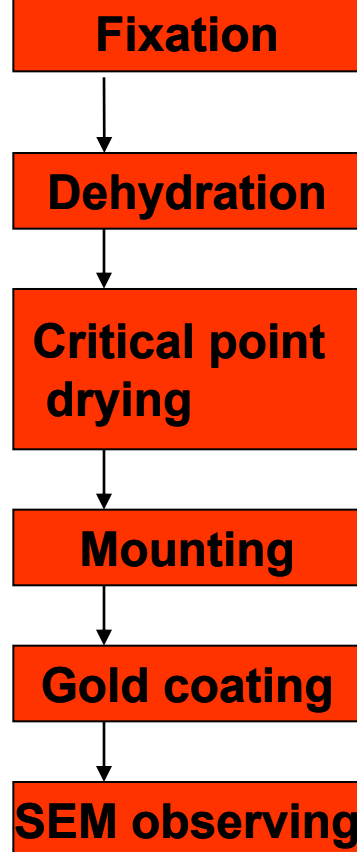
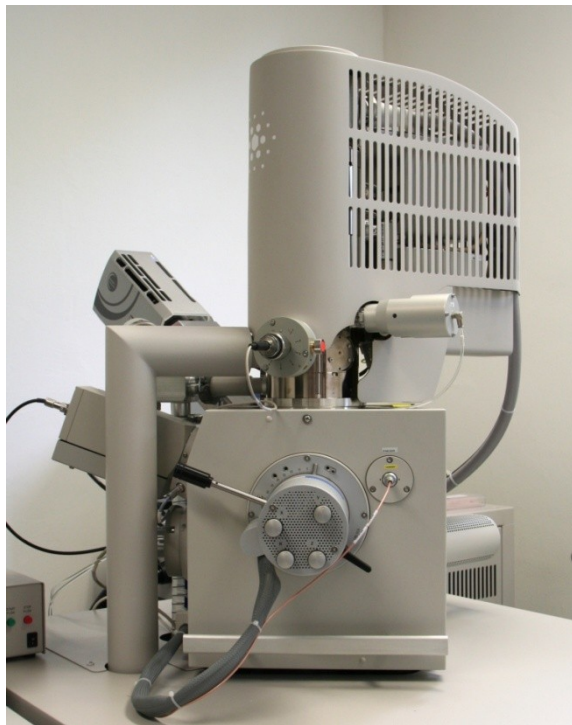
TEM – *E. nipponicum*



Muscle tissue

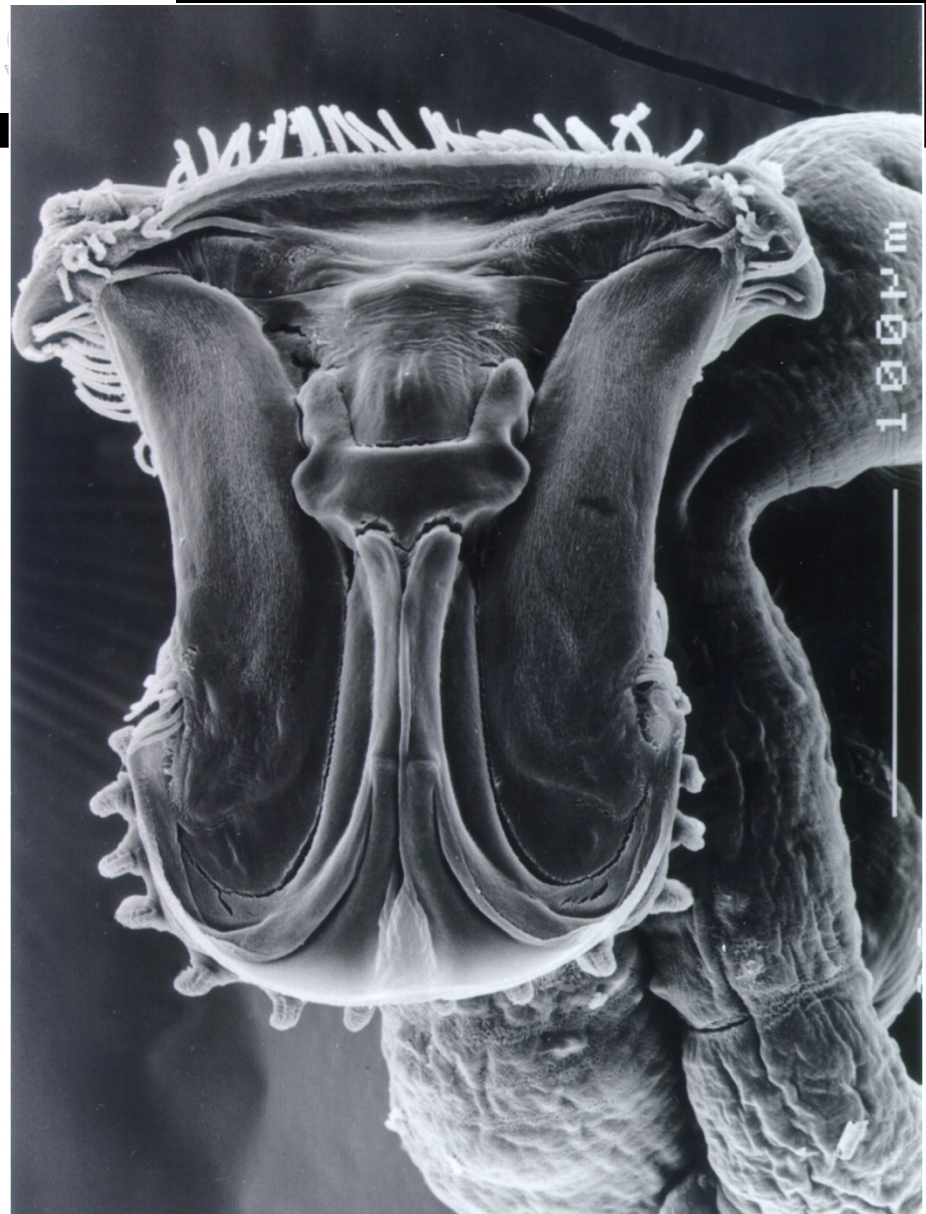
SEM

- FEI Quanta™ 3D FEG

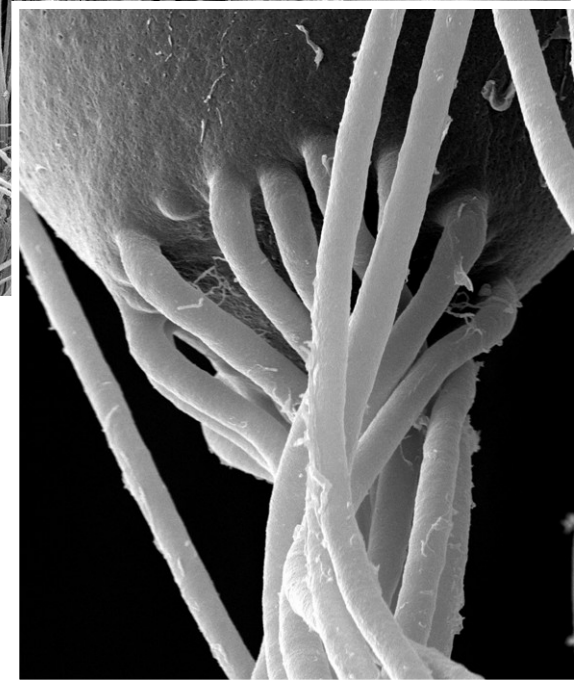
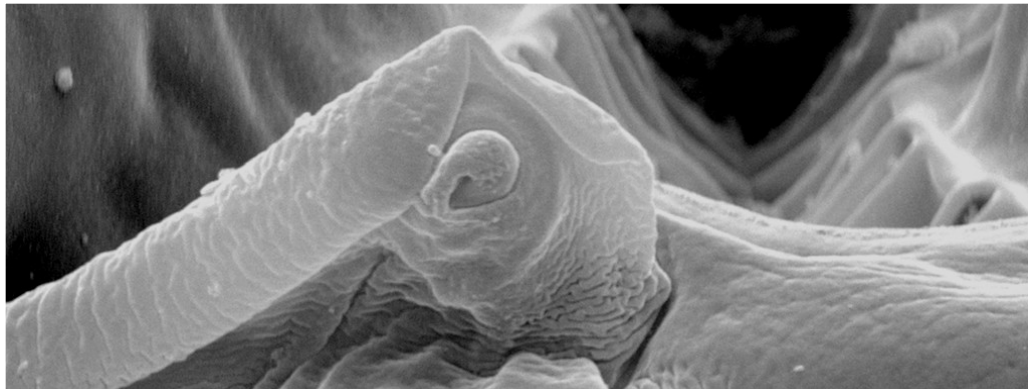
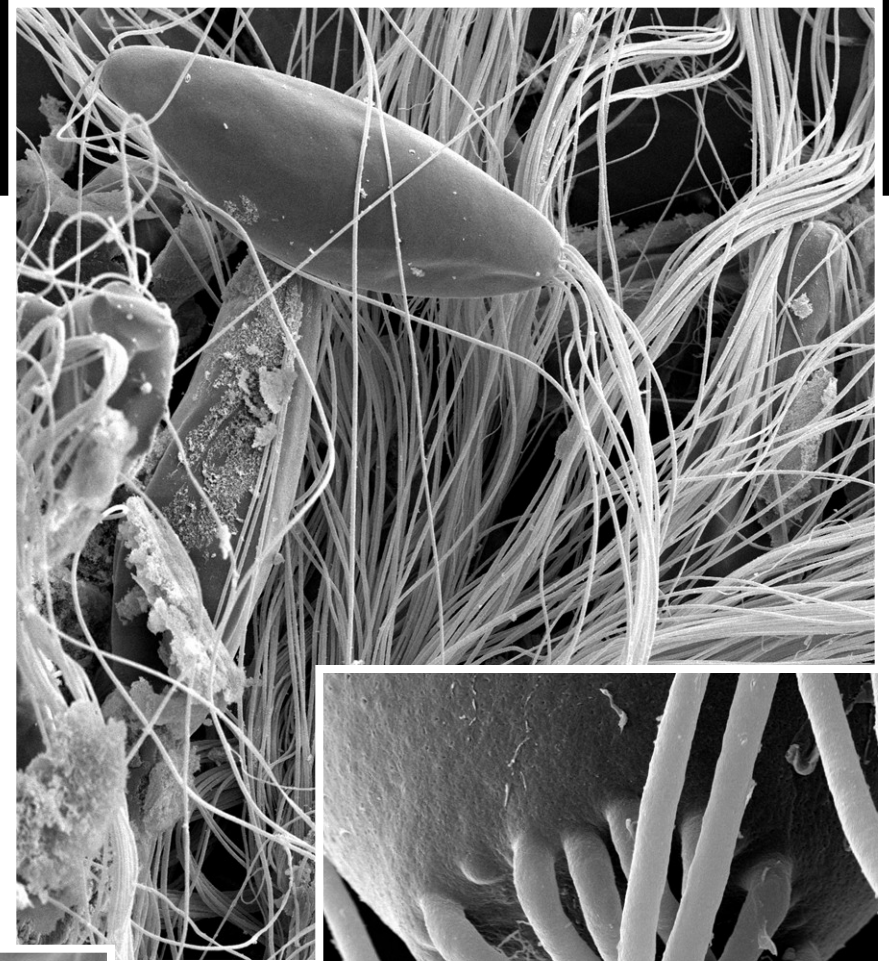
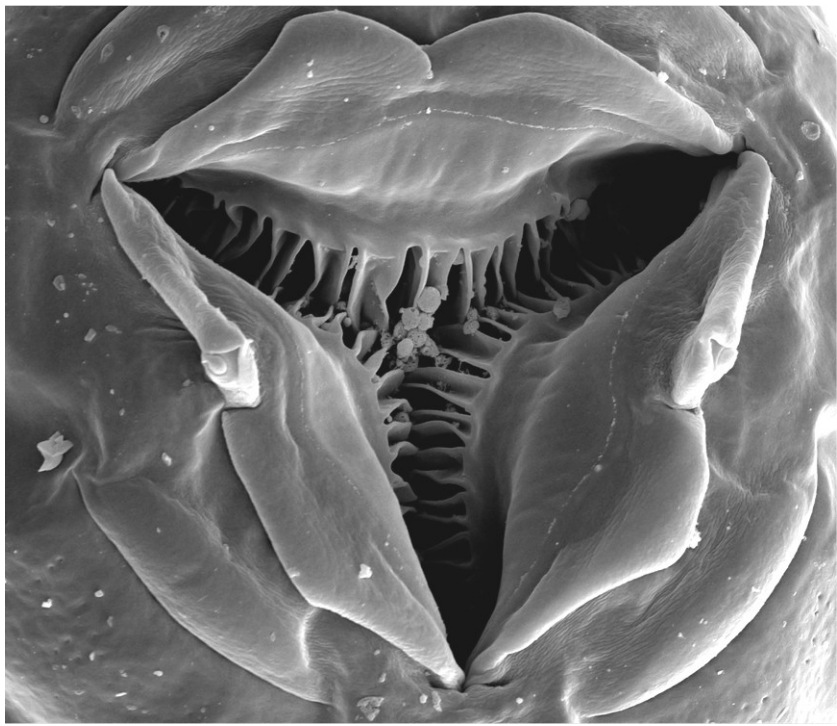


■ tescan vega and Mira

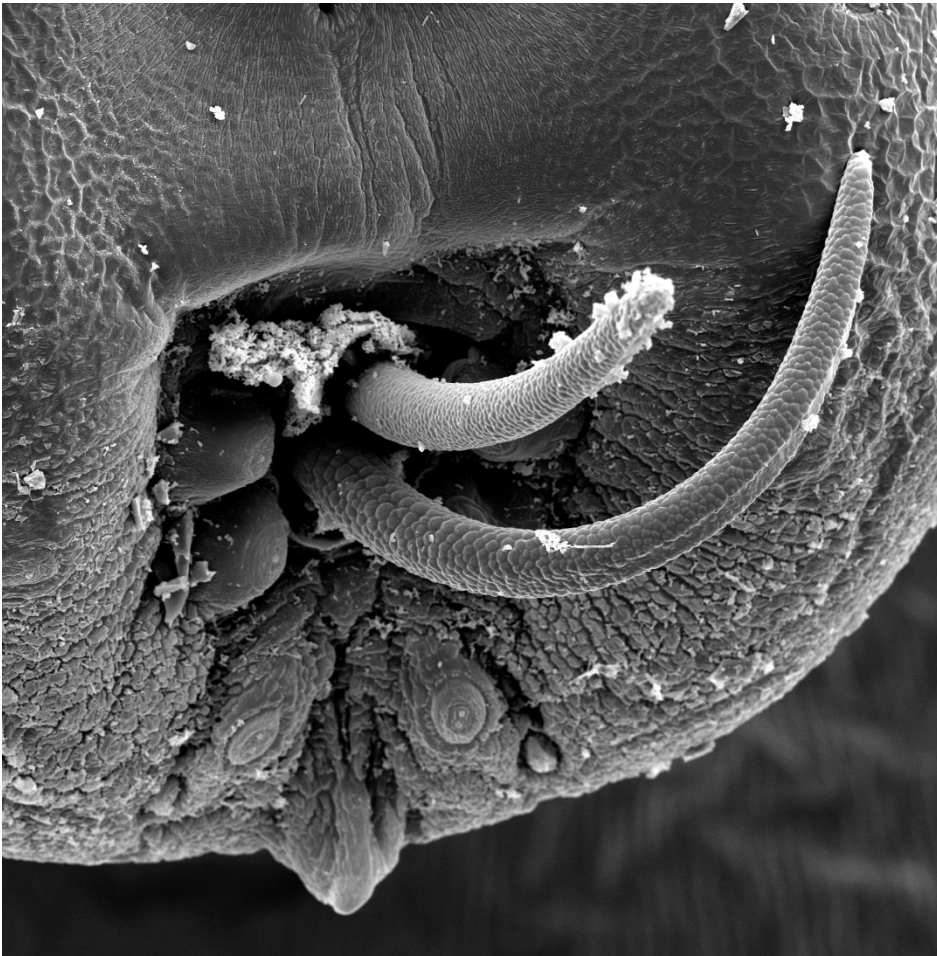
SEM - example



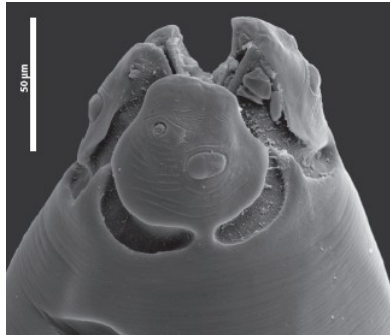
Cithariniella
khalili



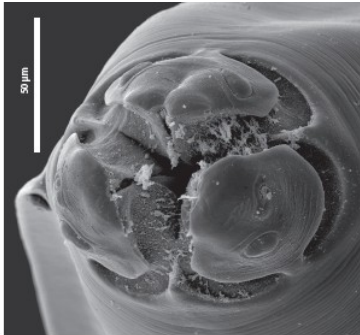
Strongyluris brevicaudata



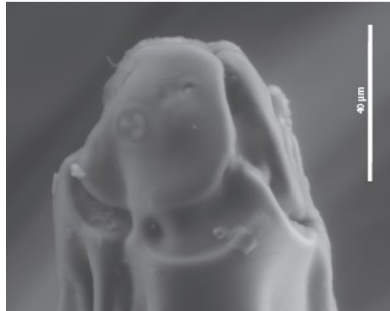
ESEM



SEM, lateral view, cephalic end



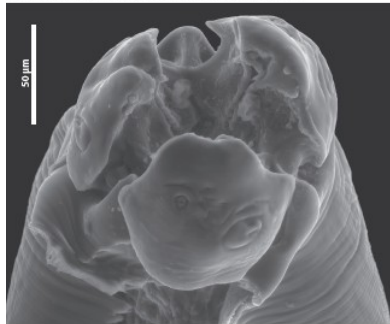
SEM, apical view, cephalic end



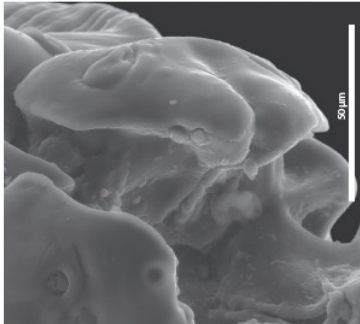
ESEM, lateral view, cephalic end with remains of water



ESEM, lateral view, cephalic end submerged in the water



ESEM, cephalic end



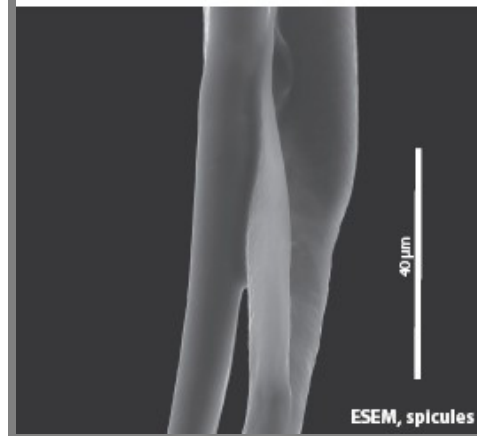
ESEM, dorsal lip with denticles



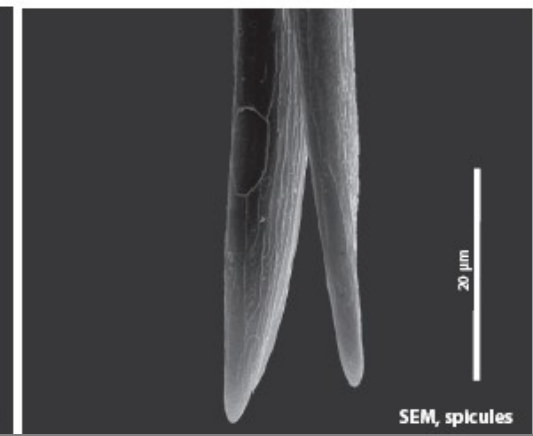
SEM, caudal part of male



ESEM, caudal part of male



ESEM, spicules

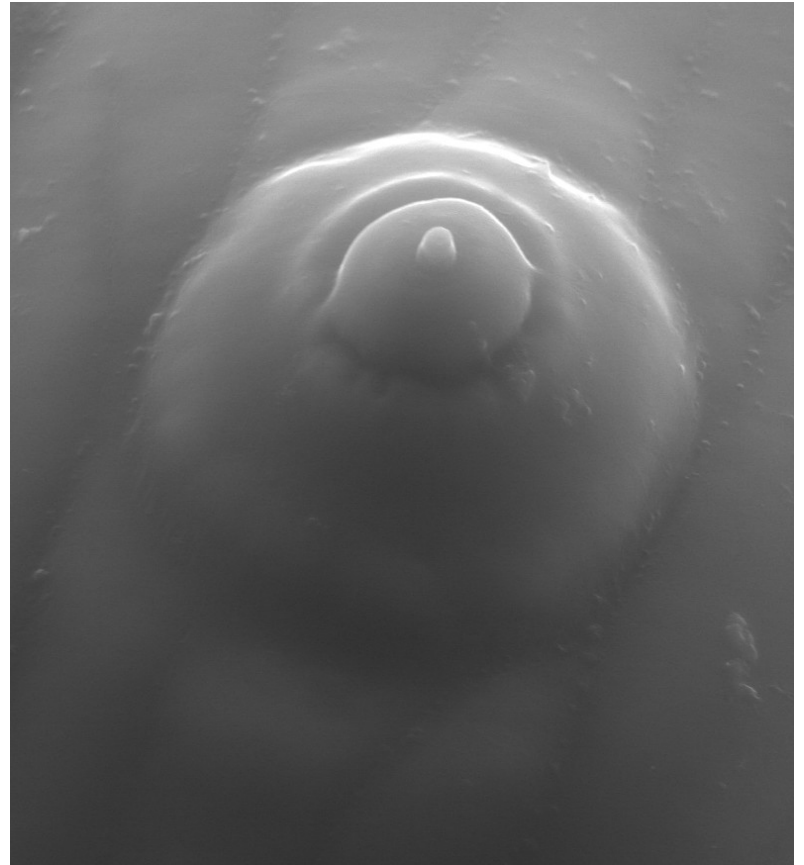
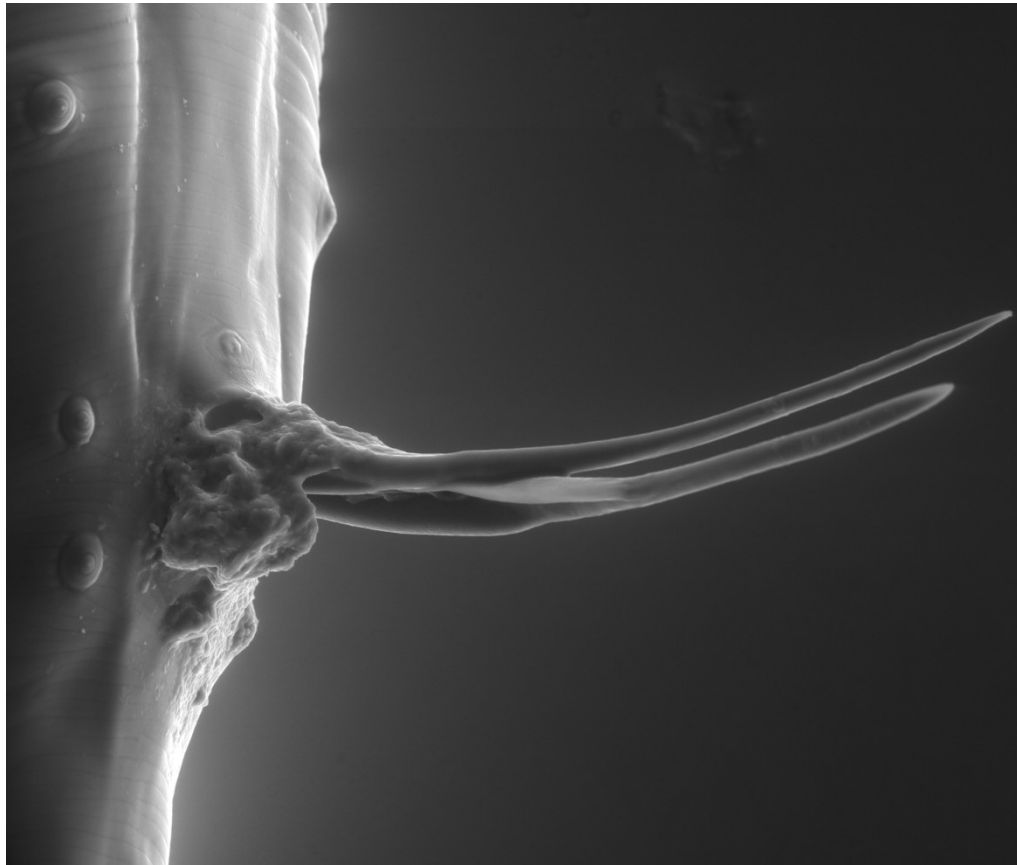


SEM, spicules

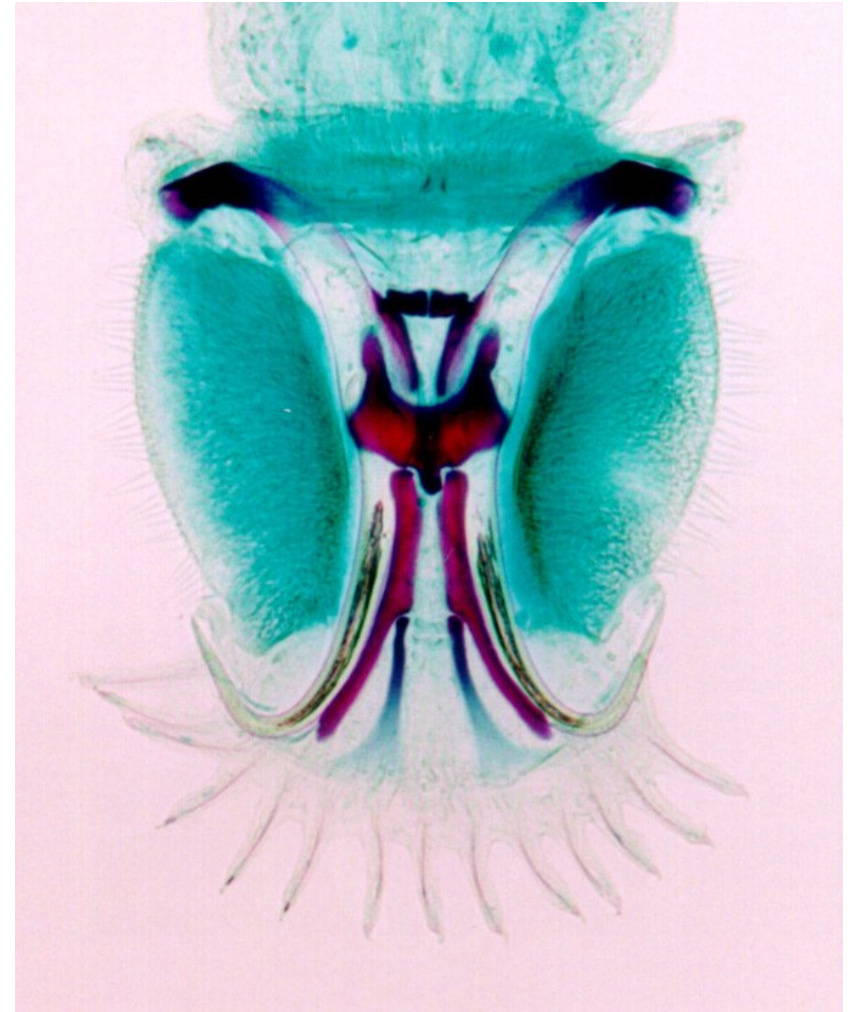
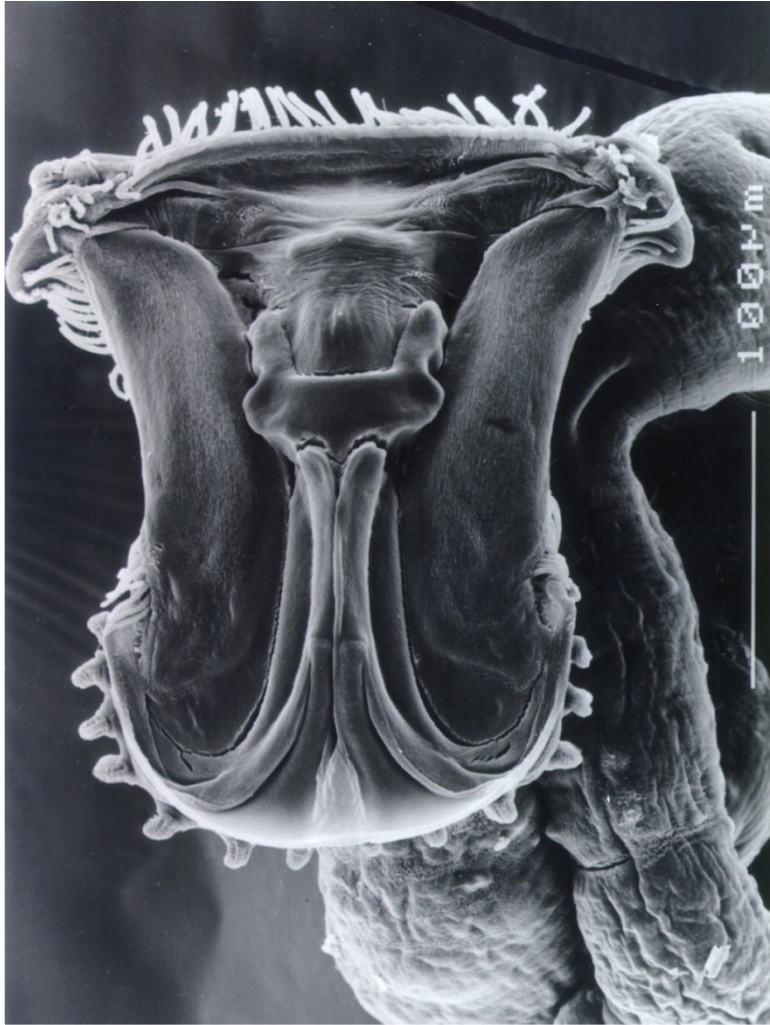
ESEM & SEM
comparison

Multicaecum heterotis

ESEM -examples



Combinations of techniques

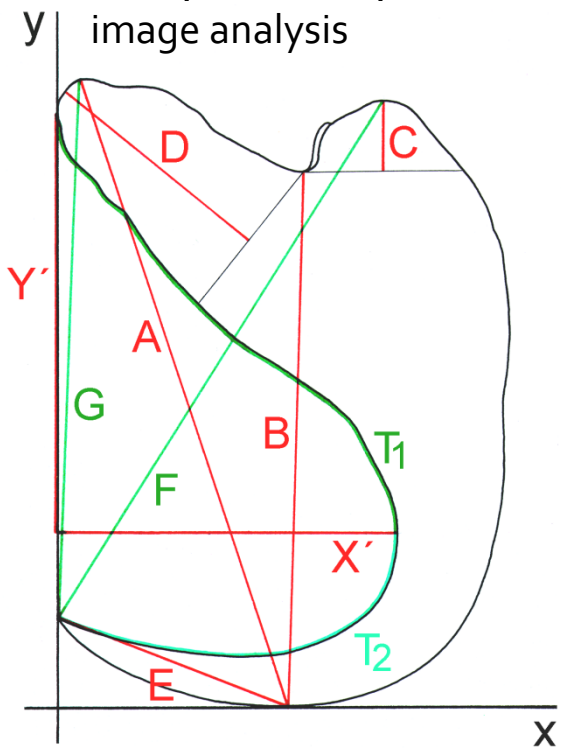


Macrogyrodactylus polypteri Malmberg, 1957

Dokumentace monogeneí

Phase contrast

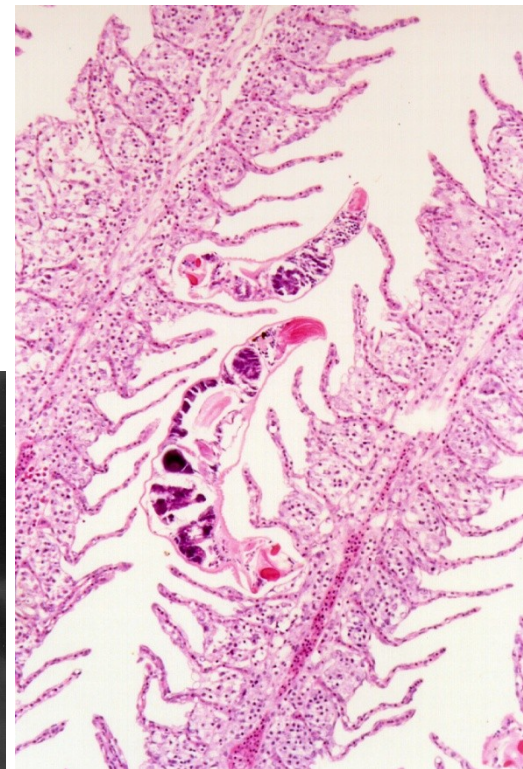
Morphometry- digital
image analysis

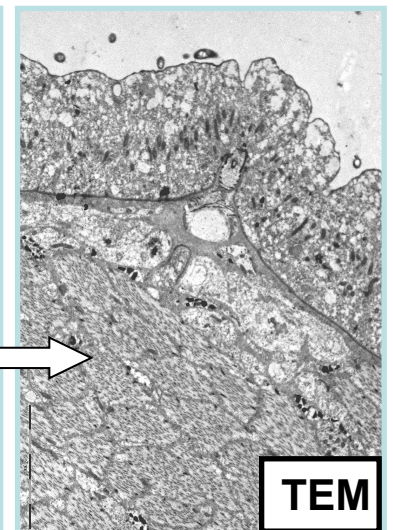
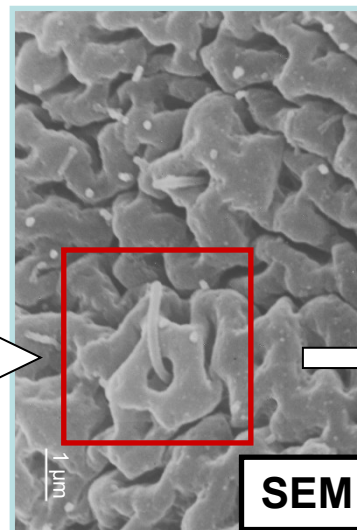
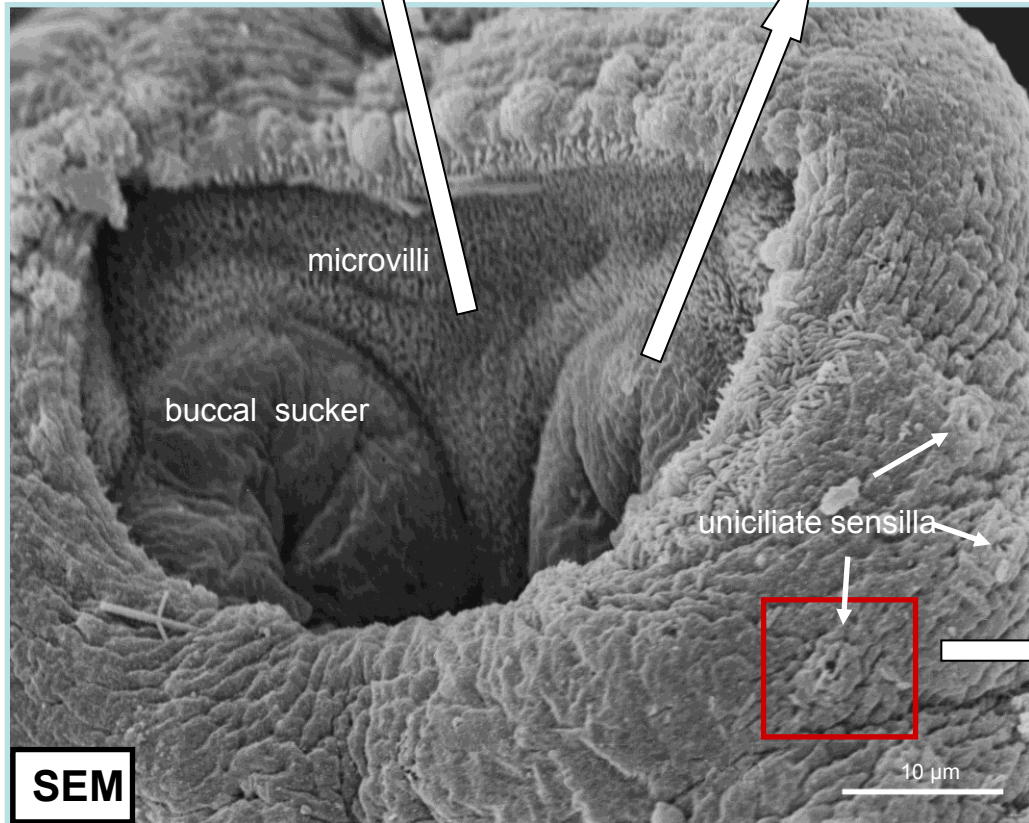
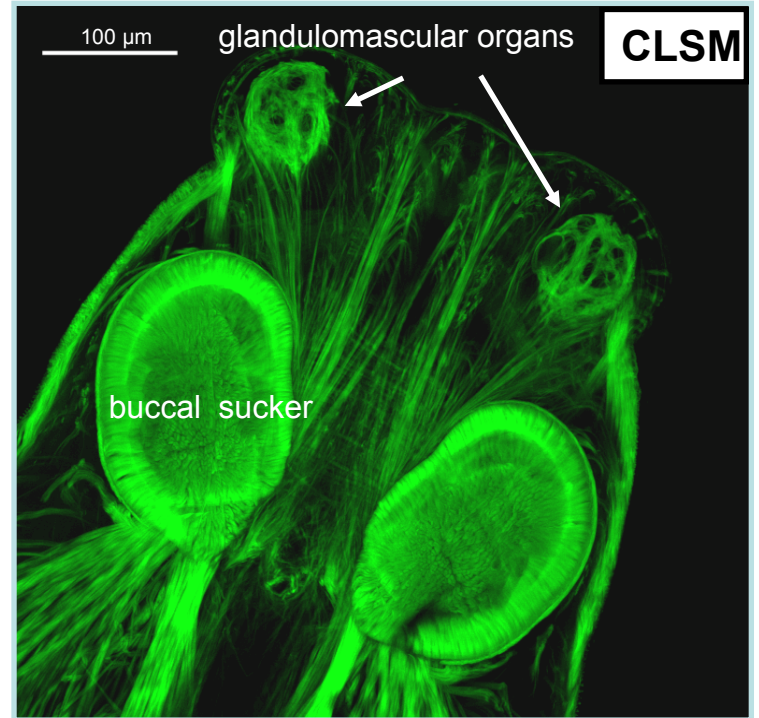
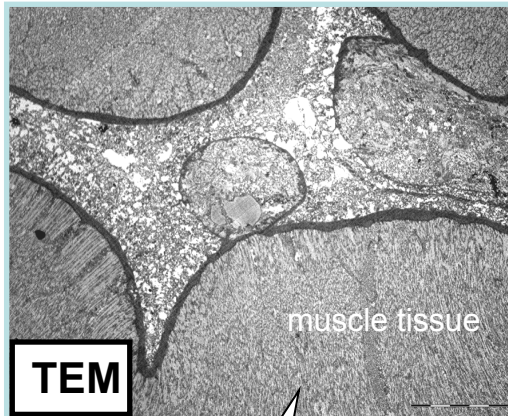
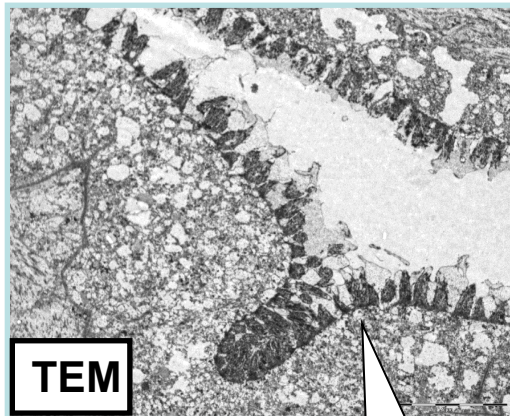


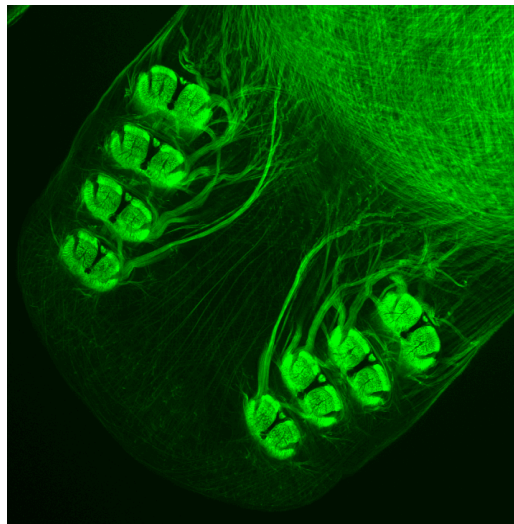
SEM



Histology (BF)







Eudiplozoon nipponicum

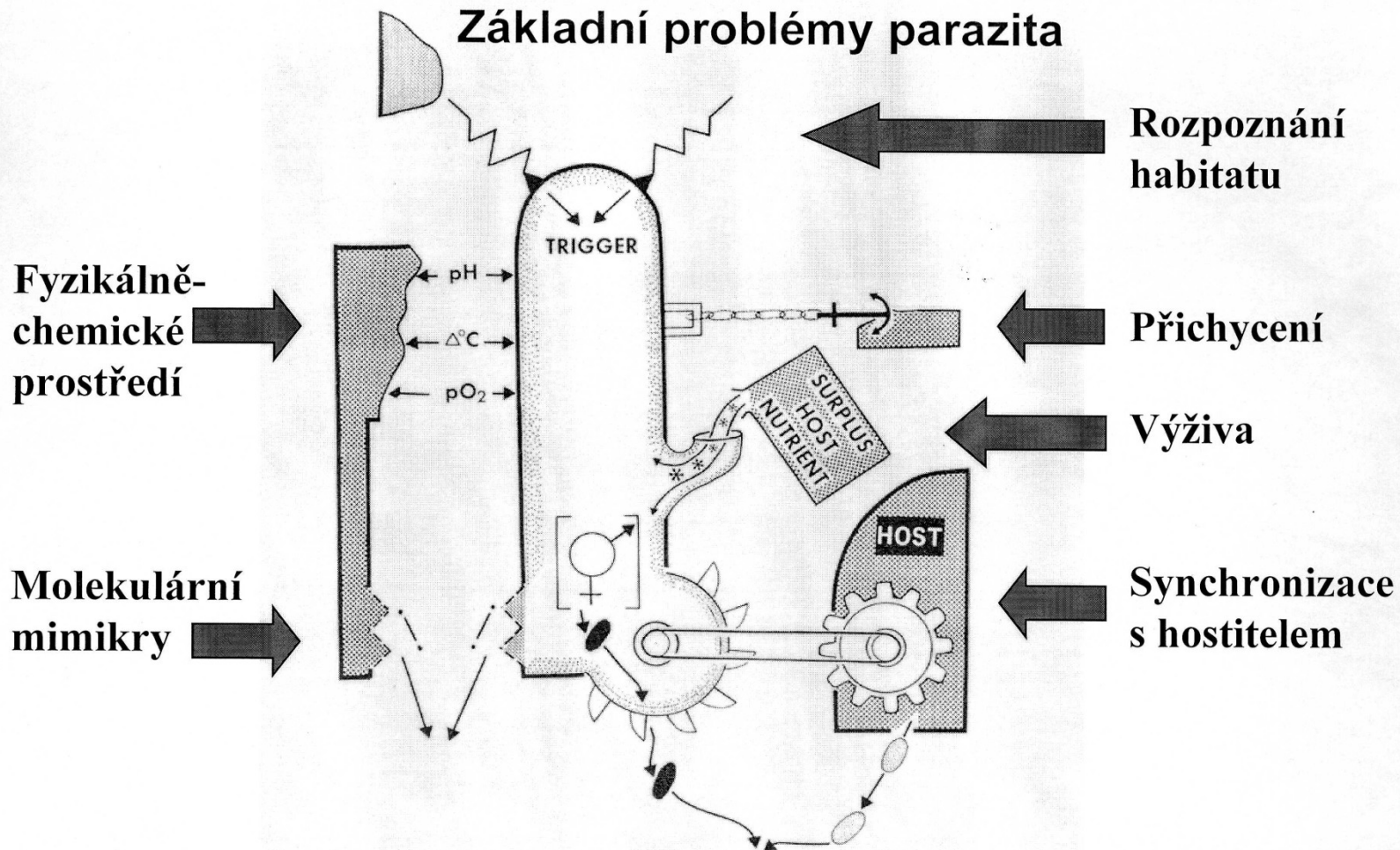
Typy prostředí: Voda
Půda
Atmosféra

Organismy → **Paraziti**

Co je to parazit ?

Raison d'être for parasitologists.

Základní problémy parazita



(upraveno podle Smytha 1994)

Jak se stát úspěšným parazitem ?

Co je potřeba umět ?

- ▶ Musí mít strategii vyhledávání hostitele
- ▶ Způsob jak proniknout do hostitele a přichytit se na/uvnitř jeho těla
- ▶ Schopnost se adaptovat na fyzikálně-chemické podmínky uvnitř organismu hostitele
- ▶ Schopnost se v těle hostitele uživit
- ▶ Schopnost se bránit vůči obranným mechanismům organismu hostitele (imunita)
- ▶ Schopnost se množit a šířit na další hostitelské organismy

Být parazitem není jednoduché !

Je to ale terno !!!

Výhody parazitismu

- ▶ (1) Po dosažení hostitele není nutno vyhledávat dalšího
- ▶ (2) Stálá dostupnost potravy
- ▶ (3) Redukovaná nezbytnost složitého získávání a zpracovávání potravy
- ▶ (4) Ochrana proti extrémům vnějšího prostředí
- ▶ (5) Ochrana proti predátorům a nemocem
- ▶ (6) Redukované mechanismy sloužící k pohybu (zajišťuje hostitel)
- ▶ (7) Vyvinutější pohlavní orgány ve srovnání s volně žijícímu příbuznými (obrovská plodnost cizopasníků)

Nevýhody parazitismu

- ▶ 1) Extrémní hostitelská specifická zvyšuje riziko vyhynutí parazita
- ▶ 2) Nezbytnost vyhledávání optimálního habitatu na/v hostiteli – často složité ontogenetické migrace cizopasníků
- ▶ 3) Nezbytnost být adaptován na podmínky uvnitř organismu hostitele
- ▶ 4) Nezbytnost se bránit vůči imunitnímu systému hostitele
- ▶ 5) Distribuce cizopasníka je limitovaná rozšířením jeho hostitele
- ▶ 6) Riskantní šíření tj. mnoho cizopasníků zahyne ještě před dosažením vhodného hostitele

Fenomén parazitismu

Typy vztahů mezi organismy	A	B
Parazitismus	+	-
Predace	+	-
Kompetice	-	-
Protokooperace	+	+
Mutualismus	+	+
Komensalismus	+	0
Amensalismus	-	0
Neutralismus	0	0

Parazitismus = forma symbiosy

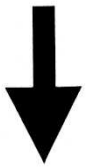
Co je to parazitismus ?

- Parazitismus – vzájemný vztah, při kterém jeden organismus (druh) získává výhodu, zatímco druhý je tímto vztahem poškozován.
- Je parazitismus symbiosa ?

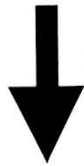
Co je to symbiosa ?

- Symbióza – jakýkoliv vztah nebo soužití dvou nebo více druhů organismů, ať prospěšné nebo nepospěšné.

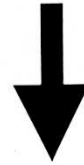
Typy symbiósy



Forezie



Komenzalizismus



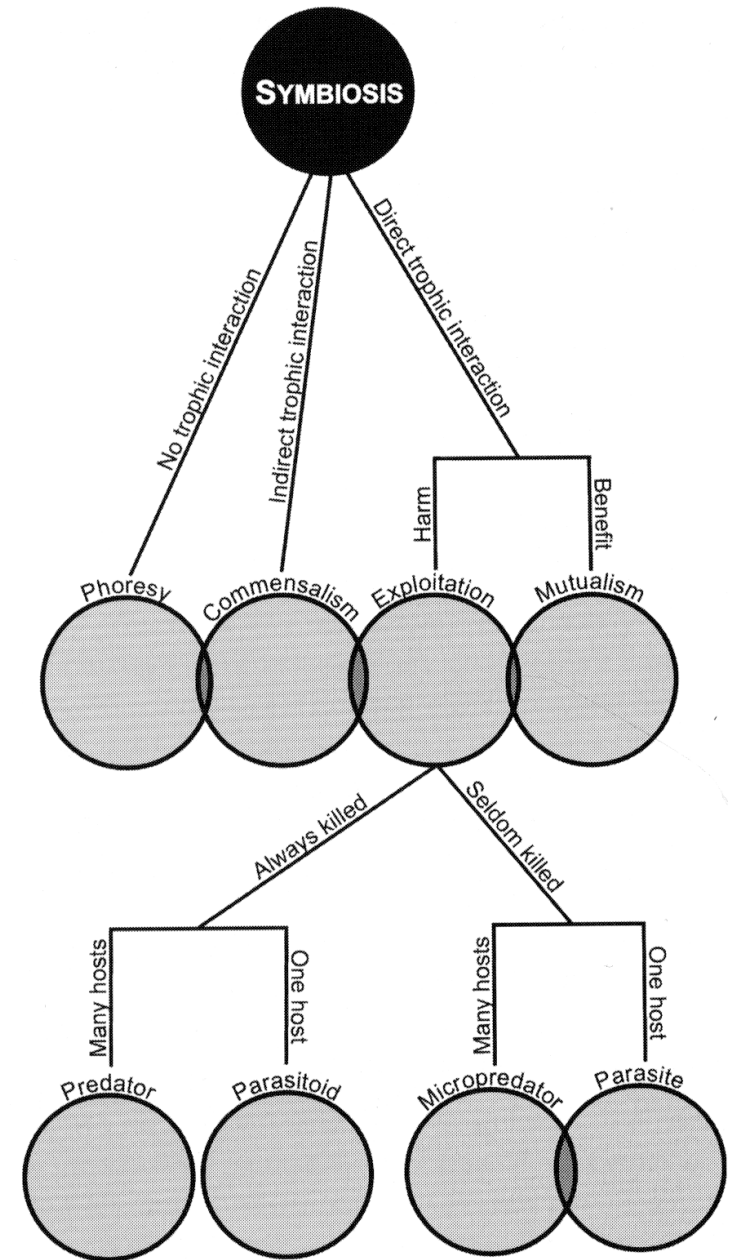
Parazitismus



Mutualismus

Parazit - symbiossa

- ▶ Celý život nebo alespoň jeho část žije na povrchu nebo uvnitř těla svého hostitele
- ▶ Živí se na jeho úkor (exploatace) → tento efekt však může být i zcela zhoubný (pro jedince hostitele - patogenita)



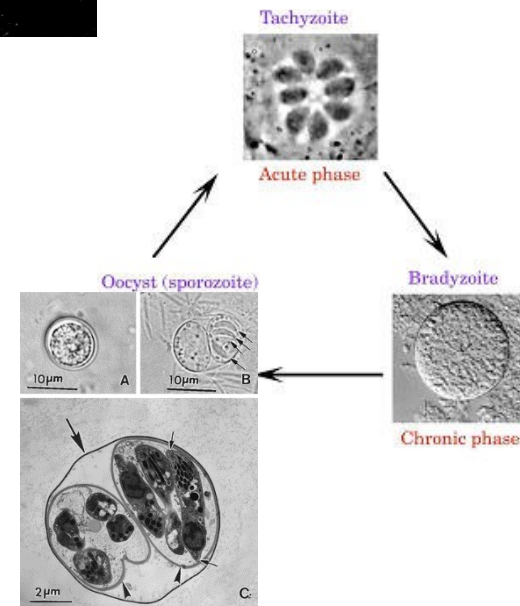
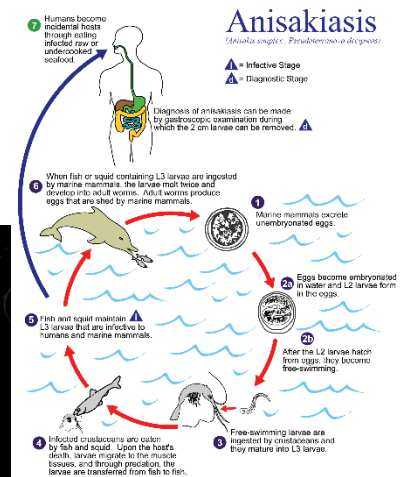
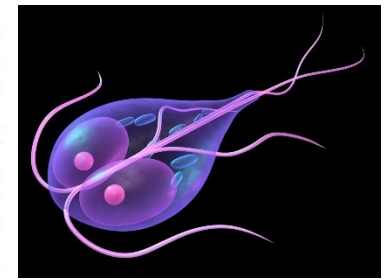
Typy parazitismu

- Parazit
- Predátor
- Parazitoid
- Mikropredátor
- Parazitický kastrátor
- Parazitičtí obratlovci
- Parazitické rostliny
- Hnízdní parazitismus
- Sociální parazitismus u hmyzu

Jak vypadá typický parazit ?

- ▶ Napadá jednoho hostitele a má slabé nebo žádné patogenní působení
- ▶ Hostitel přežívá
- ▶ Andreson & May (1979) :
typický parazit – závislý na intenzitě infekce (makroparazit)
patogen – nezávislý na intenzitě infekce (mikroparazit)

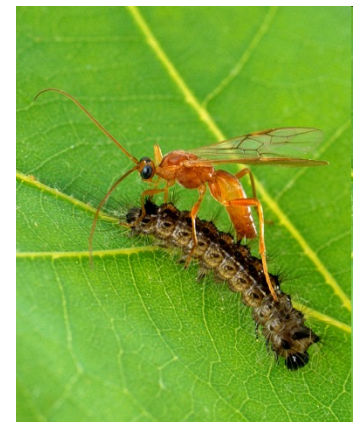
!!! Troficky přenosný typický parazit nebo patogen vyžaduje usmrcení hostitele - častá manipulace chováním hostitele



Parazitoid



- ▶ Napadá jednoho hostitele
- ▶ Usmrcení hostitele je nezbytné
- ▶ Parazitické larvy hmyzu, např. Diptera (Tachinidae) a Hymenoptera (Chalcidoidea, Braconidae), fyziologické adaptace (endosymbiotické viry)
- ▶ Samičky kladou vajíčka do hostitele, kde se pak vyvíjí parazitická larva



Parazitický kastrátor

- ▶ Energie původně určená pro reprodukci hostitele je využita ve prospěch parazita
- ▶ **Parazitický kastrátor** - zabíjí svého hostitele v evolučním smyslu
- ▶ **Částečný kastrátor** – přechod mezi typickým parazitem a parazitickým kastrátorem



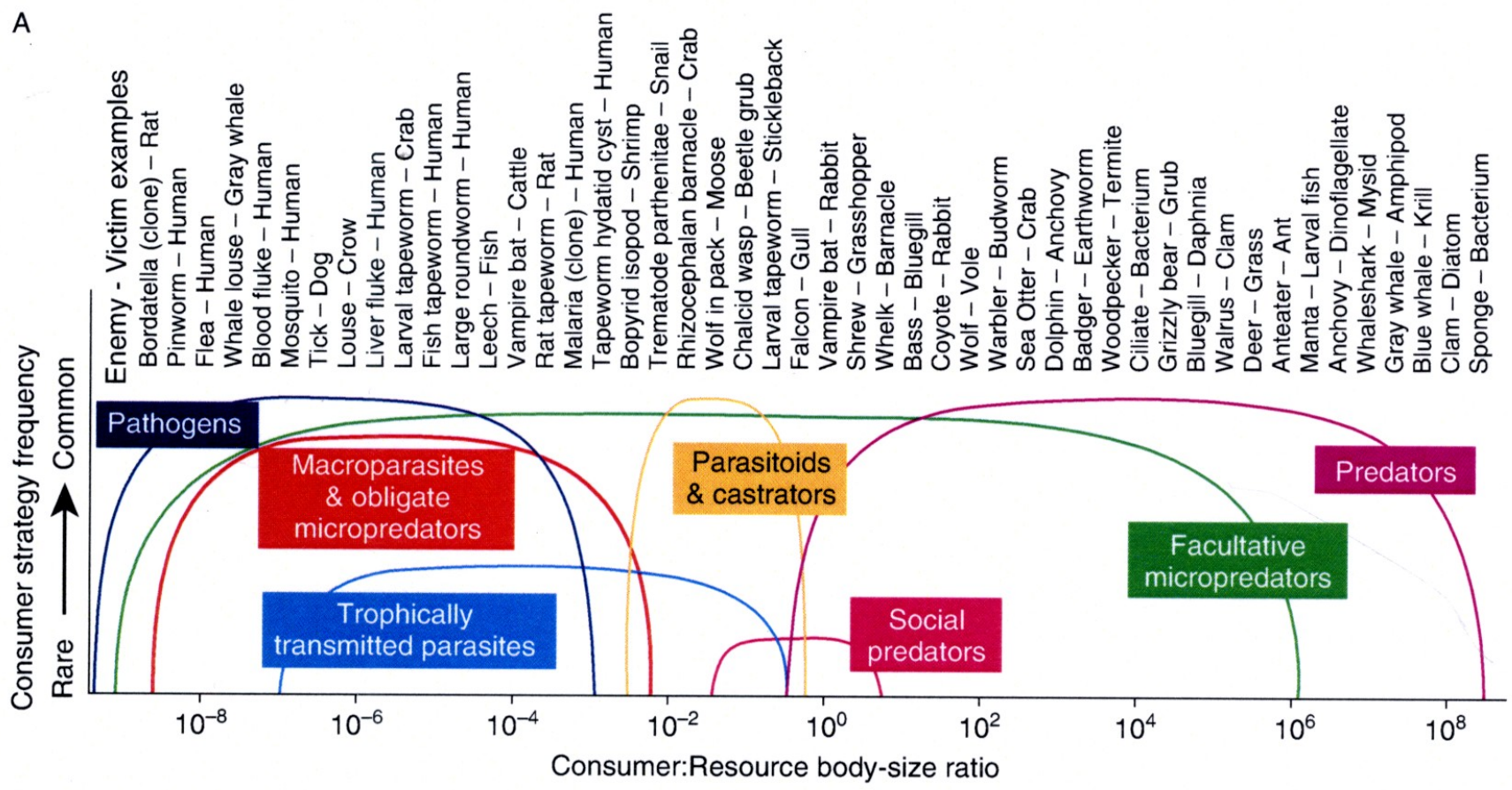
Ekologické vymezení parazita I

Effect on fitness	Number of hosts/prey attacked		
	1 host		> 1host/prey
	Death of host not required	Death of host required	
< 100%	Typical parasite	Tropically transmitted typical parasite	Micropredator
100% (prey has 0 fitness)	Parasitic castrator	Parasitoid	Predator

Ekologické vymezení parazitismu II

Effect on host fitness	Number of hosts/prey attacked		
	1 host		> 1 host/prey
	Death of host not required	Death of host required	
< 100%	typical parasite pathogen	trophically transmitted typical parasite trophic transmitted pathogen	micropredator harvest regeneration
100% (prey has 0 fitness)	partial castrator parasitic castrator	trophically transmitted castrator parasitoid	social predator predator

Frekvence poměru relativní velikosti těla konzumenta a hostitele



Ekologická klasifikace parazitů

▶ Association with host

obligate (Monogenea, Digenea, Cestoda)

facultative (nematoda *Micronema*)

hyperparasitic – *Udonella* on parasitic Crustacea

▶ Time of parasitic lifespan

permanent (*Plasmodium*, *Entamoeba*)

temporal (*Argulus*, *Ixodes*)

periodic – developmental stages (glochidie Mollusca, larvae Diptera), generation (*Rabdias bufonis*)

Ekologická klasifikace parazitů

▶ According to the life cycle

monoxenous – life cycle in one host (Monogenea)

heteroxenous (euryxenous) – life cycle in more than one host (Digenea, Cestoda)

▶ According to the range of food items (**host specificity**)

monophagous (stenophagous) – only one host species (*Eudiplozoon nipponicum*) (strictly host specific)

polyphagous (euryphagous) – more than one host species (*Posthodiplostomum cuticola*, *Ergasilus sieboldi*)



Klasifikace hostitelů

- Hostitel definitivní
- Mezihostitel
- Vektor
- Rezervoárový hostitel
- Paratenický (transportní) hostitel

Typy hostitelů

- ▶ **Definitive host** – host, in which parasite reach maturity, produce eggs or larvae
- ▶ **Intermediate host** – important for the development of larval stages, invasion stage → definitive host
one or more intermediate hosts (Digenea, Cestoda)
human as intermediate hosts (*Echinococcus*, *Taenia*)

Typy hostitelů

- ▶ **Paratenic (transport) host** – parasite does not develop, but retain the ability of invasion, not necessary for life cycle of parasite
i.e. Mollusca for the larvae of nematodes usually occurring in short-living crustacea
- ▶ **Reservoir host**
= source of infection in ecosystem, parasite lives in the conditions unsuitable for common host, epidemiological significance
i.e. rats and carnivora for *Trichinella*, *Schistosoma japonicum*
- ▶ **Vector** – usually transfers other pathogen on its hosts, i.e. mosquitos – facultative parasite, micropredator

Rozmanitost parazitů

