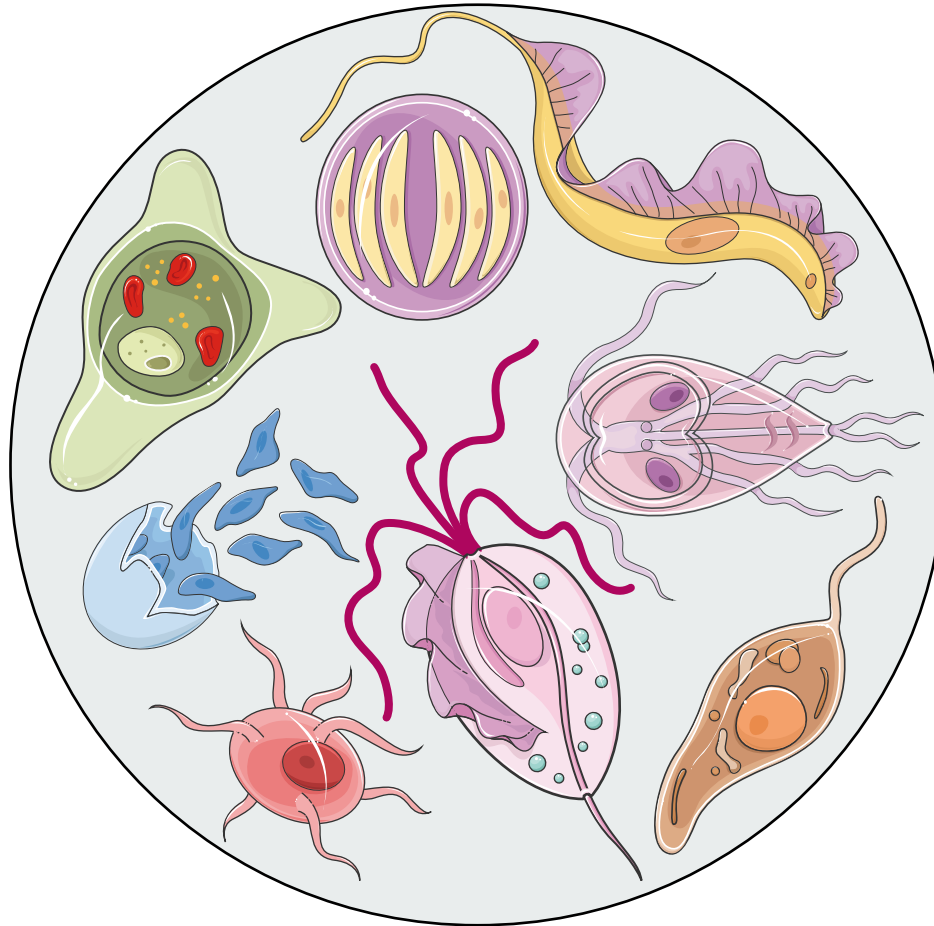


Biology of parasitic protozoa

X. Myxozoa (Opisthokonta, Animalia)



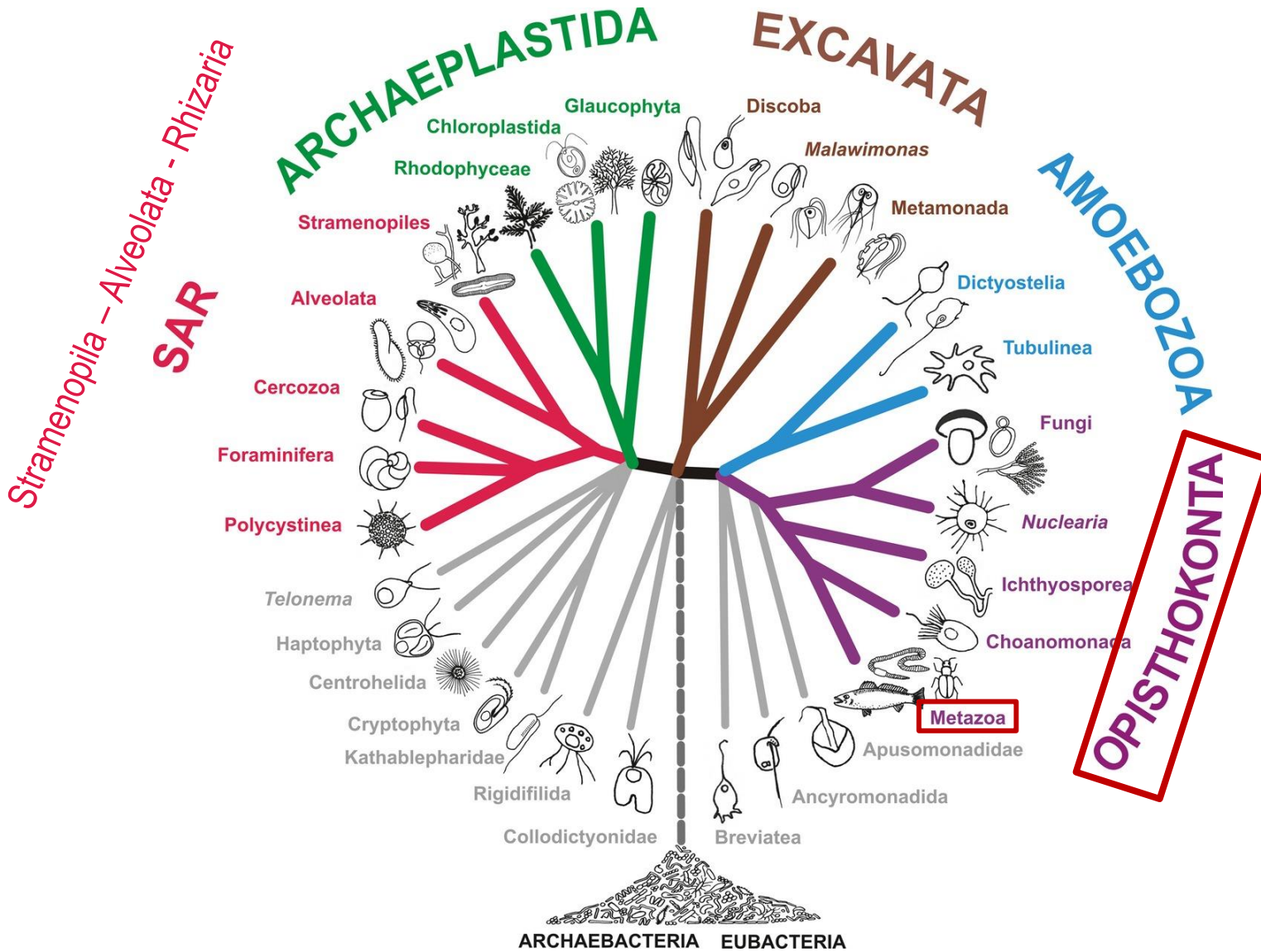
Andrea Bardůnek Valigurová

andreav@sci.muni.cz

Notice

This presentation contains some material available on the web without the permission of the creator or the copyright owner. This presentation is to be used for educational purposes only. Educational purposes are defined as a communicating material for a particular course of instruction or for the administration of such course. Educational purposes do not cover the use of copyright material in PowerPoint for public lectures or other purposes.

5 supergroups = megagroups



Myxozoa

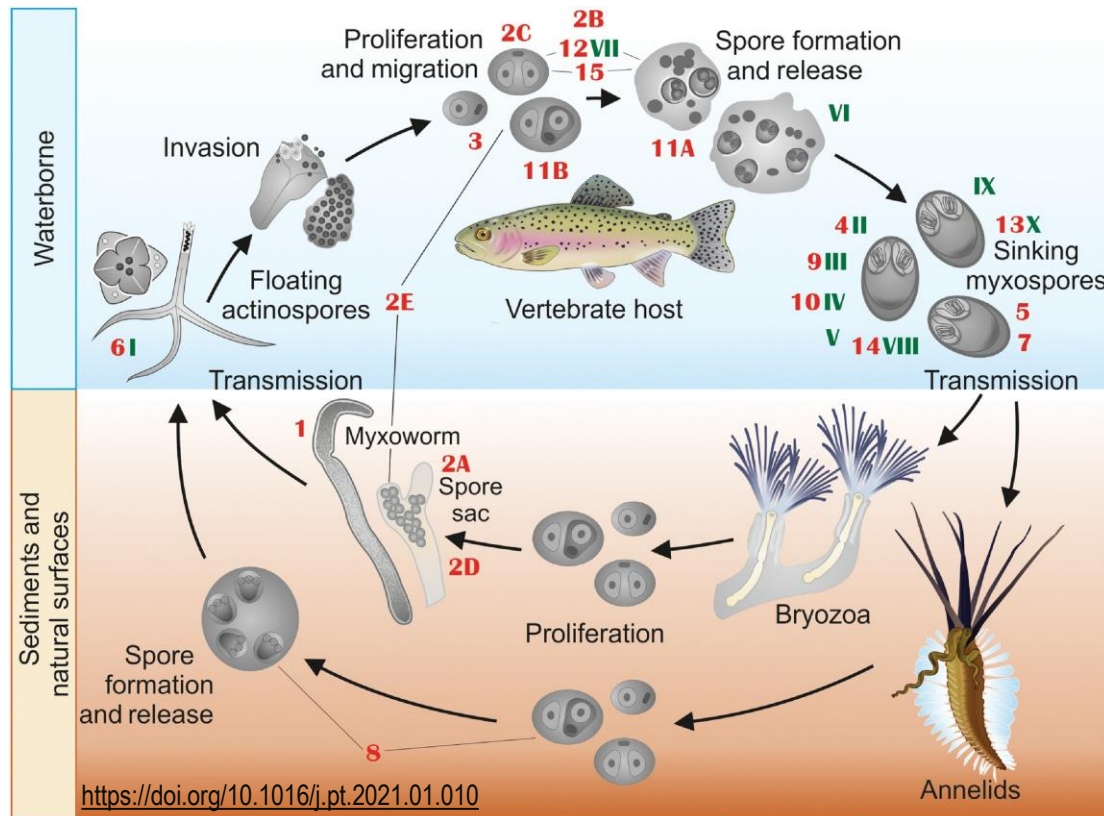
- organisms previously considered protozoa
- recently placed among Metazoa (Opisthokonta) = multicellular organisms (highly derived cnidarians) that are extremely developmentally and structurally altered by parasitic life style
- endoparasites mainly of fish and some invertebrates (annelids, polychaetes, bryophytes)
- rare findings in mammals and ducks (liver of a shrew, brain of a mole, bile ducts and liver of a duck)
- main feature = formation of multicellular spores equipped with polar capsules (= cnidaria) with coiled and ejectable fiber



https://www.youtube.com/watch?v=VG_r2KQgljQ

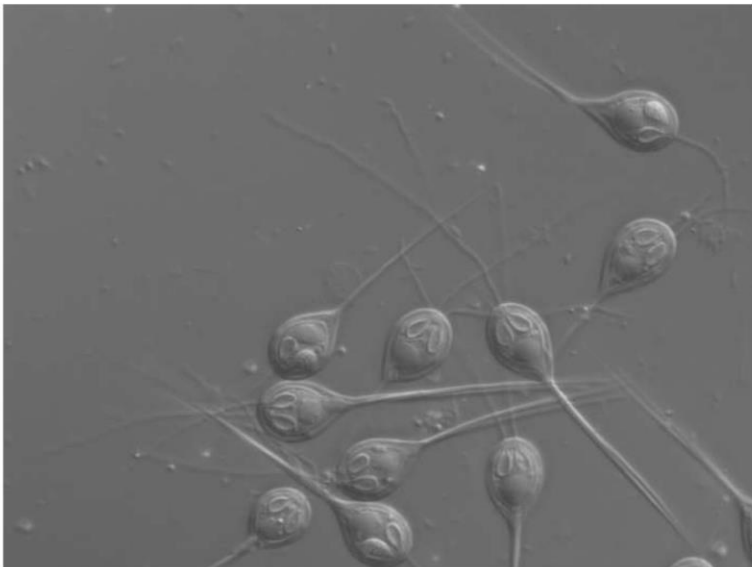
Myxozoa

- due to evolution into microscopic parasites, they lost many genes responsible for multicellular development, coordination, cell-cell communication, and even aerobic respiration (*Henneguya salminicola*)
- complex life cycle usually involving intermediate host - usually fish and rarely amphibians
- envelopment of cells by cells; differentiation of nuclei and cells into the vegetative and generative cells



Henneguya salminicola: Microscopic parasite has no mitochondrial DNA

by Bob Yirka , Phys.org



Light microscope image of spores of the parasitic cnidarian *Henneguya salminicola*, from Chinook salmon. Credit: Stephen Douglas Atkinson.

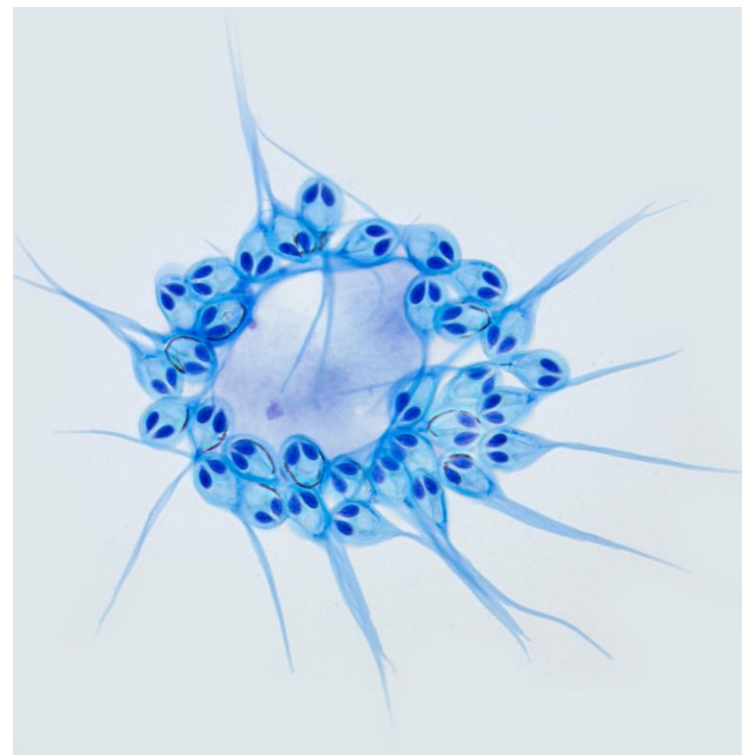
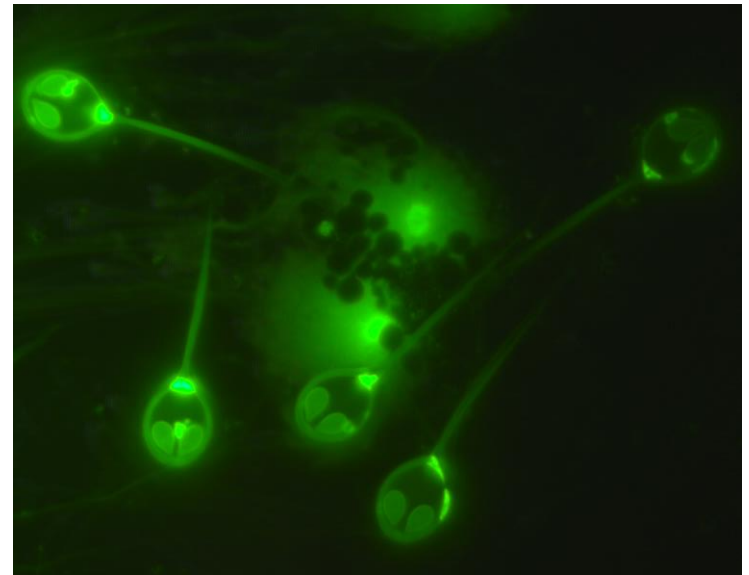
An international team of researchers has found a multicellular animal with no mitochondrial DNA, making it the only known animal to exist without the need to breathe oxygen. In their paper published in *Proceedings of the National Academy of Sciences*, the group describes their study of *Henneguya salminicola*, a microscopic, parasitic member of the group Myxozoa and its unique physiology.

<https://phys.org/news/2020-02-henneguya-salminicola-microscopic-parasite-mitochondrial.html>

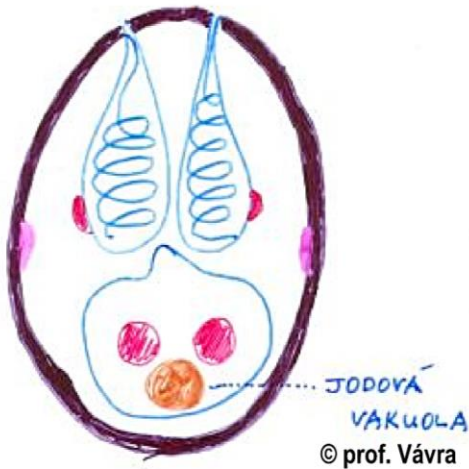
<https://www.pnas.org/doi/full/10.1073/pnas.1909907117>

<https://www.youtube.com/watch?v=sVqExF5-2Ms&t=21s>

https://www.youtube.com/watch?v=4N_HIkBAez8



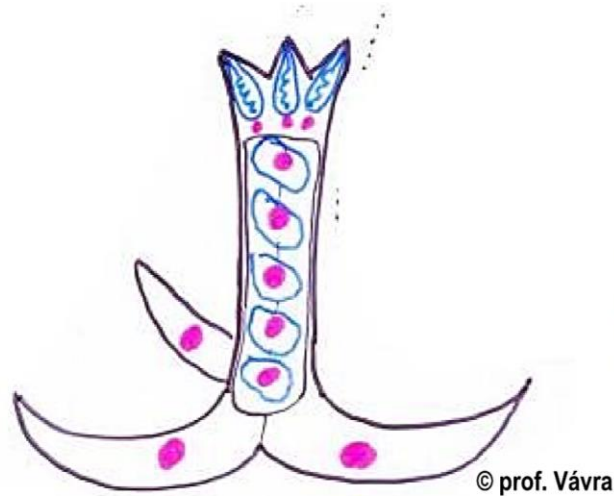
Three types of spores in Myxozoa



myxosporean spore

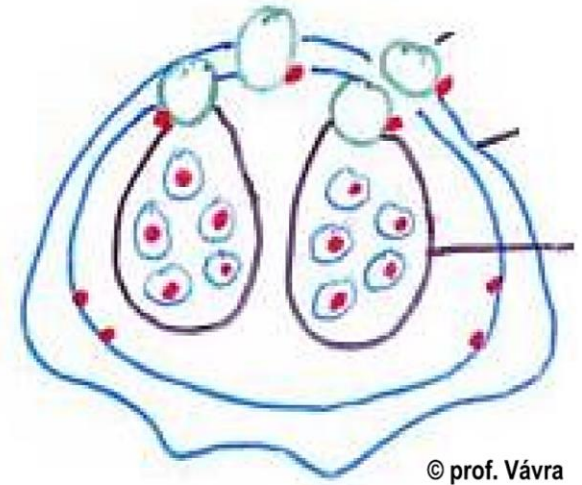
fish, rarely mammals

2 phases of the same organism



actinosporean spore

annelids



malacosporean spore

bryozoans

next phase ???

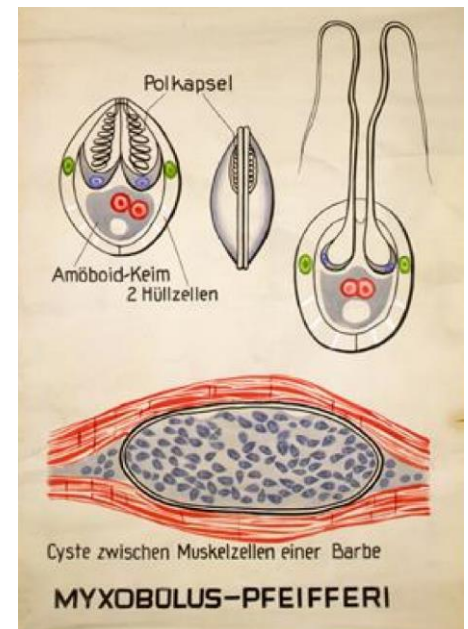
Myxozoa

Myxosporea

- myxosporean and actinosporean phases of development + corresponding spores (= developmental stages of the same organism)
- annelids (actinosporean phase) and fish (myxosporean phase), annelids are the original hosts where sexual cycle occurs
- not all myxozoa have the actinosporean phase

Malacosporea

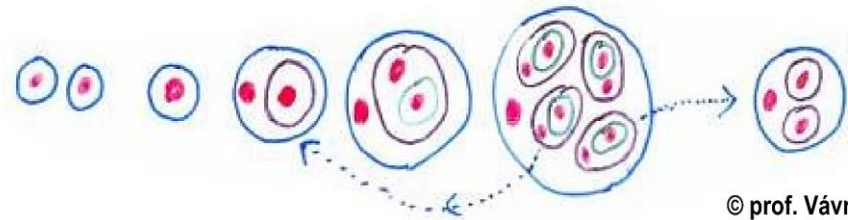
- only malacosporozoans known so far
- bryozoan parasites
- fish as a second host?



Myxosporea

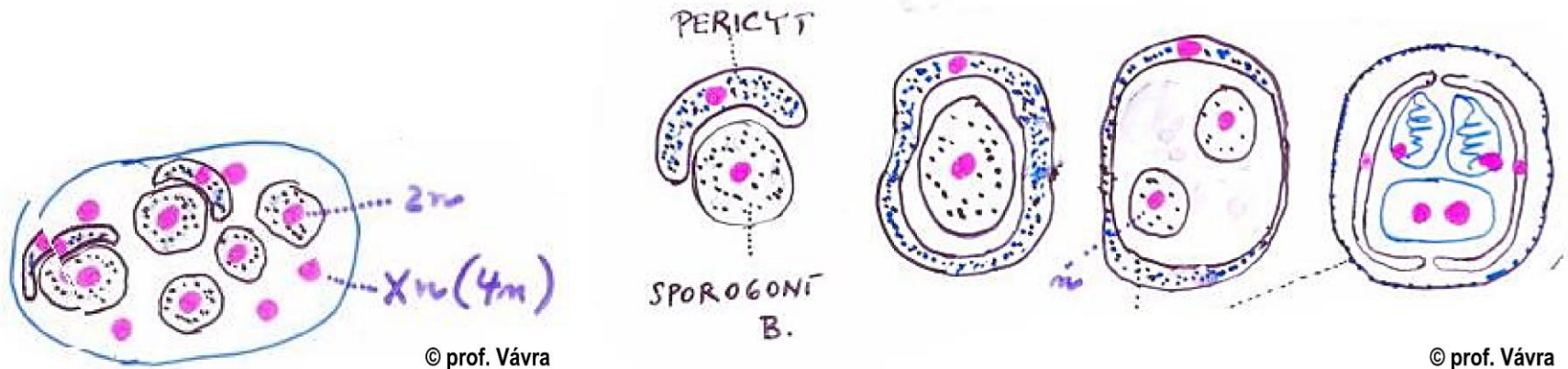
Myxosporean phase

- cavity parasites in fish (bladder, gall pouch, kidney canals, swim bladder) or histozoan parasites (gill epithelium, muscle, cartilage, etc.)
- proliferative phase via cell-in-cell system („matryoshka“) - cells forming within other cells



Sporogonial phase

- formation of sac-shaped plasmodia and multicellular spores
- nuclei and cells differentiate into vegetative and generative lines



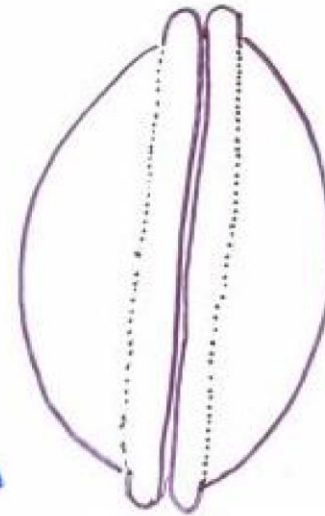
Myxosporea – myxosporean spore

Bivalvulida type

- freshwater myxozoans
- bilateral symmetry
- 2 valves
- 2 polar capsules



JODOVÁ
VAKUOLA



Spory
infekční pro
hlávenku

© prof. Vávra



HENEGUYA

© prof. Vávra



MYXIDIUM

© prof. Vávra



SPHAEROSPORA

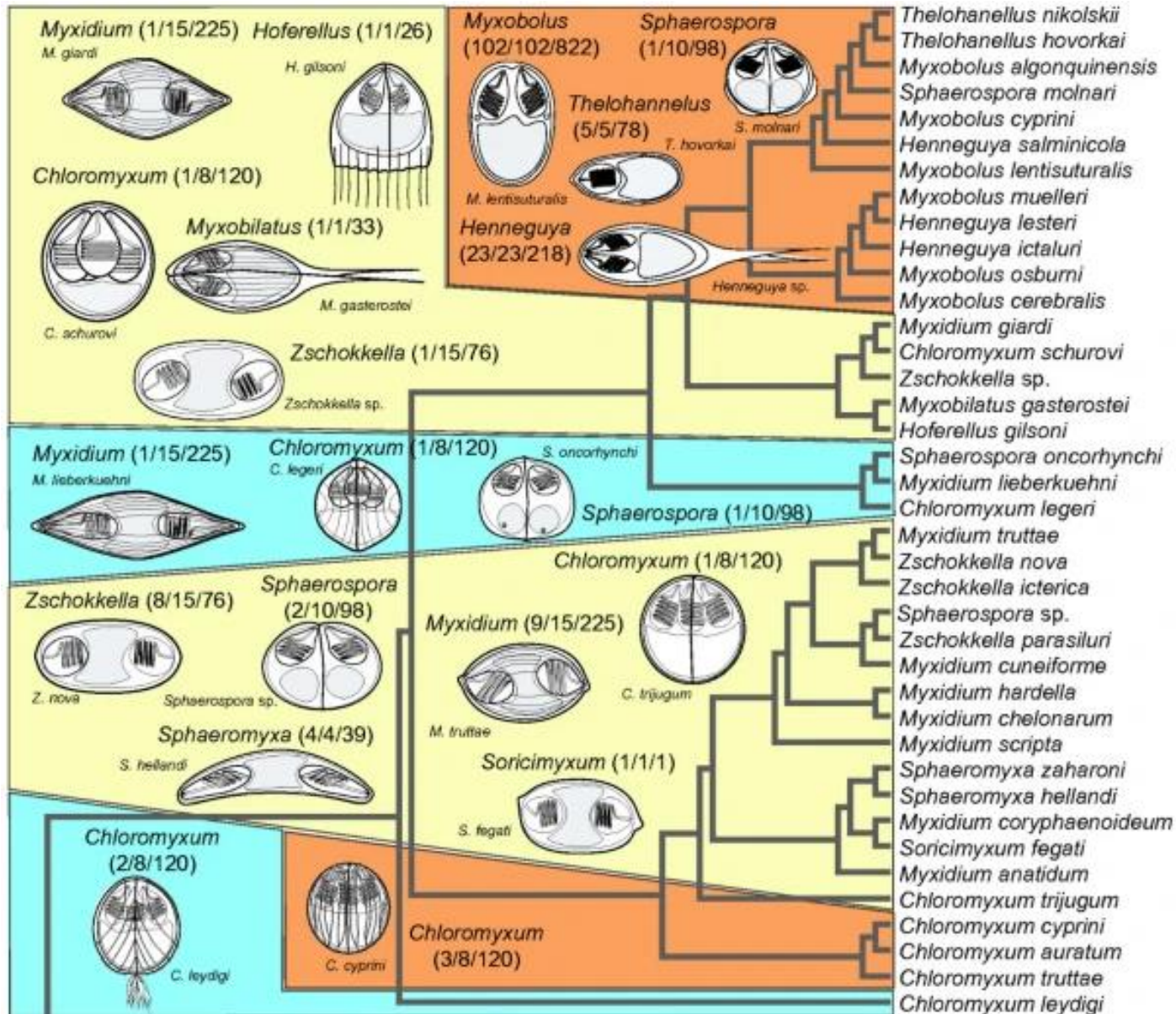
© prof. Vávra



MYXOBOLUS

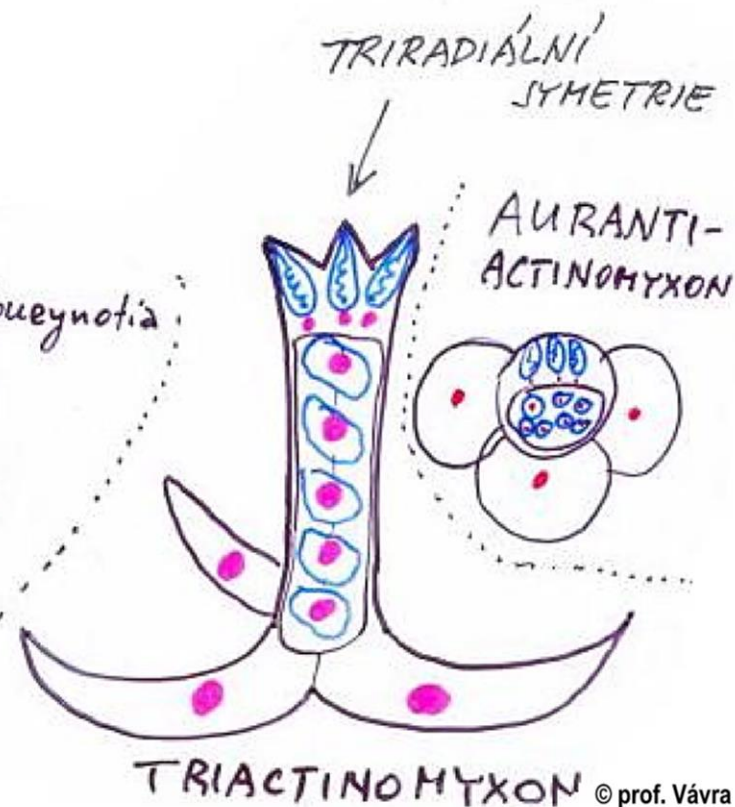
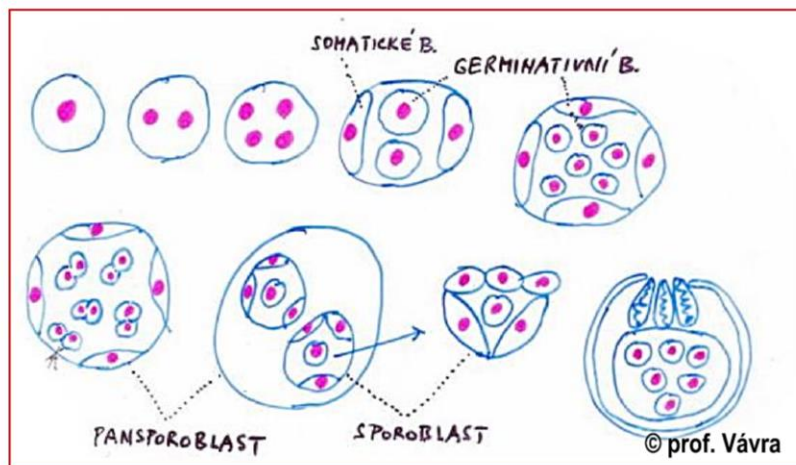
© prof. Vávra

Myxosporea – myxosporean spore

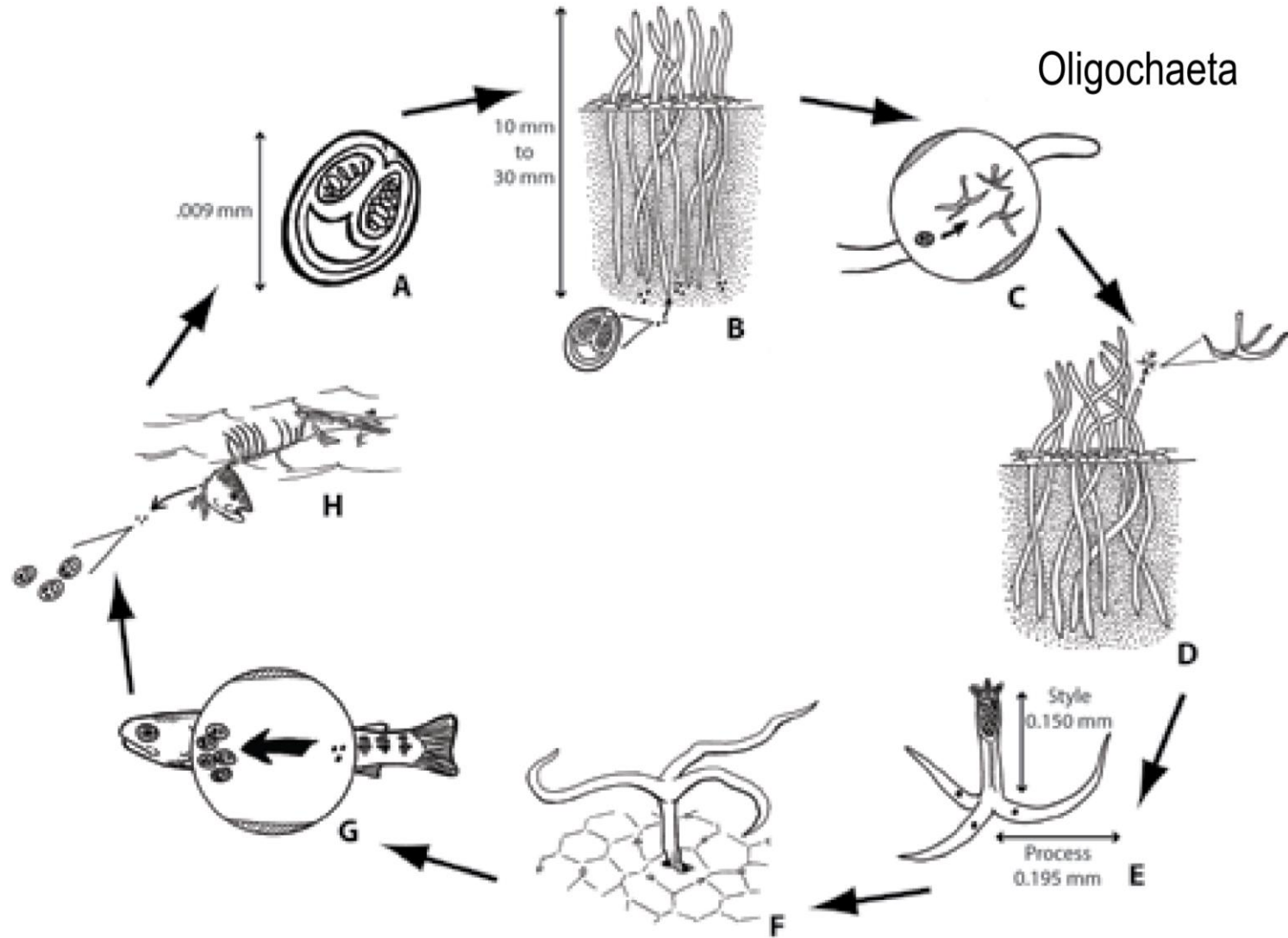


Myxosporea – actinosporean spore

- 3 valves often with floats, 3 polar capsules, sporoplasm multicellular



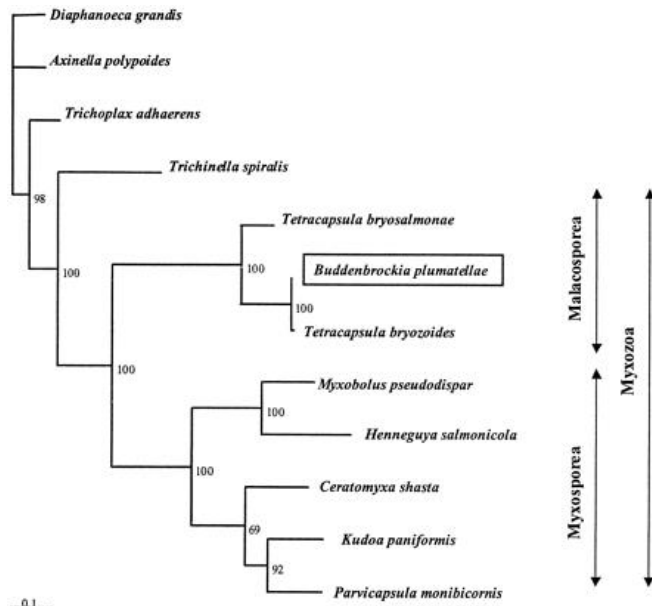
Life cycle of *Myxobolus cerebralis* (1984)



The complex life cycle of *Myxobolus cerebralis* involves multiple stages of the parasite and requires two hosts: an aquatic worm *Tubifex tubifex*, infected by the parasite's myxospore (A), and a salmonid fish, infected by the triactinomyxon (E).

Why belong Myxozoa among Metazoa

- multicellular spores
- nuclear differentiation
- cell differentiation
- cell junctions as in Metazoa (tight, adhesive)
- similarity of polar capsules with nematocysts of cnidarians
- rRNA nucleotide sequence



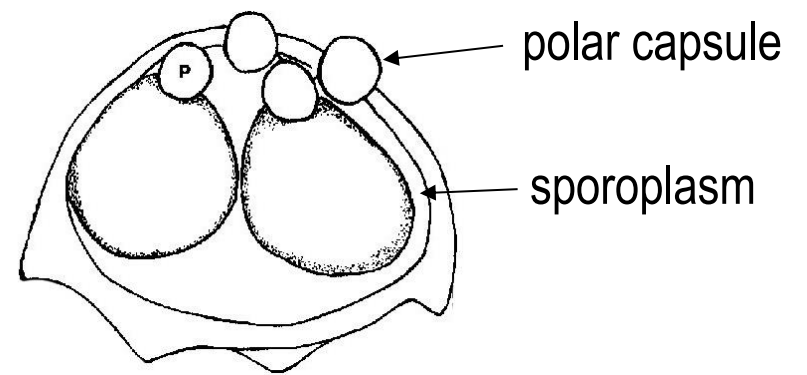
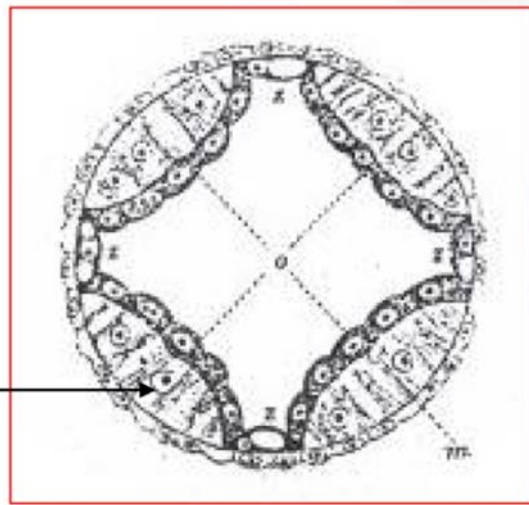
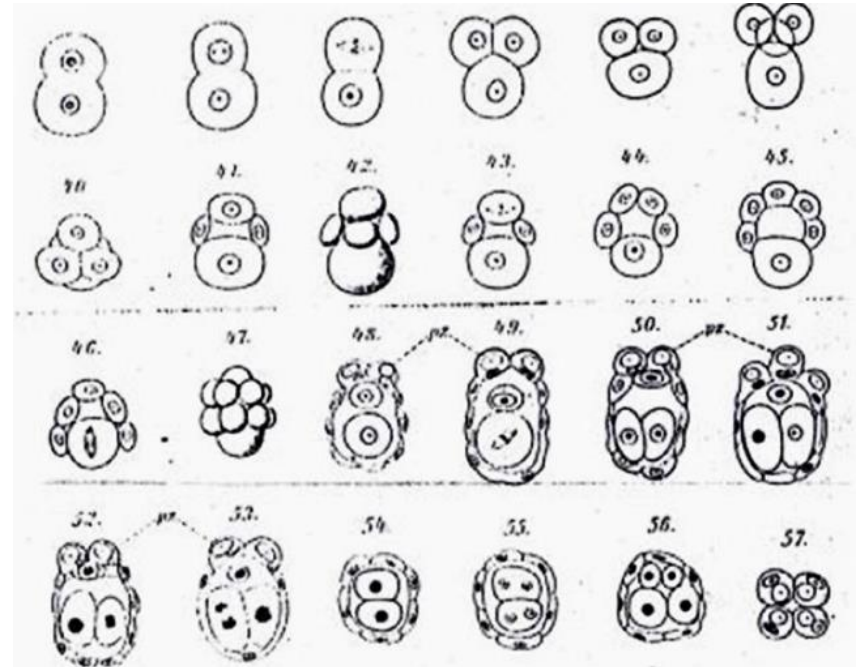
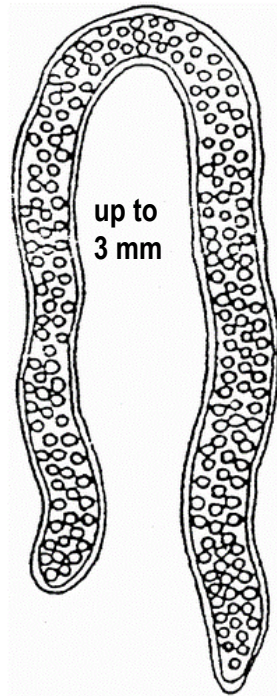
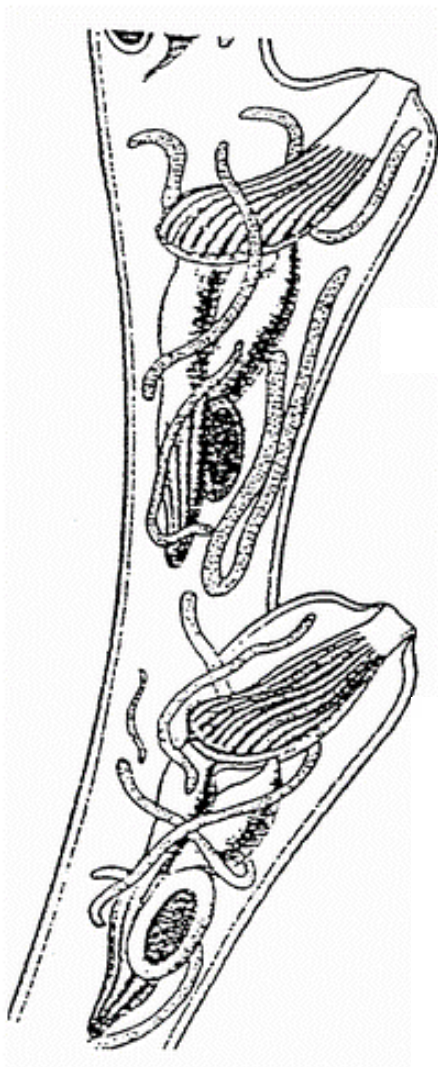
Myxozoa are:

- Cnidaria (Diblastica)? - extreme similarity of polar capsules with cnidarian nematocysts
- Bilateria (Triblastica)? - presence of HOX genes (determining the anteropodial location of a metazoan cell)
- solution???: *Buddenbrockia plumatellae* is a myxozoon with muscles and body organisation similar to nematodes!
- according to phylogenetic analysis *B. plumatellae* belongs among Cnidaria

<https://doi.org/10.1093/oxfordjournals.molbev.a004155>

<https://www.youtube.com/watch?v=Pq05yrqsQ4k>

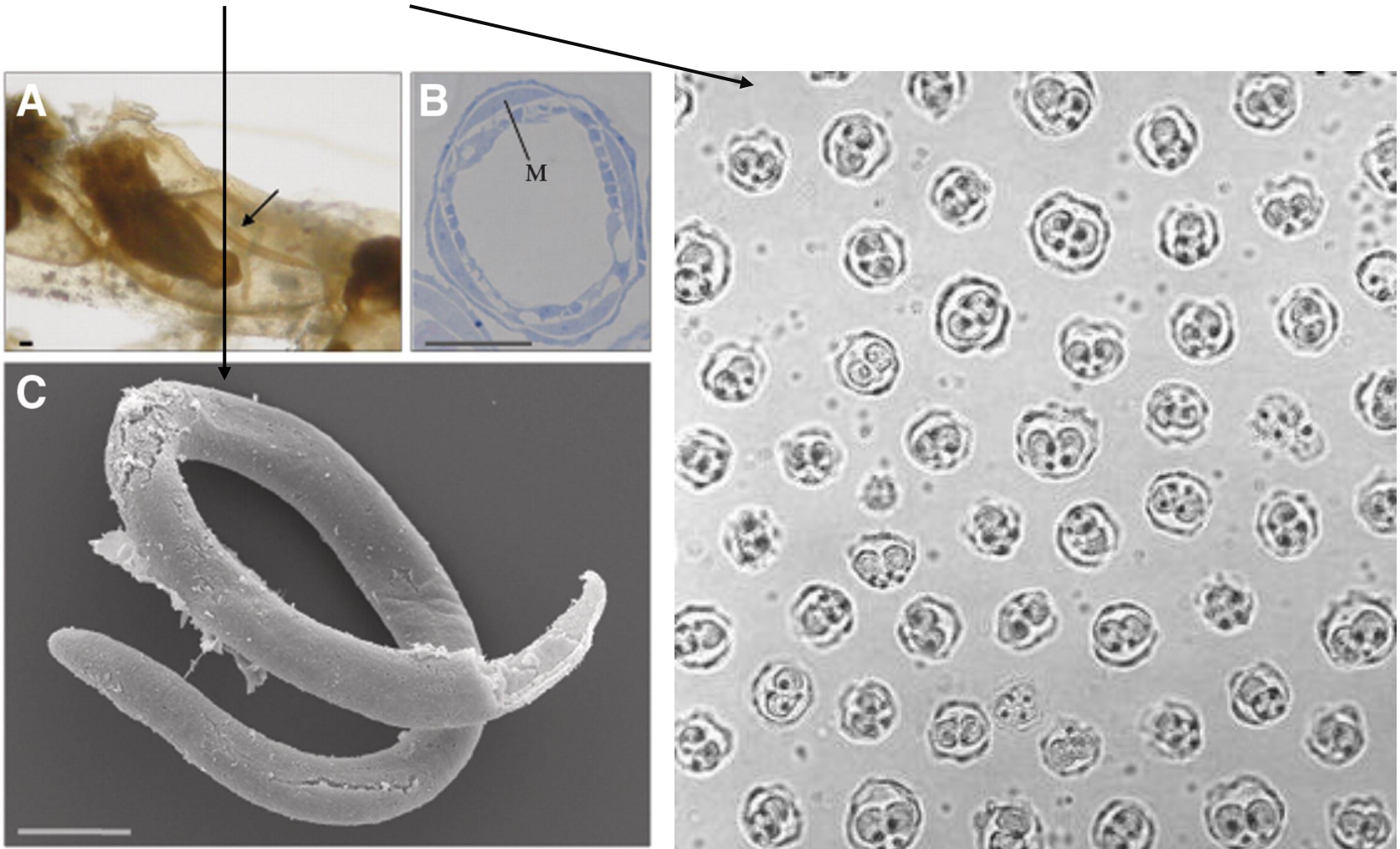
Buddenbrockia plumatellae Schroeder 1910



Malacospora = 4 soft valves

Buddenbrockia plumatellae

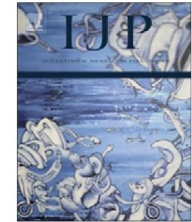
- a "worm" with spores in the cavity of bryzoans = nematode-like cnidarian





Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

A new model for myxosporean (Myxozoa) development explains the endogenous budding phenomenon, the nature of cell within cell life stages and evolution of parasitism from a cnidarian ancestor

D.J. Morris*

Institute of Aquaculture, University of Stirling, Stirling, Scotland FK9 4LA, UK

ARTICLE INFO

Article history:

Received 12 March 2012

Received in revised form 5 June 2012

Accepted 6 June 2012

Available online 26 June 2012

Keywords:

Actinosporea

Myxozoa

Myxosporea

Evolution

Malacosporea

Cnidaria

Polypodium

ABSTRACT

The phylum Myxozoa is composed of endoparasitic species that have predominately been recorded within aquatic vertebrates. The simple body form of a trophic cell containing other cells within it, as observed within these hosts, has provided few clues to relationships with other organisms. In addition, the placement of the group using molecular phylogenies has proved very difficult, although the majority of analyses now suggest that they are cnidarians. There have been relatively few studies of myxozoan stages within invertebrate hosts, even though these exhibit multicellular and sexual stages that may provide clues to myxozoan evolution. Therefore an ultrastructural examination of a myxozoan infection of a freshwater oligochaete was conducted, to reassess and formulate a model for myxozoan development in these hosts. This deemed that meiosis occurs within the oligochaete, but that fertilisation is not immediate. Rather, the resultant haploid germ cell (oocyte) is engulfed by a diploid sporogonic cell (nurse cell) to form a sporoplasm. It is this sporoplasm that infects the fish, resulting in the multicellular stages observed. Fertilisation occurs after the parasites leave the fish and enter the oligochaete host. The nurse cell/oocyte model explains previously conflicting evidence in the literature regarding myxosporean biology, and aligns phenomena considered distinctive to the Myxozoa, such as endogenous budding and cell within cell development, with processes recorded in cnidarians. Finally, the evolutionary origin of the Myxozoa as cnidarian parasites of ova is hypothesised.

© 2012 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.

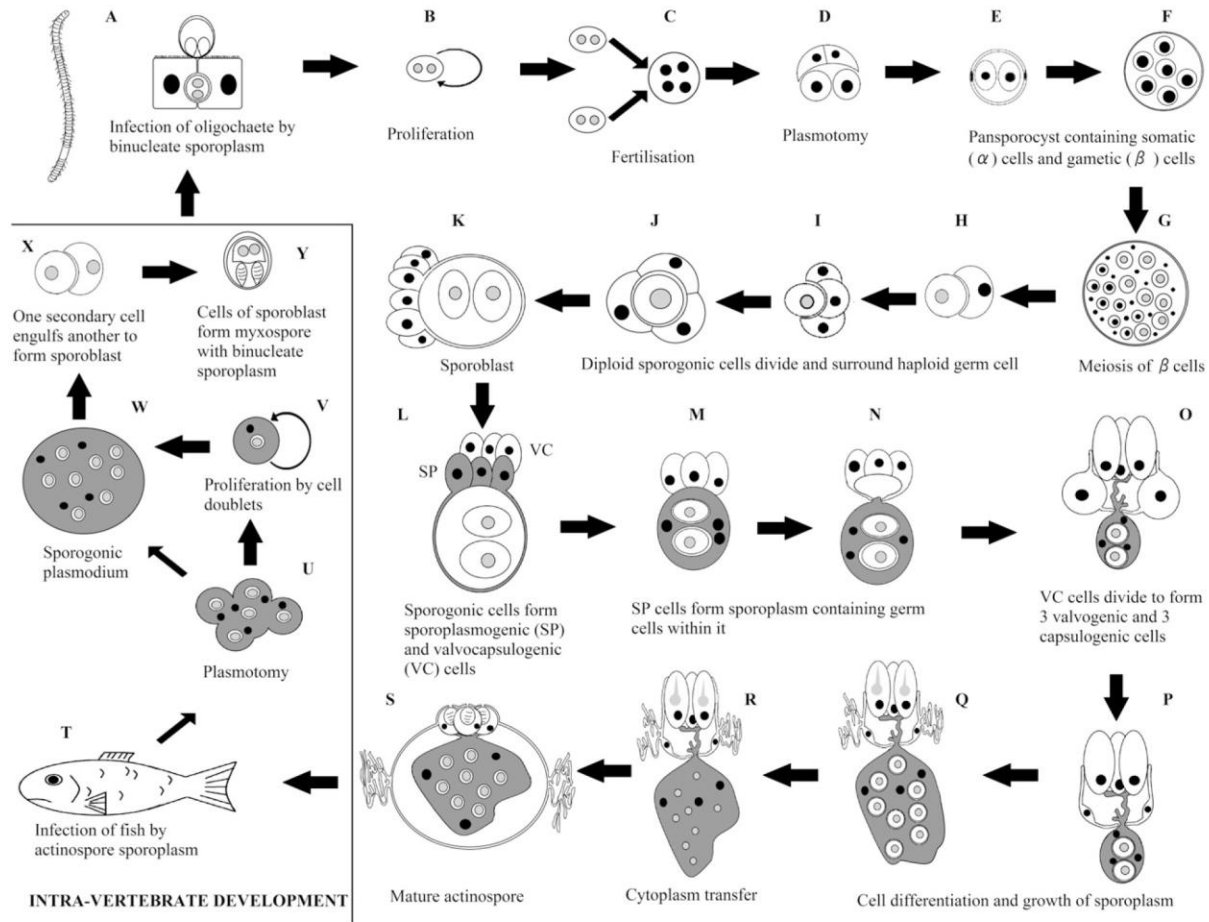


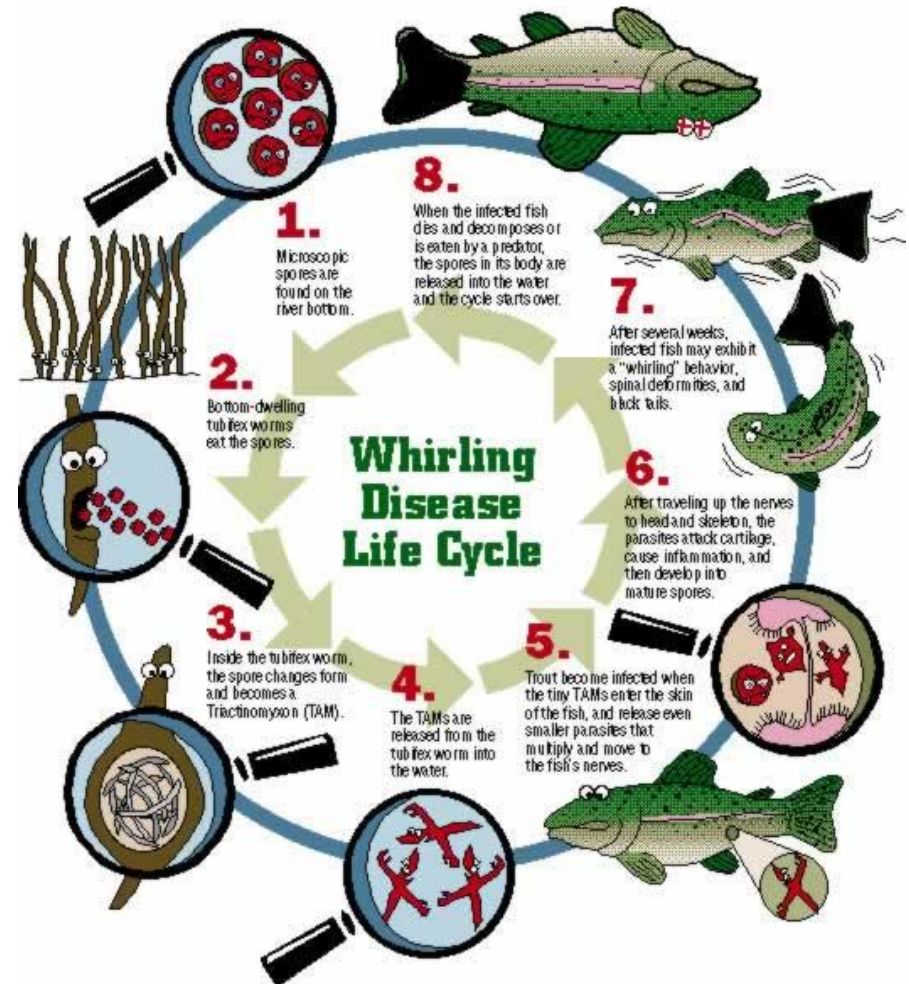
Fig. 21. Proposed model for freshwater myxosporean development from the oligochaete host to the fish. Diploid nuclei represented by black spots; haploid nuclei by light grey spots. (A) A binucleate haploid sporoplasm enters intestinal tissue of a worm after release from myxospore. (B) Proliferation and migration of binucleate cells within tissues of oligochaete. (C) Two binucleate cells fuse and then undergo karyotomy to produce the diploid tetranucleate stage. (D) The tetranucleate stage undergoes plasmotomy to form four diploid cells. (E) The early pansporocyst comprising two envelope cells and two internal cells (one α somatic cell and one gametic β cell). (F) α and β cells divide by mitosis, resulting in eight α and eight β cells. (G) β cells undergo meiosis, releasing 16 polar bodies, and become eight haploid germ cells. The somatic α cells remain diploid. (H) A diploid somatic cell attaches to a haploid germ cell. (I) Somatic cells surround a germ cell to form a sporoblast. (J) A sporoblast consists of haploid a germ cell surrounded by three diploid sporogonic cells. (K) Sporogonic and germ cells divide by mitosis. (L) Sporogonic cells rearrange to form sporoplasmogenic cells (grey cells) that surround germ cells, and valvocapsulogenic cells (clear cells). (M) Sporoplasmogenic cells fuse and the fluid surrounding the germ cells is removed, resulting in sporoplasm. (N) Valvocapsulogenic cells form constriction where they attach to sporoplasm. (O) Valvocapsulogenic cells divide to produce three valvogenic and three capsulogenic cells. All of these cells remain in contact with sporoplasm. (P) Capsulogenic cells form polar capsules within them; valvogenic cells form the spore body and caudal processes. (Q) Germ cells divide within sporoplasm. (R) Cytoplasm transfer, where membranes of germ cells become indistinct during the growth phase of the sporoplasm. This may also occur at stages H and W. (S) Mature actinospore. Sporoplasm is contained within spore body. Germ cells are visible again within this sporoplasm. Capsule cells contain mature polar capsules, the stoppers of which are free of the surrounding valve cells. The valve cells form the spore body and the caudal processes. In this representation, the spore is still within the pansporocyst, thus caudal processes still appear as a mass of membranes, rather than float cells. (T) The actinospore is released into the water column where it comes in contact with a fish host. (U) The sporoplasm enters the fish host to form the plasmodia. This undergoes plasmotomy resulting in cell doublets. (V) Each cell doublet contains one secondary (germ) cell surrounded by a diploid primary cell and can migrate and proliferate in the host. (W) Each cell doublet becomes a plasmodium containing replicating secondary cells. The plasmodium can undergo cytoplasmic transfer to resemble a syncytial cell. (X) One secondary cell engulfs another to herald the onset of sporogony. (Y) Myxospores are formed within plasmodium. These contain a binucleate, haploid sporoplasm. They are released from fish into the environment to infect the oligochaete host.

Pathological effects of Myxozoa

- generally low pathogenicity, but under certain conditions causing serious fish disease
- part of the marine fish catch is spoiled by the myxosporidian proliferative stage (having effective proteolytic enzymes) dying in tissues of dead fish becomes gelatinous after cooking consistency
- causative agents of inflammatory diseases (PKD - proliferative kidney disease in salmonids) or directly destroying important tissues of the fish ("whirling disease"), infection of gill epithelium

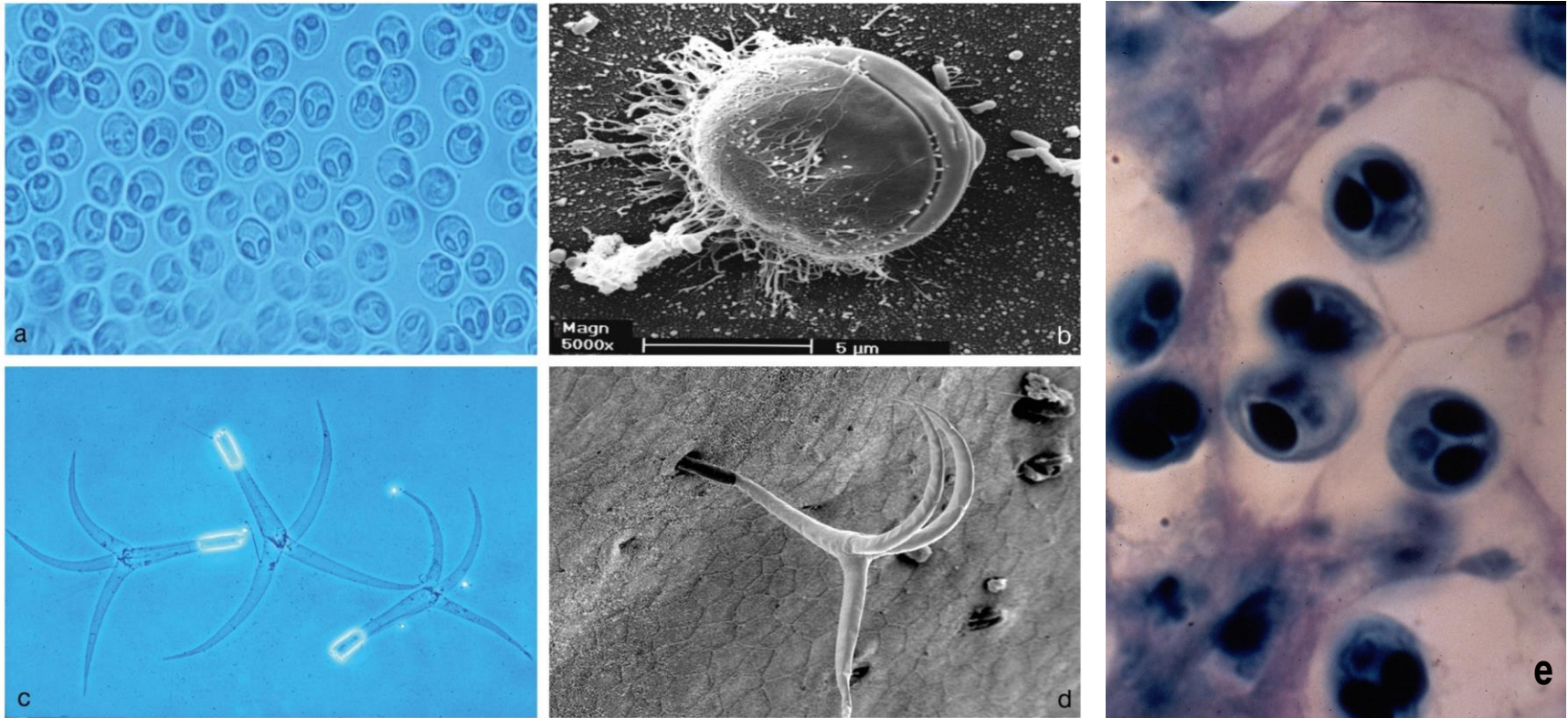
Life Cycle of Whirling Disease

Whirling Disease poses a serious threat to New Mexico's trout population. To prevent the spread of this disease it is helpful to understand its life cycle.



Myxobolus cerebralis

- agents of Whirling disease in salmonid fish, affecting juvenile fish and causes skeletal deformation and neurological damage



A) Unstained mature *M. cerebralis* myxospores obtained from rainbow trout displaying whirling disease 120 d post-exposure (p.e.). Each infective myxospore displays 2 intact polar capsules. LM. **B)** Mature myxospore. SEM. **C)** Unstained mature waterborne triactinomyxon spores released from *T. tubifex* 120 d p.e. Triactinomyxon spore is larger than the myxospore and contains a minimum of 64 spherical sporoplasm cells (sporozoites) Triactinomyxon spores elicit refractile sporoplasm and transparent processes. LM. **D)** Mature triactinomyxon penetrating the epidermis of fish host. SEM. **E)** Histological changes caused by *M. cerebralis* infection of salmonid cartilage. Giemsa, LM.

Life cycle of *Myxobolus cerebralis*

1-3) Tricapsulate spore of the *Triactinomyxon* stage contains several uninucleate sporoplasms (amoebic stage). If these stages are eaten by trout with their hosts (a tubificid worm, e.g., *Limnodrilus*), they give rise to multinucleate trophozoites within the cartilage (3).

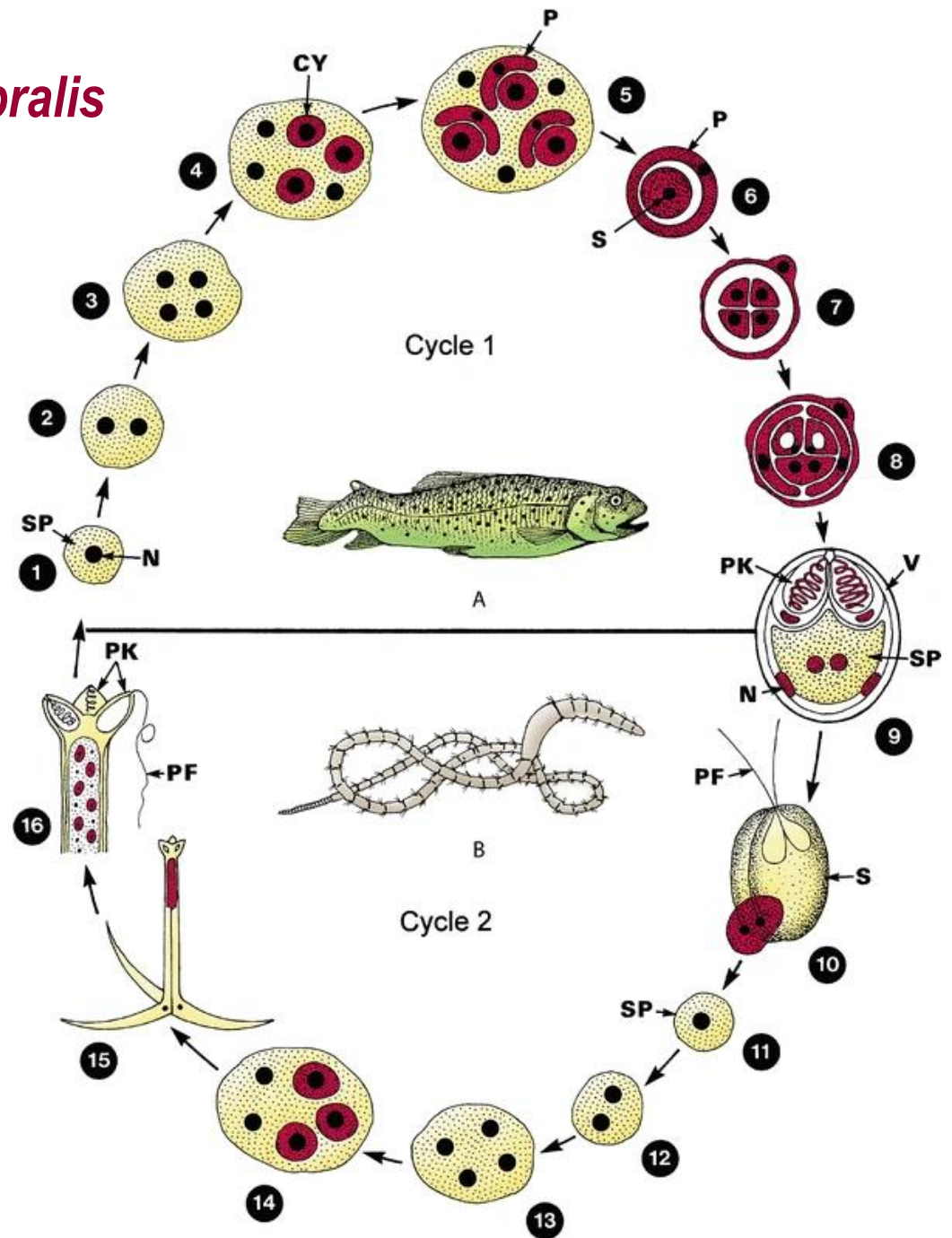
4) Occurrence of uninuclear cytomeres (CY) within the trophozoites.

5-6) One cell (pericytic cell, P) surrounds the other (sporogonic cell, SP).

7-8) Sporogonic cell divides and gives rise to two valvogenic cells, two capsulogenic cells, and 1, 2-nucleate sporoplasm.

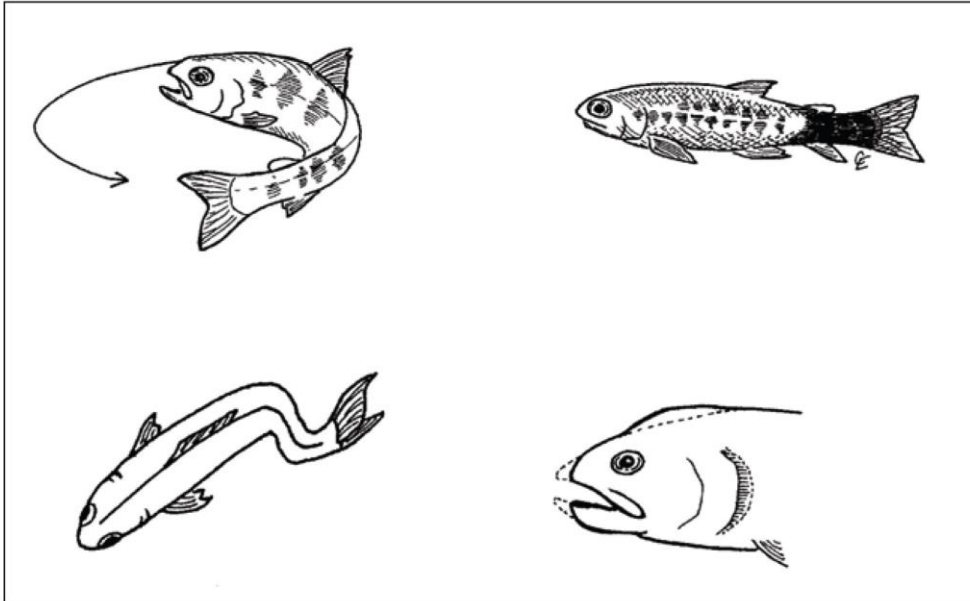
9) Fully differentiated multicellular spore (*Myxobolus* stage) which becomes free after death of fish.

10) If a tubificid worm eats such spores, the two valves open in its intestine and the sporoplasm (SP) creeps into the body cavity...



Whirling disease

Clinical signs: whirling behaviour, blackened tail, skeletal deformities of spine and head, mortality



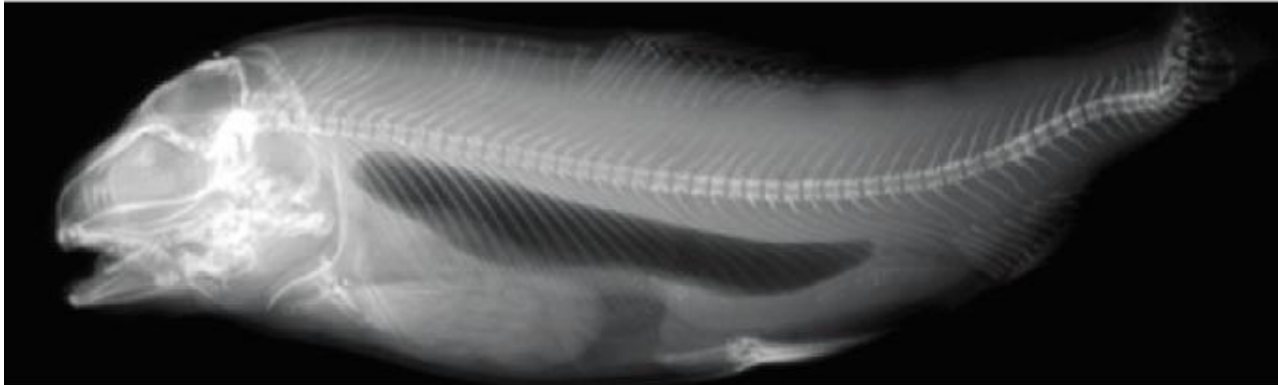
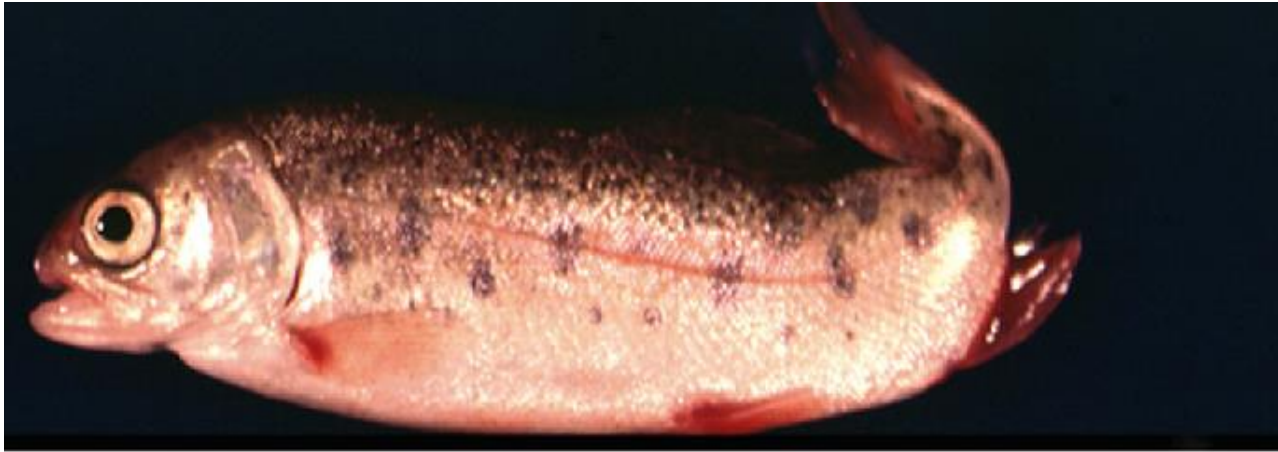
Brown Trout with black, deformed tail.



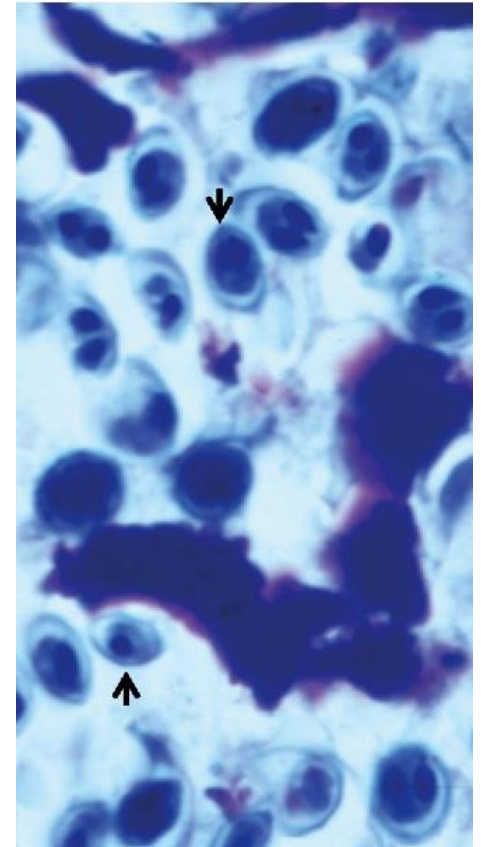
<https://www.youtube.com/watch?v=SLjgDeX8xGs>

Whirling disease

Etiology of whirling disease

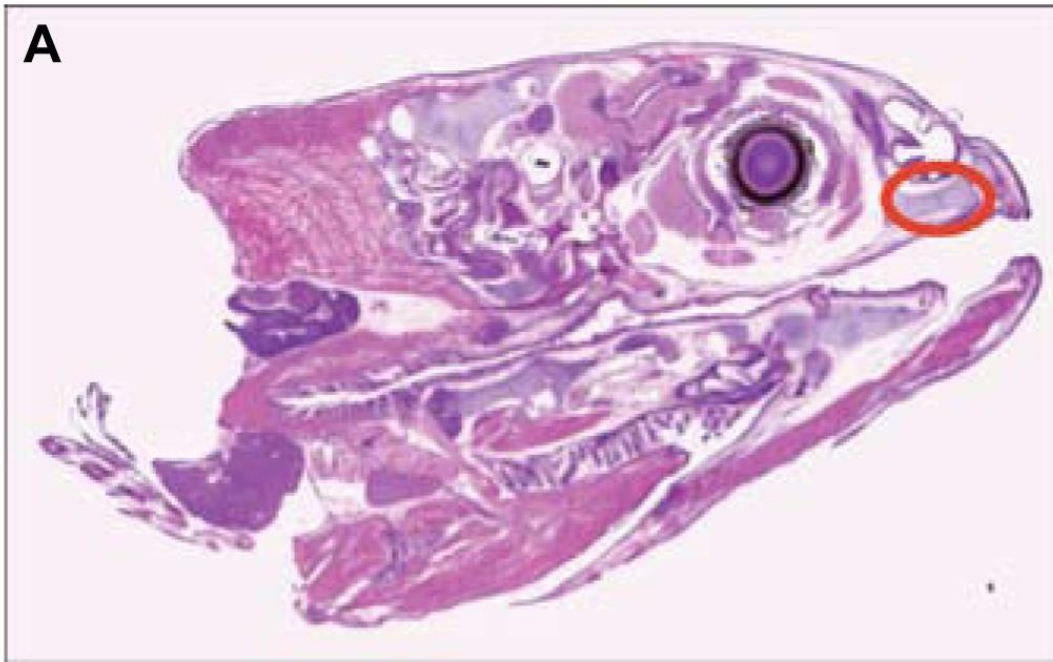


Representative X-ray image displaying characteristic skeletal deformity.



Aggregation of *M. cerebralis* (black arrows) accompanied by digestion and destruction of ossification by trophozoites leading to skeletal deformities.

Whirling disease



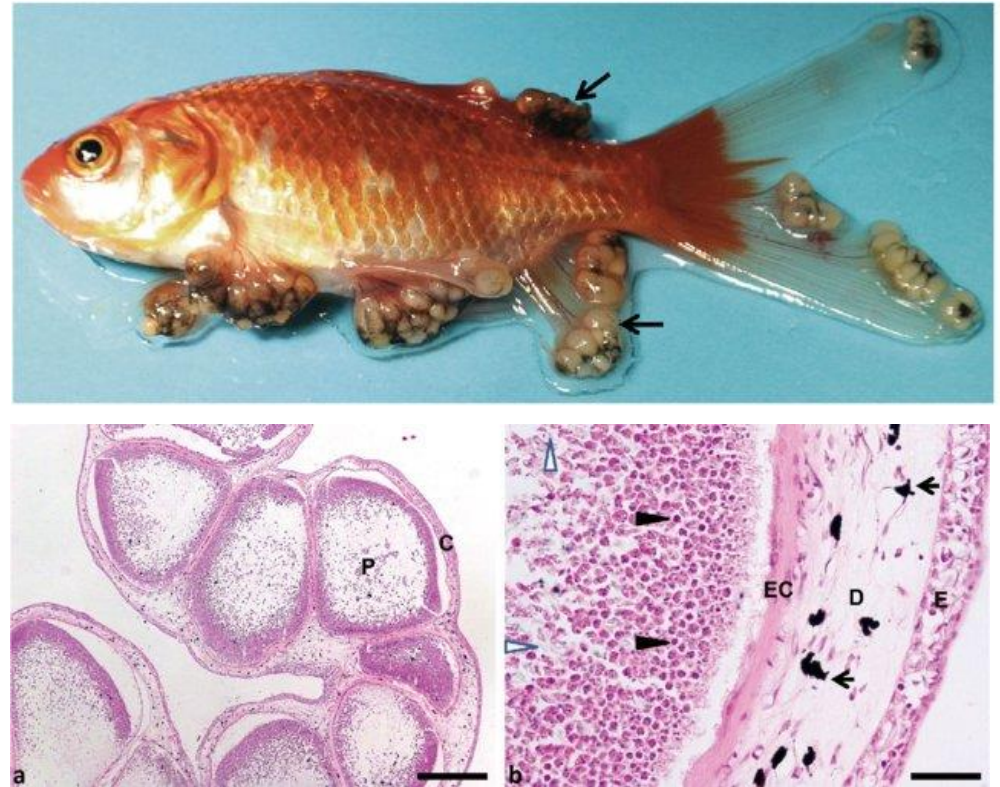
Thinly sliced cross sections of infected fish tissues reveal microscopic damage caused by *Myxobolus cerebralis* to cartilage when compared to healthy fish. **A)** Cross section through rainbow trout head showing location of cartilage (red circle) in smaller images. **B)** Cartilage section from an uninfected fish. **C)** Cartilage section from a heavily infected fish.

Myxobolus turpisrotundus

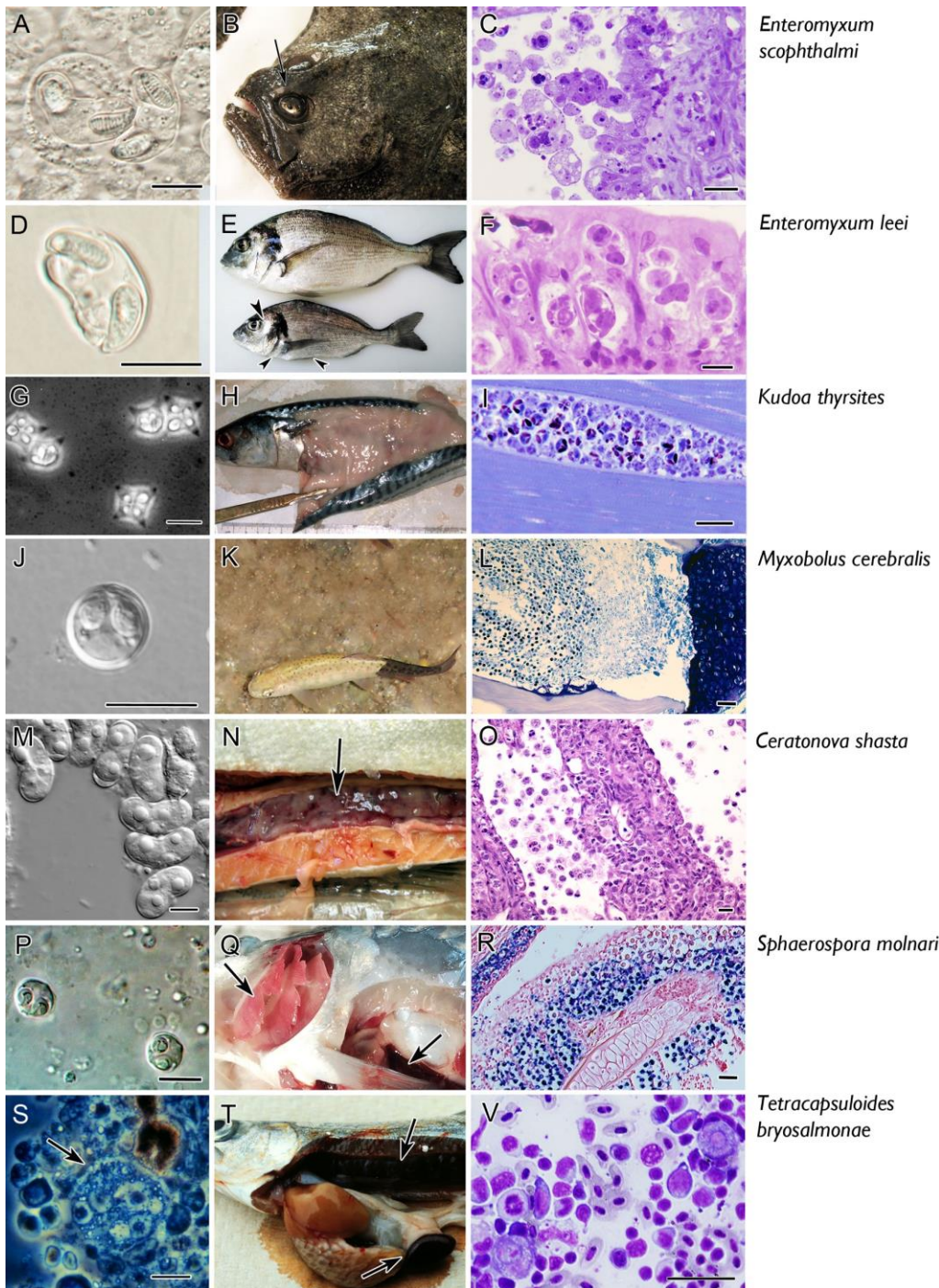


Gibel carp heavily infected with *Myxobolus turpisrotundus*. **A)** Disfigured appearance of fish from infection on body surface. **B)** Infected intestine with 3 large plasmodia in the foregut (arrow).

Thelohanellus hoffmanni



Histopathology of the caudal fin of goldfish with nodules (plasmodia). HE. A) Plasmodia (P) are filled with different developmental stages of *T. hoffmanni*. C - cyst wall. **B)** Higher magnification of a nodule from A; D - dermis; E - epidermis; EC - eosinophilic cyst wall. Melanocytes (arrows) are gathering together around the plasmodia. Note that the early developmental stages are in the periphery of the plasmodium (black arrowheads), and the mature spores are in the centre (white arrowheads).

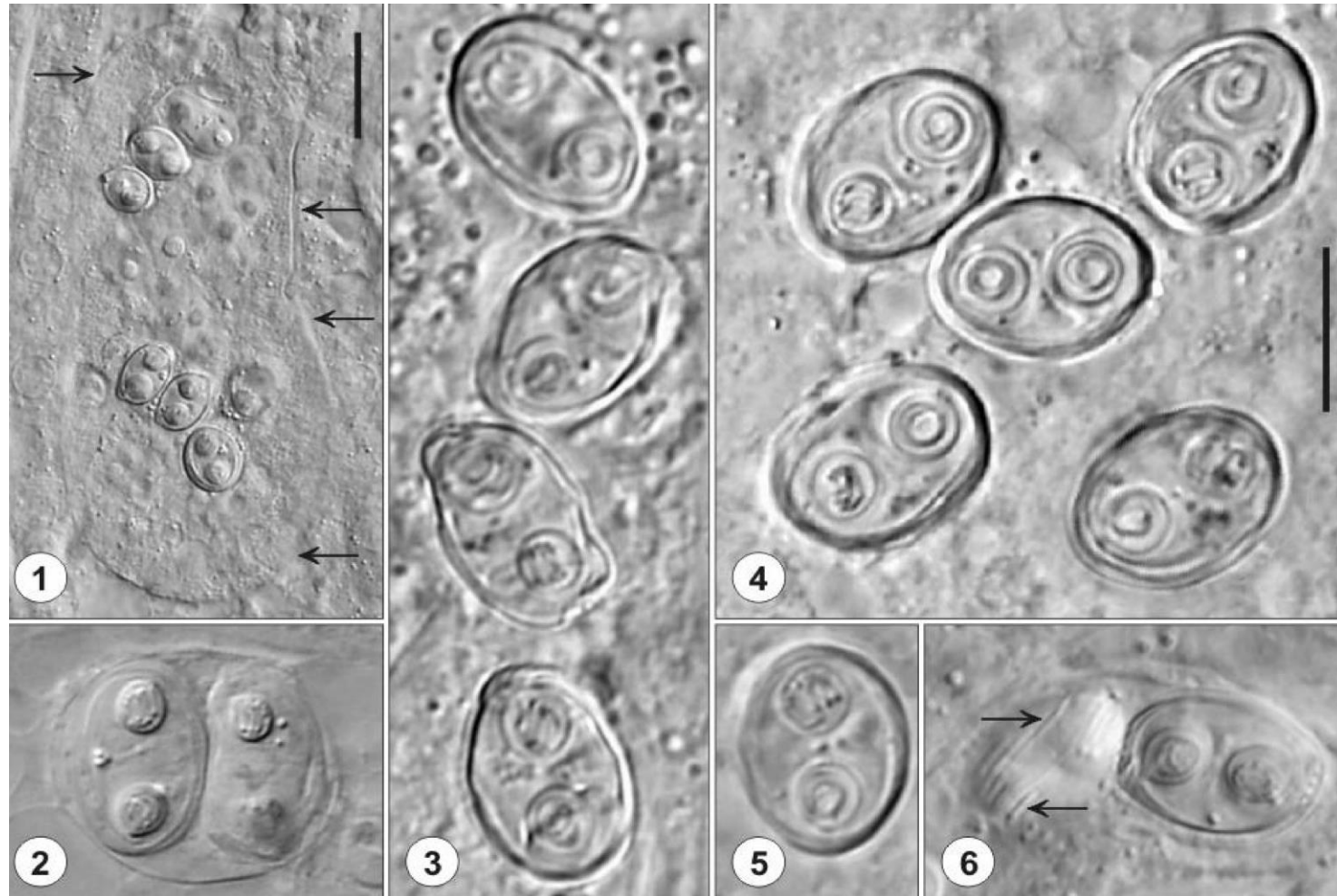


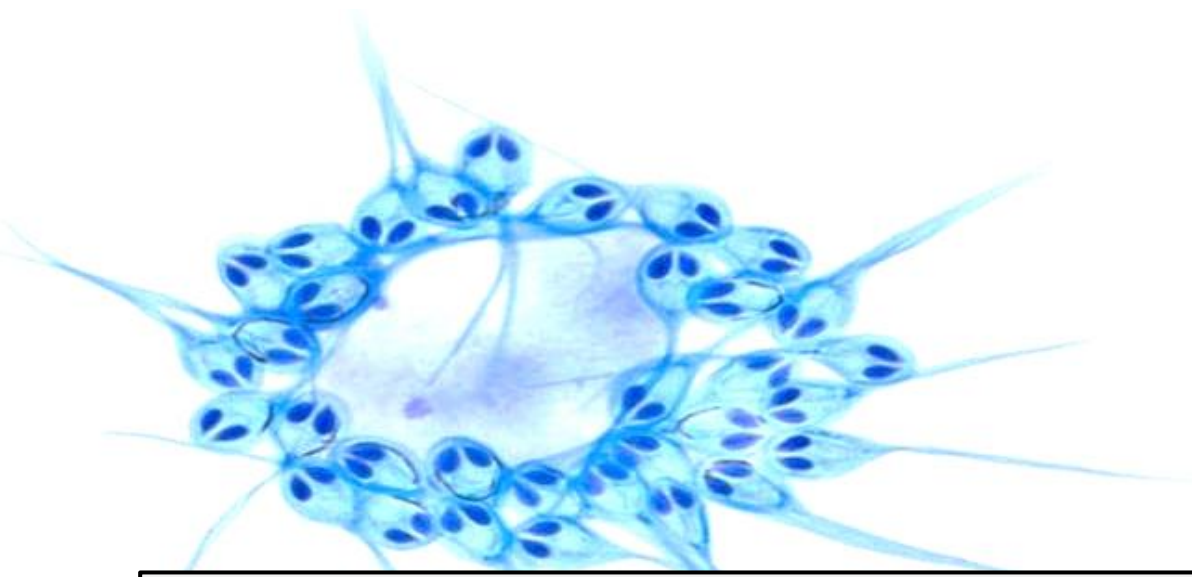
Main myxozoan species for which information is available on fish adaptive immune response

Pictures in the left column (A, D, G, J, M, P, S) fresh smears of myxospores, except for *T. bryosalmonae* in which a proliferative stage is shown (S). Middle column refers to clinical signs: note the sunken head syndrome in turbot (arrow in B), the sunken belly and prominent head bones in gilthead sea bream (arrowheads in E), the myoliquefaction in a mackerel (H), the black tail in rainbow trout (K), the swollen and ascitic digestive tract in rainbow trout (N), the pale gills and splenomegaly in common carp (arrows in Q), the swollen kidney and splenomegaly in rainbow trout (arrows in T). The right column depicts histopathological aspects. Catarrhal enteritis in turbot (C, Giemsa); invasion of the paracellular space of the gut (Giemsa, F); hypertrophy of myocytes (I, toluidine blue); invasion of the cartilage of rainbow trout (L, Giemsa); destruction of the intestine with detachment of stages to the lumen (Q, H, E); sporogony in the gill epithelium [R, in situ hybridisation with parasites labelled in by VectorBlue, counterstained with Neutral red; interstitial stages in a kidney imprint (V, Diff-Quick).

Soricimyxum fegati

- parasitising liver of shrews in Czechia





Thank you for your attention 😊

