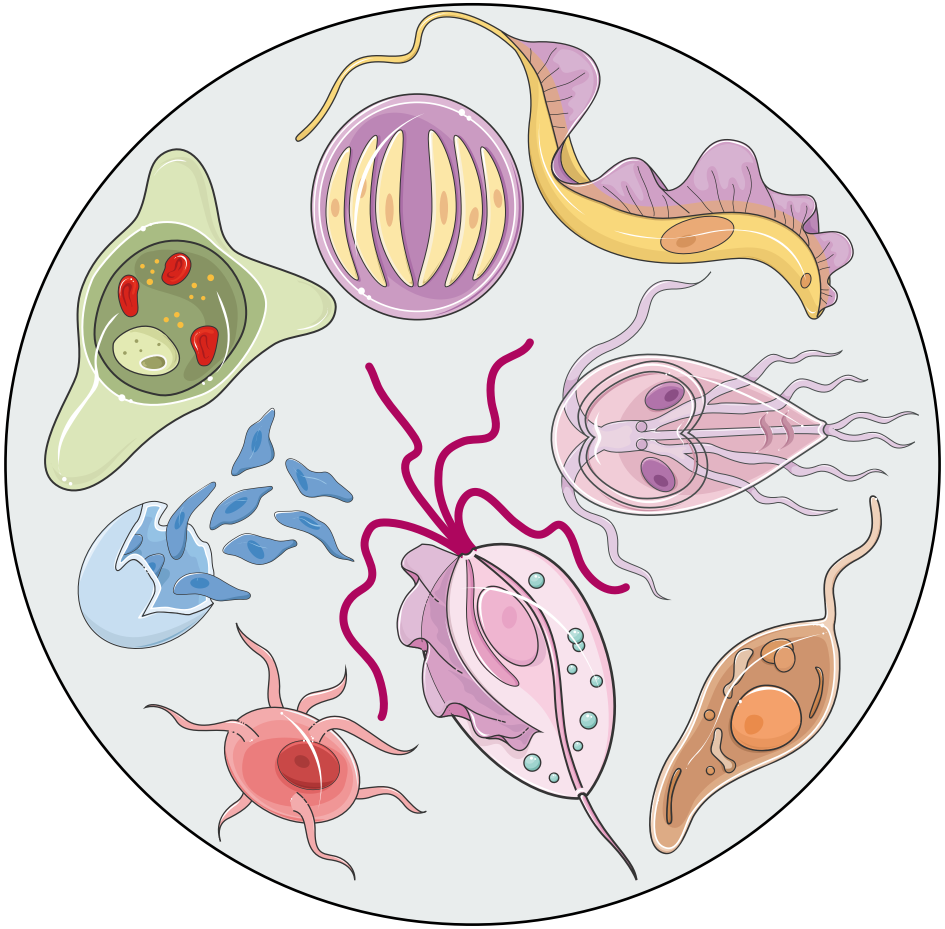
Biology of parasitic protozoa

III. Fornicata, Parabasala, Preaxostyla (Excavata)



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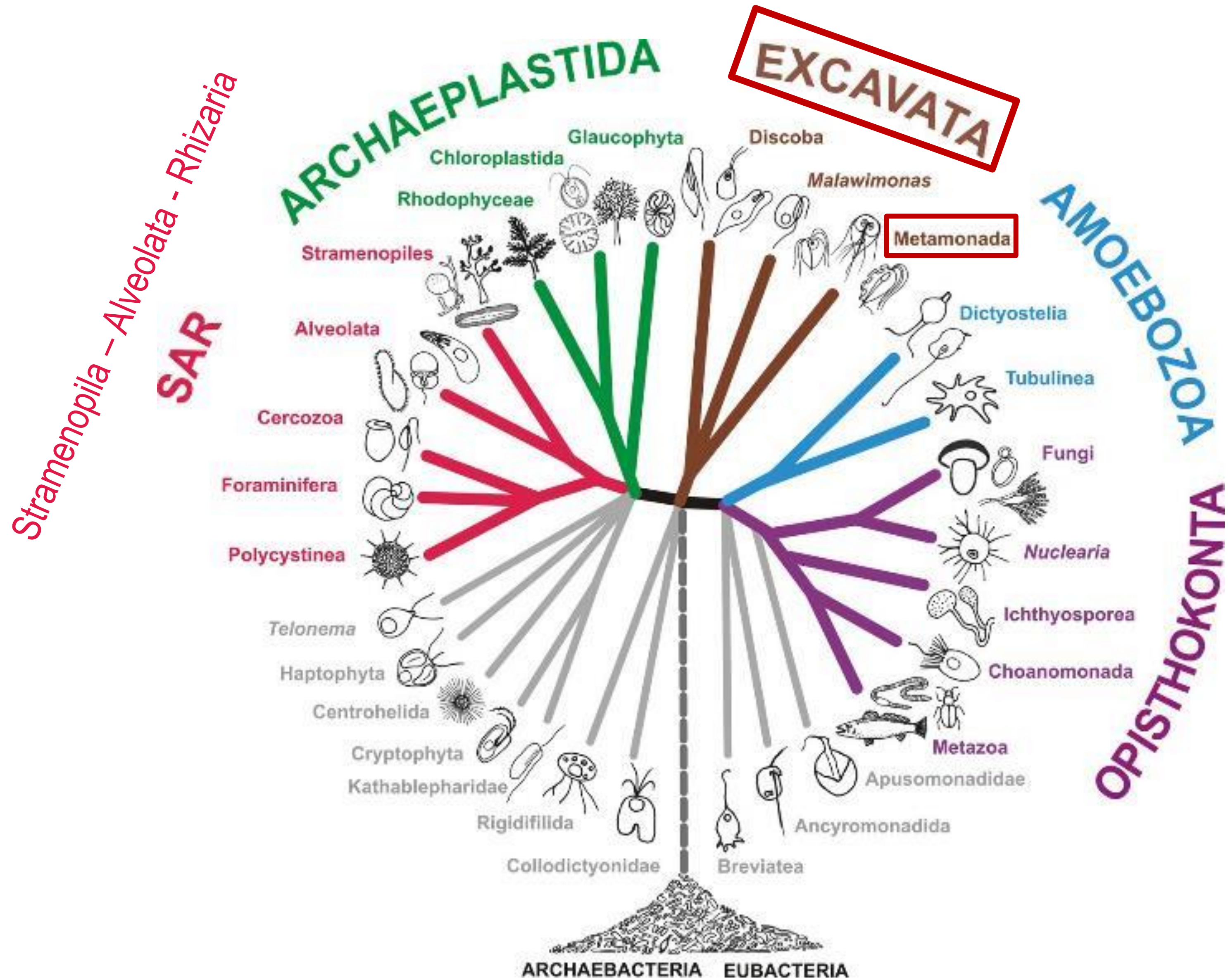
5 supergroups = megagroups



J. EUKARYOT. MICROBIOL., 59, NO. 5, SEPTEMBER-OCTOBER 2012

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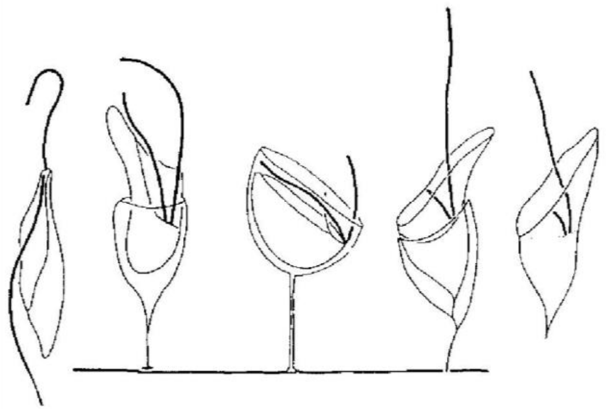
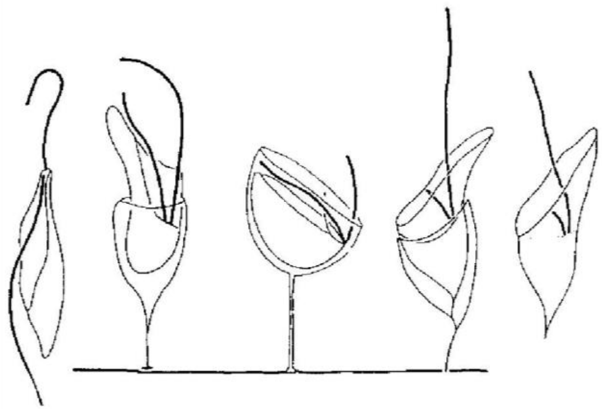
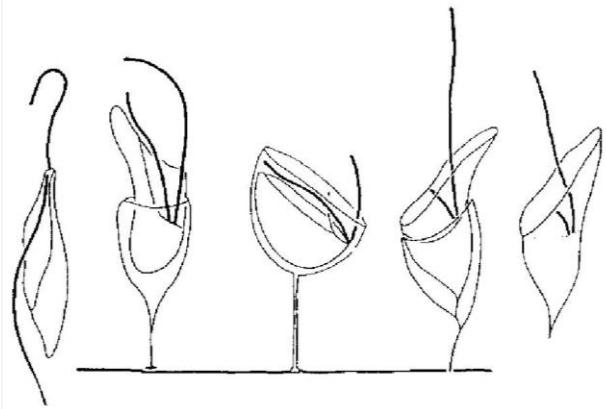
Excavata

- conspicuous **ventral feeding groove** that is "excavated" from one side and through which pass one or more recurrent flagella; the ventral groove has characteristic ultrastructure and is supported by microtubules
- originally 2 flagellated state - many changes: multiplication of flagella, reduction or disappearance of the ventral groove
- variety of free-living and symbiotic forms
- not a monophyletic group
- paraphyletic group with the ancestors of other living eukaryotes
- parasitic species in **Metamonada•**: **Fornicata•••**, **Parabasalia•••**, **Preaxostyla•••**







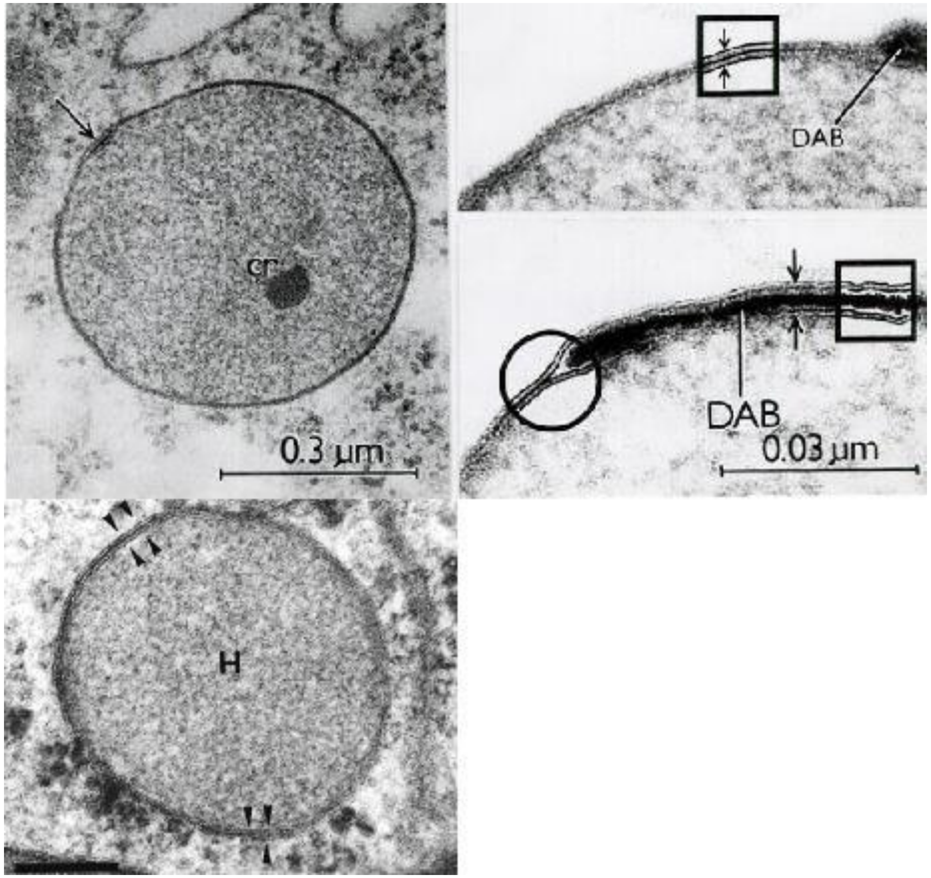
Fornicata

- together with Preaxostyla (oxymonads) and Parabasalia named as **metamonads** = group of flagellate amitochondriate protozoa
- unicellular heterotrophic flagellates
- anaerobic / microaerophilic
- mostly flagellated cells, usually with 4 kinetosomes per kinetid;
- some are free-living, many endobiotic or parasitic
- modified atypical, non-respiratory mitochondria, lacking the cristae and genome (DNA) (e.g. **hydrogenosomes** or **mitosomes**)

Fornicata – atypical mitochondria

Hydrogenosome

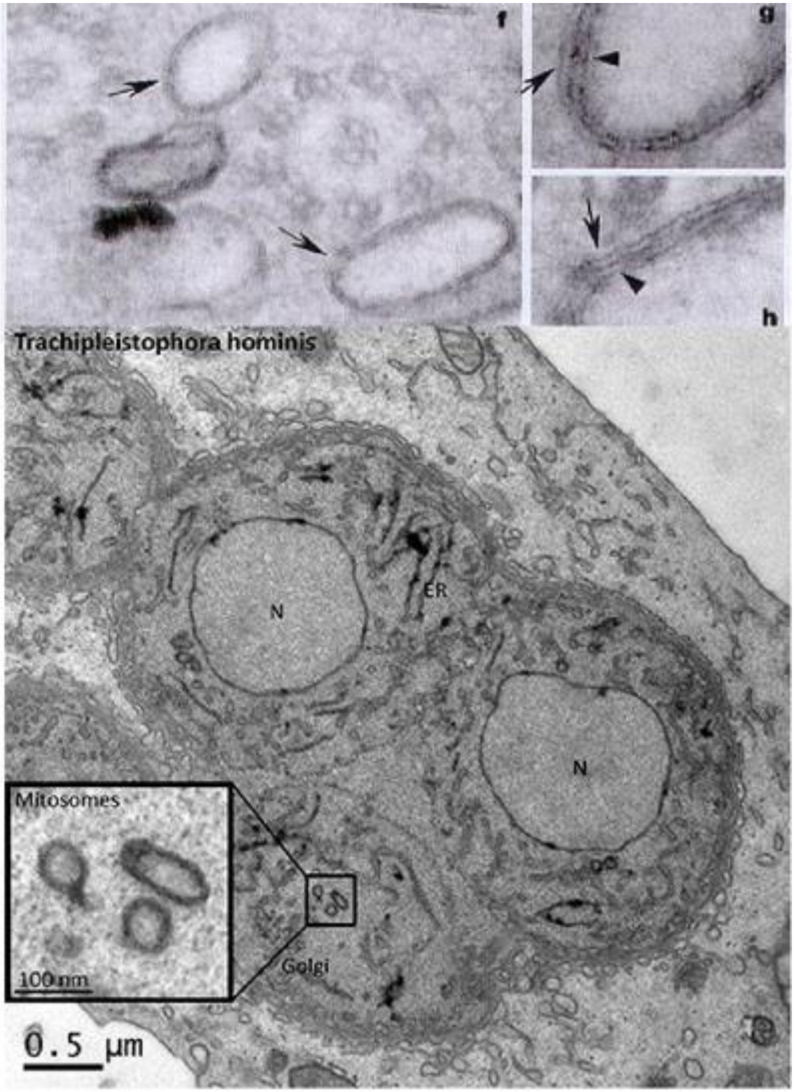
- membrane-enclosed organelles of some anaerobic ciliates, trichomonads, fungi, and animals
- approximately 1 µm in diameter, under stress conditions up to 2 µm
- are so called because they produce molecular hydrogen
- bound by distinct double membranes and one has an inner membrane with some cristae-like projections
- genes for hydrogenosomal components in the nuclear genome



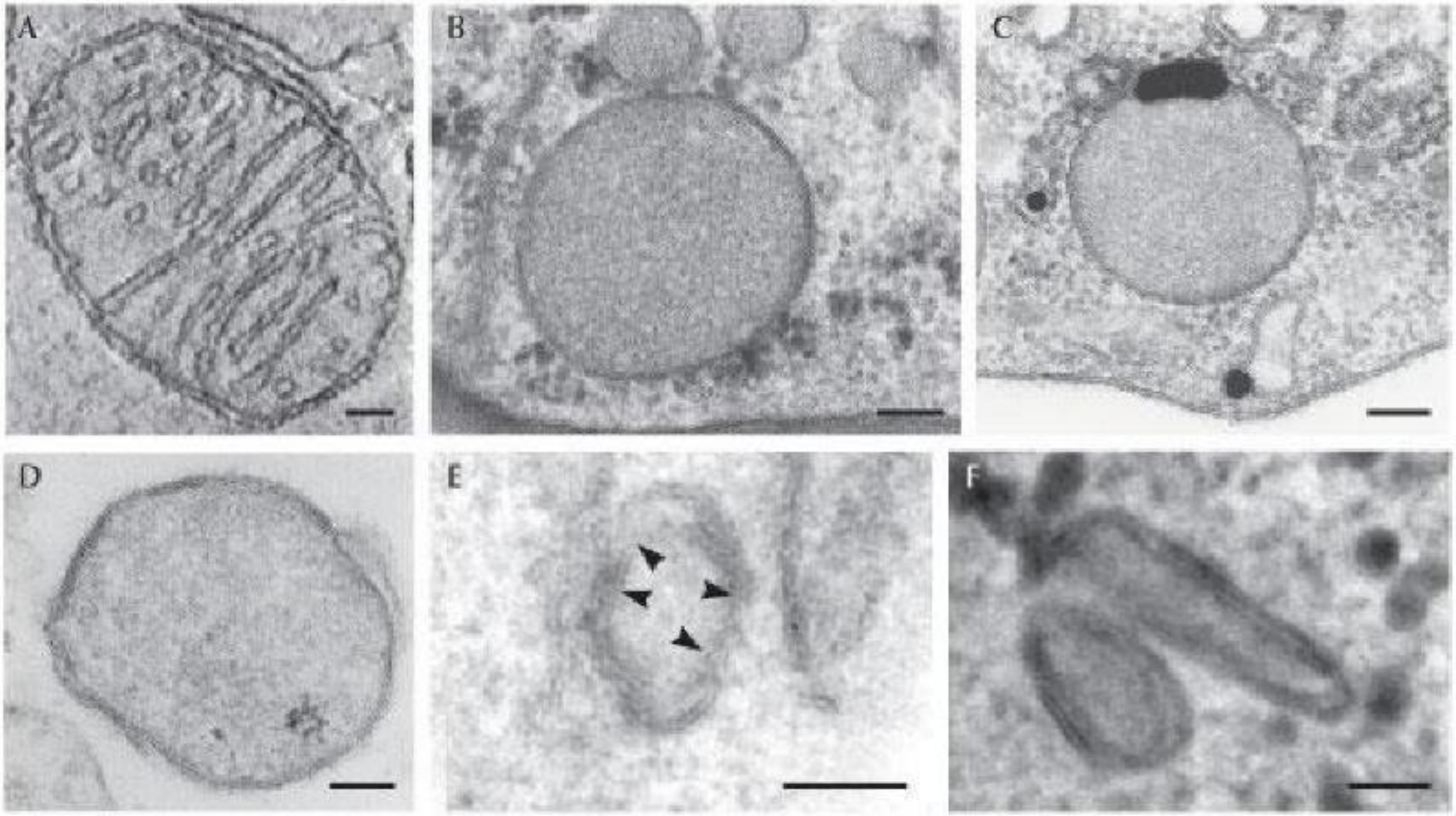
Fornicata – atypical mitochondria

Mitosomes

- detected only in anaerobic or microaerophilic organisms that do not have mitochondria (microsporidia, *Giardia intestinalis*, *Entamoeba* spp.)
- about 0,1 μm in diameter
- just like mitochondria surrounded by a double membrane
- genes for mitosomal components are contained in the nuclear genome



Electron micrographs of different mitochondrial manifestations



Electron micrographs of different mitochondrial manifestations. (A) Mitochondrion from chicken cerebellum. (B,C) Hydrogenosomes from (B) the anaerobic fungus *Neocallimastix patriciarum* and (C) the cattle parasite *Tritrichomonas foetus*. (D–F) Mitosomes from (D) the intestinal parasite *Entamoeba histolytica*, (E) the microsporidian *Trachipleistophora hominis* and (F) the diplomonad *Giardia intestinalis*. Scale bars: (A–D) 100 nm and (E,F) 50 nm.

Mark van der Giezen, and Jorge Tovar EMBO Rep. 2005;6:525-530

EMBO *reports*

Fornicata

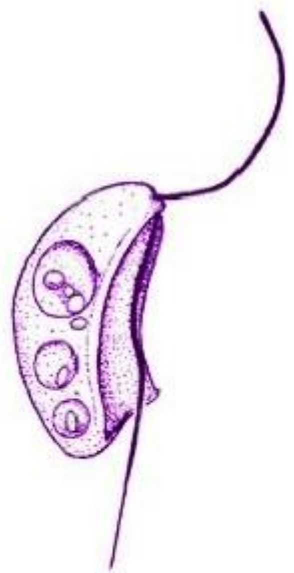
- monophyletic taxon
- unicellular heterotrophic flagellates with one or two karyomastigonts per cell
- **karyomastigont** – first used by Janicki (1915) - conspicuous organellar system observed in certain protozoans
- **mastigont** by definition is present in all ciliated or flagellated eukaryotes and bears the basal body, cilium or flagellum (undulipodium) and in some cases the parabasal body (Golgi complex supported by a parabasal fiber)
- karyomastigont (= mastigont associated with nucleus) bears 1-4 flagella

  - ***Carpediemonas*-like** organisms (carpediemonads) - free-living
  - **Retortamonadida** (retortamonads) - almost exclusively parasitic
  - **Diplomonadida** (diplomonads) - parasitic

Fornicata

Carpediemonas-like organisms

- marine, free-living flagellates
- <u>uninucleate</u> (unikaryotic)
- anaerobic / microaerophilic flagellated cells with a broad ventral suspension = the feeding groove
- almost always biflagellated
- *Carpediemonas bialata* and other „*Carpediemonas*-like“ organisms (= CLOs)



Fornicata

Carpediemonas-like organisms

a-b) *Carpediemonas membranifera*

c-d) *Kipferlia bialata*

e) *Dysnectes brevis*

f) *Dysnectes* sp.

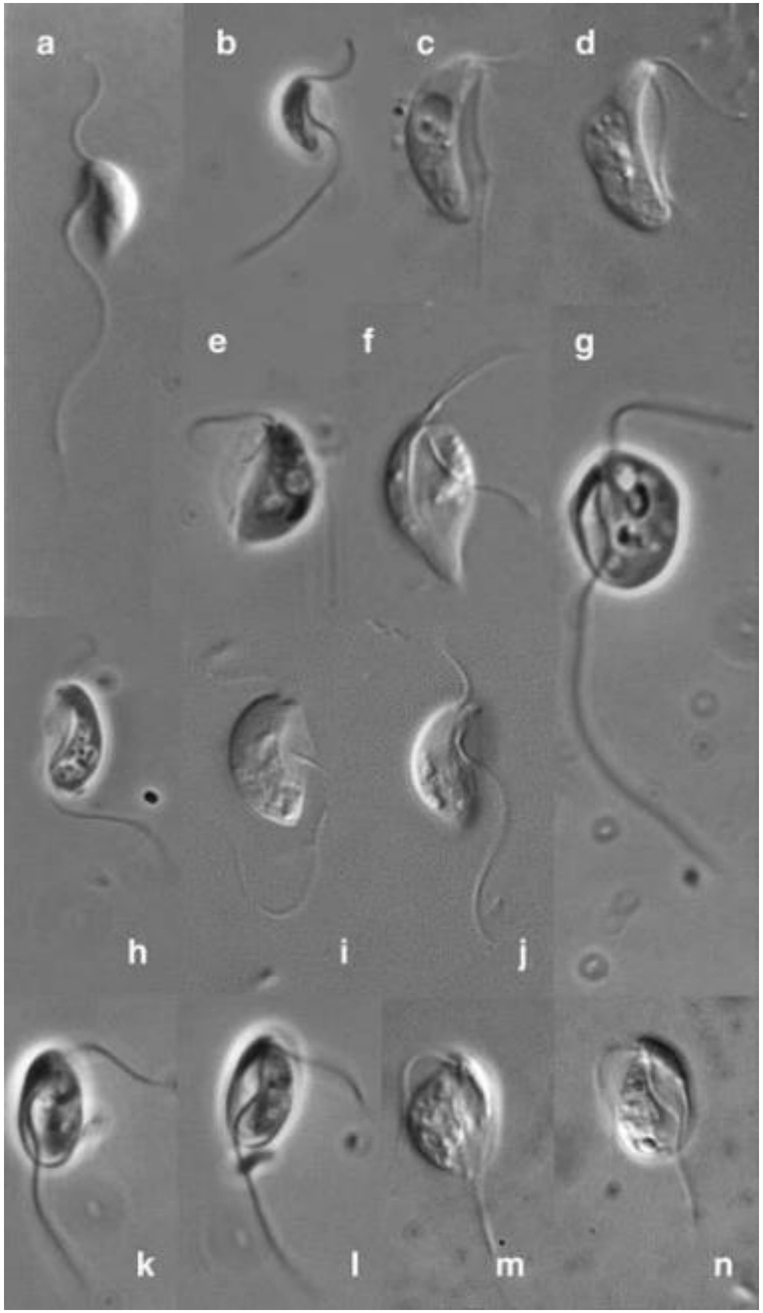
g) *Hicanonectes teleskopos*

h) *Ergobibamus cyprinoides*

i-j) *Carpediemonas*-like organism

k-l) *Aduncisulcus* sp.

m-n) *Aduncisulcus paluster*



Fornicata

Retortamonadida

- uninucelate
- phagotrophic
- conspicuous apical cytostome associated with the posterior flagellum
- system of microtubules underlying the plasmatic membrane-microtubular cytoskeleton/corset
- genera ***Retortamonas, Chilomastix***, both with trophozoites in host intestinal tract and piriform shaped cysts in environment

genus ***Retortamonas***

- biflagellate, more than 20 described species

genus ***Chilomastix***

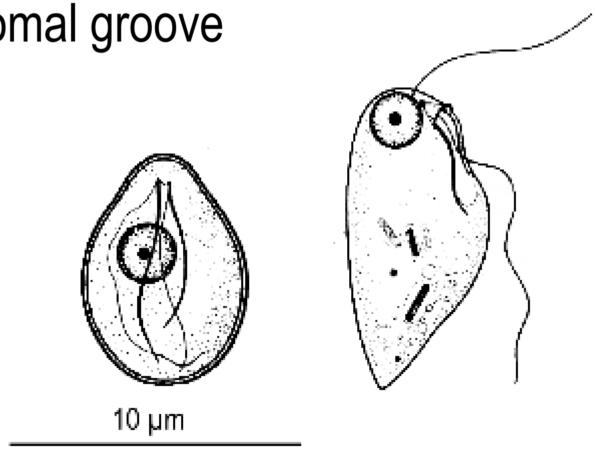
- quadriflagellate, about 30 described species

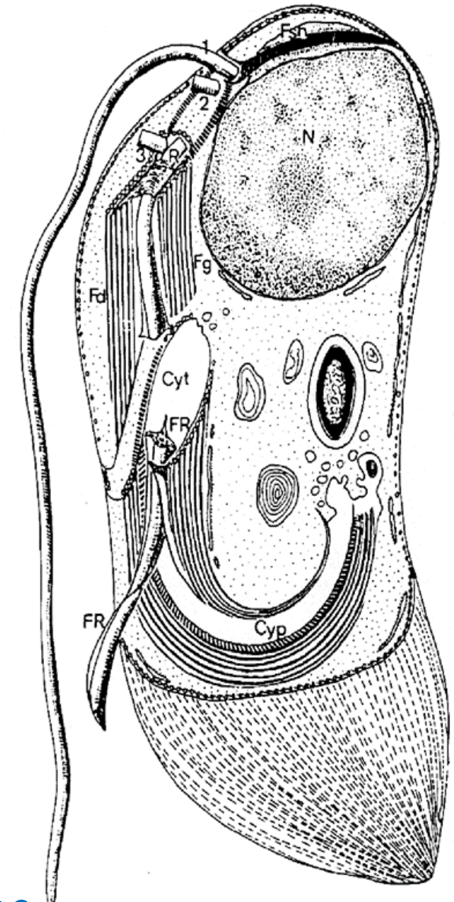
genus ***Retortamonas***

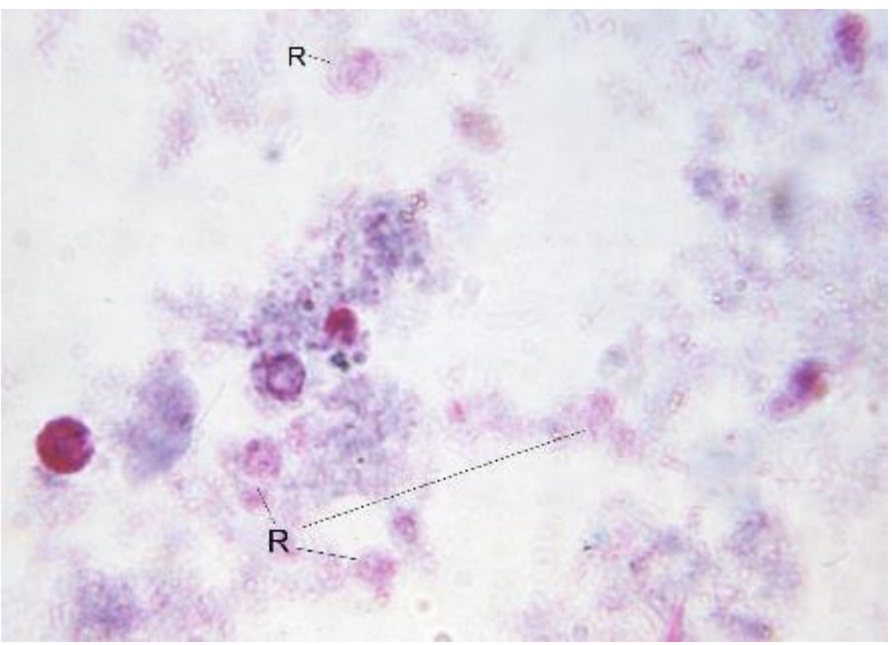
- <u>biflagellate</u> - 1 anterior + 1 recurrent (trailing) flagellum extending through the cytostomal groove
- 4 kinetosomes
- intestinal commensals

Retortamonas intestinalis

- colon of man and monkeys







Retortamonas caviae

- guinea-pigs

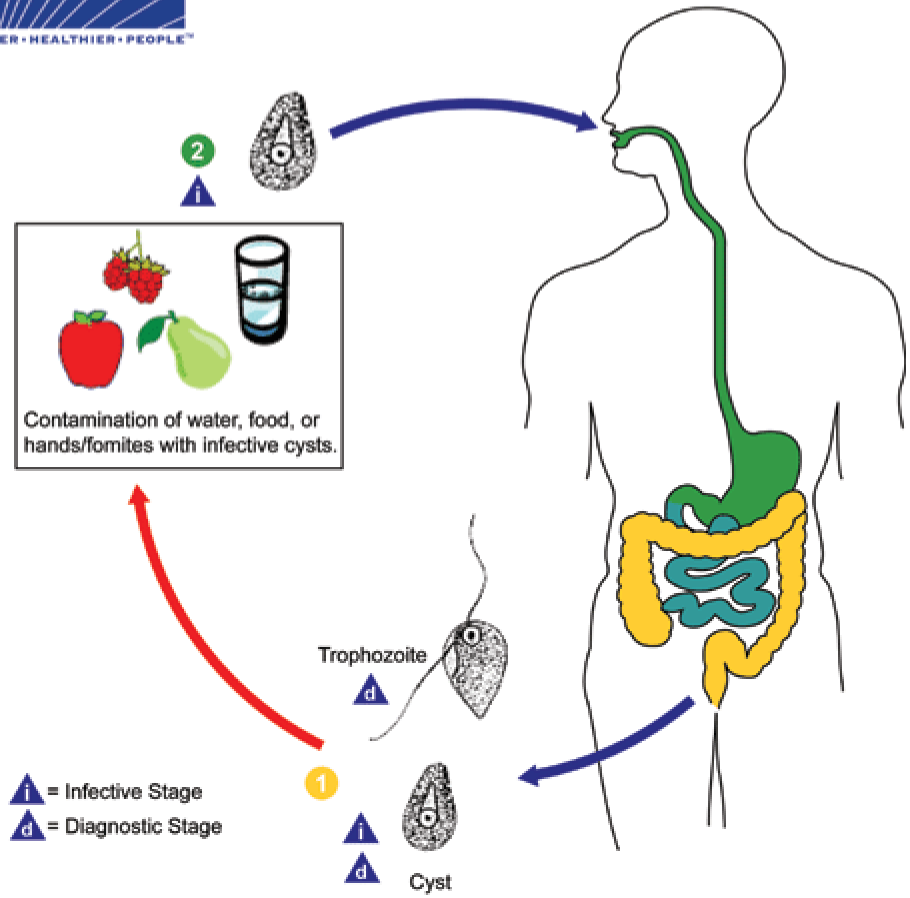
Retortamonas blattae

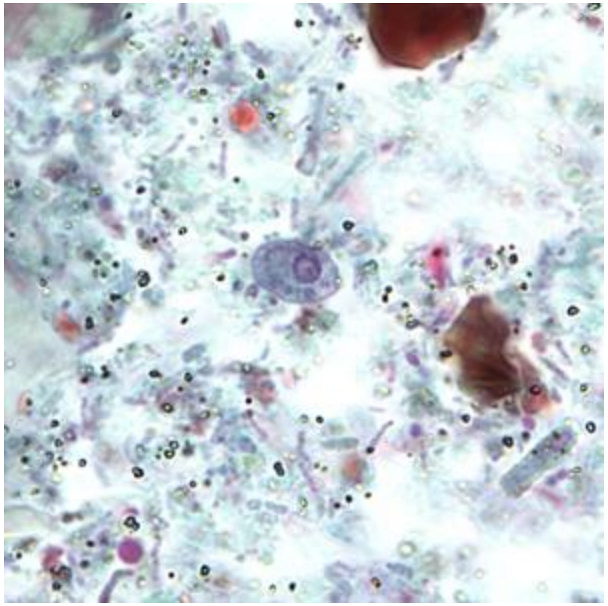
- cockroaches

Retortamonas testudae

- tortoises

Life cycle of *Retortamonas intestinalis*



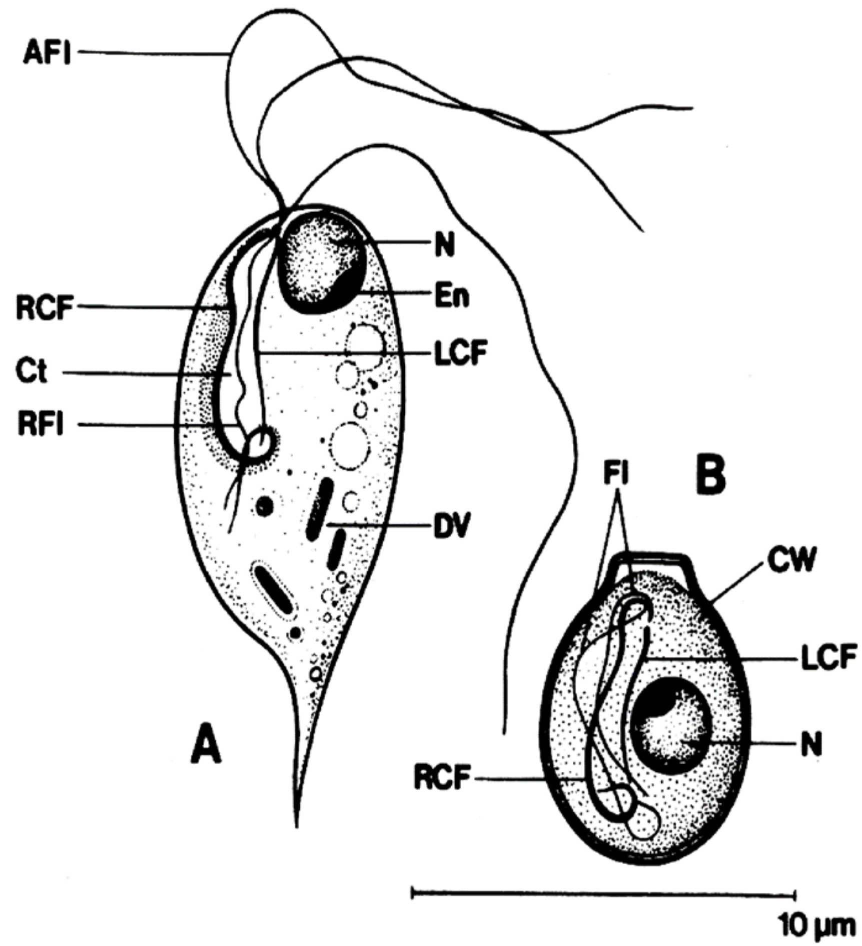


ovoid or pyriform cysts measuring 4-7 x 3-5 μm

Cysts and trophozoites of *R. intestinalis* are shed in faeces. Infection occurs after the ingestion of cysts in faecal-contaminated food/water, or on fomites. In the large (and possibly small) intestine it excysts and releases trophozoites and then resides in colon as a commensal (not known to cause disease). The presence of trophozoites and/or cysts in stool specimens can be an indicator of faecal contamination of a food or water source, and thus does not rule-out other parasitic infections.

genus ***Chilomastix***

- <u>quadriflagellate</u> - 3 anterior and 1 short recurrent (cytostomal) flagellum entirely located in the <u>cytostomal pouch</u>
- spiral groove crossing over the middle half of the body
- the beating of anterior flagella moves the cell, while the undulation of the recurrent flagellum propels food into the cytopharynx

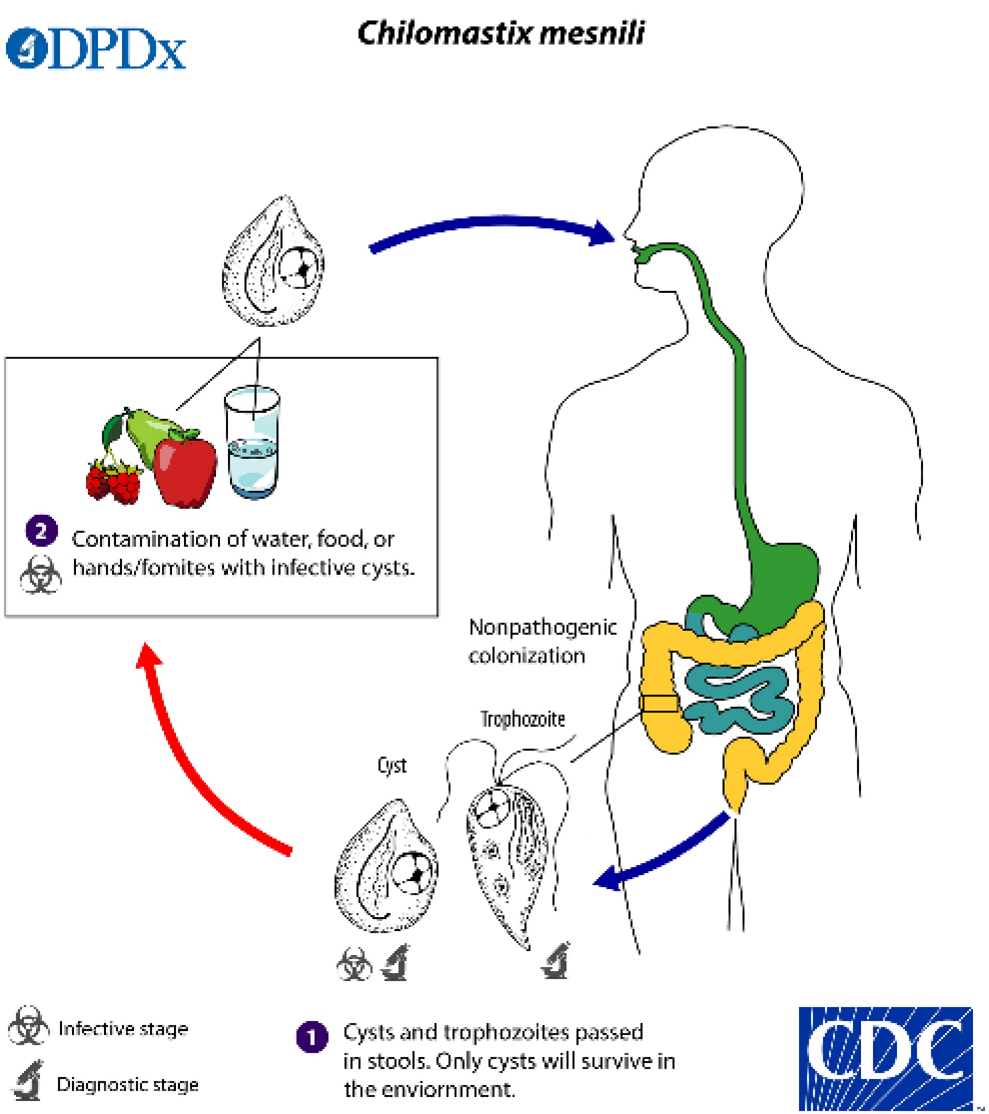


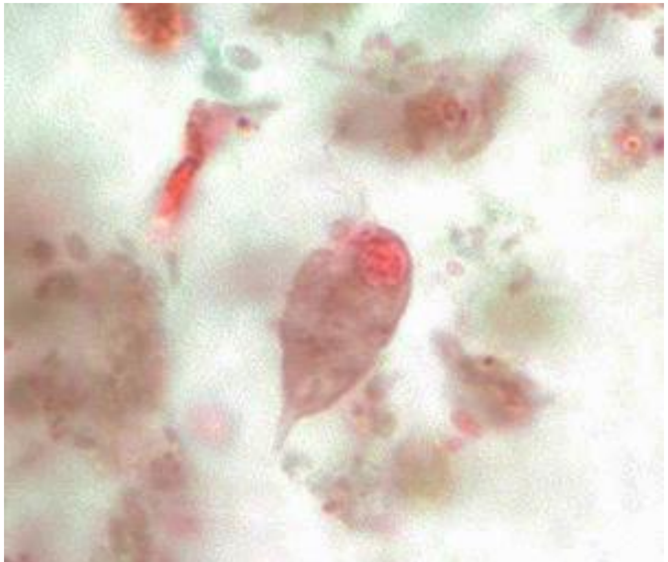
pear-shaped trophozoites measuring 6-24 x 3-10 µm

piriform /lemon-shaped cysts measuring 6-10 µm

Chilomastix mesnili

- human non-pathogenic flagellate, found also in non-human primates and pigs

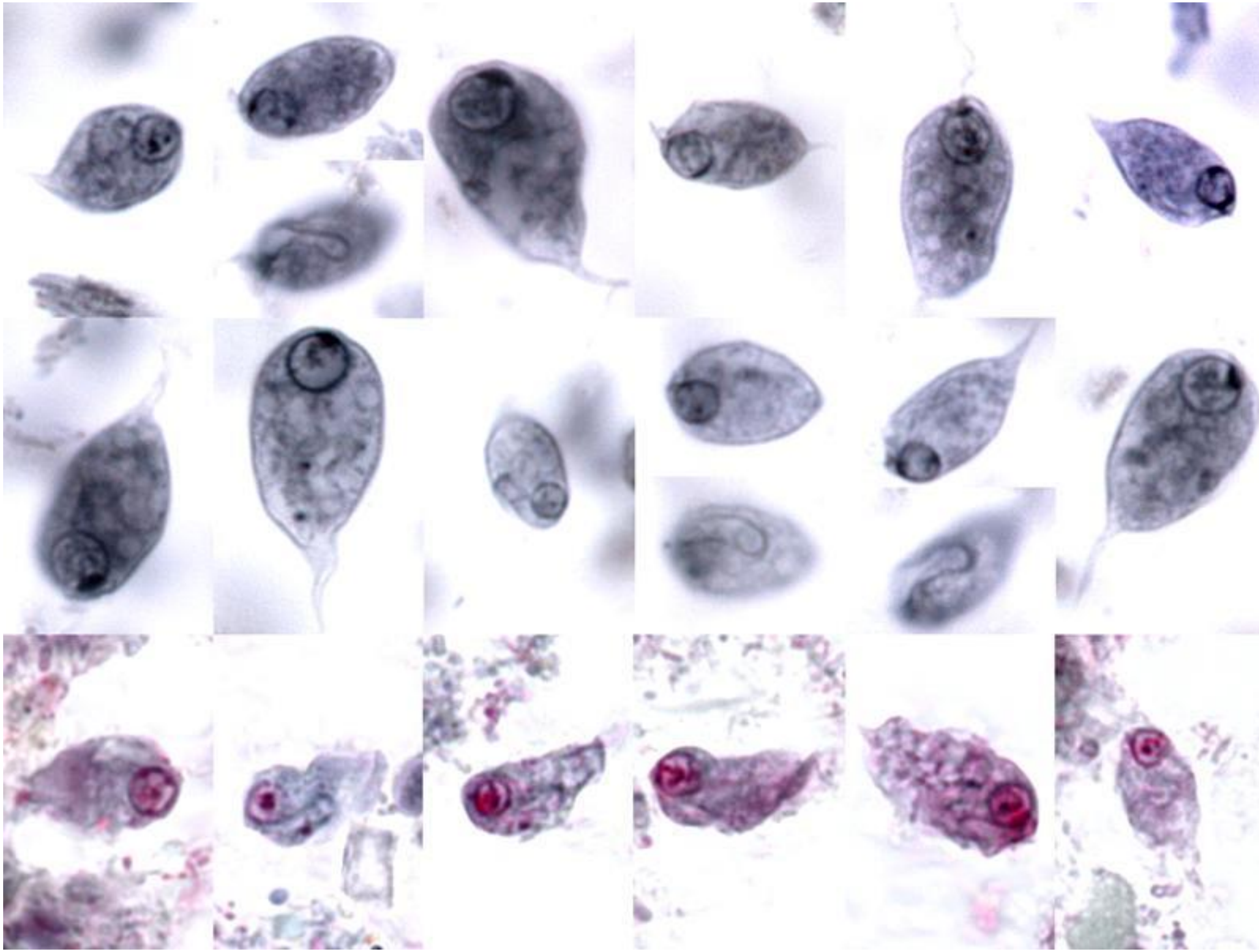




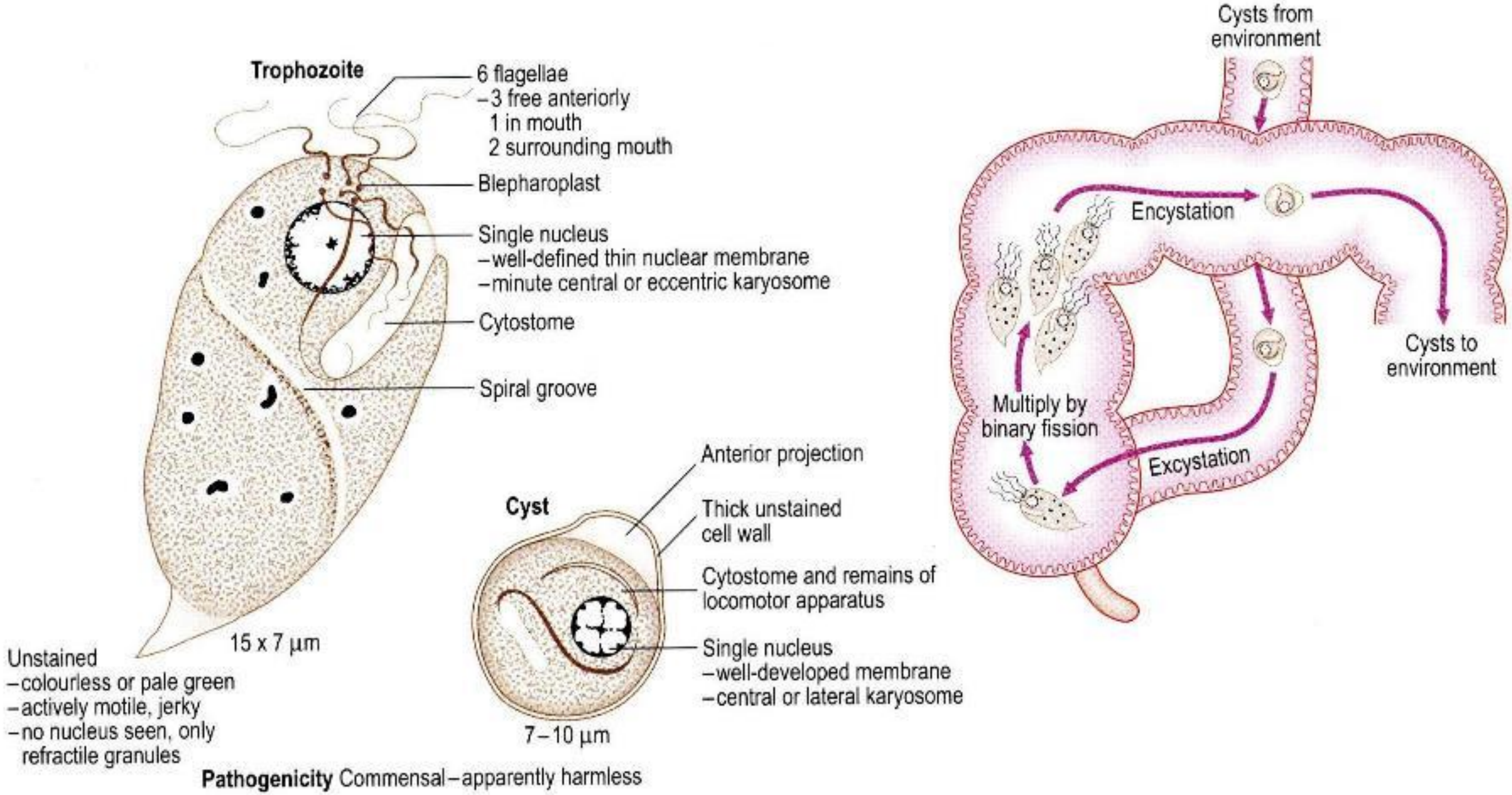
Trophozoite of *C. mesnili* from a stool specimen stained with trichrome

C. mesnili cysts - Iodine stain

Trophozoites of *Chilomastix mesnili*

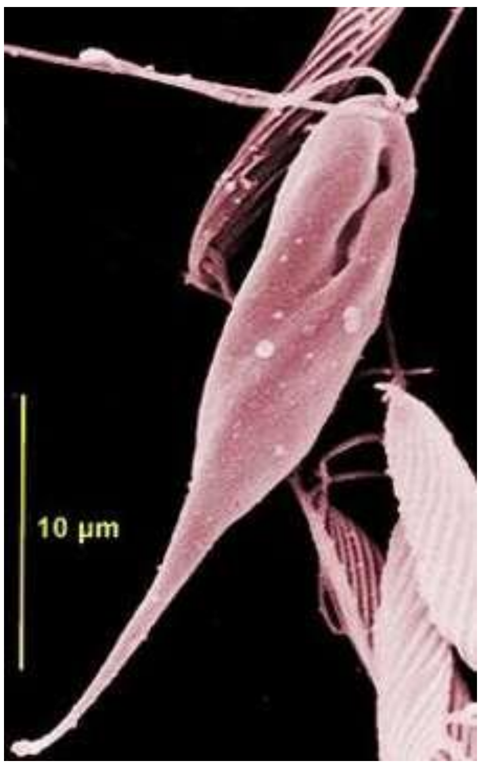


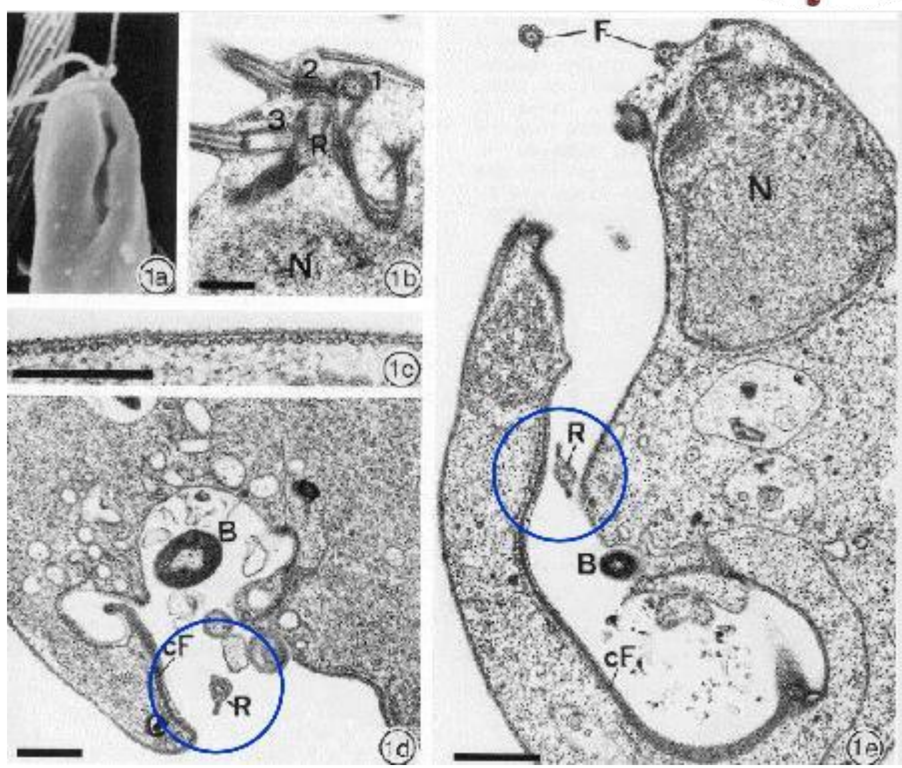
Morphology and life cycle of *Chilomastix mesnili*



Chilomastix caulleryi

- amphibians and leeches





Chilomastix bandicooti
C. bettencourti
C. rosenbuchi
- rodents

C. wenrichi
- guinea pigs

C. caprae
- goats, cattle

C. cuniculi
- rabbits

C. equi
- horses

C. gallinarum
- birds

C. bursa
- lizards

C. motellae
- marine fish

Fornicata

Diplomonadida

- most diplomonads are diplozoic (= 2 karyomastigonts), i.e. all cellular structures are doubled and the cell is axially symmetric

Hexamitidae

- unizoic diplomonads: genus ***Enteromonas*** (enteromonads)
- diplozoic diplomonads: genera ***Trepomonas***, ***Hexamita***, ***Spironucleus***
- tube-like cytostomes passing through the entire cell and opening posteriorly or pocket-like grooves harbouring 3 flagella
- trophozoite x cyst

Giardiidae

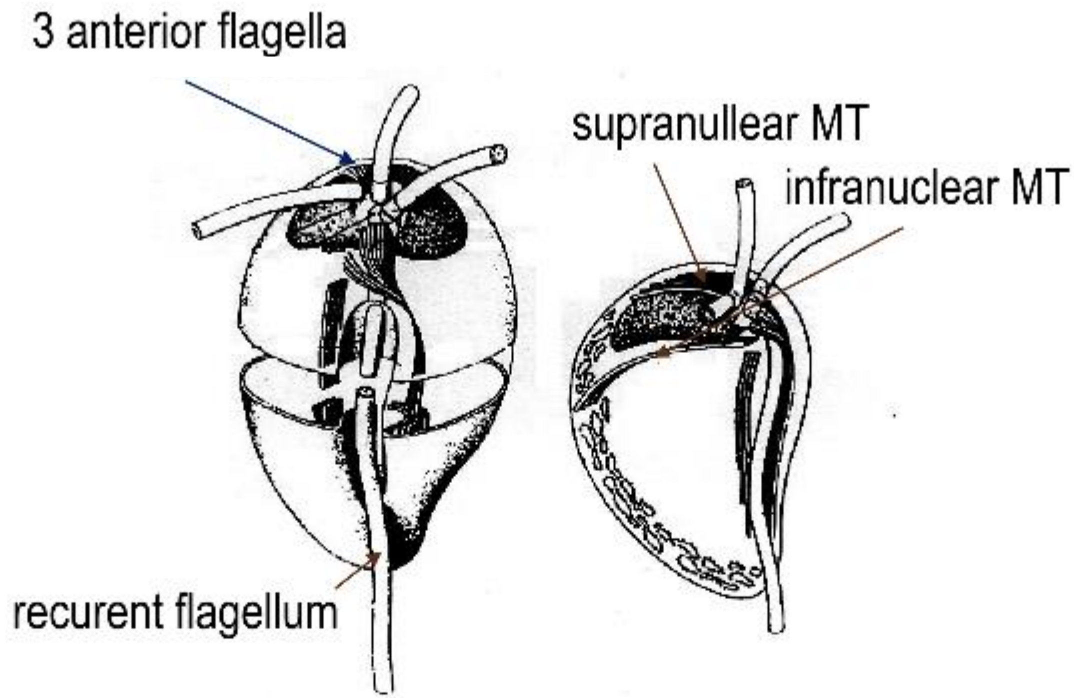
- dorsoventrally flattened cell and the anterior part of the ventral surface is modified into an adhesive disc
- lacking cytostomes (feeding by pinocytosis)
- recurrent flagella passing through the cytoplasm as naked axonemes
- genera ***Octomitus***, ***Giardia***
- trophozoite x cyst

Fornicata

Diplomonadida - Hexamitidae

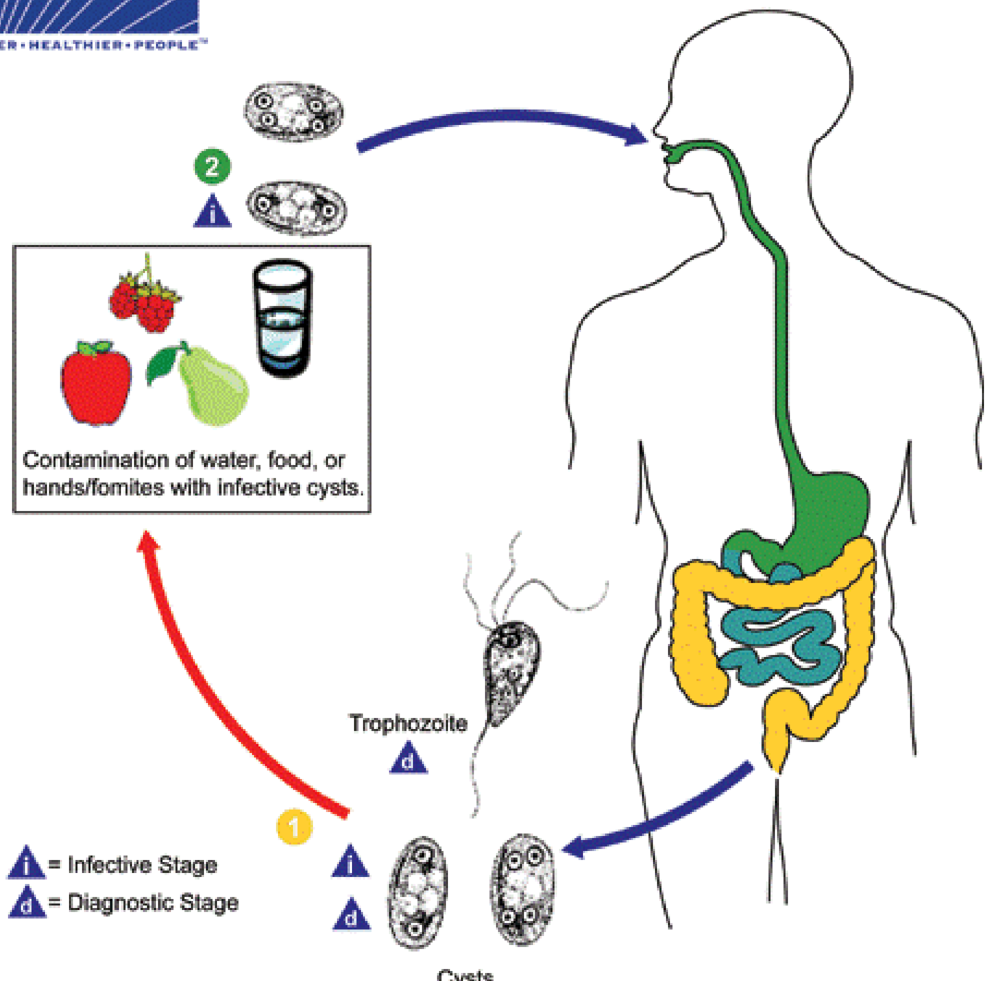
genus ***Enteromonas***

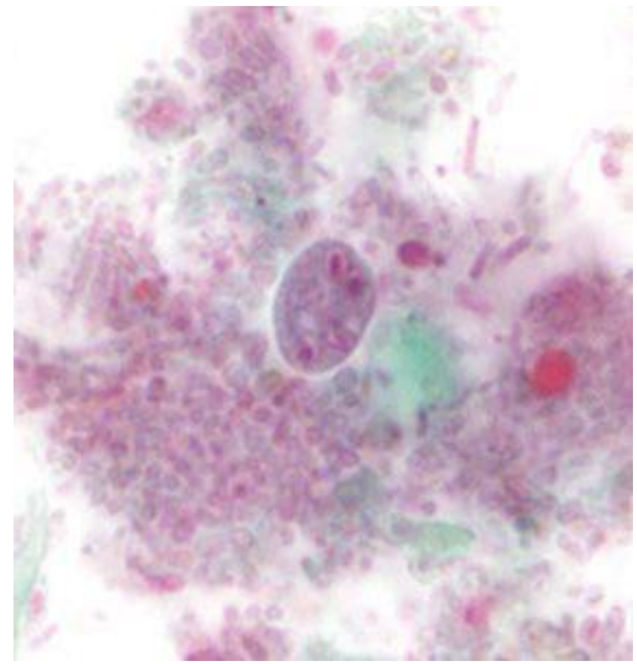
- oval trophozoites, 4-10 μm
- quadriflagellate - 3 anterior flagella + 1 recurrent flagellum that adheres to the body ending in a tail and produces a rotational movement
- no cytostome, only shallow oral gutter
- cysts with 1, 2 or 4 nuclei, binucleate forms being the most common



Enteromonas hominis

- human non pathogenic flagellate,
- indicator of faecal contamination of a food and water source





Cyst of *E. hominis* in a stool specimen stained with trichrome

Enteromonas intestinalis
- appendix of rabbits

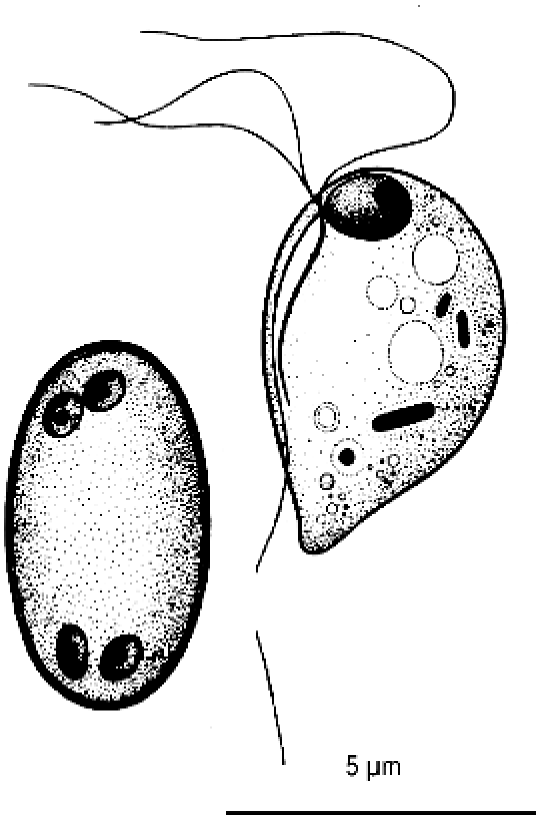
E. ratti
- rats

E. caviae
- guinea-pigs

E. suis
- pigs

E. lagostomi
- viscacha *Lagostomus maximus*

E. wenyoni
- big-eared opossum *Didelphis aurita*



Fornicata

Diplomonadida - Hexamitidae

genus ***Trepomonas***

- all free-living, in ponds rich in decaying organic matter and infusions
- oval to elliptical trophozoites, 7-15 µm
- octaflagellate – 2 lateral flagella for rotational movements + 2 lateral pocket-like grooves each harbouring 3 flagella
- 2 cytostomes

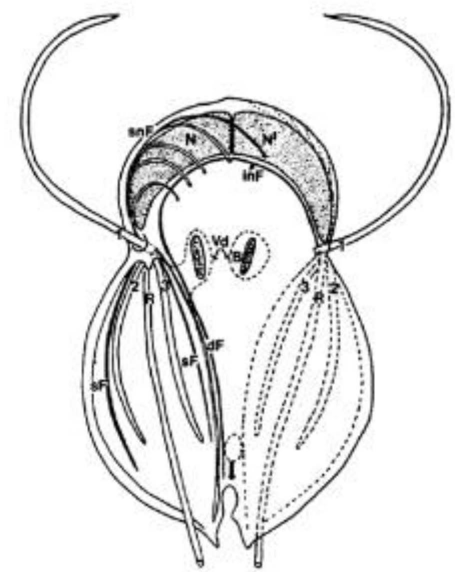
Trepomonas agilis

T. angulatus

T. rotans

T. simplex

T. steinii



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BMC Biology

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RESEARCH ARTICLE Open Access

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On the reversibility of parasitism: adaptation to a free-living lifestyle via gene acquisitions in the diplomonad *Trepomonas* sp. PC1

Feifei Xu[1], Jon Jerlström-Hultqvist[1,5], Martin Kolisko[2,3,6], Alastair G. B. Simpson[2,4], Andrew J. Roger[3,4], Staffan G. Svärd[1] and Jan O. Andersson[1*]

Abstract

Background: It is generally thought that the evolutionary transition to parasitism is irreversible because it is associated with the loss of functions needed for a free-living lifestyle. Nevertheless, free-living taxa are sometimes nested within parasite clades in phylogenetic trees, which could indicate that they are secondarily free-living. Herein, we test this hypothesis by studying the genomic basis for evolutionary transitions between lifestyles in diplomonads, a group of anaerobic eukaryotes. Most described diplomonads are intestinal parasites or commensals of various animals, but there are also free-living diplomonads found in oxygen-poor environments such as marine and freshwater sediments. All these nest well within groups of parasitic diplomonads in phylogenetic trees, suggesting that they could be secondarily free-living.

Results: We present a transcriptome study of *Trepomonas* sp. PC1, a diplomonad isolated from marine sediment. Analysis of the metabolic genes revealed a number of proteins involved in degradation of the bacterial membrane and cell wall, as well as an extended set of enzymes involved in carbohydrate degradation and nucleotide metabolism. Phylogenetic analyses showed that most of the differences in metabolic capacity between free-living *Trepomonas* and the parasitic diplomonads are due to recent acquisitions of bacterial genes via gene transfer. Interestingly, one of the acquired genes encodes a ribonucleotide reductase, which frees *Trepomonas* from the need to scavenge deoxyribonucleosides. The transcriptome included a gene encoding squalene-tetrahymanol cyclase. This enzyme synthesizes the sterol substitute tetrahymanol in the absence of oxygen, potentially allowing *Trepomonas* to thrive under anaerobic conditions as a free-living bacterivore, without depending on sterols from other eukaryotes.

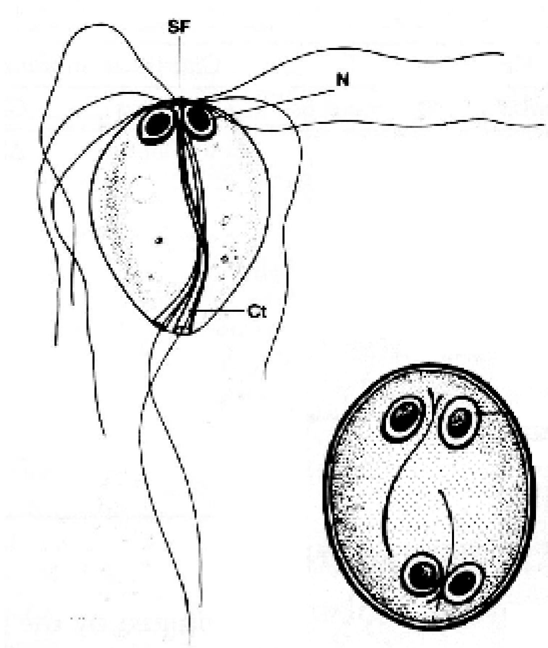
Conclusions: Our findings are consistent with the phylogenetic evidence that the last common ancestor of diplomonads was dependent on a host and that *Trepomonas* has adapted secondarily to a free-living lifestyle. We believe that similar studies of other groups where free-living taxa are nested within parasites could reveal more examples of secondarily free-living eukaryotes.

Fornicata

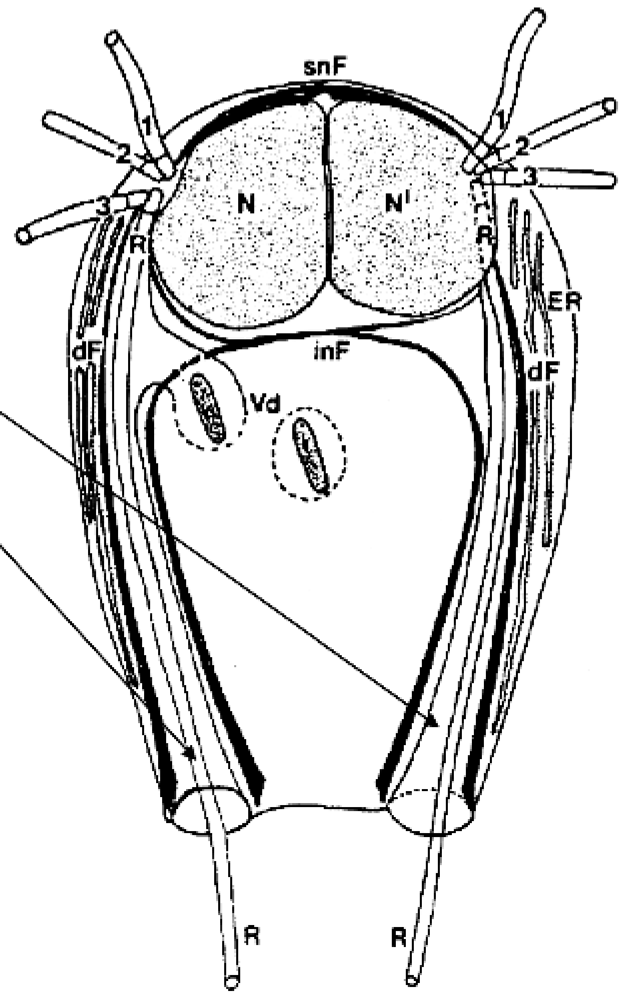
Diplomonadida - Hexamitidae

genus ***Hexamita***

- <u>octaflagellate</u> – 6 anterior + 2 posterior flagella
- <u>2 cytostomes</u>



cytostomes



https://www.int-res.com/articles/dao/25/d025p229.pdf

Hexamita salmonis

- significant anorexia in infected salmonids

Parasite host relations: *Hexamita salmonis* in rainbow trout *Oncorhynchus mykiss*

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ABSTRACT: The relationship between host size and infection, the site selection in the host and the association between health status and infection were studied in *Hexamita salmonis* (Moore, 1923) infected rainbow trout from commercial trout farms. During a 1 yr survey it was shown that the flagellates occur primarily in the smallest fry. The site occupied by the parasite is preferentially the pyloric region and the anterior intestine but in heavily infected fish the parasites occur throughout the entire length of the gut. Infected fish exhibited significant anorexia and significant body weight reduction.

KEY WORDS: Diplomonadida · *Hexamita salmonis* · Rainbow trout · *Oncorhynchus mykiss* · Site selection · Body weight reduction · Host size

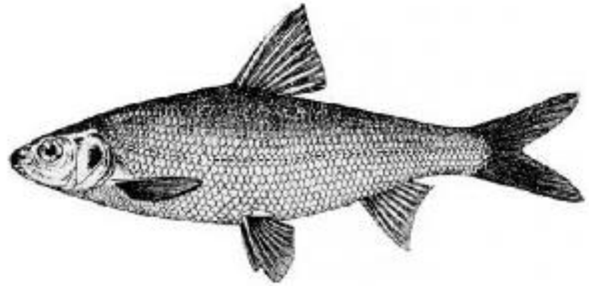
Hexamita axostyles

H. giganti

H. globulus

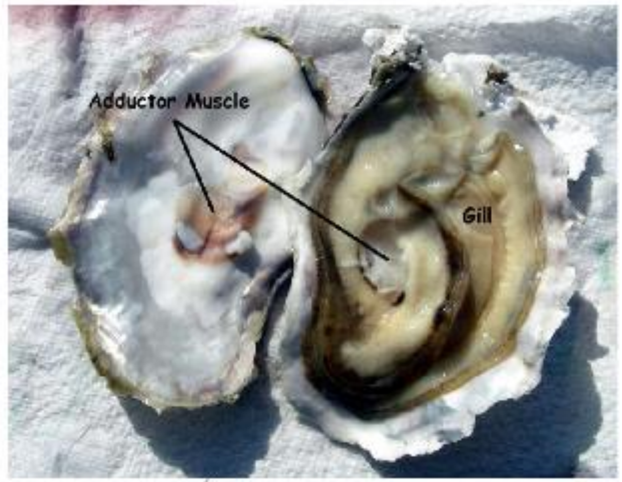
H. guanqiaoensis

- fish



H. nelsoni

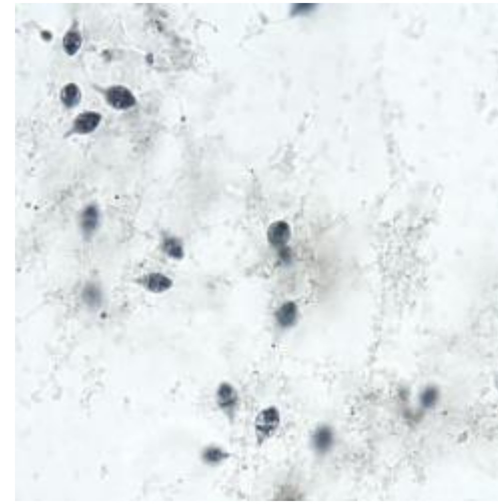
- oysters

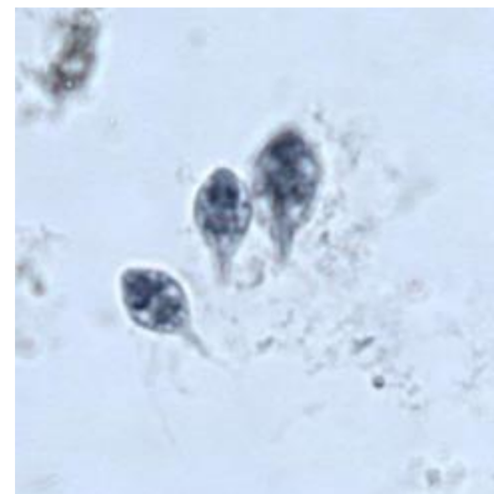


H. cryptocerci

H. periplanetae

- cockroaches

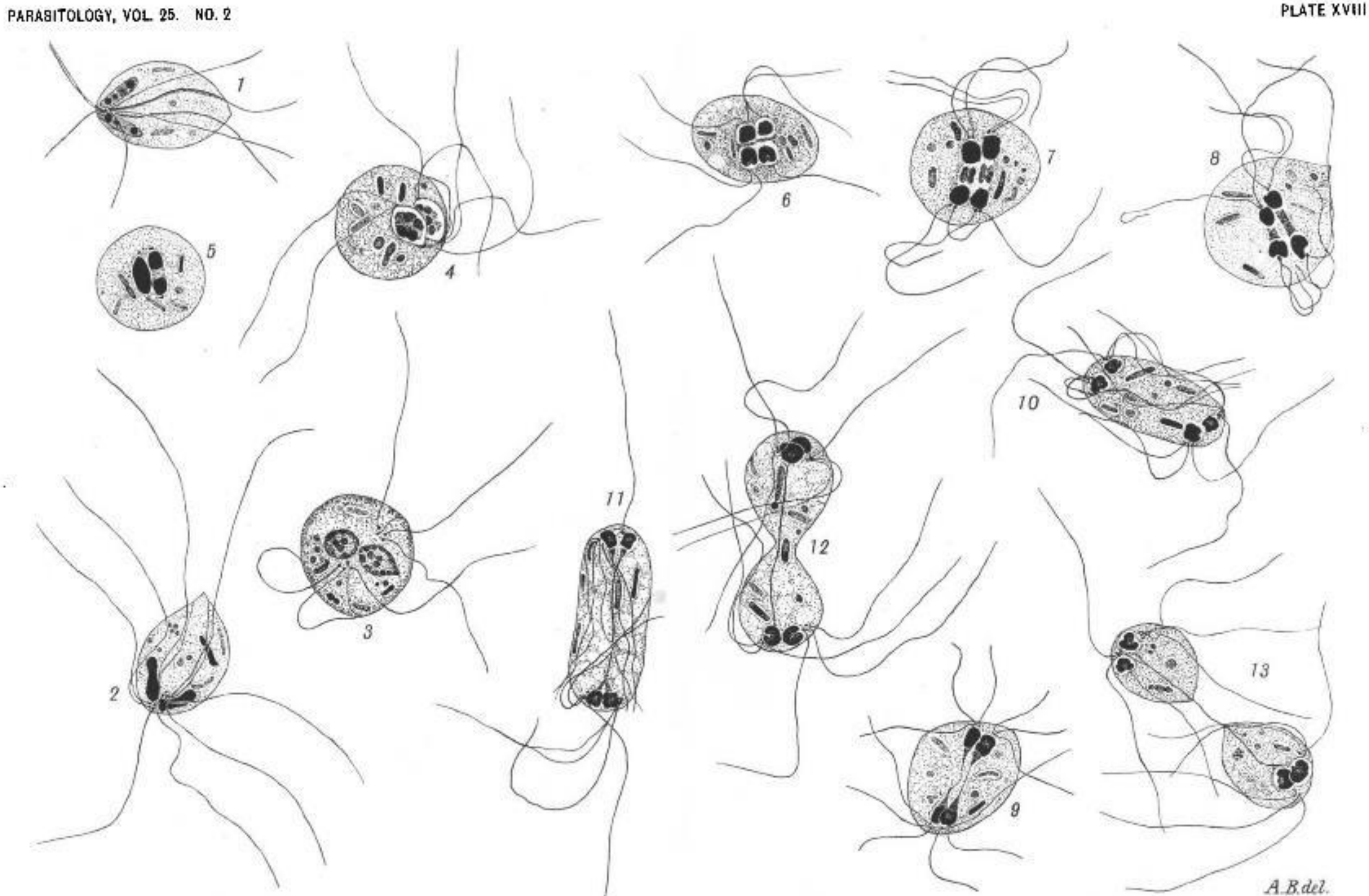




Hexamita gigas

- hind-gut of horse-leech (*Haemopis sanguisugae*)

Phases of cell division



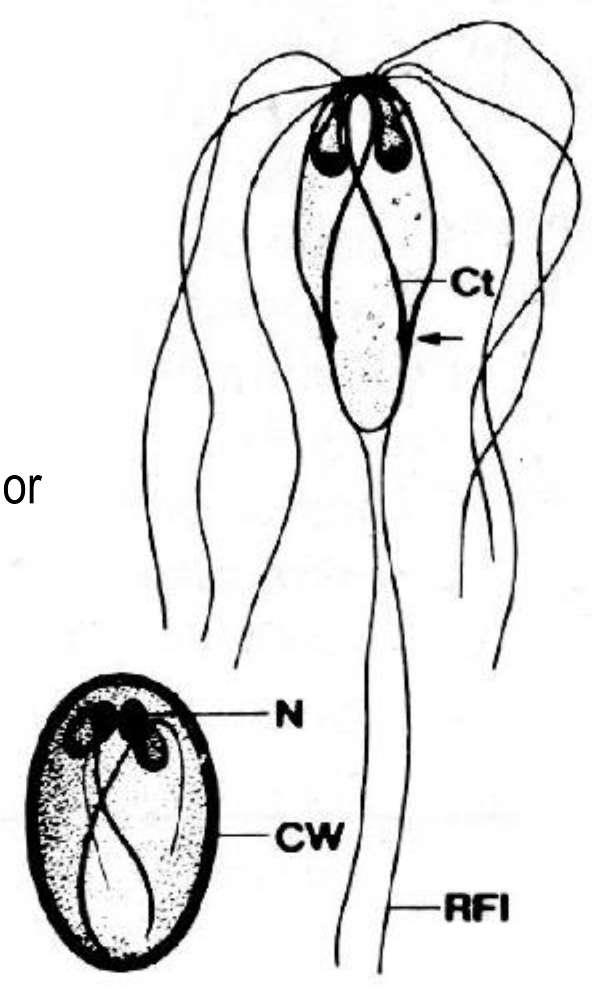
https://doi.org/10.1017/S0031182000019351

Fornicata

Diplomonadida - Hexamitidae

genus ***Spironucleus***

- octaflagellate – 6 anterior flagella arising back to the anterior pole + 2 posterior flagella emerging posteriorly - recurrent flagella toward the posterior cytostomal apertures
- 2 slender cytostomes
- elongate kidney-shaped nuclei
- elliptical cyst



Spironucleus elegans

- amphibians and fish

S. salpae

- marine fish *Box salpa*

Spironucleus anguillae

S. barkhanus

S. torosa

S. vortens

S. salmonicida

S. mobilis

- fish

Spironucleus columbae

- pigeons

S. meleagridis

- birds

S. muris

- rodents

Spironucleus vortens

Vol. 45: 197–202, 2001	DISEASES OF AQUATIC ORGANISMS Dis Aquat Org	Published August 2

https://www.int-res.com/abstracts/dao/v45/n3/p197-202/

Spironucleus vortens, a possible cause of hole-in-the-head disease in cichlids

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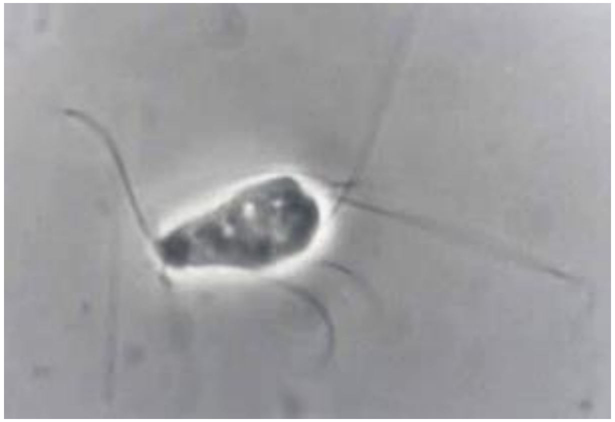
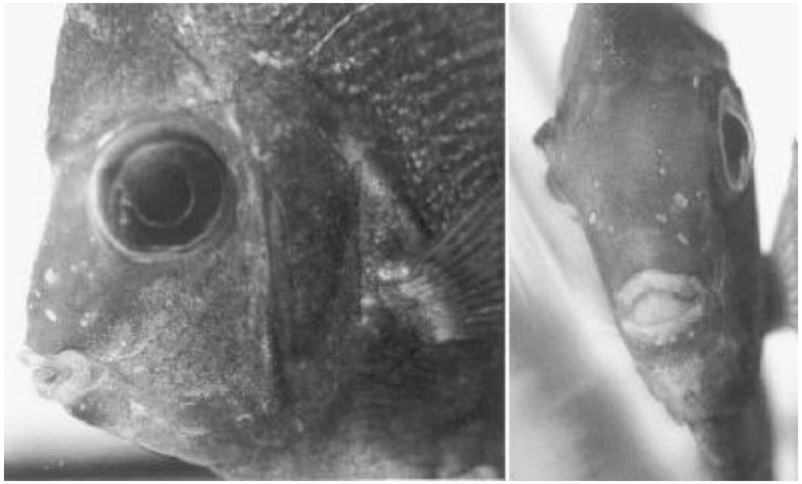
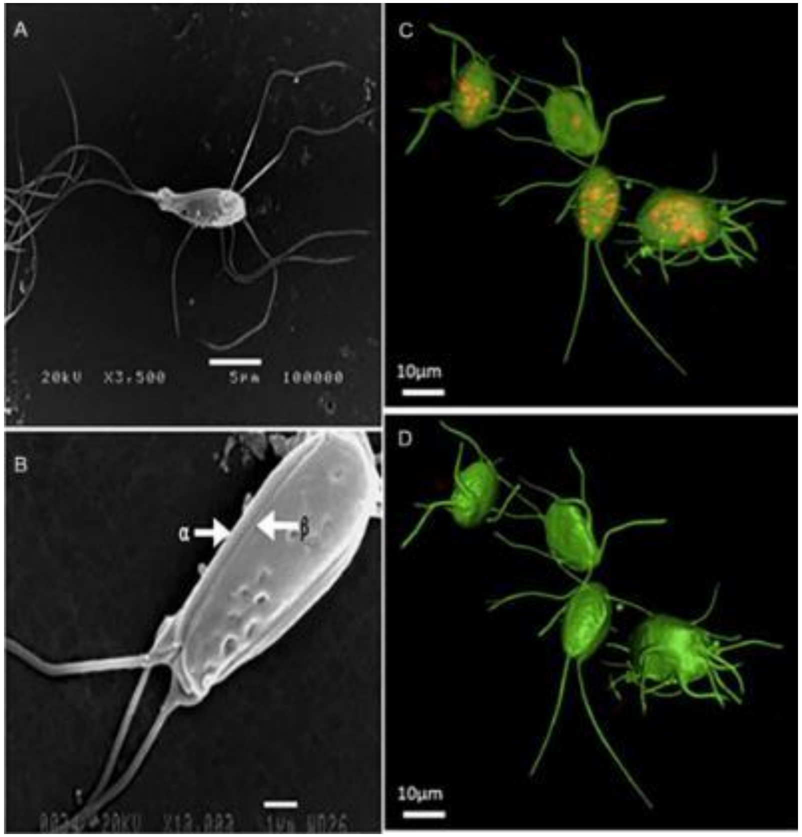
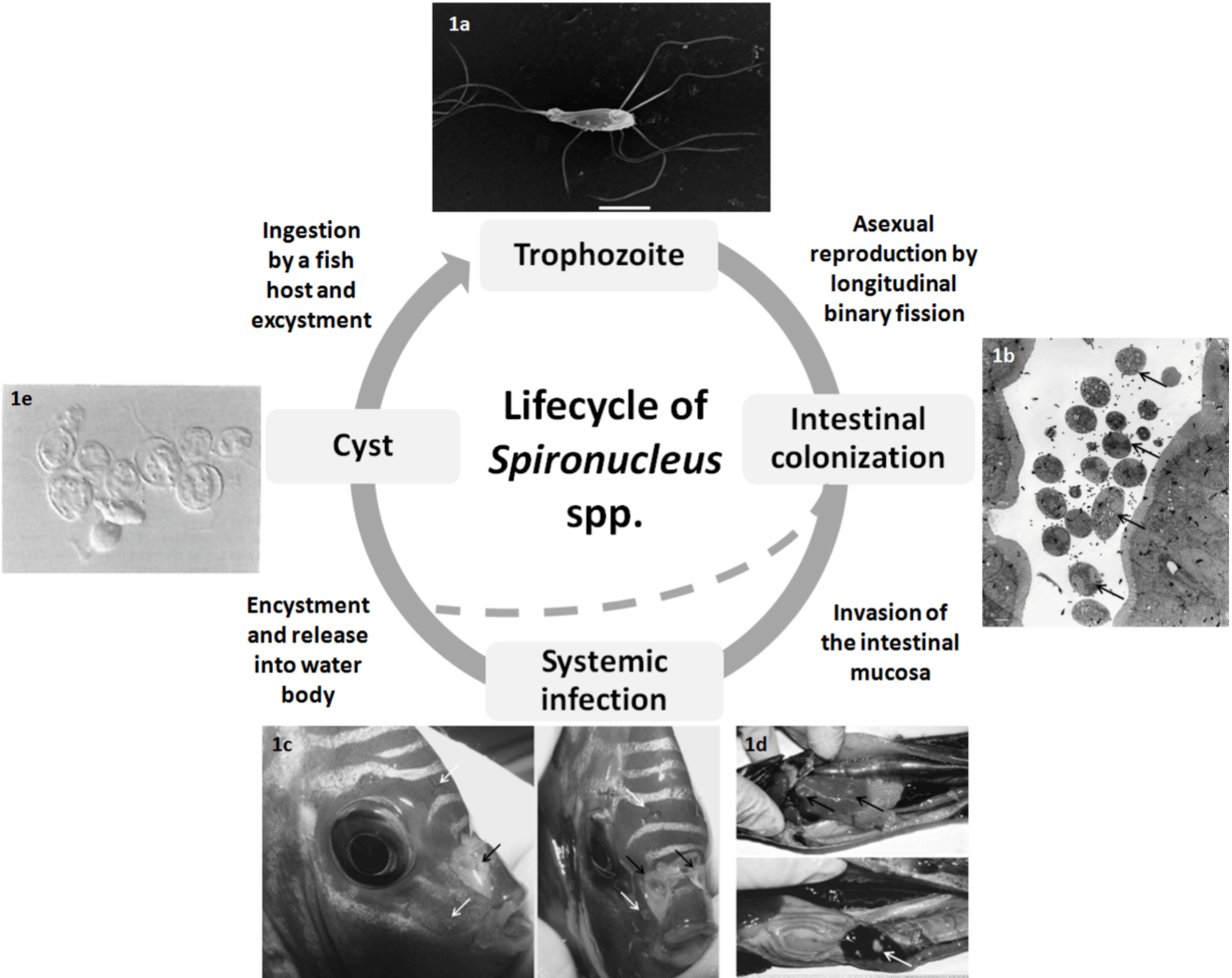
Department of Biological Sciences, University of Plymouth, Plymouth, Devon PL4 8AA, United Kingdom

ABSTRACT: Hole-in-the-head disease is recorded in 11 discus *Symphysodon discus* Heckel, 1840 and 1 angelfish *Pterophyllum scalare* Lichtenstein, 1823 obtained from local aquarists within the Southwest of the UK. *Spironucleus vortens* Poynton et al. 1995, was isolated from the kidney, liver, spleen and head lesions of discus showing severe signs of the disease and from the intestines of all fish. The hexamitid was also recorded from the head lesions of the angelfish. The identity of these flagellates was confirmed as *S. vortens* on the basis of topographical features seen with the aid of SEM. A modified *in vitro* culture method was successfully developed for the detection, isolation and long-term maintenance of *S. vortens*. The flagellate was sub-cultured at 3 to 5 d intervals, new media being supplemented with fresh liver from *Oreochromis niloticus* (Linnaeus, 1757) free from infection. The results are discussed in relation to *S. vortens* as the causative agent for hole-in-the-head disease following systemic infection via the digestive tract.








1a
Trophozoite
Asexual reproduction by longitudinal binary fission
1b
Intestinal colonization
Invasion of the intestinal mucosa
1d
Systemic infection
1c
Encystment and release into water body
Cyst
1e
Ingestion by a fish host and excystment
Lifecycle of *Spironucleus* spp.

Spironucleus meleagridis

https://doi.org/10.1637/7250-080204R
AVIAN DISEASES 48:138–143, 2004

Research Note—

Spironucleosis (Hexamitiasis, Hexamitosis) in the Ring-Necked Pheasant (*Phasianus colchicus*): Detection of Cysts and Description of *Spironucleus meleagridis* in Stained Smears

A. M. Wood[A] and H. V. Smith[B]

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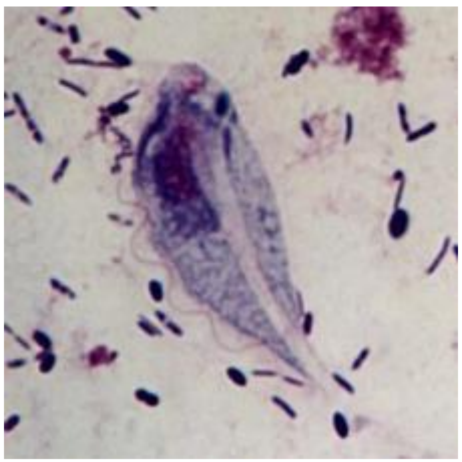
Received 6 May 2004; Accepted 16 August 2004

SUMMARY. Trophozoites and cysts of *Spironucleus* (*Hexamita*) *meleagridis* were detected in the intestinal fluid and mucus of pheasant poults with spironucleosis (hexamitiasis, hexamitosis) following staining with Heidenhain iron hematoxylin (HIH) and the Romanowsky-type stain Hemacolor®. Their morphology was consistent with that of flagellates of the genus *Spironucleus*, and bright-field morphologic observations were confirmed by transmission electron microscopy. Cysts occurred mostly within intestinal mucus, which was firmly compressed between microscope slides prior to staining. The internal structures of cysts were similar to those of trophozoites, allowing them to be confidently recognized. Hemacolor provided differential color staining of trophozoites and cysts, allowing accurate identification of *S. meleagridis* life cycle stages, even in smears in which there was heavy background staining. While HIH often produced clearer and more detailed staining of protozoan structures, in the context of a diagnostic laboratory its use was outweighed by the ease of use, rapidity of results, and differential color staining provided by Hemacolor. The possible significance of a resistant cystic stage in the life cycle of *S. meleagridis* is discussed.



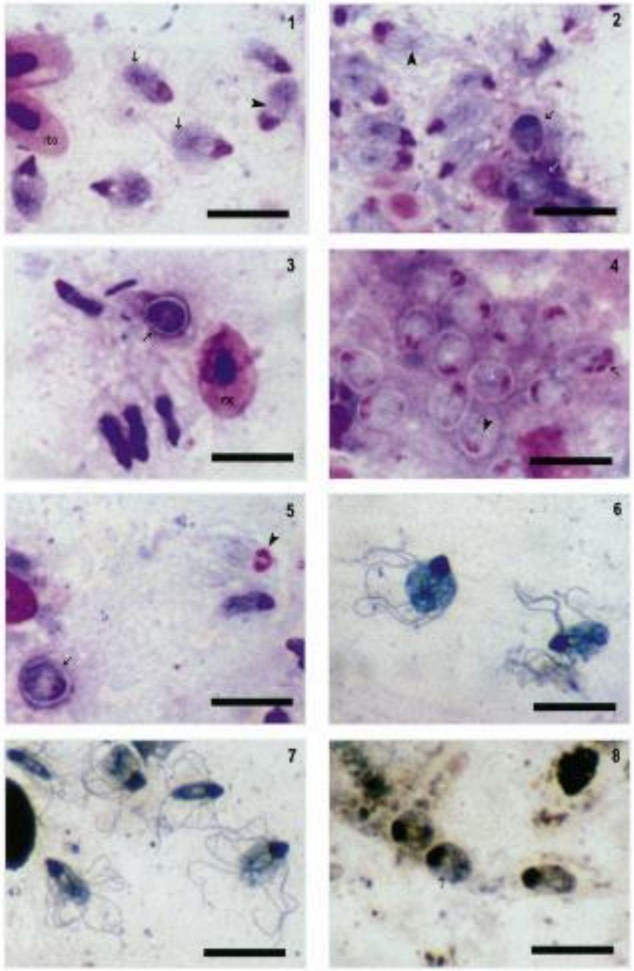


Fig. 1. Trophozoites of *Spironucleus meleagridis* present in pheasant intestinal fluid smear. Note the caudolateral location of cytostomes (arrows) and tubular cytopharyngeal canal (arrowhead); rbc = red blood cell. Hemacolor® stain. Bar = 10 µm.

Fig. 2. Several *Spironucleus meleagridis* trophozoites and two *S. meleagridis* cysts (arrows) present in pheasant intestinal fluid smear. Note the "crossover" of the cytopharyngeal canals in one trophozoite (arrowhead); rbc = red blood cell. Hemacolor® stain. Bar = 10 µm.

Fig. 3. Several *Spironucleus meleagridis* trophozoites and a single cyst (arrow) in pheasant intestinal fluid smear; rbc = red blood cell. Hemacolor® stain. Bar = 10 µm.

Fig. 4. Cysts of *Spironucleus meleagridis* present in pheasant intestinal mucus smear. Note cyst with four nuclei (black arrow). Trophozoite structures are visible inside some cysts: for example, cytopharyngeal canals (white arrow) and transversely wound flagella (arrowhead). The unstained cyst walls are clearly visible against the blue-stained mucus. Some cysts appear slightly distorted. Hemacolor® stain. Bar = 10 µm.

Fig. 5. Two trophozoites and one cyst (arrow) of *Spironucleus meleagridis* present in pheasant intestinal fluid smear. Note the good separation of the nuclei in one of the trophozoites and the resulting "horseshoe-shaped" nuclear complex (arrowhead). Hemacolor® stain. Bar = 10 µm.

Figs. 6, 7. Trophozoites of *Spironucleus meleagridis* present in pheasant intestinal fluid smear. Note the variation in size and body shape. Flagella and cytopharyngeal canals stain strongly. It is not possible to distinguish the two nuclei in these trophozoites. Heidenhain iron hematoxylin stain. Bar = 10 µm.

Spironucleus meleagridis ???

Vet Pathol. 2010 May ; 47(3): 488–494. doi:10.1177/0300985810363704.

Systemic Spironucleosis In Two Immunodeficient Rhesus Macaques (*Macaca mulatta*)

C Bailey[*], J Kramer[*], A Mejia, J MacKey, KG Mansfield, and AD Miller
Harvard Medical School, Department of Pathology, New England Regional Primate Center, Division of Comparative Pathology, Southborough, MA (CB, JK, AM, JM, KGM, ADM)

Abstract

Spironucleus spp. are parasites of fish and terrestrial vertebrates including mice and turkeys that rarely cause extraintestinal disease. Two rhesus macaques (*Macaca mulatta*) were experimentally inoculated with simian immunodeficiency virus mac251 (SIVmac251). Both progressed to simian acquired immune deficiency syndrome (SAIDS) within one year of inoculation and, in addition to common opportunistic infections including rhesus cytomegalovirus, rhesus lymphocryptovirus, and rhesus adenovirus, developed systemic protozoal infections. In the first case, the protozoa were associated with colitis, multifocal abdominal abscessation, and lymphadenitis. In the second case they one of a number of organisms associated with extensive pyogranulomatous pneumonia and colitis. Ultrastructural, molecular, and phylogenetic analysis revealed the causative organism to be a species of *Spironucleus* closely related to *Spironucleus meleagridis* of turkeys. This is the first report of extraintestinal infection with *Spironucleus* sp. in higher mammals and further expands the list of opportunistic infections found in immunocompromised rhesus macaques.

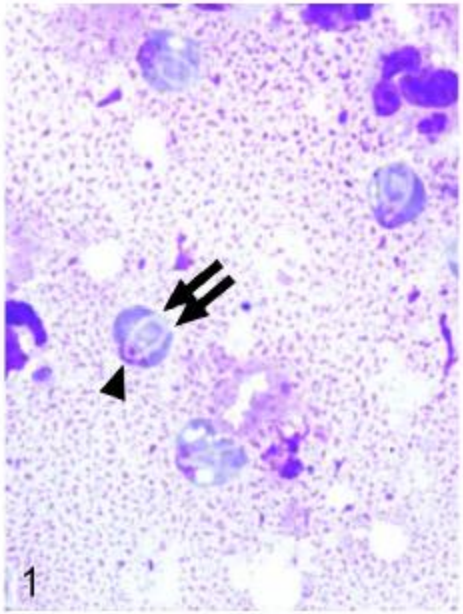


Fig. 1.
Abscess; Animal No. 1. Organisms contain apically oriented and closely associated nuclei (arrowheads). Two cytopharynxes, visible as negatively staining lines, transverse the cell posteriorly (arrows). The flagella do not stain. Wright's stain.

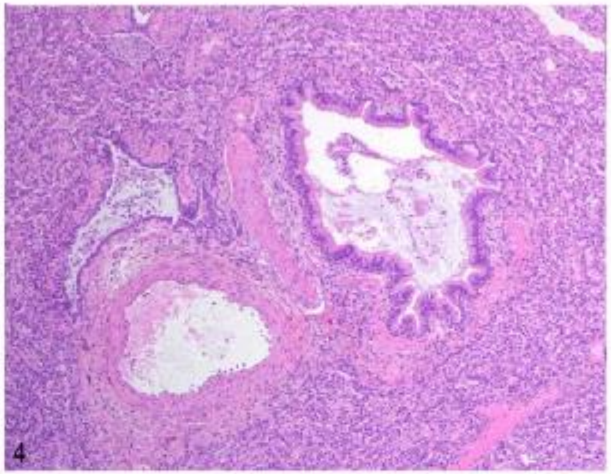


Fig. 4.
Lung; Animal No. 2. Extensive regions of lung are obliterated by an inflammatory infiltrate of neutrophils and macrophages. HE.

https://doi.org/10.1177/03009858103637

Fornicata

Diplomonadida - Giardiidae

genus ***Octomitus***

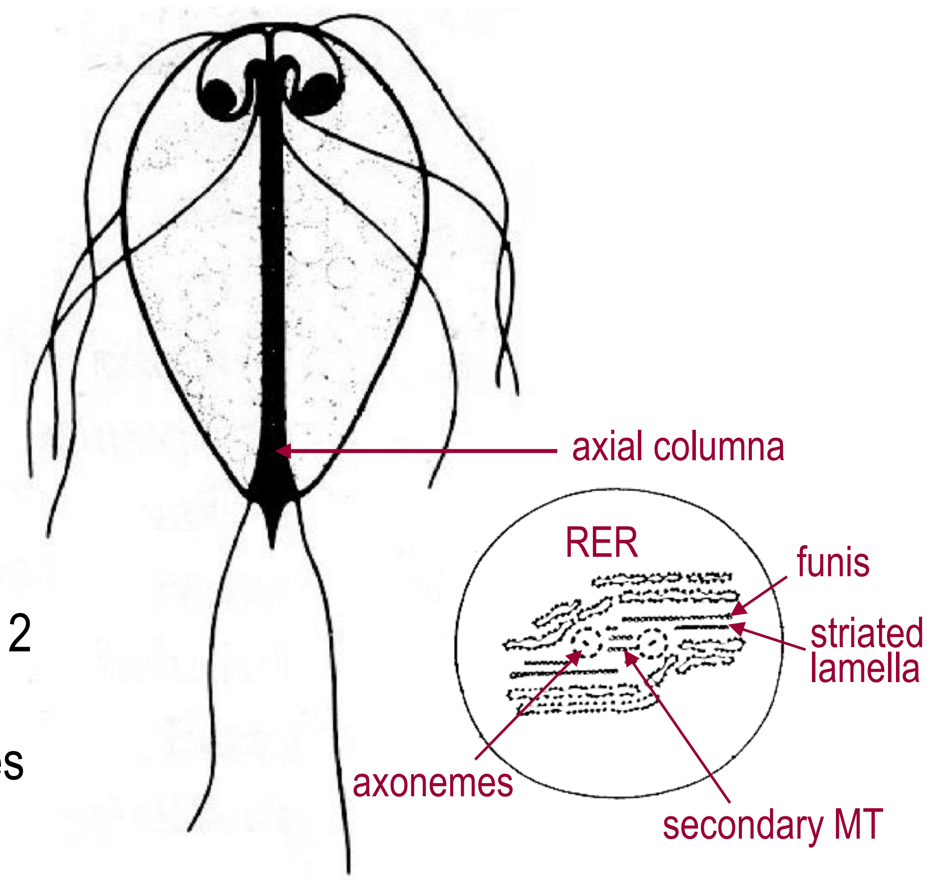
- 12-17 µm, <u>no adhesive disc</u>
- 2 anterior nuclei
- <u>octaflagellate</u> – 6 antero-lateral flagella + 2 posterior flagella which traverse the cell axially and are lined by microtubular fibres
- <u>no cytostomal opening</u> at the emergence of the trailing flagella

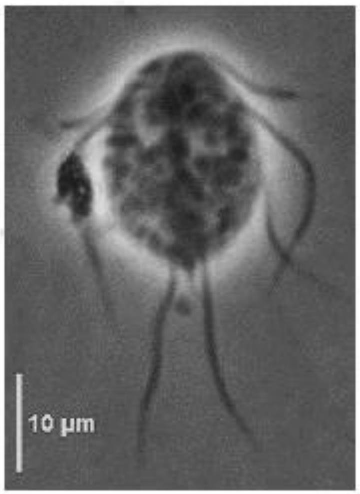
Octomitus intestinalis

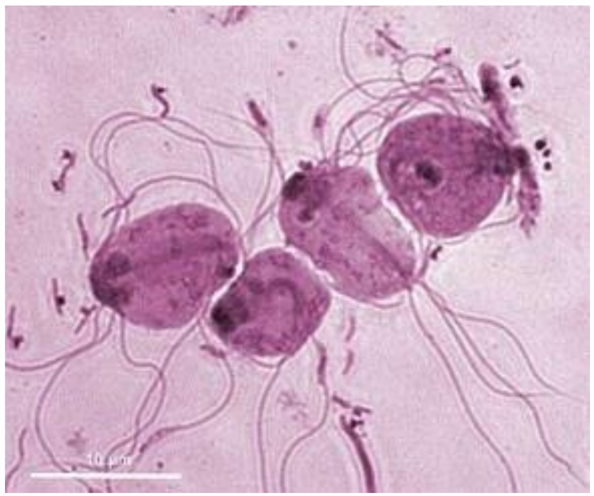
- intestine of rodents

O. neglectus

- amphibians



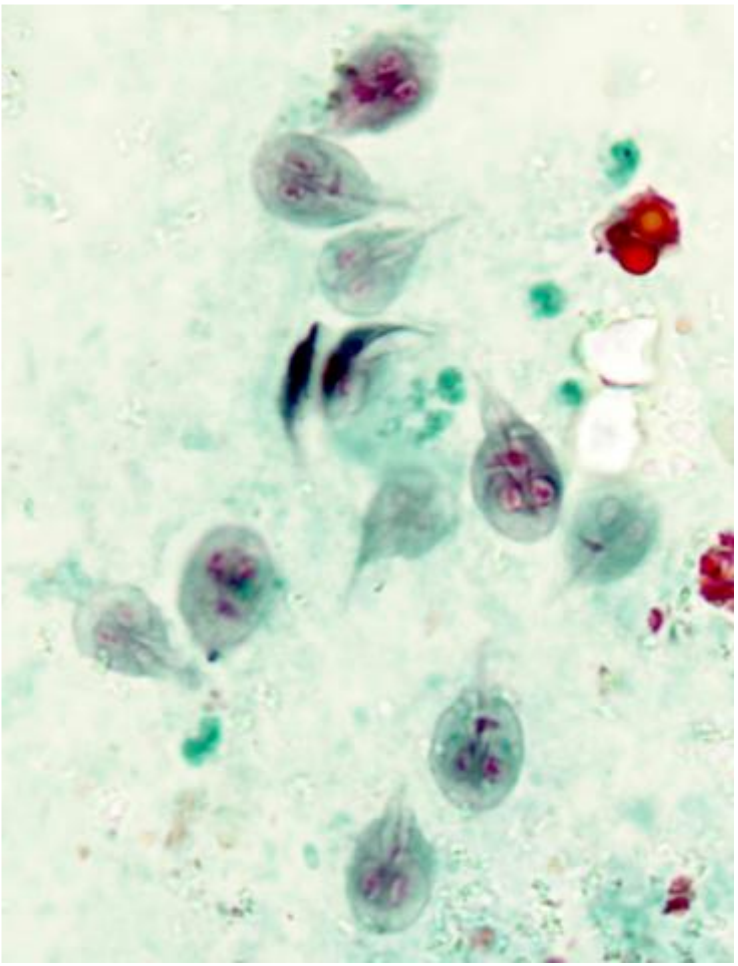




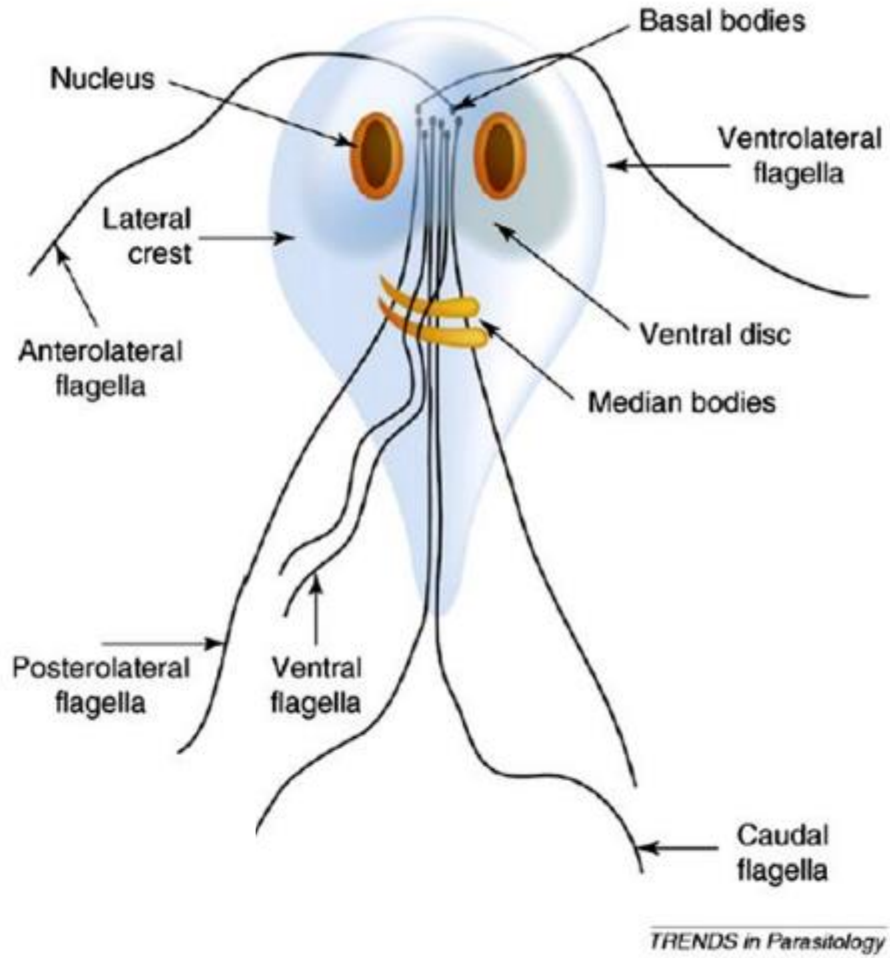
Fornicata

Diplomonadida - Giardiidae

genus ***Giardia***

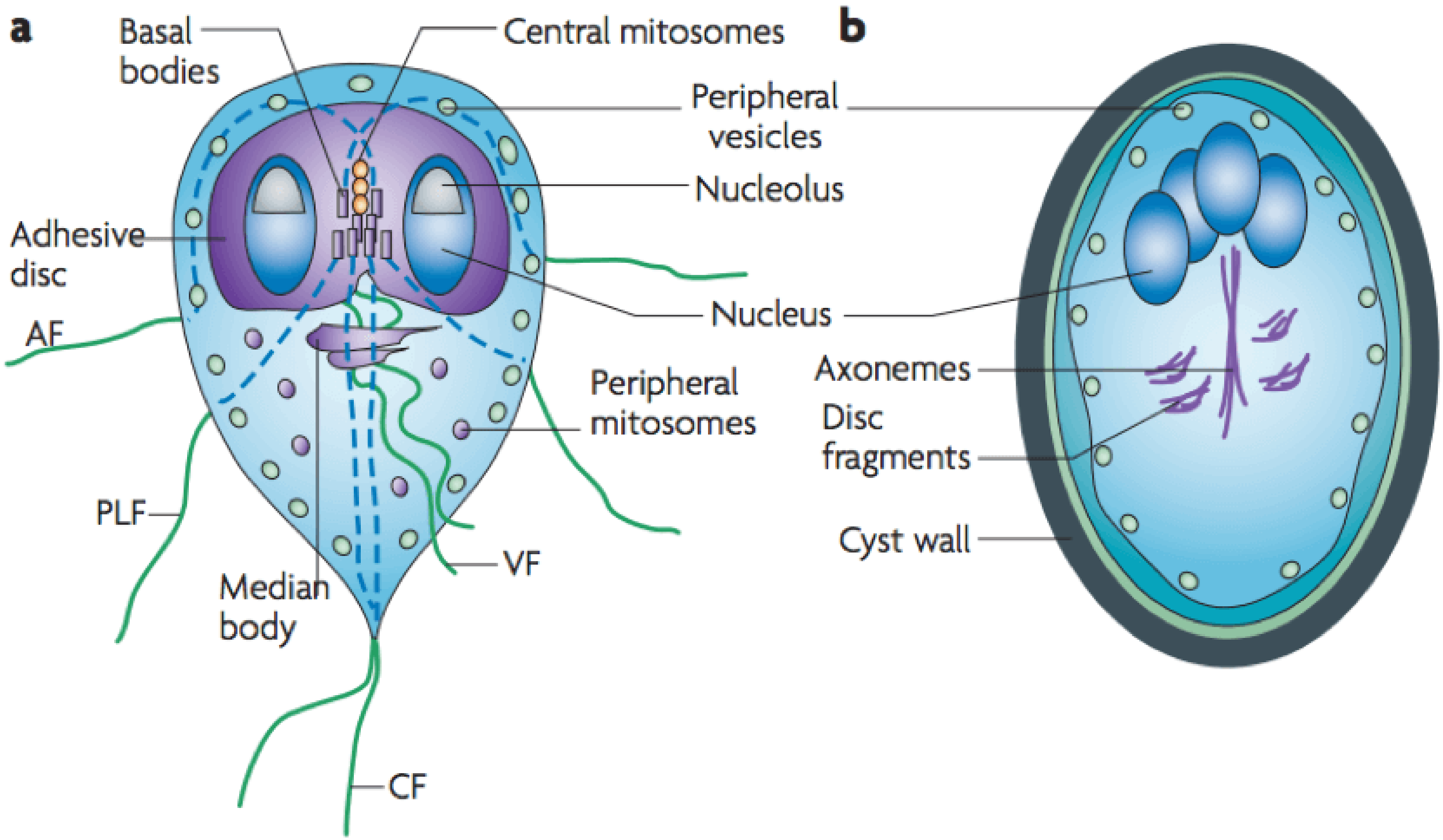


Giardia intestinalis, trichrome stained stool specimen



https://www.youtube.com/watch?v=2R7fTTsXQzY
https://www.youtube.com/watch?v=UbSUE0gYfxI

Giardia intestinalis

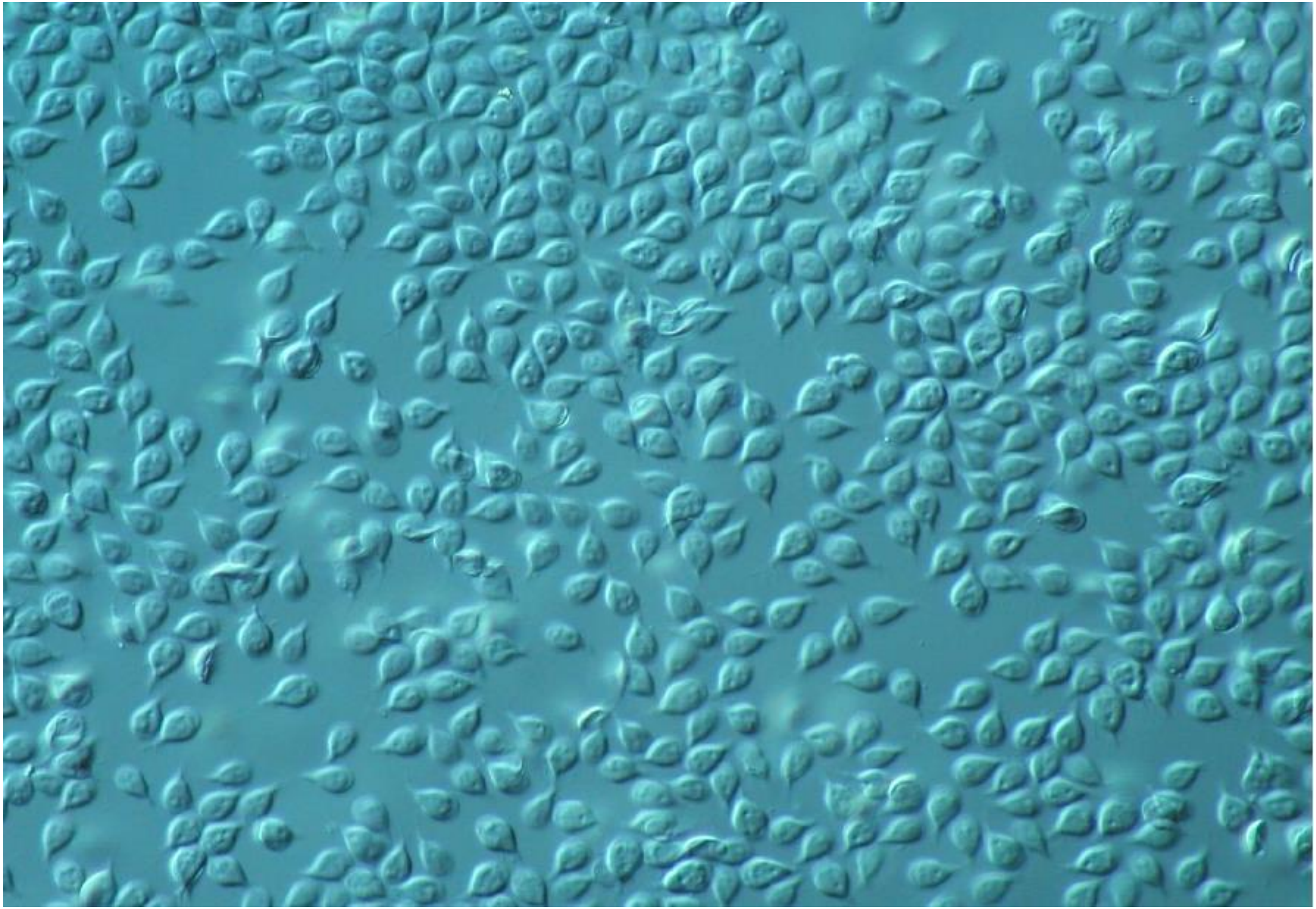


trophozoites 10-20 x 7-10 µm

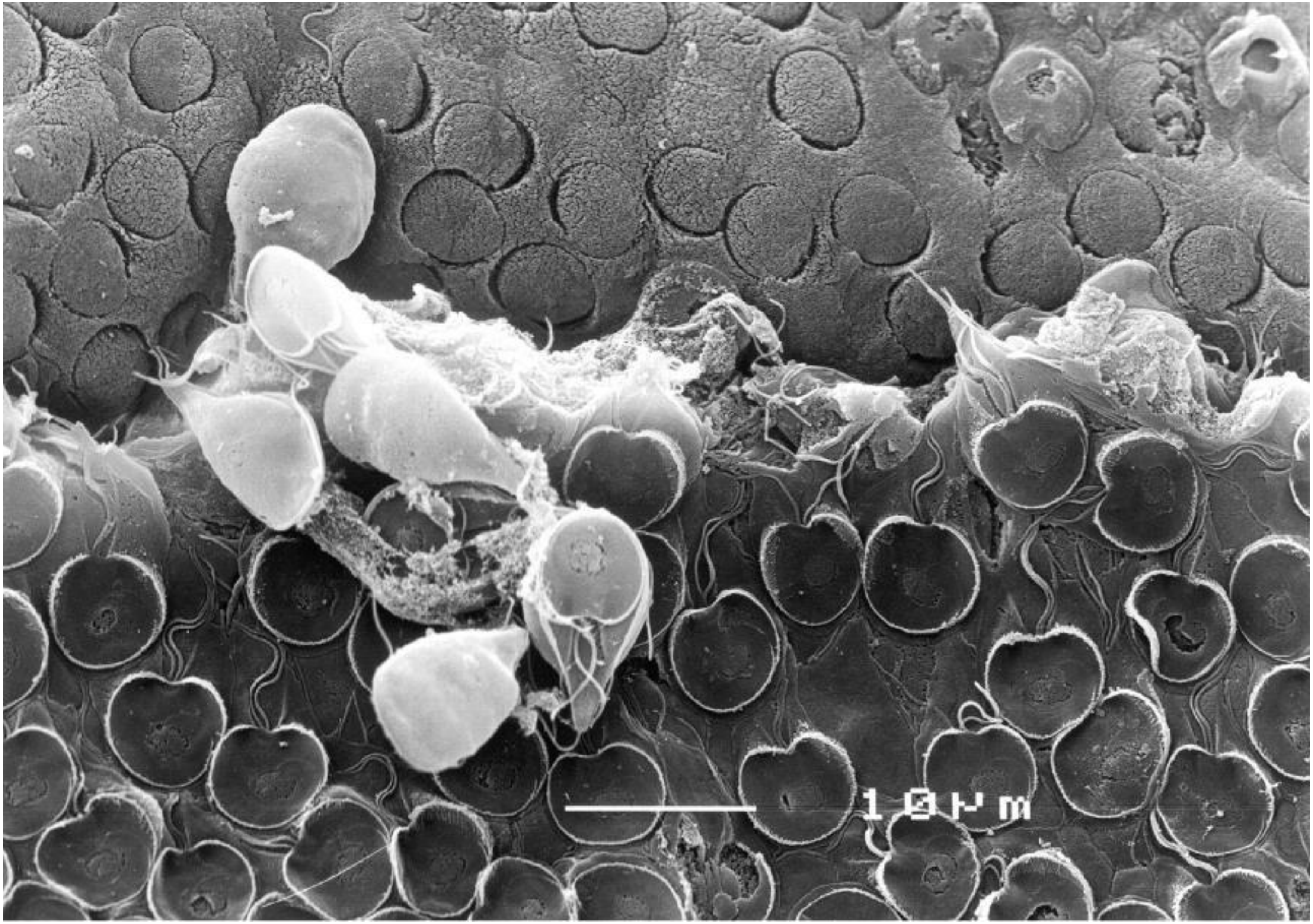
cysts 11-14 x 7-10 µm

Giardia intestinalis

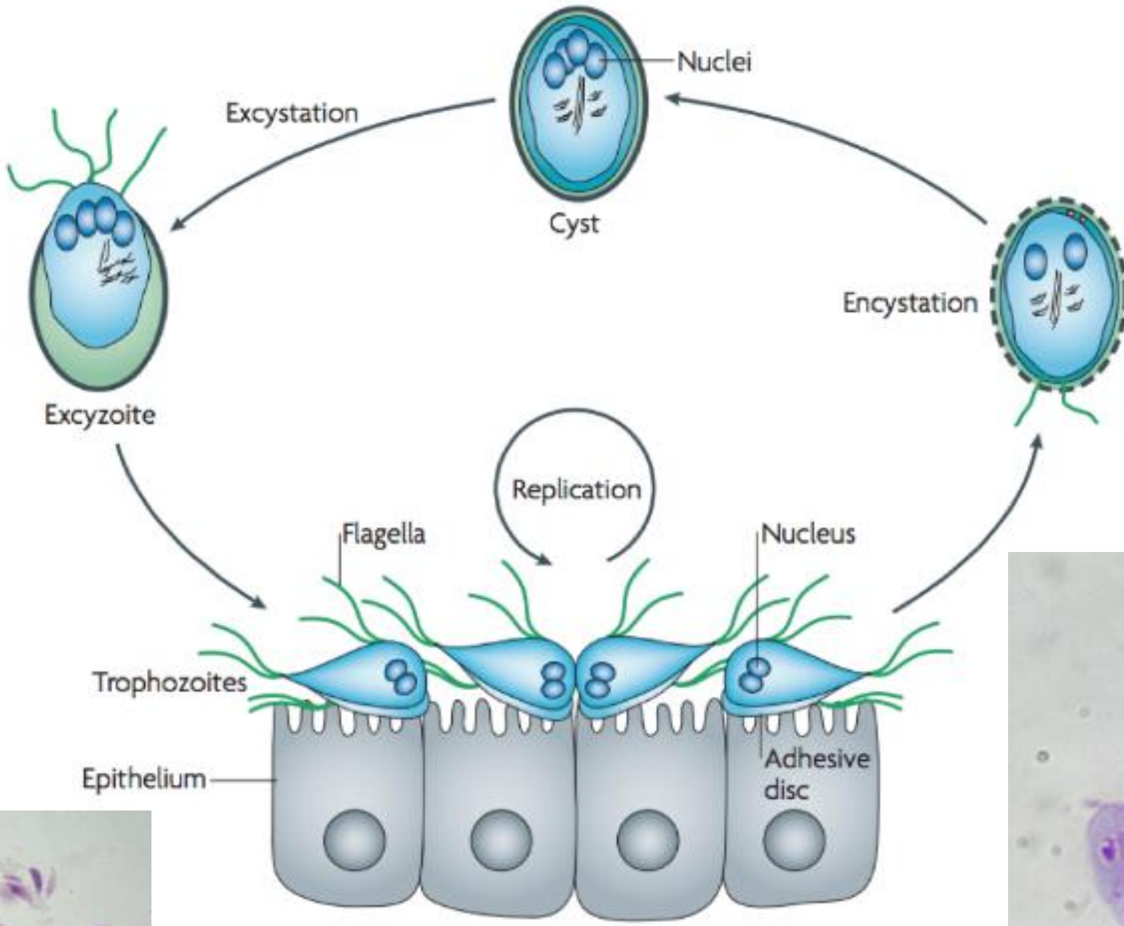
https://www.youtube.com/watch?v=8X4bxITNPeg

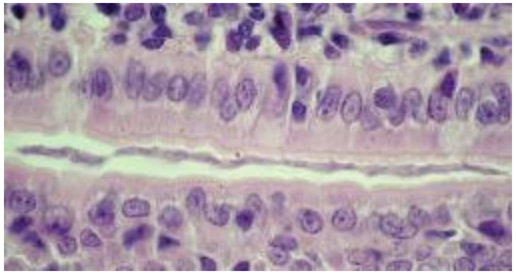


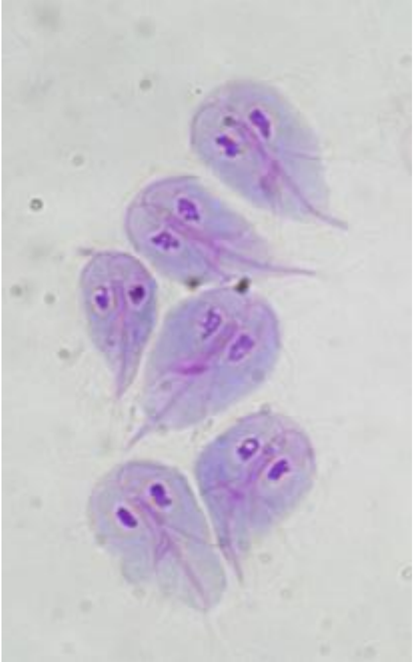
Giardia intestinalis



Life cycle and infection of *Giardia intestinalis*

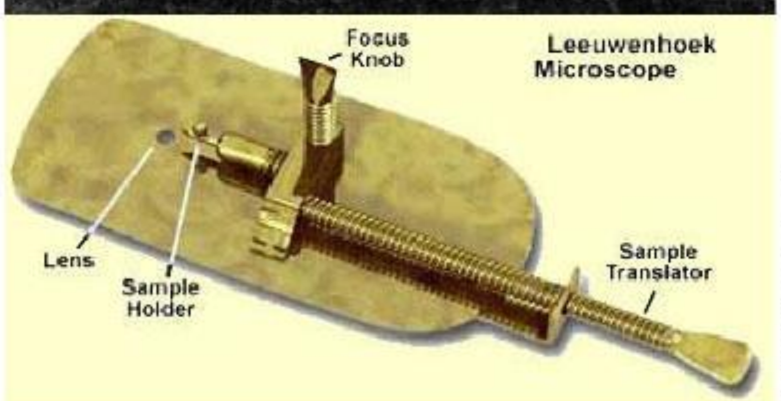






Discovery of *Giardia*

Correspondence between Anthoni van Leeuwenhoek and Robert Hooke, a curator of experiments of the Royal Society in London



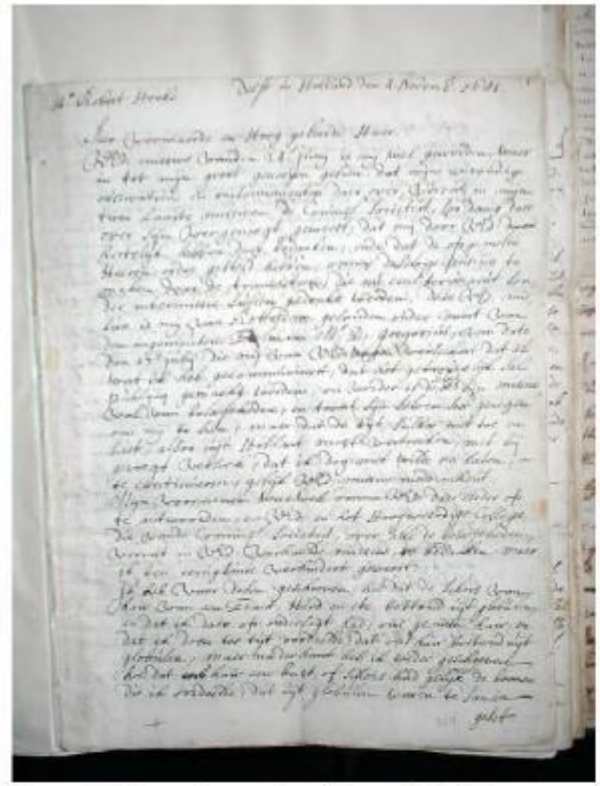


Figure 1. The opening section of Leeuwenhoek's letter describing the discovery of Giardia. *This is letter No 66 addressed to Robert Hooke dated November 4 1681, in Book L1 in the Leeuwenhoek archive of the Royal Society of London. I am grateful to Mr Harry Leechburch of Leiden for bibliographical advice and to the President and Council of the Royal Society for their assisting my access to the original documentation.*

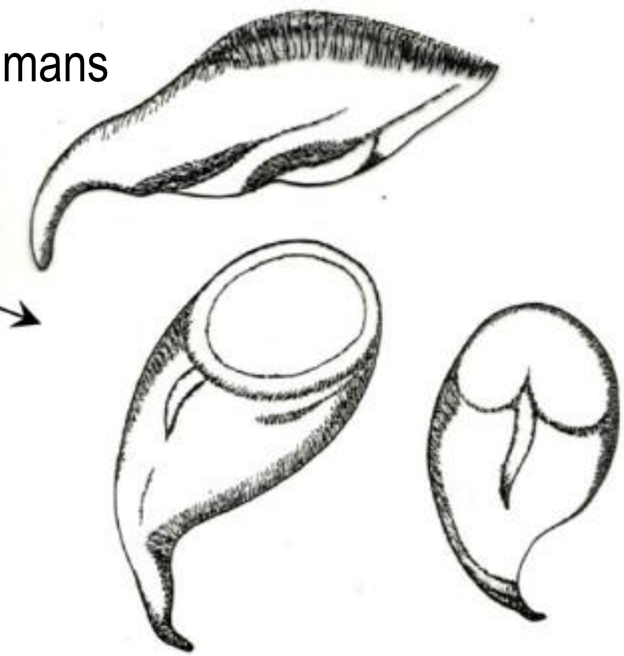
Letter to Robert Hook (1681)

I weigh 160 pounds and have been of the same weight for some 30 years, and ordinarily in the morning I have a well-formed stool; however, now and then I have had a looseness at intervals of 2, 3, or 4 weeks when I went to stool 2, 3, or 4 times a day. My excrement being so thin I was at diverse times persuaded to examine it. I have generally seen in my excrement many irregular particles of sundry sizes..

All lay in a clear transparent medium, wherein I have sometimes seen **animalcules** moving very prettily; some of them a bit bigger, others a bit less than a blood globule…their bodies were somewhat longer than broad, and their belly, which was flat like, furnished with sundry little paws, wherewith they made such a stir in the clear medium and among the globules…they made a quick motion with their paws, yet for all that they made but slow progress.

History of *Giardia intestinalis*

1859 Vilém Lambl, Praha - *Cercomonas intestinalis* in humans



1875 Daviane - *Hexamita duodenalis*

1882 Kunstler - *Giardia agilis*

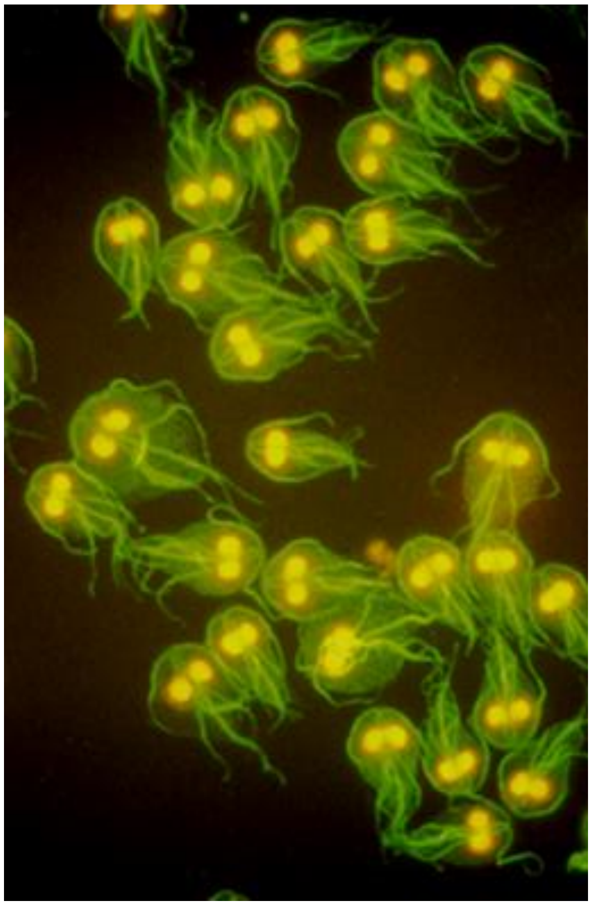
1888 Blanchard - *Lamblia intestinalis*

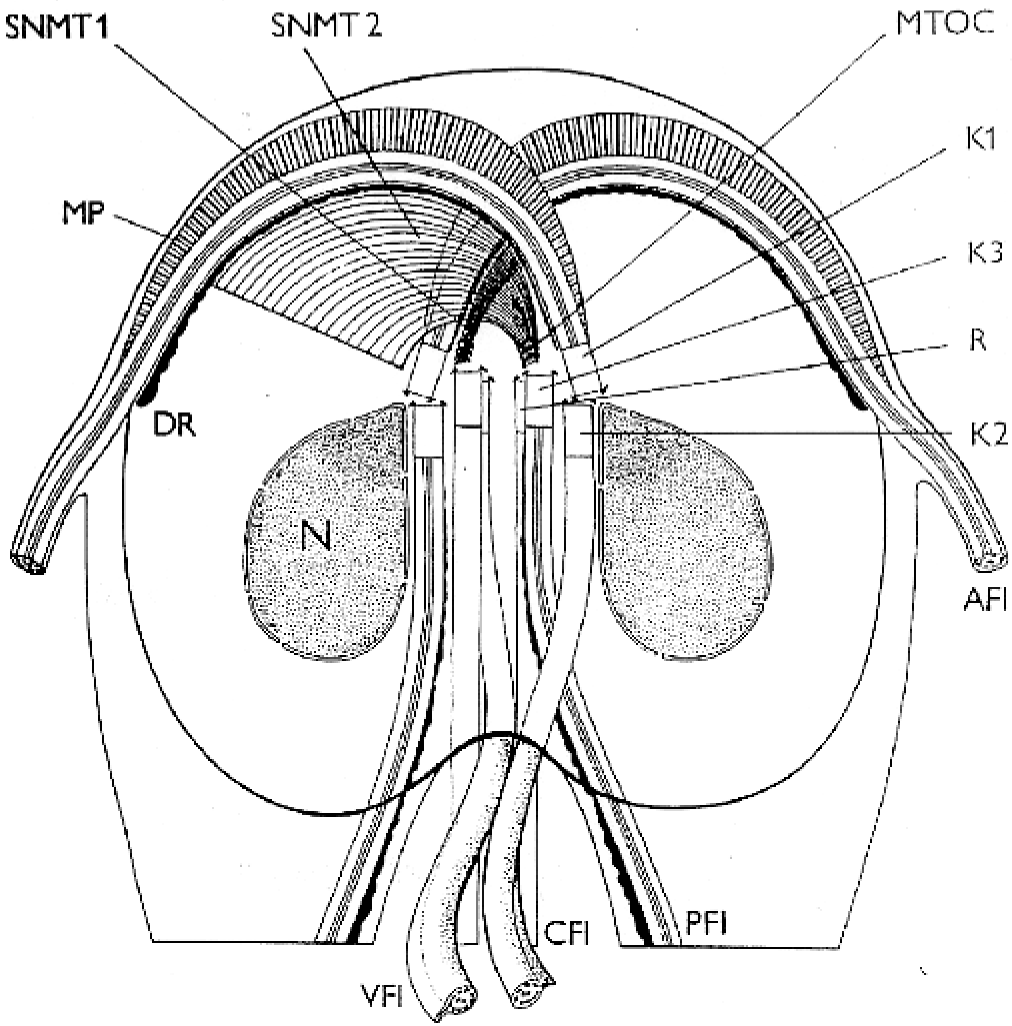
1914 Alexeieff - *Lamblia / Giardia*

1952 Filice - ***Giardia duodenalis***

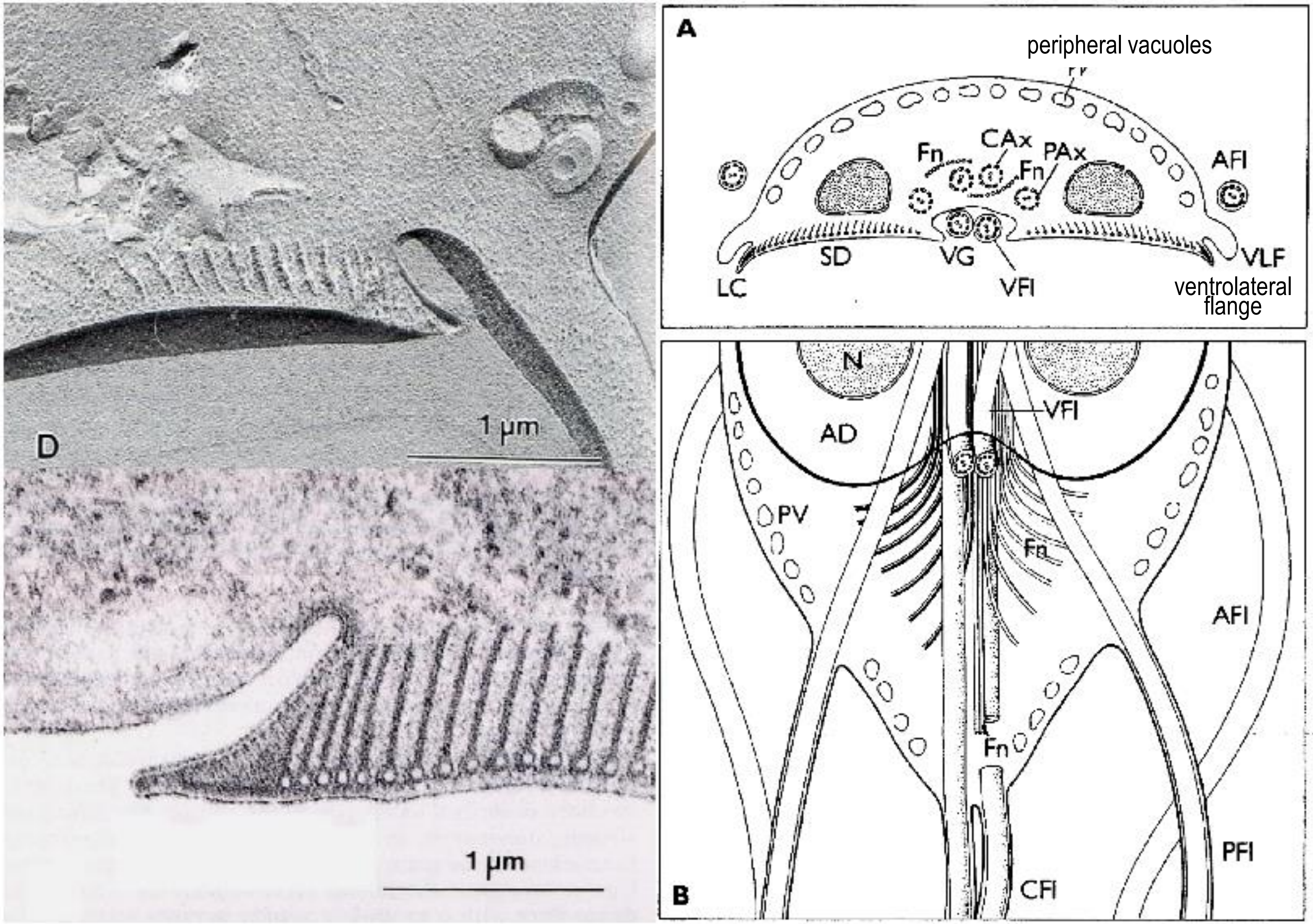
Current synonyms: *Giardia intestinalis*, *Giardia duodenalis*, *Giardia lamblia*, *Lamblia intestinalis*, *Lamblia lamblia*

Karyomastigont of *Giardia intestinalis*



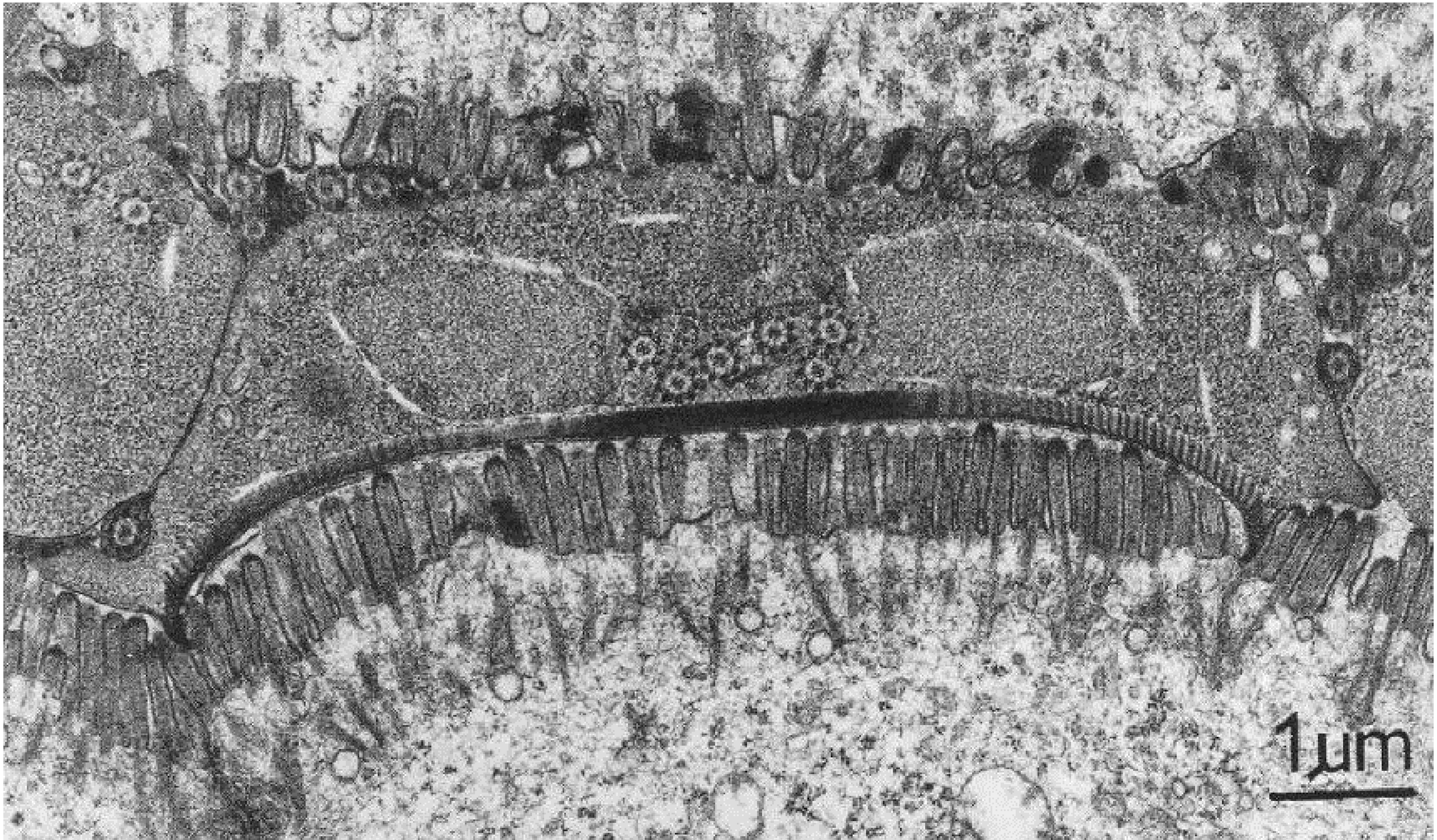


Subcellular organisation of *Giardia intestinalis*

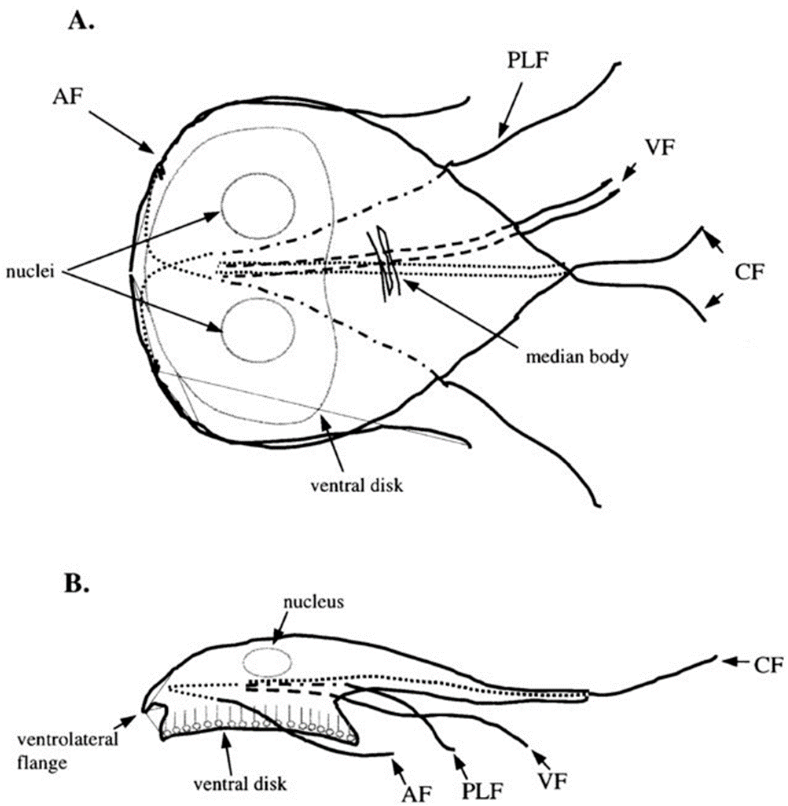


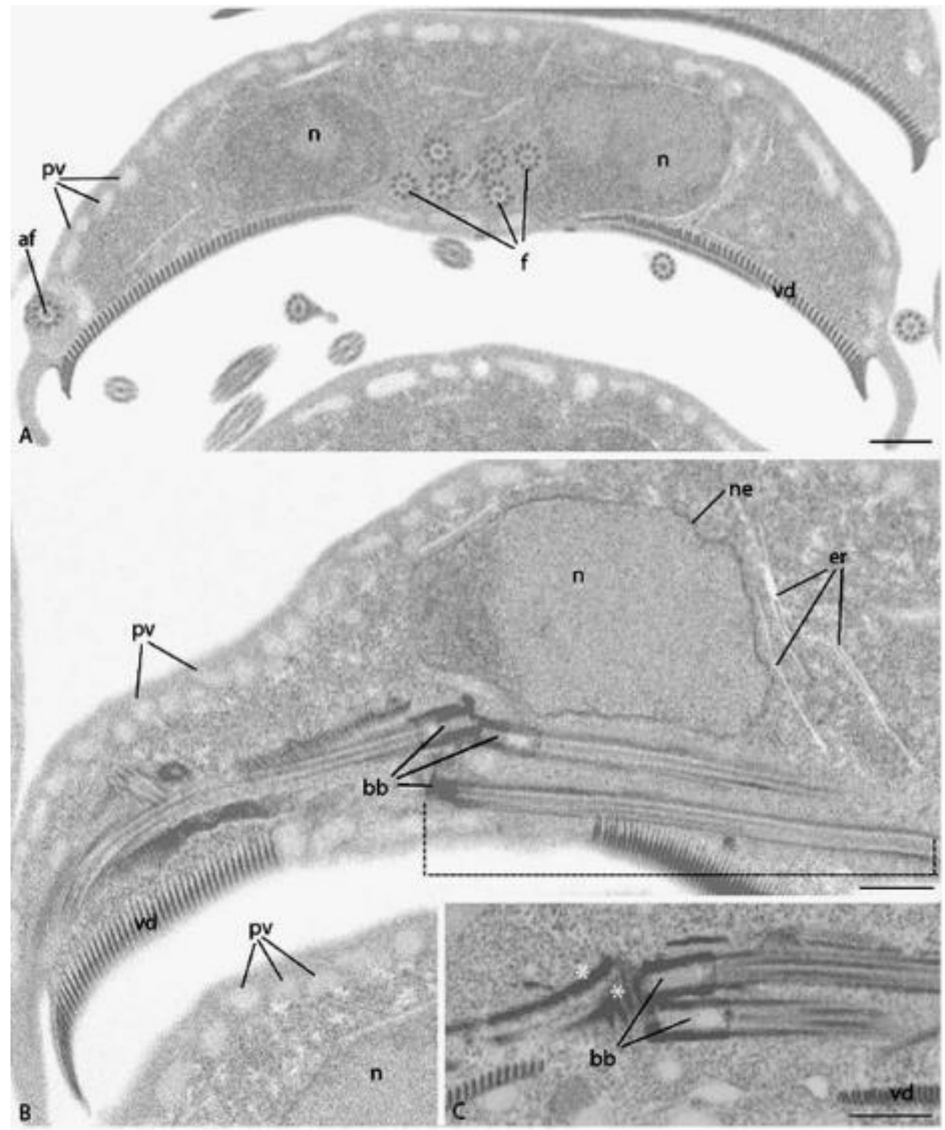
TEM visualisation of funis: https://doi.org/10.1016/j.jsb.2004.01.017

Subcellular organisation of *Giardia intestinalis*



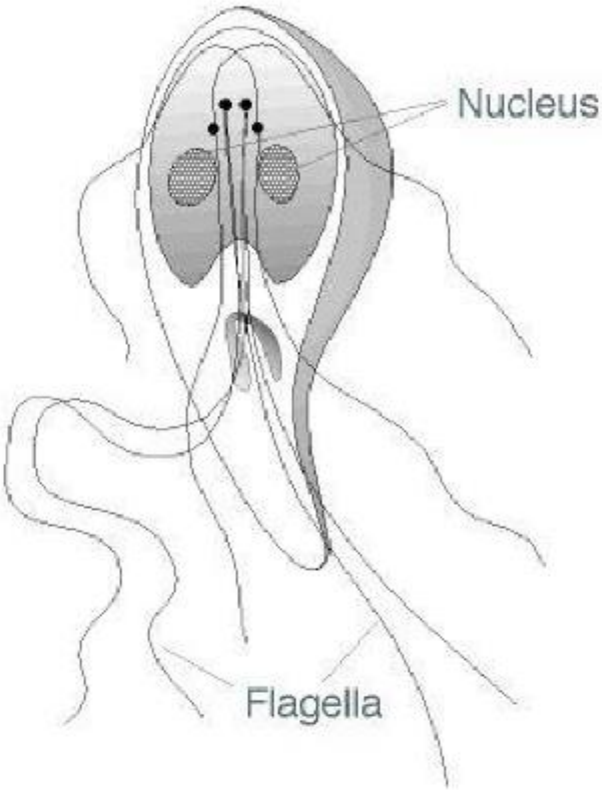
Subcellular organisation of *Giardia intestinalis*

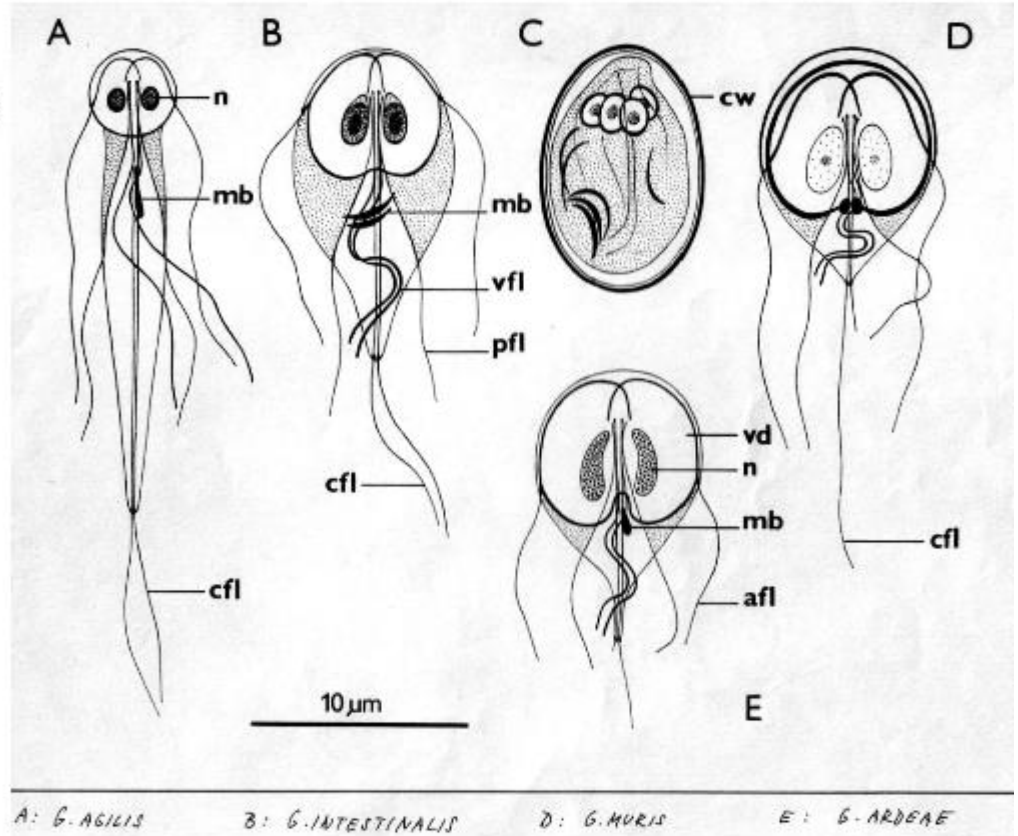




https://doi.org/10.1016/S0020-7519(02)00228-X

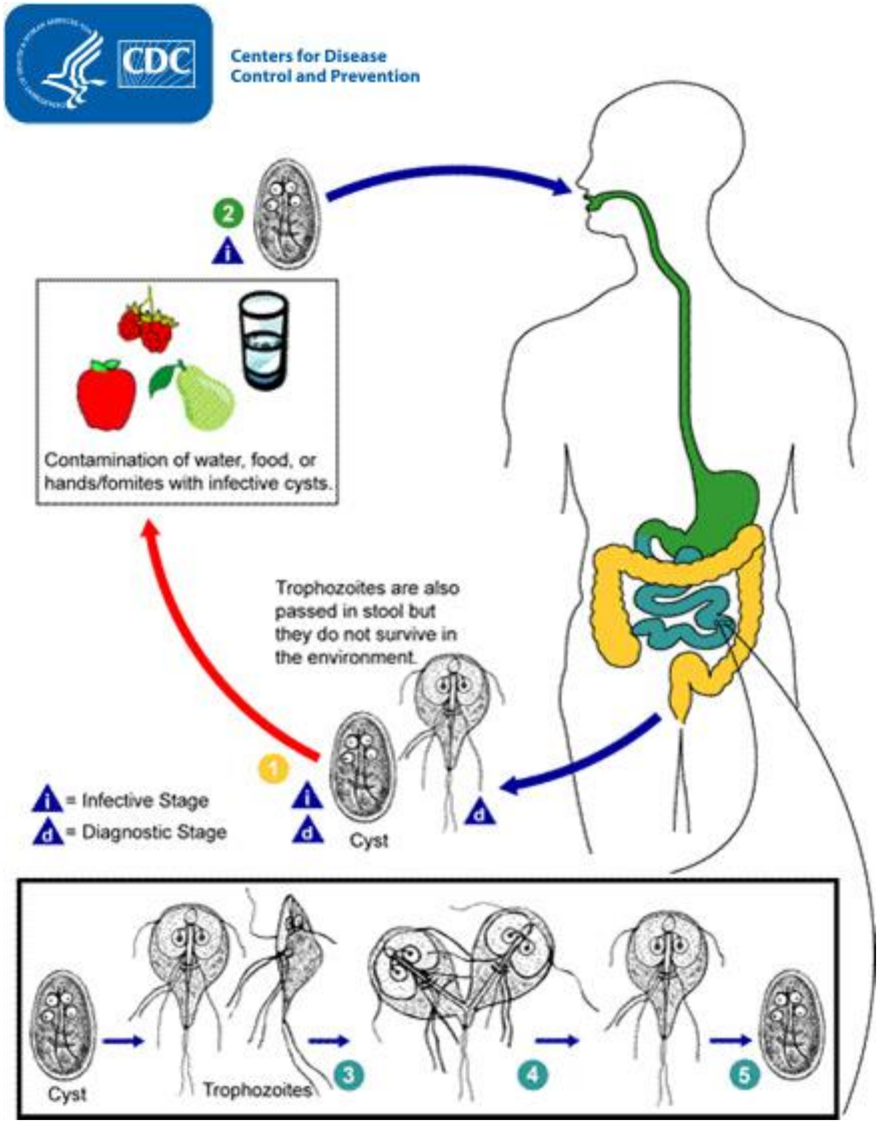
Morphology of *Giardia* spp.





Species	Hosts	Morphological characteristics	Trophozoite dimensions Length	Width
G. duodenalis	Wide range of domestic and wild mammals, including humans	Pear-shaped trophozoites with claw-shaped median bodies	12–15 µm	6–8 µm
G. agilis	Amphibians	Long, narrow trophozoites with club-shaped median bodies	20–30 µm	4–5 µm
G. muris	Rodents	Rounded trophozoites with small round median bodies	9–12 µm	5–7 µm
G. ardeae	Birds	Rounded trophozoites, with prominent notch in ventral disc and rudimentary caudal flagellum. Median bodies round-oval to claw shaped.	~10 µm	~6.5 µm
G. psittaci	Birds	Pear-shaped trophozoites, with no ventro-lateral flange. Claw-shaped median bodies.	~14 µm	~6 µm
G. microti	Rodents	Trophozoites similar to *G. duodenalis*. Mature cysts contain fully differentiated trophozoites.	12–15 µm	6–8 µm

Giardiosis



https://www.youtube.com/watch?v=xjYgfjVZ-Vk

- known as beaver fever
- *Giardia* inhabits the digestive tract of a wide variety of domestic and wild animal, as well as humans
- one of the most common pathogenic parasitic infections in humans worldwide
- in 2013, there were about 280 million people worldwide with symptomatic giardiasis
- incubation period 12-19 days – acute phase symptoms: diarrhoea, greasy stool without blood, weakness, convulsions, vomiting, malabsorption
- most infected people are asymptomatic, only about a third of infected people exhibit symptoms
- zoonotic potential

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Invited Review

Zoonotic potential of *Giardia*

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ABSTRACT

Giardia duodenalis (syn. *Giardia lamblia* and *Giardia intestinalis*) is a common intestinal parasite of humans and mammals worldwide. Assessing the zoonotic transmission of the infection requires molecular characterization as there is considerable genetic variation within *G. duodenalis*. To date eight major genetic groups (assemblages) have been identified, two of which (A and B) are found in both humans and animals, whereas the remaining six (C to H) are host-specific and do not infect humans. Sequence-based surveys of single loci have identified a number of genetic variants (genotypes) within assemblages A and B in animal species, some of which may have zoonotic potential. Multi-locus typing data, however, has shown that in most cases, animals do not share identical multi-locus types with humans. Furthermore, interpretation of genotyping data is complicated by the presence of multiple alleles that generate "double peaks" in sequencing files from PCR products, and by the potential exchange of genetic material among isolates, which may account for the non-concordance in the assignment of isolates to specific assemblages. Therefore, a better understanding of the genetics of this parasite is required to allow the design of more sensitive and variable subtyping tools, that in turn may help unravel the complex epidemiology of this infection.

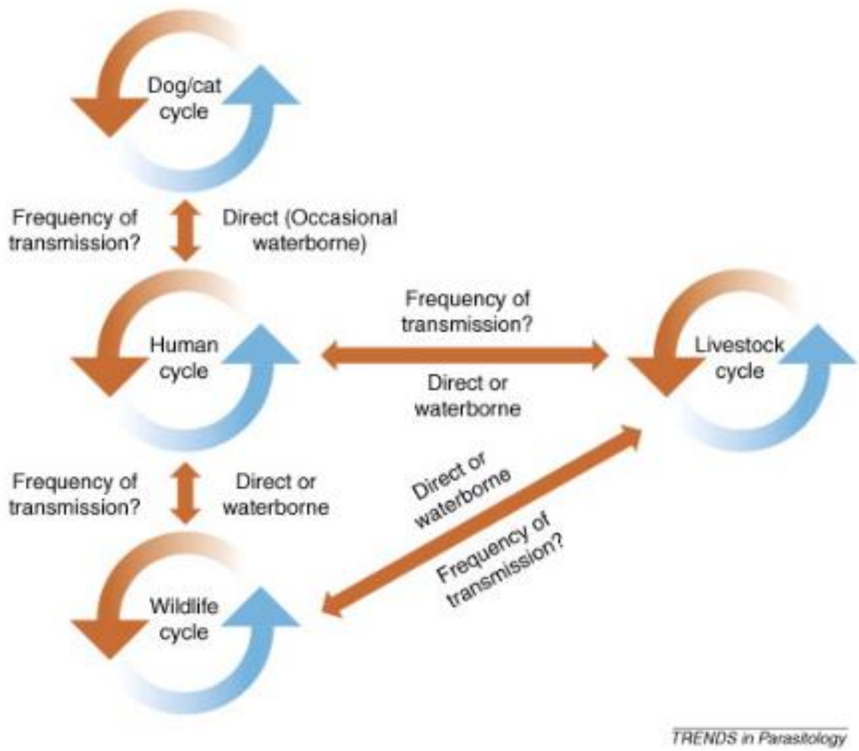
https://doi.org/10.1016/j.ijpara.2013.06.001

Zoonotic potential of *Giardia intestinalis*

Table 2. Genotypic groupings (assemblages) of *Giardia duodenalis* and species[a]

Species (= assemblage)	Host
G. duodenalis (= assemblage A)	Humans and other primates, dogs, cats, livestock, rodents and other wild mammals
G. enterica (= assemblage B)	Humans and other primates, dogs, some species of wild mammals
G. agilis	Amphibians
G. muris	Rodents
G. psittaci	Birds
G. ardeae	Birds
G. microti	Rodents
G. canis (= assemblages C/D)	Dogs, other canids
G. cati (= assemblage F)	Cats
G. bovis (= assemblage E)	Cattle and other hoofed livestock
G. simondi (= assemblage G)	Rats

[a]Designation based on original taxonomic descriptions.



Molecular Characterization

Assemblages	Some Species Commonly Infected
A-I	Humans and animals (cats, dogs, livestock, deer, muskrats, beavers, voles, guinea pigs, ferrets)
A-II	Humans (more common than A-I)
A-III and A-IV	Exclusively animals
B	Humans and animals (livestock, chinchillas, beavers, marmosets, rodents)
C and D	Dogs, coyotes
E	Alpacas, cattle, goats, pigs, sheep
F	Cats

https://doi.org/10.1016/j.pt.2008.11.006

Zoonotic potential of *Giardia intestinalis*

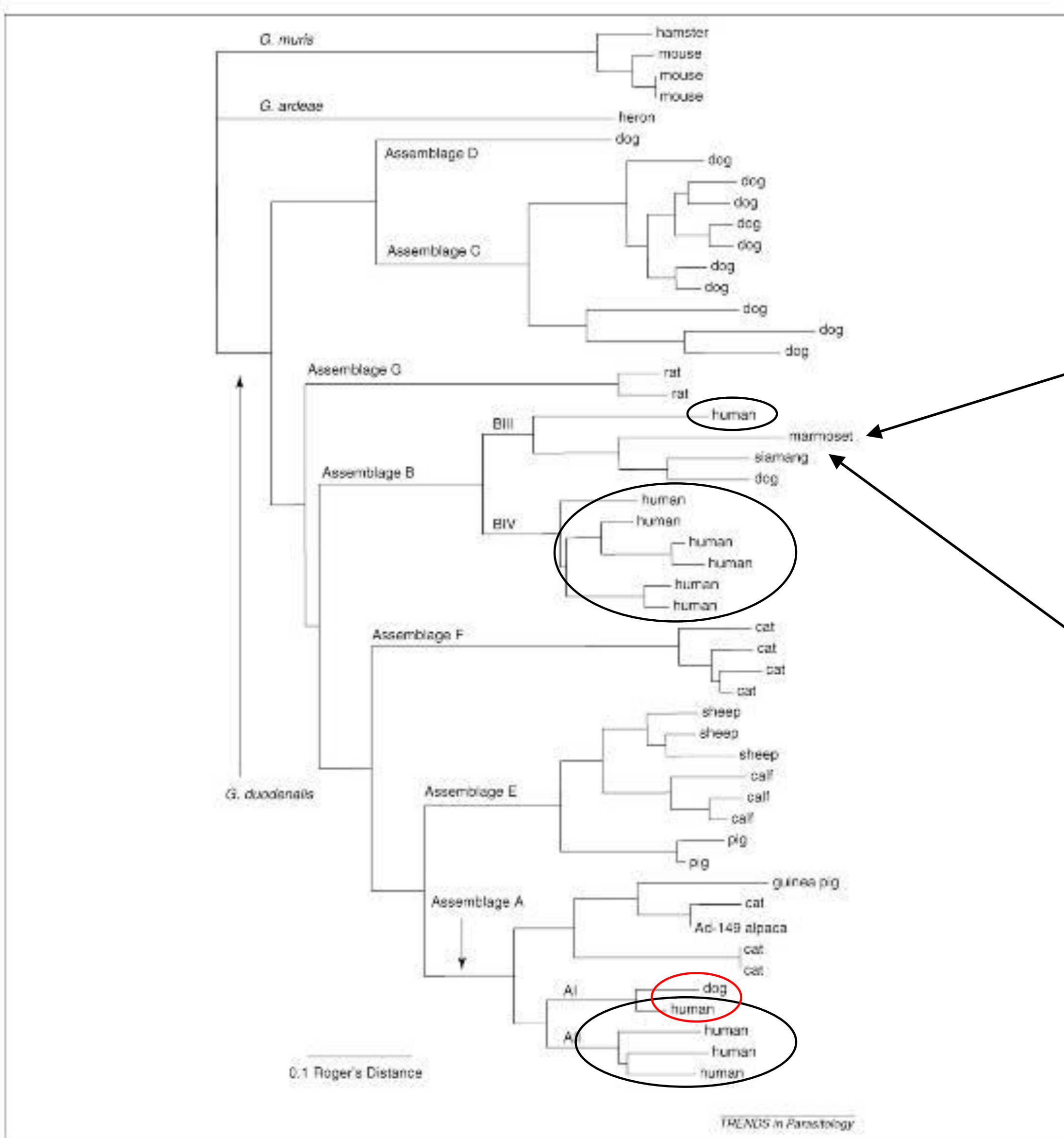
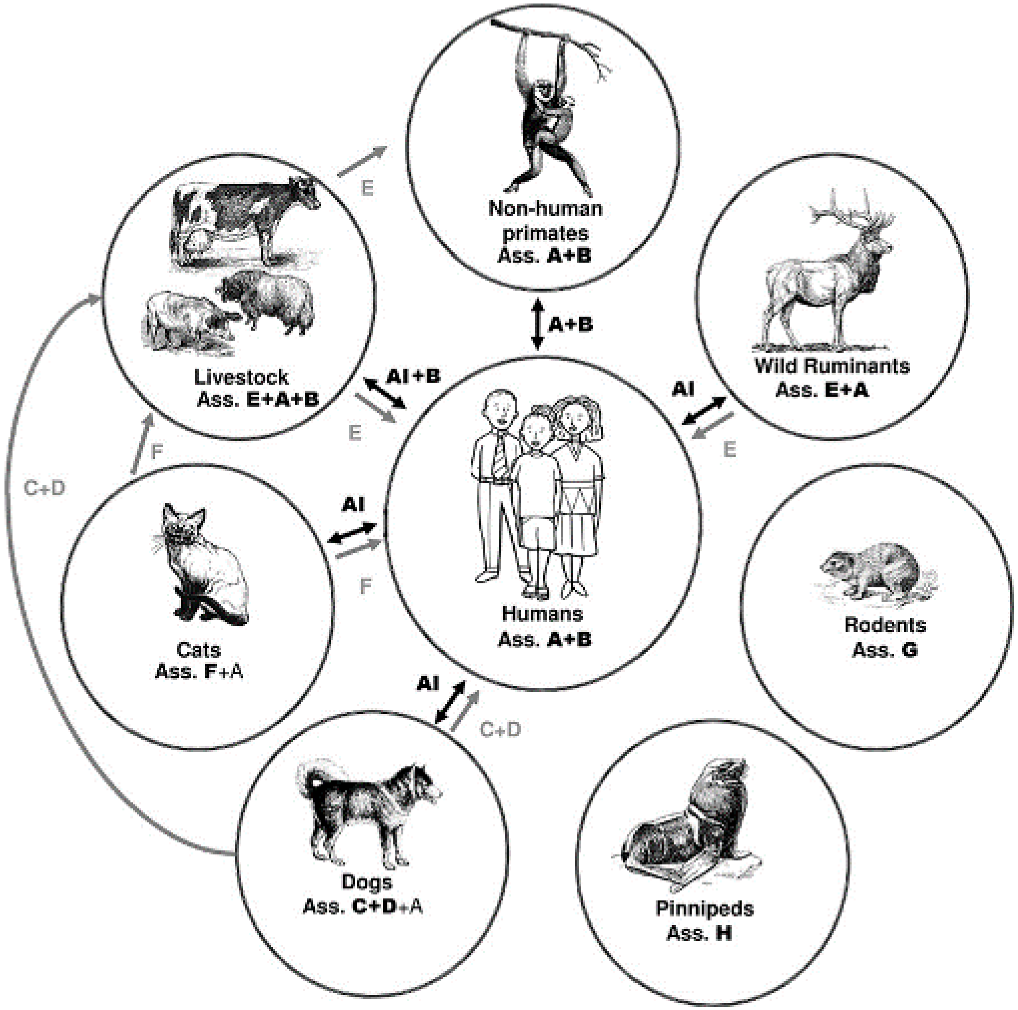


Figure 3. Dendrogram depicting the genetic relationships of isolates of *G. duodenalis* determined by NJ analysis of Roger's distances calculated from enzyme electrophoretic data. The host origin of each isolate is in parentheses. Modified, with permission, from Ref. [14].

https://doi.org/10.1016/j.pt.2008.11.006

Zoonotic potential of *Giardia intestinalis*

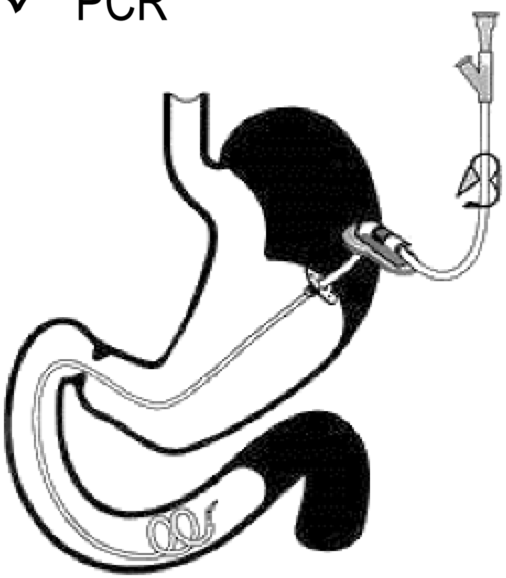


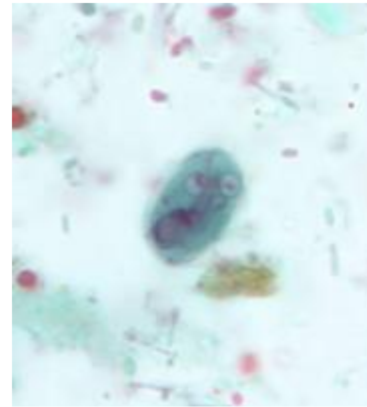
Giardia assemblages and transmission to humans. Most human infections are anthroponotic, and involve sub-assemblages **AI**, **AII**, **BIII** and **BIV**. Sub-assemblage **AI** has zoonotic potential, and involve livestock, wild ruminants, non-human primates, cats and dogs. Occasional reports of transmission of assemblages **C**, **E** and **F** have been reported, likely originating from the animals predominantly infected by these assemblages.

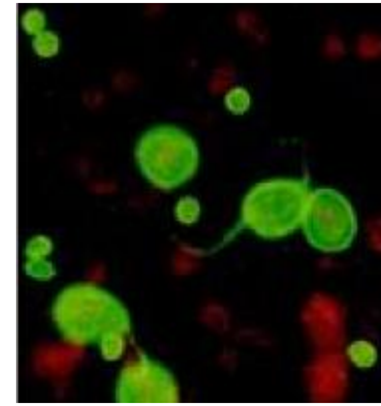
https://doi.org/10.1016/j.meegid.2017.12.001

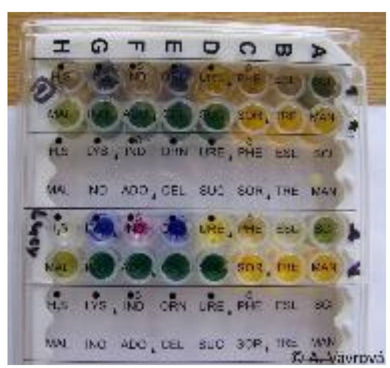
Diagnosis of giardiosis

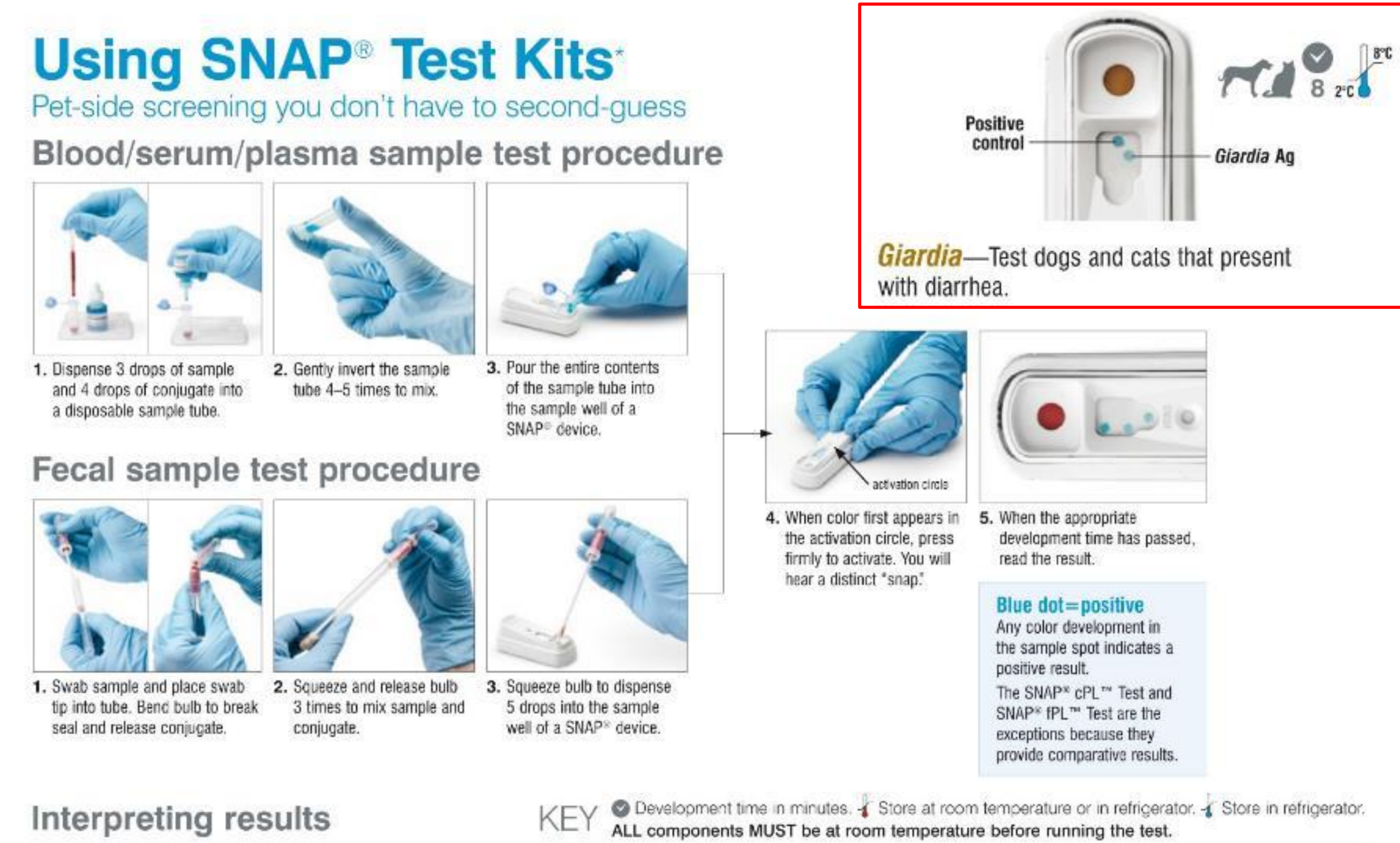
- ✓ microscopy
- ✓ detection of coproantigens
- ✓ examination of duodenal fluid
- ✓ ENTEROtest
- ✓ ELISA
- ✓ PCR











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

SNAP Giardia Test

- ✓ detects soluble *Giardia* antigens, no more slide-scanning for evasive cysts
- ✓ no sample-prep time = easy to set up alongside faecal floats
- ✓ the first USDA-approved in-clinic rapid assay for the detection of *Giardia*-solution antigen

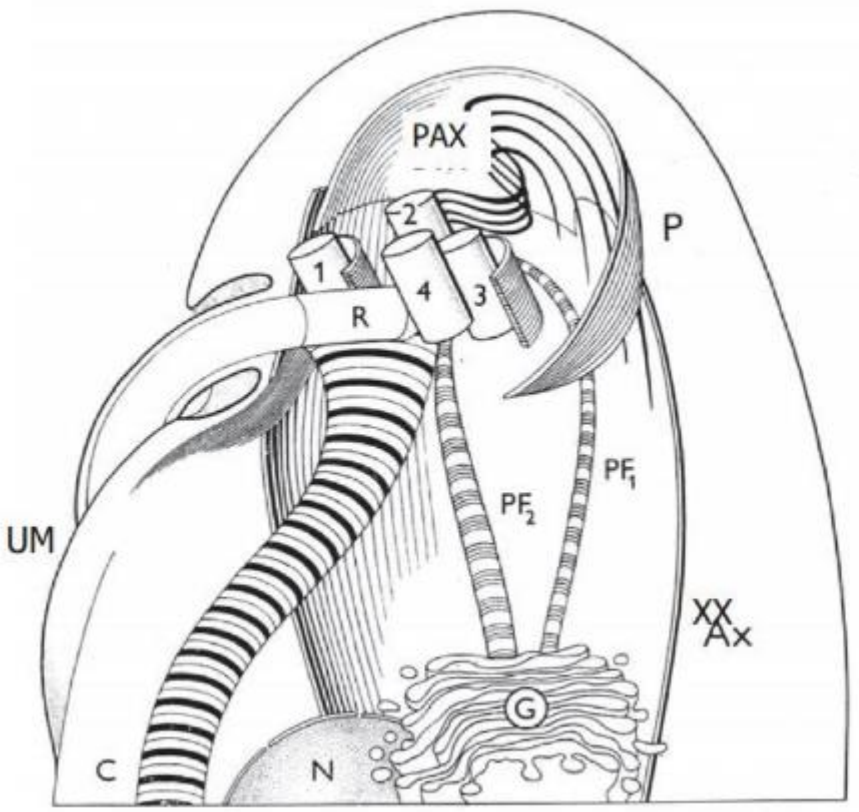
Parabasalia

- monophyletic group
- parabasal apparatus = massively developed or even multiplied Golgi apparatus associated with 2 or more transversely striated microfibrils extending from the kinetosome = parabasal fibres
- generally with 4 flagella / kinetosomes, but frequently with additional flagella (one to thousands)
- 1 kinetosome bears sigmoid fibres that connect to a pelta–axostyle complex
- reduction or loss of the flagellar apparatus in some taxa x multiplication of all or of parts of the flagellar apparatus in several taxa
- closed mitosis with an external spindle, including a conspicuous microtubular bundle
- hydrogenosomes instead of mitochondria
- no cytostome
- anaerobic organisms, commensals, mutualists and parasites of vertebrates and invertebrates

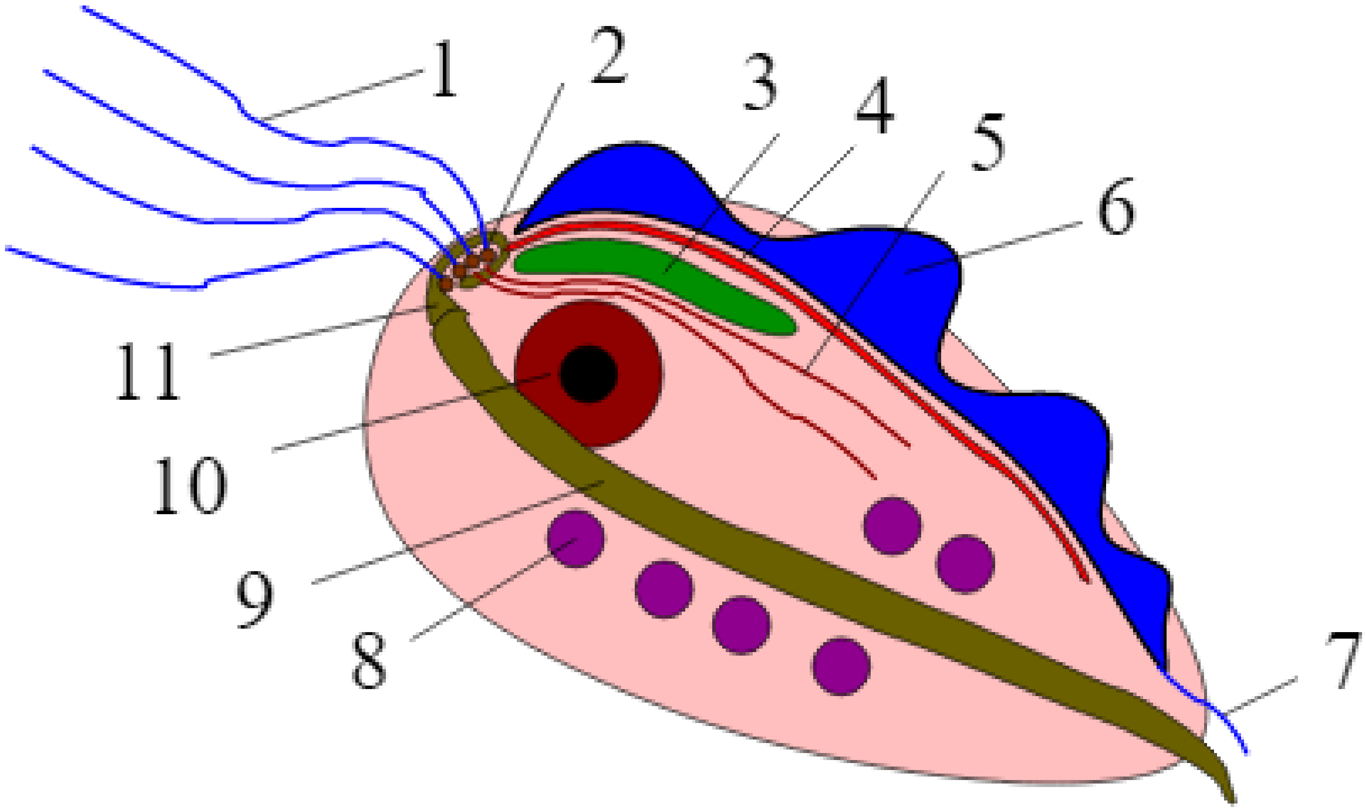
Parabasalia

karyomastigont / parabasal apparatus

- basal bodies(**1, 2 3, R**) (kinetosomes)
- parabasal fibres (**PF1, PF2**)
- pelta-axostyle complex (**PAX)**
- pelta (**P**) sheet microtubules (**helmet**)
- axostyle (**Ax**) sheet microtubules that runs down the centre of the cell and in some cases projects past the end
- undulating membrane (**UM**)
- costa (**C**) microfibrillar structure to support undulating membrane
- Golgi apparatus (**G**)
- nucleus (**N**)



Parabasalia

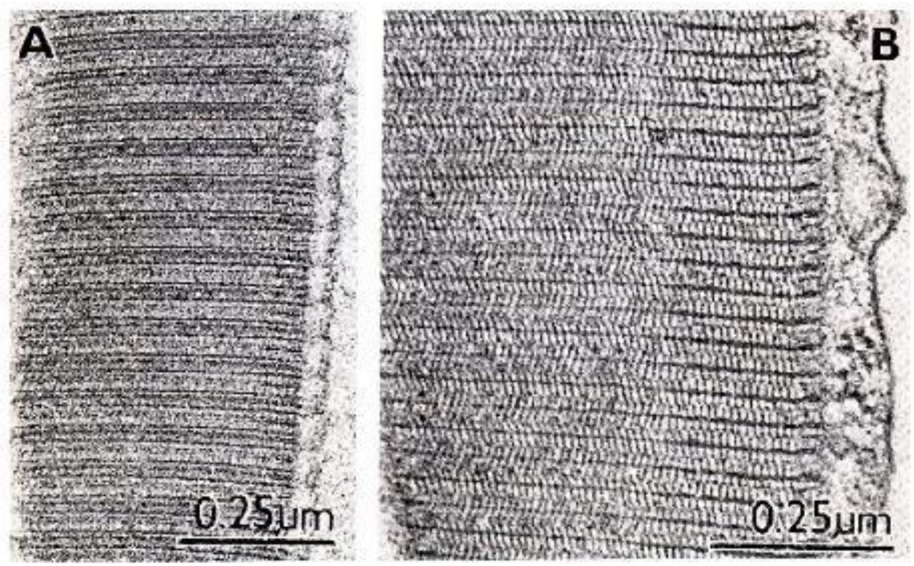


Cell organisation in Parabasala (*Trichomonas*): 1) anterior flagella, **2)** kinetosomes, **3)** parabasal body, **4)** costa, **5)** parabasal fibers, **6)** undulating membrane, **7)** posterior flagellum, **8)** hydrogenosomes, **9)** axostyle, **10)** nucleus, **11)** pelta

Parabasalia

Karyomastigont – costa

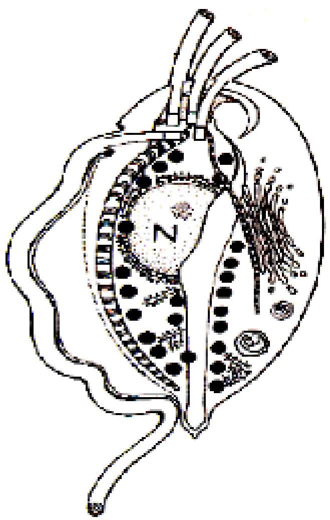
A-type costa

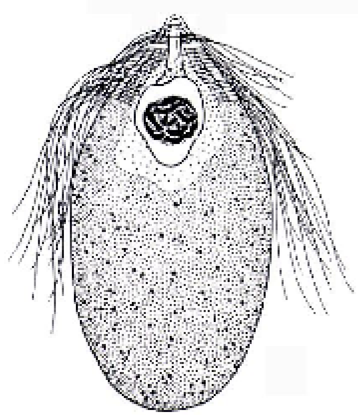


B-type costa

- mechanical support to the undulating membrane
- different striations (A-type x B-type)
- A-type (*Tritrichomonas*) - attached to kinetosome 2
- B-type (*Trichomonas*, *Pentatrichomonas*) - connected to kinetosome R (kinetosome of the recurrent flagellum)

Parabasalia





https://doi.org/10.1016/j.protis.2009.11.005

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Protist

ORIGINAL PAPER

Critical Taxonomic Revision of Parabasalids with Description of one New Genus and three New Species

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Monitoring Editor: C. Graham Clark

We propose a new classification of Parabasalia which is congruent with both ultrastructural and molecular-phylogenetic studies. We identify six main parabasalid lineages and give them the rank of class: Hypotrichomonadea, Trichomonadea, Tritrichomonadea, Cristamonadea, Trichonymphea, and Spirotrichonymphea. Trichomonadea is characterized by a single mastigont and by the absence of both a comb-like structure and an infrakinetosomal body. Most representatives also possess a lamelliform undulating membrane. Trichomonadea is divided into two monophyletic orders, Trichomonadida (family Trichomonadidae; with a B-type costa) and Honigbergiellida (families Honigbergiellidae, Hexamastigidae and Tricercomitidae; without a costa). The class Tritrichomonadea, with a single order Tritrichomonadida, is ancestrally characterized by a single mastigont with four flagella, and both a comb-like structure and an infrakinetosomal body. The morphologically most complex representatives (family Tritrichomonadidae) possess in addition a rail-type undulating membrane, an A-type costa, and a suprakinetosomal body. These last three characters are absent in families Monocercomonadidae and Simplicimonadidae. The remaining tritrichomonadids, Dientamoebidae, have undergone reductive evolution. Cristamonads (Cristamonadea) are morphologically derived from tritrichomonads. Because we are unable to determine morphologically homogenous monophyletic lineages within cristamonads, we classify all cristamonads into a single family, Lophomonadidae. Hypotrichomonadea, comprising the genera *Trichomitus* and *Hypotrichomonas*, resembles Tritrichomonadea by an A-type costa, and by the presence of a comb-like structure in the mastigont. However, they do not possess an infrakinetosomal body, and are not specifically related to Tritrichomonadea in molecular-phylogenetic analyses. Moreover, unlike Tritrichomonadea, Hypotrichomonadea possesses a lamelliform undulating membrane. The remaining parabasalids are of complex morphology and belong to the classes Trichonymphea and Spirotrichonymphea. A new parabasalid genus, *Simplicimonas* (Tritrichomonadea), and three new species, *Tetratrichomonas undula*, *Hexamastix coercens* and *Simplicimonas similis*, are described.

Parabasalia

Trichomonadea
Hypotrichomonadea
Tritrichomonadea

Cristamonadea
Spirotrichonymphea
Trichonymphea

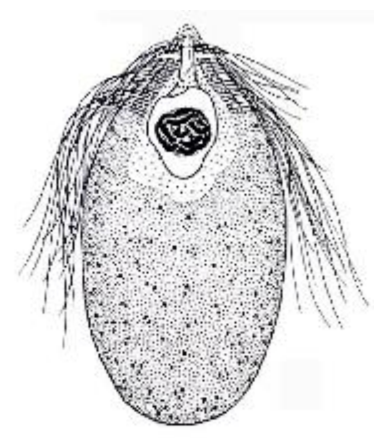
Parabasalids were traditionally divided in orders Trichomonadida and Hypermastigida:

„trichomonads“

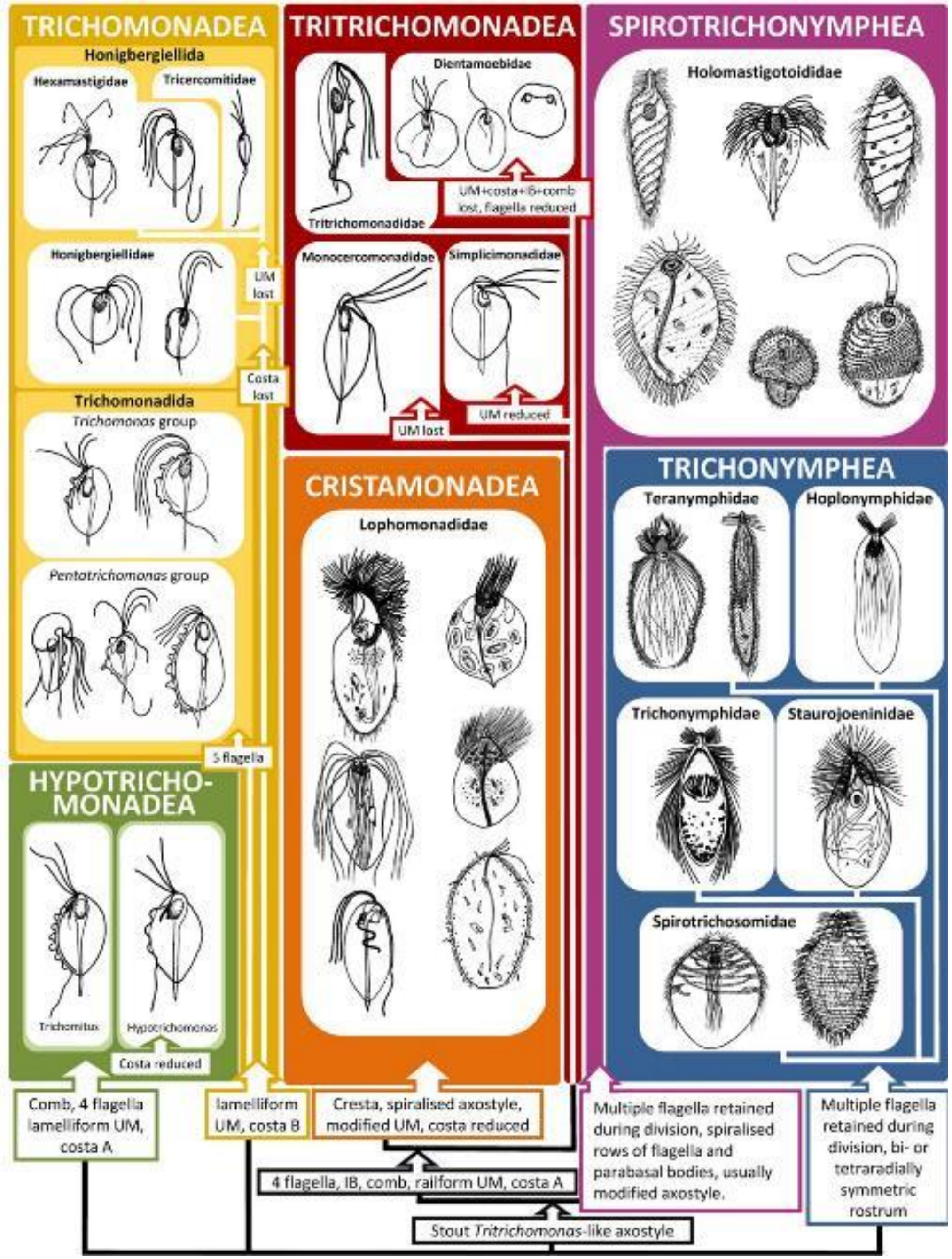
- 10-40 μm
- up to 6 flagella
- symbionts or parasites
- urogenital tract, oral cavity, intestine of vertebrates

„hypermastigids“

- up to 500 μm
- thousands flagella
- exclusively in the hindgut of termites and wood-eating *Cryptocercus* cockroaches



Parabasalia



Čepička et al. 2010:
Critical taxonomic revision of Parabasalids with description of one new genus and three new species.
Protist 161, 400–433

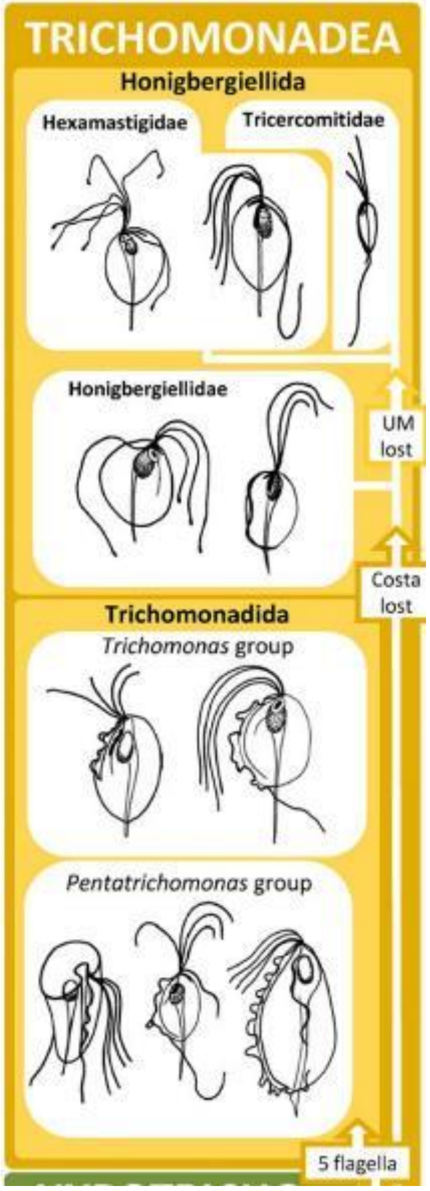
https://doi.org/10.1016/j.protis.2009.11.005

Parabasalia

Trichomonadea

- 4-6 flagella with 1 axoneme supporting lamelliform undulating membrane
- B-type costa, sometimes absent
- axostyle usually of "*Trichomonas* type"
- ***Hexamastix***, ***Pseudotrichomonas***, ***Tricercomitus***, ***Trichomonas***, ***Tetratrichomonas***, ***Pentatrichomonas***, ***Cochlosoma***

Čepička et al. 2010: Protist 161



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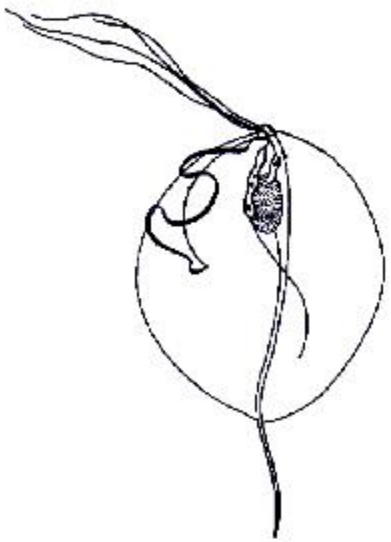
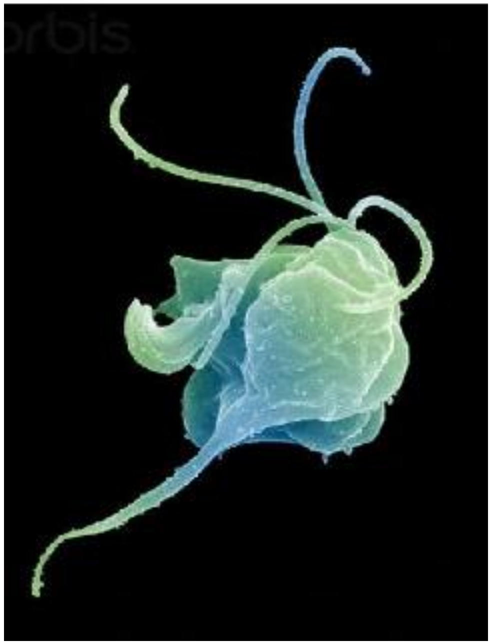
Trichomonas vaginalis

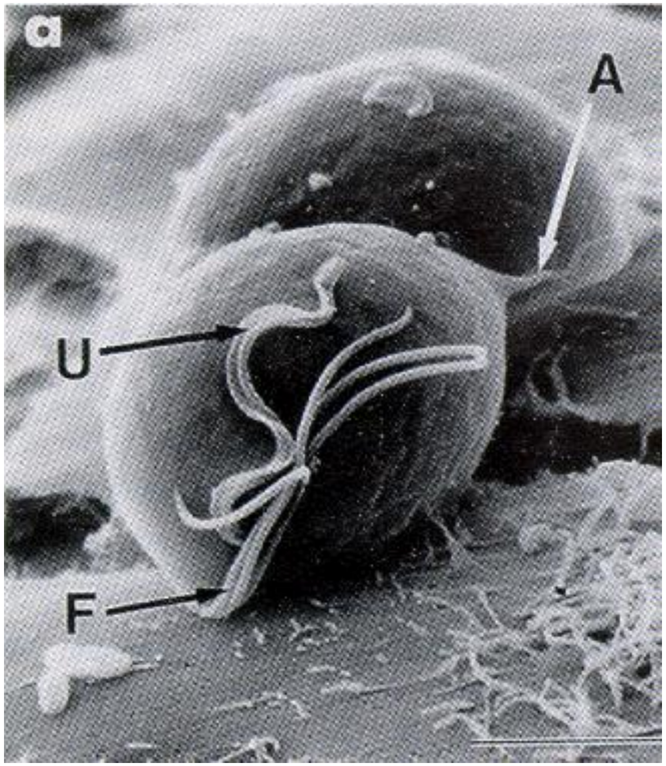


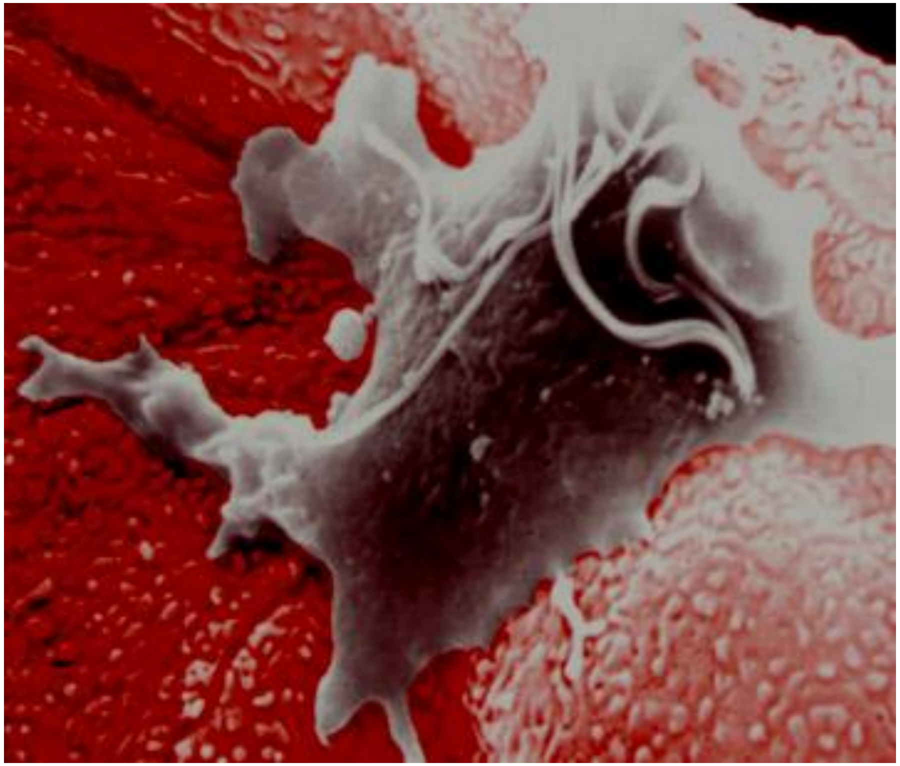
- causative agent of human trichomoniasis
- 10-20 ×2-14 μm, typically pyriform
- <u>pentaflagellate</u> - 4 flagella from the anterior cell portion + 1 flagellum backwards to the middle of the organism, forming undulating membrane
- undulating membrane without free flagellum
- axostyle extending from the posterior aspect of the cell
- V-shaped parabasal apparatus
- large genome (strain G3, 176,441,227 bp) with ~ 60,000 protein coding genes organized into 6 chromosomes
- obligate parasite phagocytosing bacteria, vaginal epithelial cells and erythrocytes and is itself ingested by macrophages



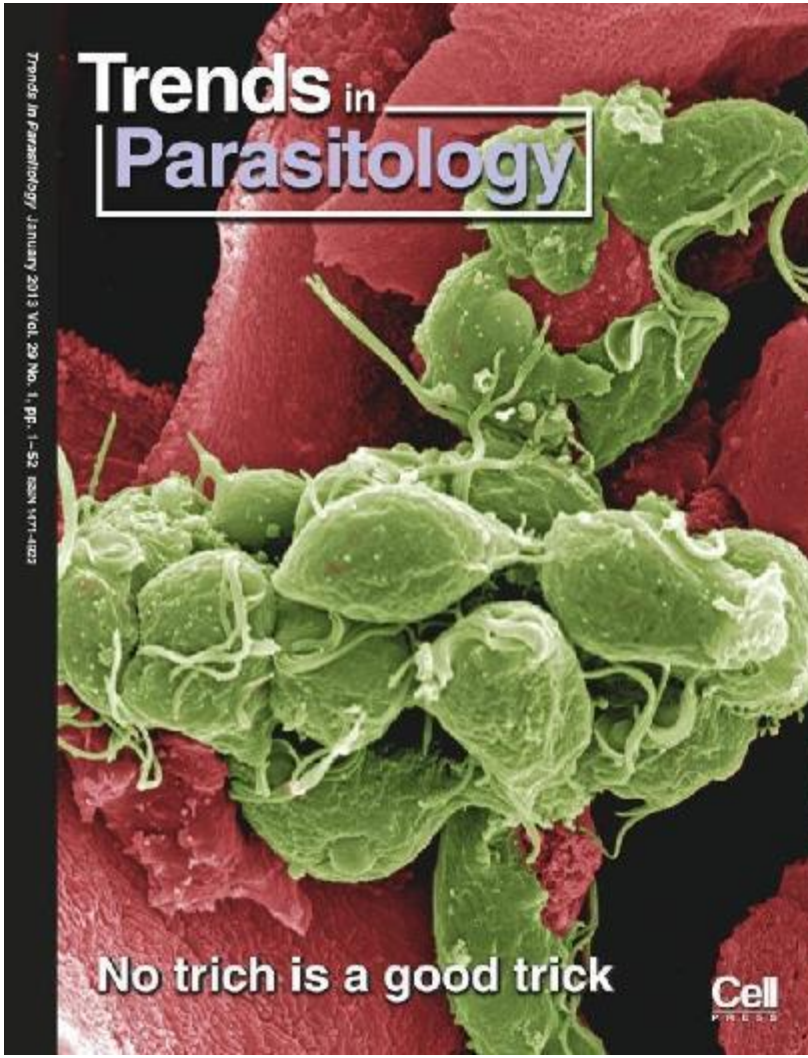
Virulence factors in *Trichomonas vaginalis*

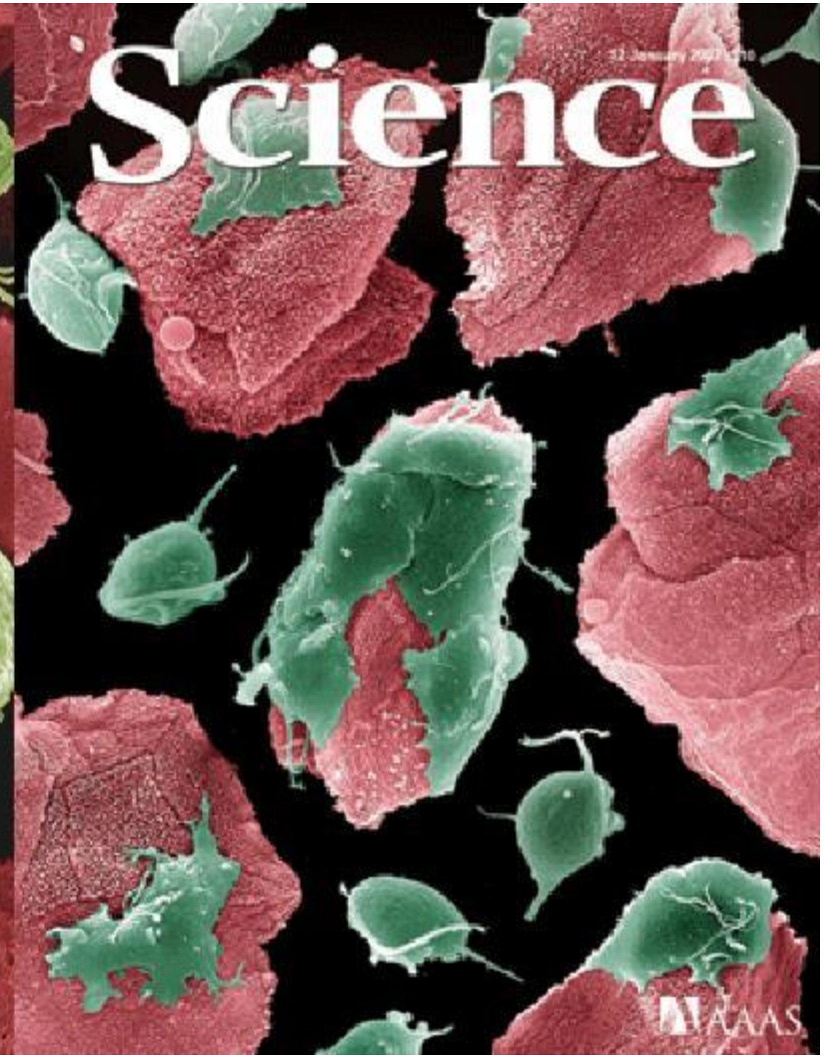
- **cytoadherence**
- level of iron intake
- defence against complement and leukocytes





Cytoadherence of *Trichomonas vaginalis*



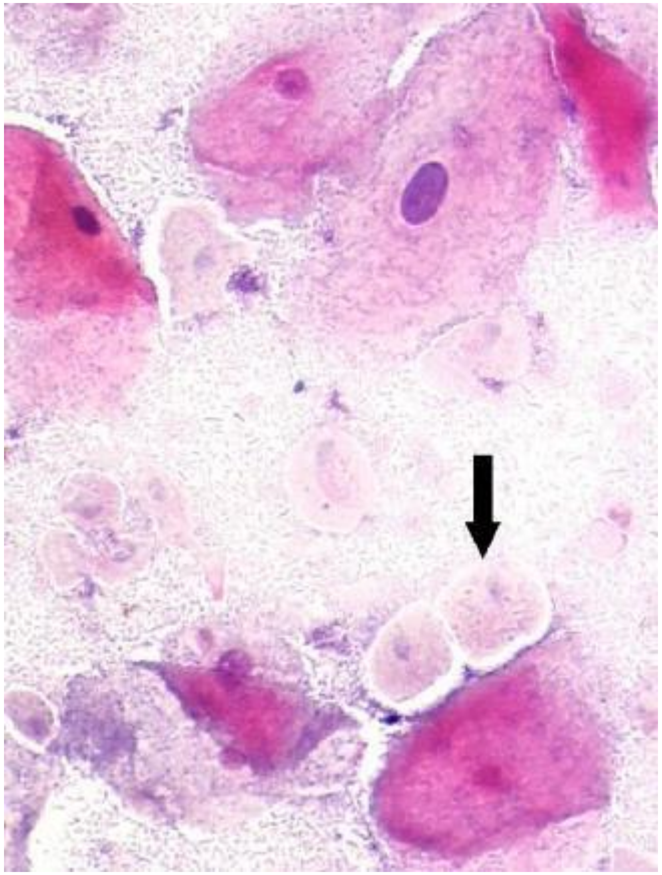


https://doi.org/10.1016/j.pt.2012.10.004

https://www.science.org/doi/10.1126/science.1132894

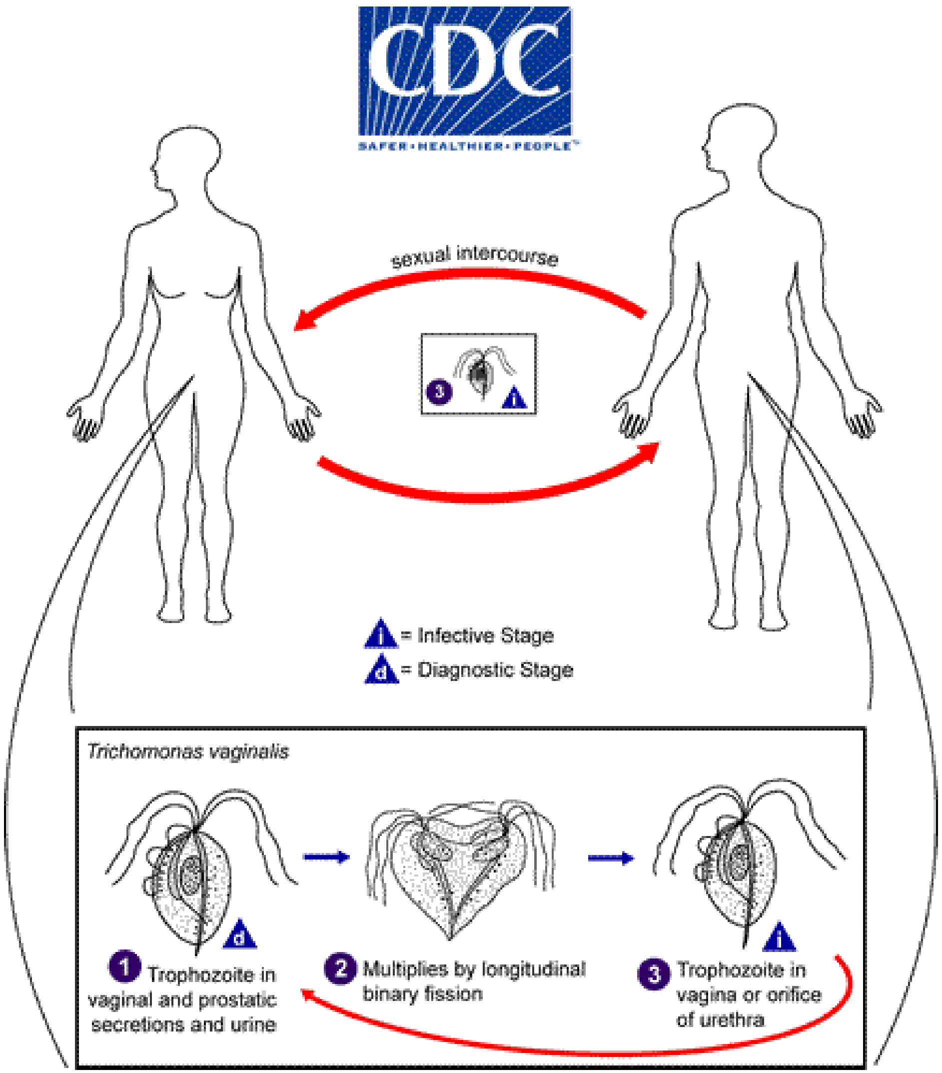
Human urogenital trichomoniasis

- humans are the only known host of *T. vaginalis*
- infection of the urogenital tract
- cosmopolitan, sexually transmitted diseases (STD)
- possible contaminant transmission and transmission to newborns during childbirth
- estimated 248 million new cases per year in the world
- treatment with metronidazole
- most infected people are asymptomatic
- sexual partners, even if asymptomatic, should also be treated
- without treatment, can persist for months to years in women, and is thought to improve without treatment in men



microbial vaginal smear (MVS)

Trichomoniasis



https://www.youtube.com/watch?v=ze8jA2y1kIQ

Table 1: Overview and characteristics of diagnostic assays for *Trichomonas vaginalis**

Diagnostic Test	Technique	Time to Result	Specimen	Sensitivity	Specificity	Comments
Wet mount	Microscopic visualization	Minutes	Vaginal or urethral discharge	51–65%	up to 100%	Inexpensive; technician-dependent
Culture	Culture media	24–120 hours	Vaginal or urethral swab	75–98%	up to 100%	Considered diagnostic gold standard in the past
OSOM Trichomonas Rapid Test	Immunochromatographic capillary-flow enzyme immunoassay dipstick	10 minutes	Vaginal swabs or saline for wet mount	82–95%	97–100%	CLIA-waived for females; can be used at the point-of-care
Affirm VPIII Microbial Identification Test	Nucleic acid probe test	45 minutes	Vaginal swabs	63%	99.9%	Moderately complex same-day test; FDA-cleared for use with specimens from females; also detects Gardnerella vaginalis and Candida albicans
APTIMA Trichomonas vaginalis Assay	Transcription Mediated Amplification (TMA)	Hours	Urine specimens, endocervical and vaginal swabs, and specimens collected in PreservCyt Solution	95–100%	95–100%	NAATs are the most sensitive tests; FDA-cleared for use with specimens from symptomatic or asymptomatic females
BD ProbeTec Trichomonas vaginalis Q[x] Amplified DNA Assay	Strand Displacement Amplification (SDA)	Hours	Not an FDA-cleared product			Variety of female specimens have been tested
PCR	Polymerase Chain Reaction	Hours	No FDA-cleared kit			Variety of male and female specimens have been tested

Kissinger *BMC Infectious Diseases* (2015) 15:307
DOI 10.1186/s12879-015-1055-0

BMC
Infectious Diseases

REVIEW **Open Access**

CrossMark

Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues

Patricia Kissinger

Abstract

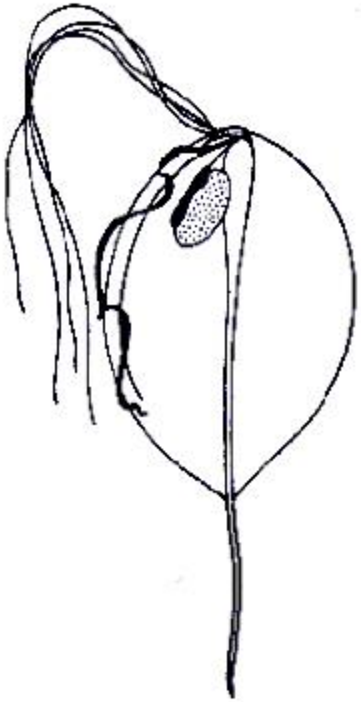
Trichomonas vaginalis (TV) is likely the most common non-viral sexually transmitted infection (STI) in the world. It is as an important source of reproductive morbidity, a facilitator of HIV transmission and acquisition, and thus it is an important public health problem. Despite its importance in human reproductive health and HIV transmission, it is not a reportable disease and surveillance is not generally done. This is problematic since most persons infected with TV are asymptomatic. Metronidazole (MTZ) has been the treatment of choice for women for decades, and single dose has been considered the first line of therapy. However, high rates of retest positive are found among TV infected persons after single dose MTZ treatment. This has not been explained by drug resistance since in vitro resistance is only 2–5 %. Treatment failure can range from 7–10 % and even higher among HIV+ women. Treatment efficacy may be influenced by vaginal ecology. The origins of repeat positives need further explanation and better treatment options are needed.

Keywords: *Trichomonas vaginalis*, Trichomoniasis, Epidemiology, Treatment

https://doi.org/10.1186/s12879-015-1055-0

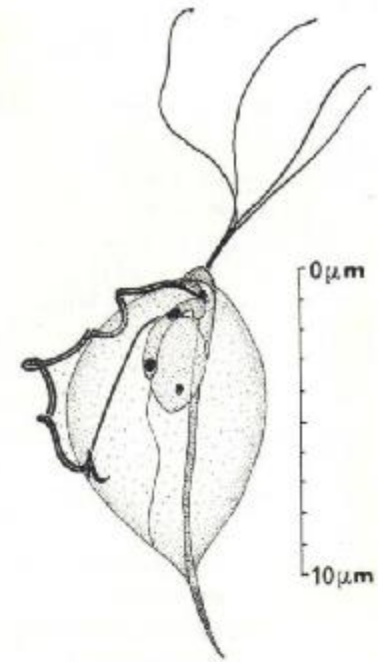
Trichomonas tenax

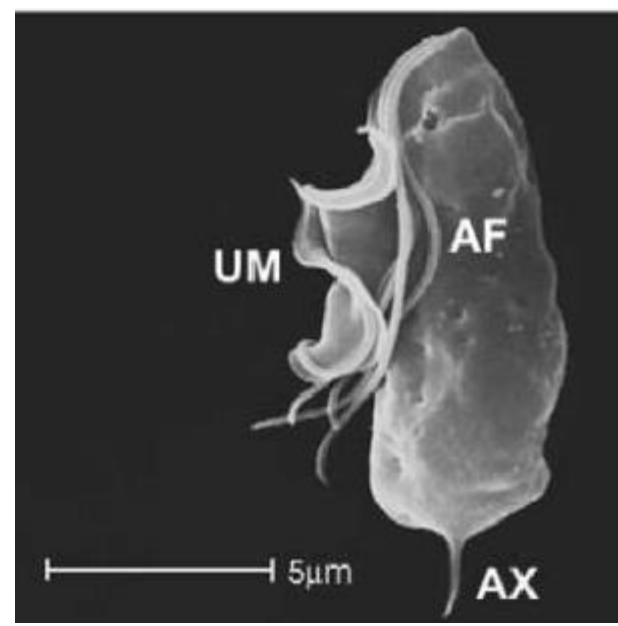
- 12-20 x 5-6 µm
- long axostyle and tail
- 4 anterior flagella + 1 recurrent flagellum raising an undulating membrane to 2/3 of the cell
- oral cavity and bronchi of humans
- likely involved in the degradation of periodontal tissue
- affecting more than 50 % of the population
- routine hygiene is generally not sufficient to eliminate the parasite



Trichomonas gallinae

- 6-15 x 4-8 µm
- cosmopolitan parasite of pigeons and doves
- 4 anterior flagella and undulating membrane
- undulating membrane extending 2/3 of the cell length
- lacking the free posterior flagellum
- commonly found in the mouth, throat, gastro-intestinal tract and upper respiratory tract of pigeons, doves, turkeys, chickens, canaries, raptors (predatory birds) and a variety of psittacine (parrot) birds including budgerigars, cockatiels and Amazon parrots





Trichomonas gallinae

- causative agent of bird trichomonosis / trichomoniasis in young birds
- <u>yellow necrotic lesions</u> in mouth and oesophagus
- known as „canker“ (doves, pigeons) and as „frounce“ (raptors)
- fatal for young pigeons aged 1-3 weeks
- adult bird as carriers - infection via regurgitated crop content („pigeon milk“)
- infection via contamination of food and water
- transmission to the raptors
- avirulent strains x virulent strains

canker in pigeon

Trichomonas gallinae

- survives at least 5 days on some moist grains and several hours in water
- extremely sensitive to desiccation (drying)
- <u>no cysts or resistant stage</u>
- <u>caseous necrotic masses</u> in the upper digestive tract and occasionally in the visceral organs
- diagnosis based on the history, clinical signs, lesions and identification of the organism microscopically and by culturing

frounce in falcon

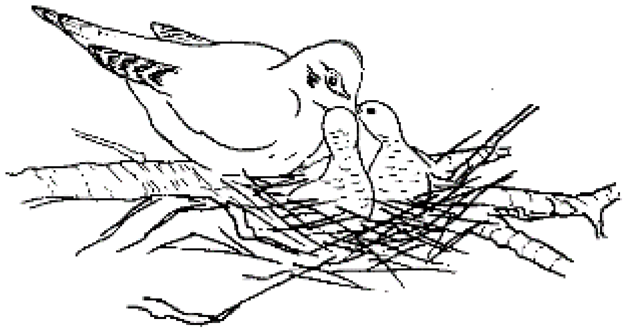
Trichomonas gallinae

- recent appearance of deadly infections in wild Passeriformes with *T. gallinae* obtained greater attention
- trichomonosis in Europaen green finch (*Carduelis chloris*)
  https://doi.org/10.1051/parasite/2019022

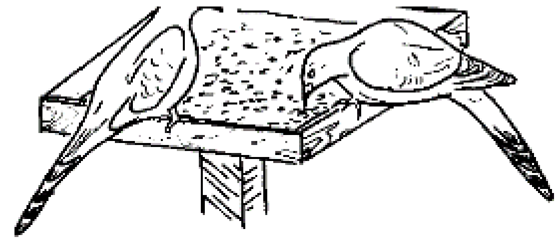
The bird sat on the ground. It had ruffled feathers and some mucus dropped out of its beak.

The bird was in poor nutritional condition and had a moderately developed pectoral muscle. Thick yellowish exudate covered part of the oral cavity, the crop and the upper oesophagus.

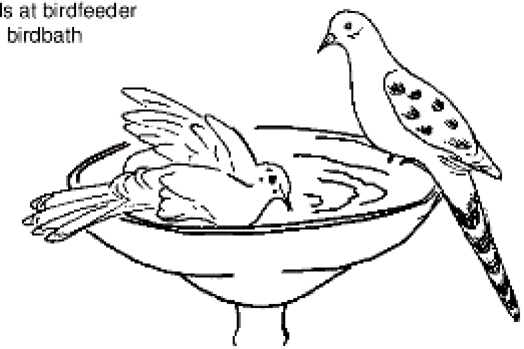
Transmission of *Trichomonas gallinae*

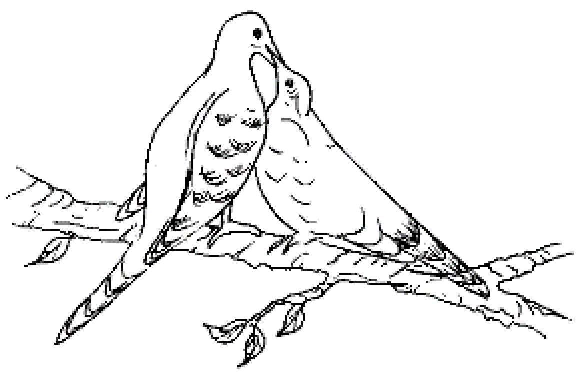


Birds in nest being fed by dove or pigeon

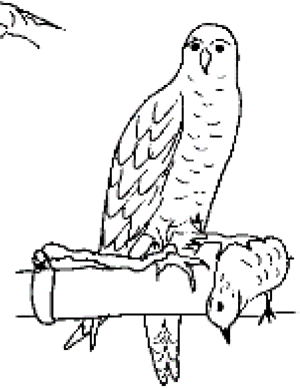


Birds at birdfeeder
and birdbath

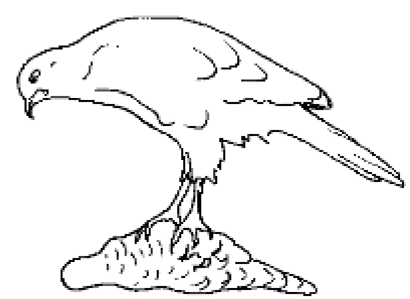




Billing/feeding courtship



Captive raptor being provided
infected dove or pigeon



Wild raptor catching or eating an
infected dove

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Trichomonas stableri n. sp., an agent of trichomonosis in Pacific Coast band-tailed pigeons (*Patagioenas fasciata monilis*) ☆

Yvette A. Girard [a,*], Krysta H. Rogers [b], Richard Gerhold [c], Kirkwood M. Land [d], Scott C. Lenaghan [e], Leslie W. Woods [f], Nathan Haberkern [d], Melissa Hopper [d], Jeff D. Cann [g], Christine K. Johnson [a]

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Phylogeny
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Fe-hydrogenase
rpb1

ABSTRACT

Trichomonas gallinae is a ubiquitous flagellated protozoan parasite, and the most common etiologic agent of epidemic trichomonosis in columbid and passerine species. In this study, free-ranging Pacific Coast band-tailed pigeons (*Patagioenas fasciata monilis*) in California (USA) were found to be infected with trichomonad protozoa that were genetically and morphologically distinct from *T. gallinae*. In microscopic analysis, protozoa were significantly smaller in length and width than *T. gallinae* and were bimodal in morphology. Phylogenetic analysis of the ITS1/5.8S/ITS2, *rpb1*, and hydrogenosomal Fe-hydrogenase regions revealed that the protozoan shares an ancestor with *Trichomonas vaginalis*, the sexually-transmitted agent of trichomoniasis in humans. Clinical and pathologic features of infected birds were similar to infections with *T. gallinae*. Evidence presented here strongly support taxonomical distinction of this parasite, which we hereby name *Trichomonas stableri* n. sp. This work contributes to a growing body of evidence that *T. gallinae* is not the sole etiologic agent of avian trichomonosis, and that the incorporation of molecular tools is critical in the investigation of infectious causes of mortality in birds.

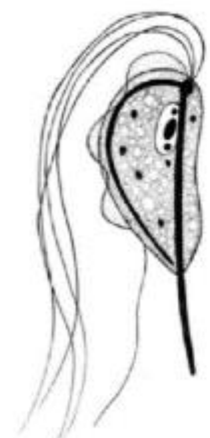
https://doi.org/10.1016/j.ijppaw.2013.12.002

Tetrarichomonas canistomae

- oral cavity of dogs

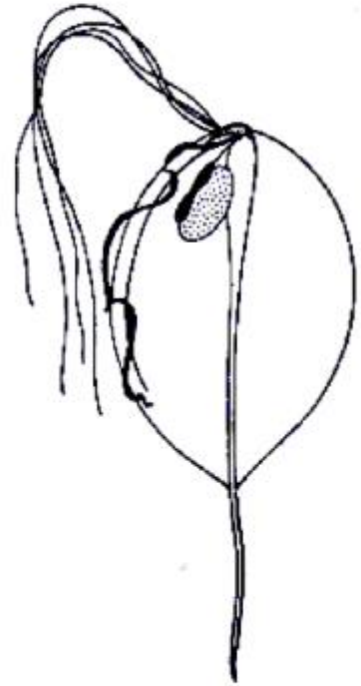
Tetratrichomonas felistomae

- oral cavity of cats



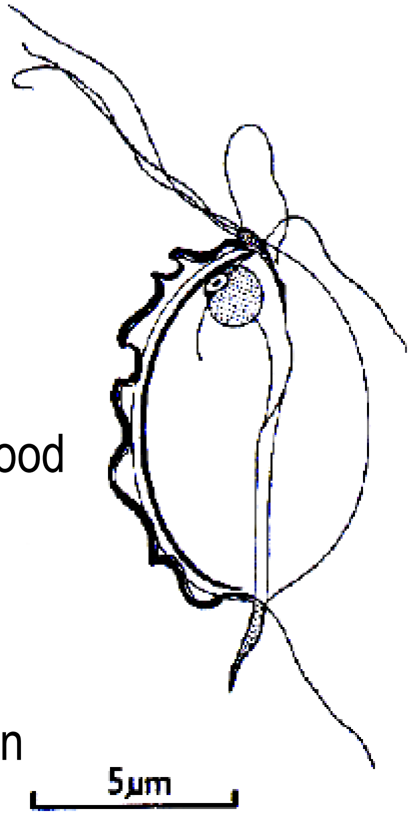
Tetratrichomonas brixi

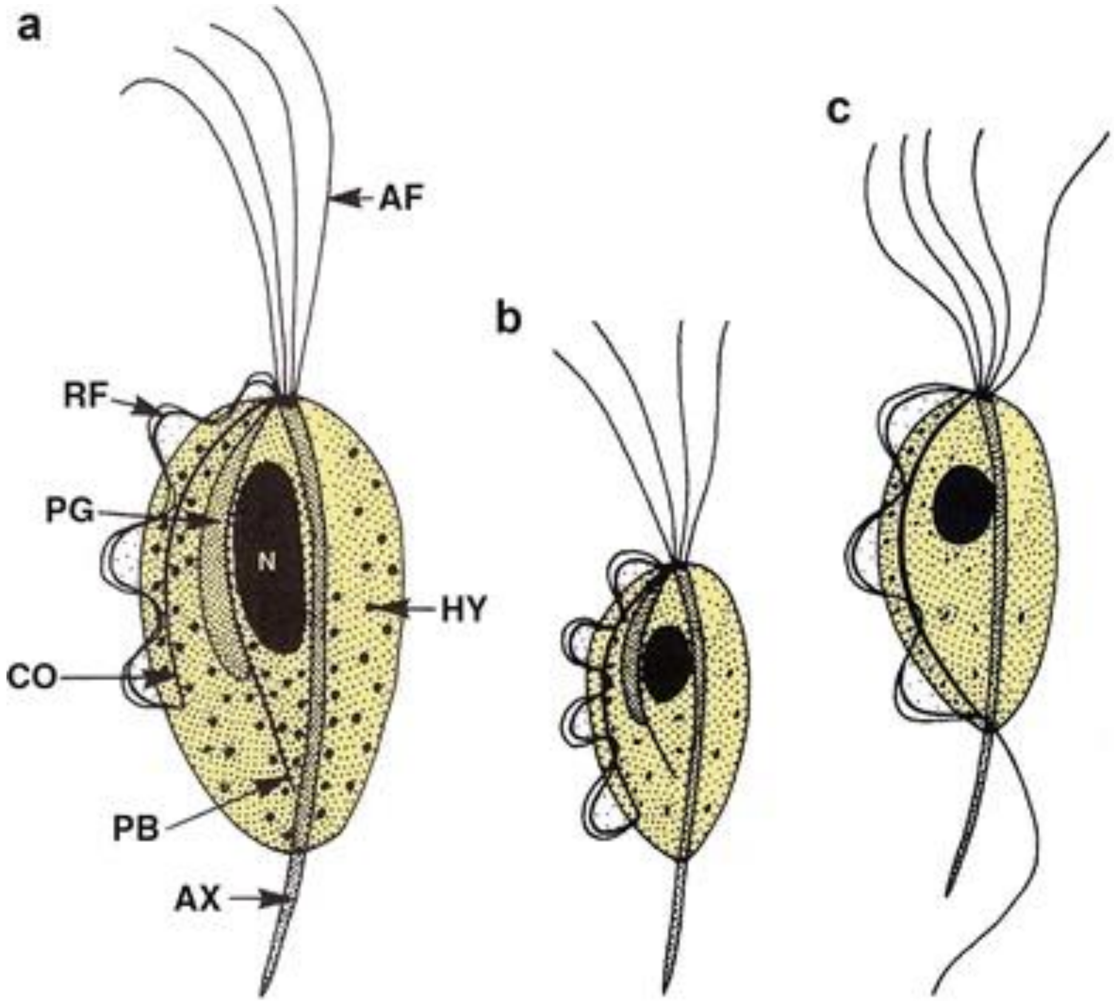
- oral cavity of cats and dogs
- morphologically identical with *T. tenax*
- new species description based on sequencing and phylogenetic analysis of ITS1-5.8S-ITS2 region
- prevalence of *T. brixi* from the mouth of dogs and cats were 30,6 % (34/111) and 6,6% (8/122)



Pentatrichomonas hominis

- 4 anterior flagella + 1 trailing flagellum
- long undulating membrane
- no known cyst
- infection via ingestion of trophozoites in faecal-contaminated food or water, or on fomites
- non-pathogenic commensal in colon of humans and various mammals
- human isolates are capable of infecting cats via oral inoculation with trophozoites

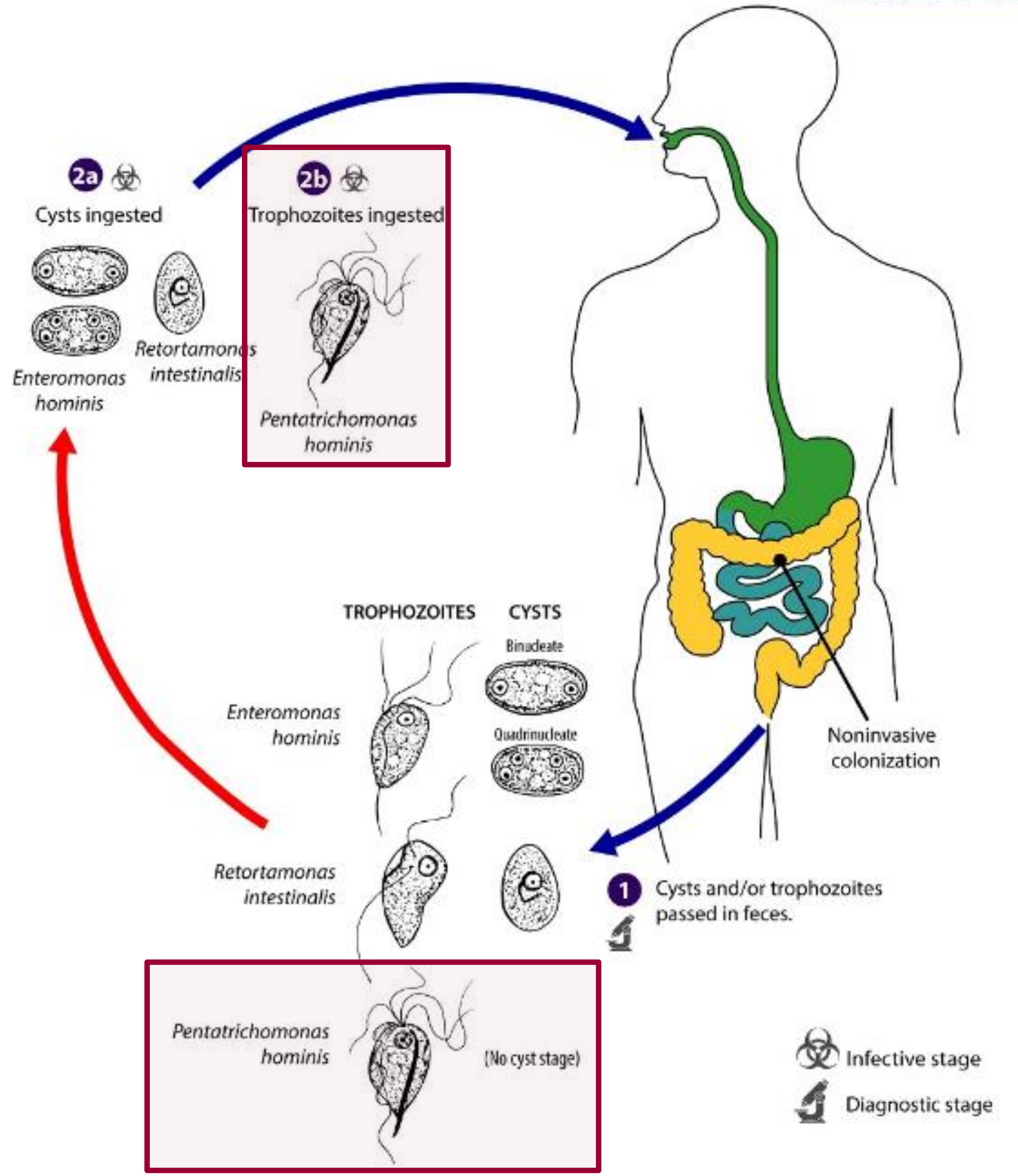




Schematic view of **(a)** ***Trichomonas vaginalis***, **(b)** ***T. tenax*** and **(c)** ***Pentatrichomonas hominis***

AF - free flagella, **AX** - axostyle, **CO** - costa, **HY** - hydrogenosome, **N -** nucleus, **PP** - parabasal body, **PG** - parabasal body + Golgi apparatus, **RF** - recurrent flagellum

Nonpathogenic Flagellates



Trends in Parasitology July 2014, Vol. 30, No. 7
https://doi.org/10.1016/j.pt.2014.05.005

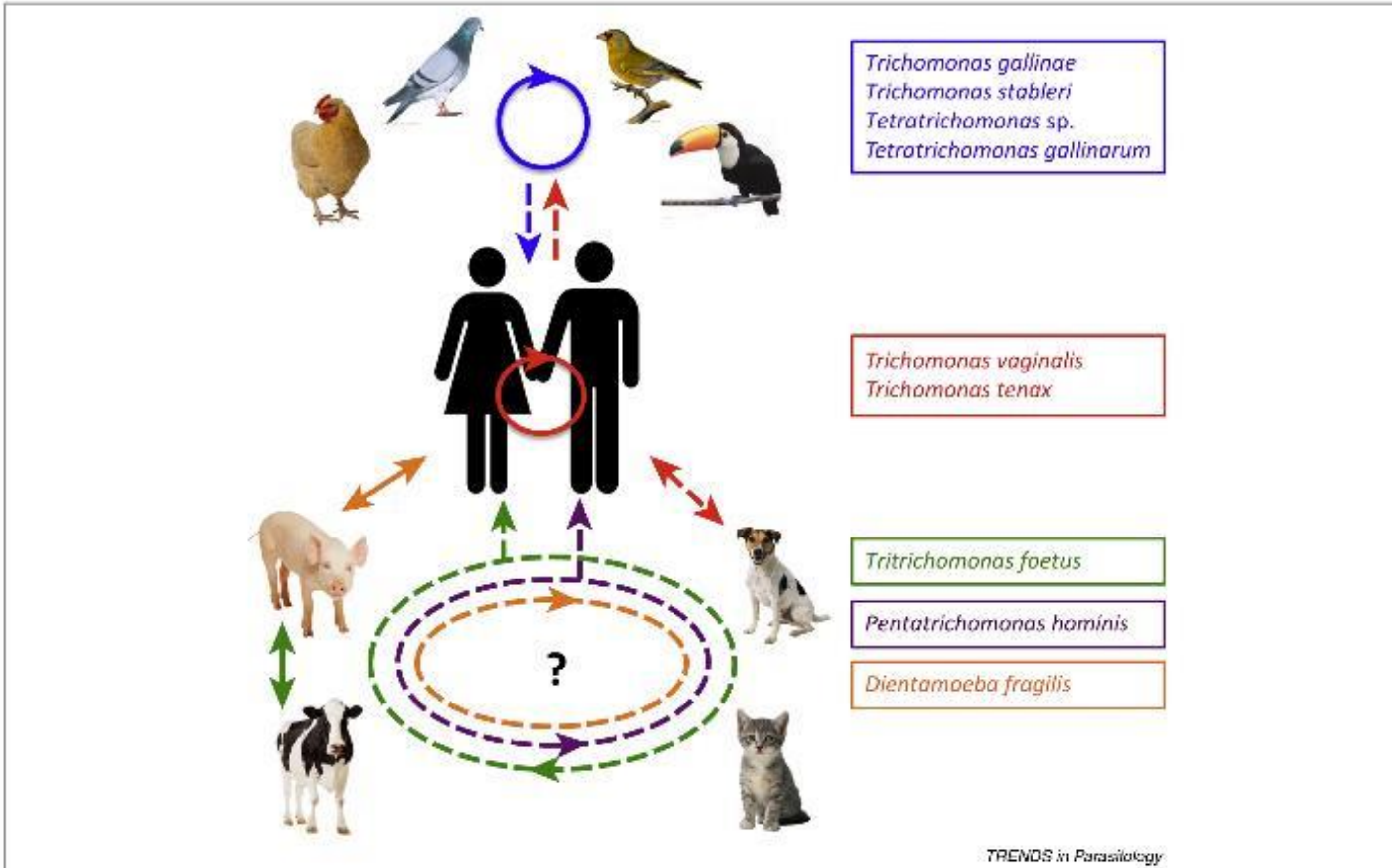
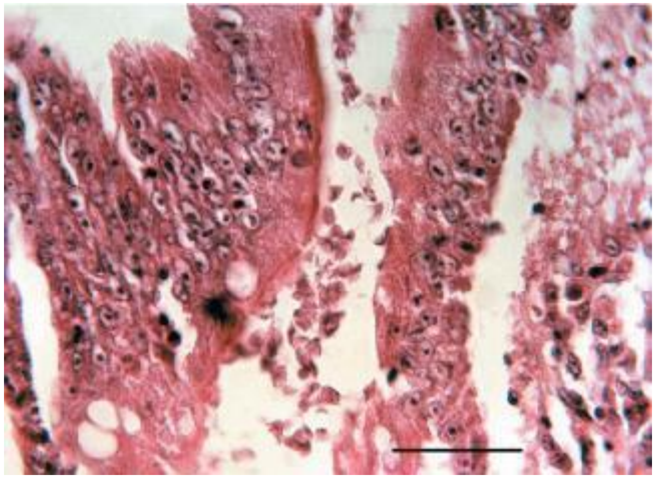
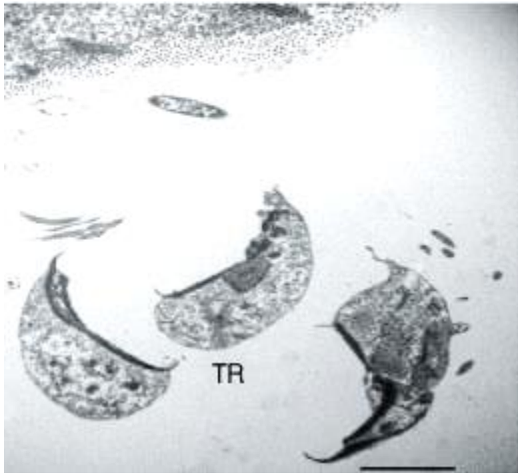


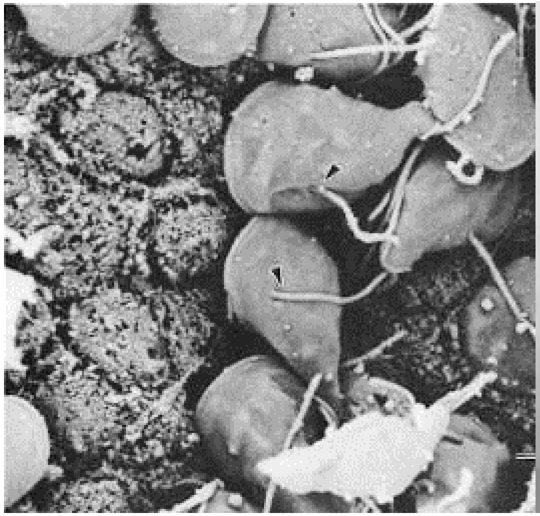
Figure 3. Speculative models of zoonoses caused by trichomonads. Trichomonads are listed on the right and colored according to primary hosts assigned historically in the literature. Unbroken lines represent known infections or transmission routes, and broken lines represent speculative infections or transmission routes for which data are lacking. The relationships are represented as follows: (blue box) trichomonads identified in wild bird species (e.g., green finch [16] and toucan [81]) in partially domesticated species (rock dove) and in fully domesticated species (chicken) circulate within these populations with variable host specificity [17] (blue unbroken circle with arrow). Two of the four avian trichomonads listed (*Tetratrichomonas* sp. and *Tetratrichomonas gallinarum*) have been identified in human lungs [24], and *Trichomonas gallinae* and *Trichomonas stableri* are also included owing to their close relationship to *Trichomonas tenax* and *Trichomonas vaginalis* [5,49]. (Red box) *T. vaginalis* and *T. tenax* are the two species considered human-specific, with known human-to-human infections (unbroken red circle). The close genetic relationship of the human and avian trichomonads (Figure 2) suggests either independent zoonotic acquisitions from avian sources (broken blue arrow) or transfer of the parasites from humans to birds through environmental contamination (broken red arrow). (Green box) *Tritrichomonas foetus* has been isolated from a variety of pets and farm animals, with the same strain known to infect cattle and pigs (unbroken green arrow) [26], but different genotypes infecting cattle and cats [29,31]; the origins of dog infections remain unclear [32]. Thus, there are at least two *T. foetus* genotypes capable of colonizing an extensive range of hosts, including humans [41] (broken green circle and arrow). The lack of precise epidemiological data is indicated by '?'. (Purple box) *Pentatrichomonas hominis* has been isolated from a variety of pets and farm animals [22], but little is known about its infection route and epidemiology; the same strain could be circulating between all identified hosts (broken purple circle). (Orange box) *Dientamoeba fragilis* has been isolated from farm animals (pigs) and non-human primates (gorillas), with the same strain known to infect pigs and humans [21] (unbroken orange arrow). Recent evidence suggests that household pets do not play a role in transmission [82]; however, the origins remain unclear and multiple strains could be circulating in animal hosts (broken orange circle and arrow). Additionally, given recent prevalence and transmission data it seems unlikely that transmission from non-human hosts represents a significant proportion of infections. Contaminated surfaces and water [83], uncooked meat, or direct contact with pets and farm animals could lead to animal-to-human transmissions of trichomonads. Initial infections were presumably through the digestive tract (via oral ingestion) with further progression to the lungs for some (various) species or the urogenital tract (*T. vaginalis*).

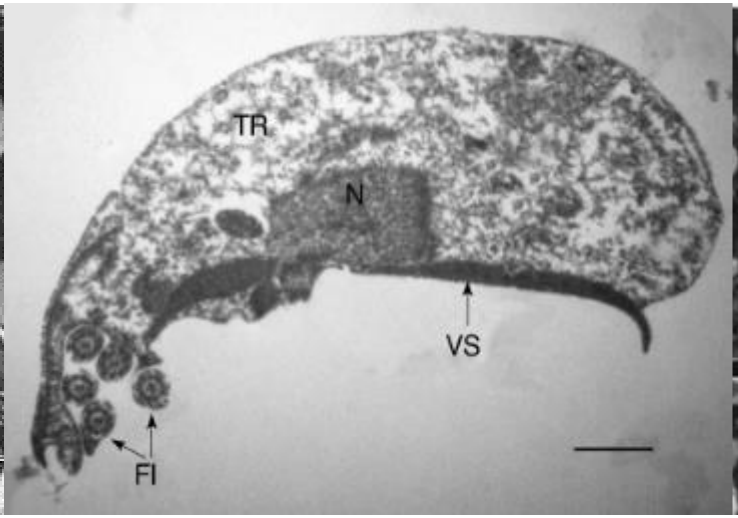
Cochlosoma anatis

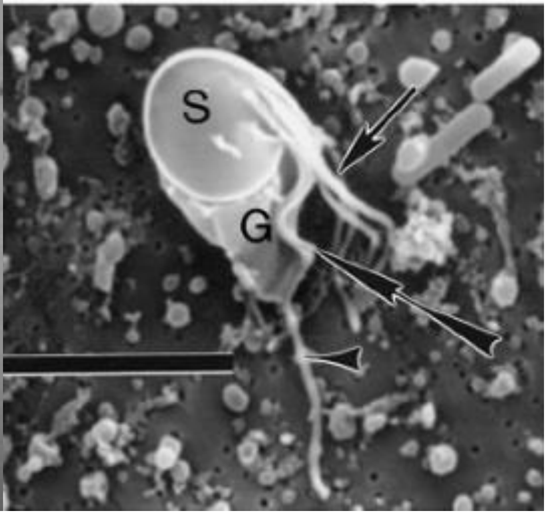
- associated with enteritis in ducklings, turkeys and finches







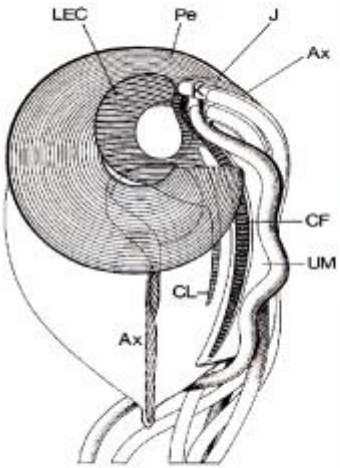


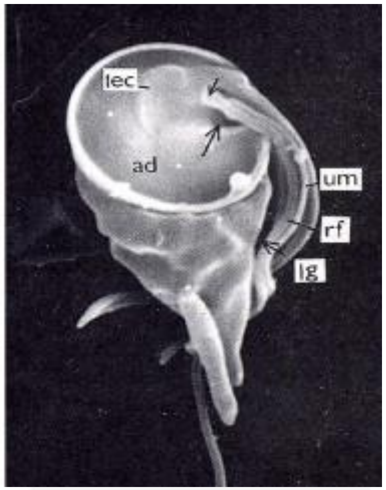


Cochlosoma anatis

https://doi.org/10.1099/ijs.0.63754-0

International Journal of Systematic and Evolutionary Microbiology (2006), 56, 305–312 DOI 10.1099/ijs.0.63754-0





Affiliation of *Cochlosoma* to trichomonads confirmed by phylogenetic analysis of the small-subunit rRNA gene and a new family concept of the order Trichomonadida

Vladimír Hampl,[1] Martin Vrlík,[1] Ivan Cepicka,[1] Zdeněk Pecka,[2] Jaroslav Kulda[1] and Jan Tachezy[1]

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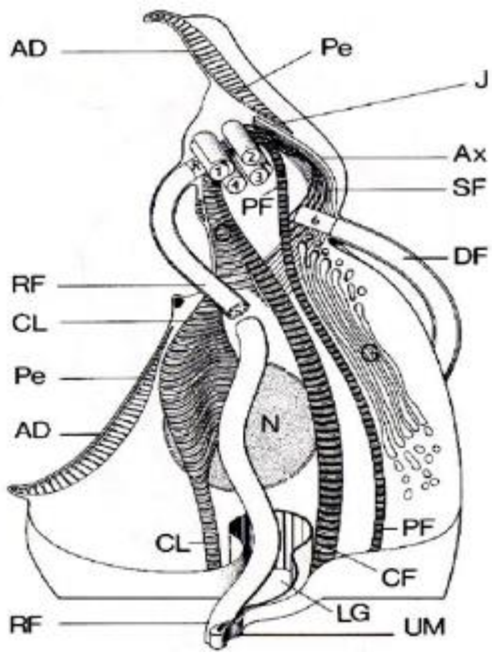


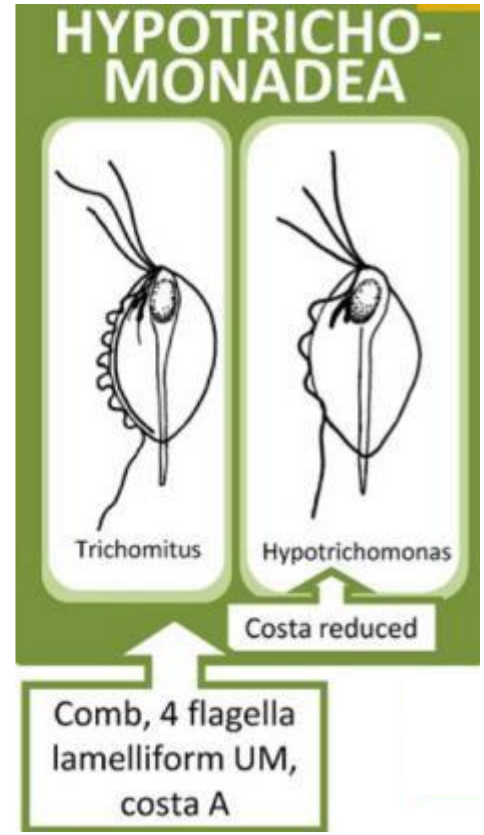
The protozoan genus *Cochlosoma* includes parasitic intestinal flagellates of birds and mammals of uncertain taxonomic classification. The presence of an adhesive disc, superficially similar to that of *Giardia*, led to a proposal that *Cochlosoma* should be classified as diplomonads. Careful morphological and ultrastructural observations, however, revealed conspicuous homologies to trichomonads. We addressed the question of classification and phylogenetic affiliation of *Cochlosoma* using the methods of molecular phylogenetics. Analyses based on the 16S rRNA gene sequence of the species *Cochlosoma anatis* very robustly placed *Cochlosoma* in the clade of the parabasalid subfamilies Trichomonadinae, Trichomitopsiinae and Pentatrichomonoidinae of the order Trichomonadida (bootstraps >94 %). The data did not provide robust support for any particular position of *Cochlosoma* within this clade because the sequence suffered from mutational saturation and produced a long branch. The most probable sister taxon of *Cochlosoma* is the genus *Pentatrichomonas*, because their relationship was supported specifically by the slowest-mutating, least-saturated positions as determined using the method slow–fast. Classification of the order Trichomonadida was revised to accommodate knowledge about its phylogeny – the family Cochlosomatidae and subfamilies Trichomitopsiinae and Pentatrichomonoidinae were abandoned, Trichomonadidae was amended and new families Tritrichomonadidae (formerly a subfamily) and Trichomitidae were proposed.

Parabasalia

Hypotrichomonadea

- 4 flagella with 1 axoneme supporting a lamelliform undulating membrane
- comb-like structure present, but no infrakinetosomal body, biramous parabasal body
- A-type costa
- axostyle of "*Trichomonas* type"
- symbionts in intestines of lizards, tortoises, kangaroos, pigs and cockroaches
- ***Hypotrichomonas***, ***Trichomitus***

Čepička et al. 2010: Protist 161



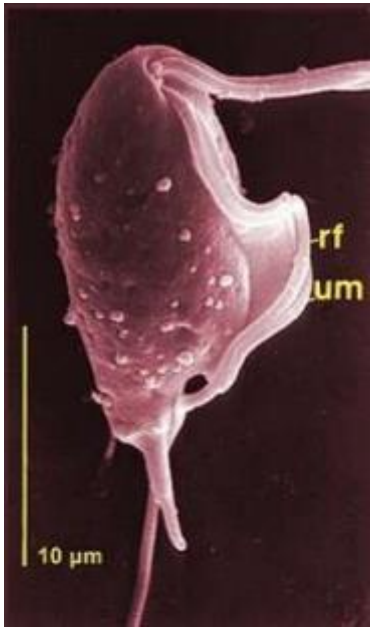
https://doi.org/10.1016/j.protis.2009.11.005

Parabasalia

Hypotrichomonadea

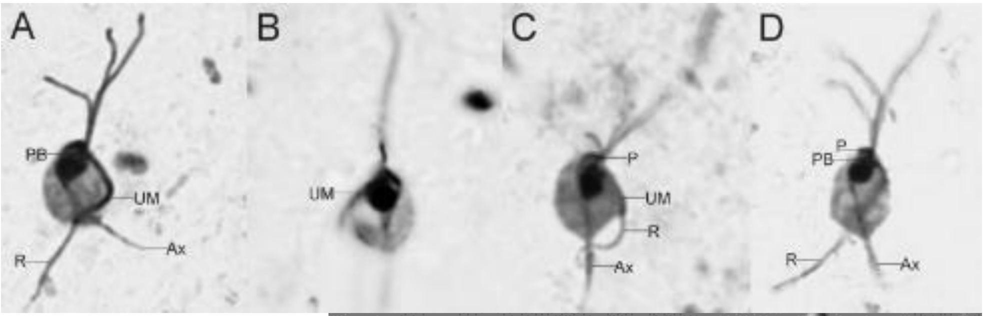
Trichomitus batrachorum

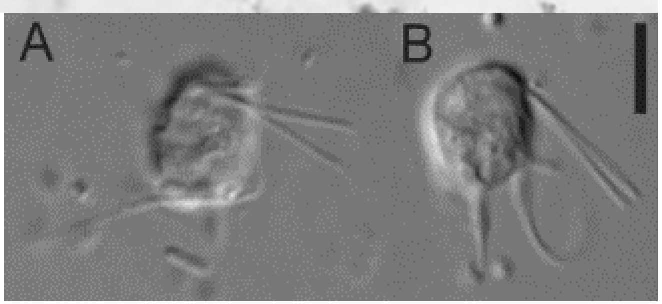
- commensal in intestine of frogs



Hypotrichomonas blattarum

- commensal in hindgut of blaberid cockroaches





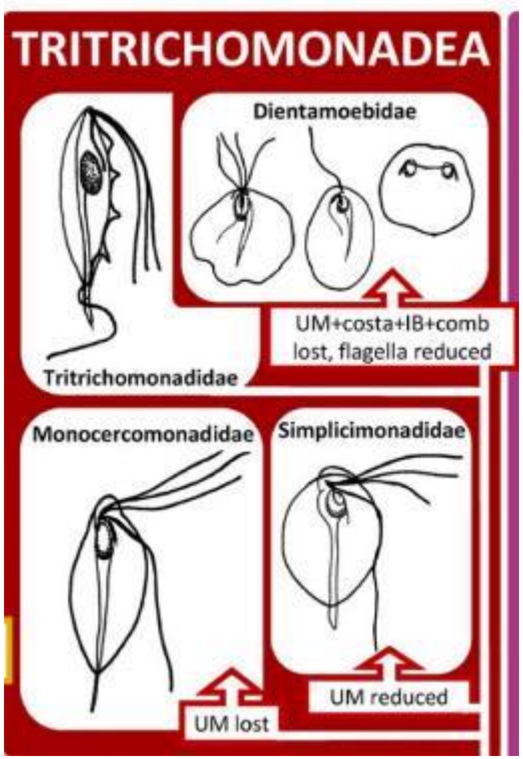
http://dx.doi.org/10.1016/j.ejop.2015.02.003

Parabasalia

Tritrichomonadea

- uninucleate or binucleate; 0–5 (4 ancestrally) flagella; ancestrally with comb-like structure
- undulating membrane typically of a rail type (if present)
- A-type costa
- suprakinetosomal and infrakinetosomal body
- axostyle of "*Tritrichomonas* type" or" *Trichomonas* type"
- ***Histomonas***, ***Dientamoeba***, ***Monocercomonas***, ***Tritrichomonas***

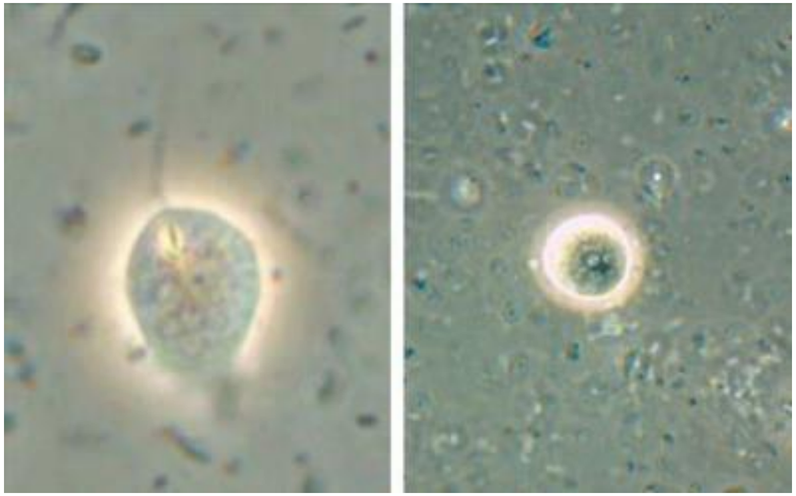
Čepička et al. 2010: Protist 161

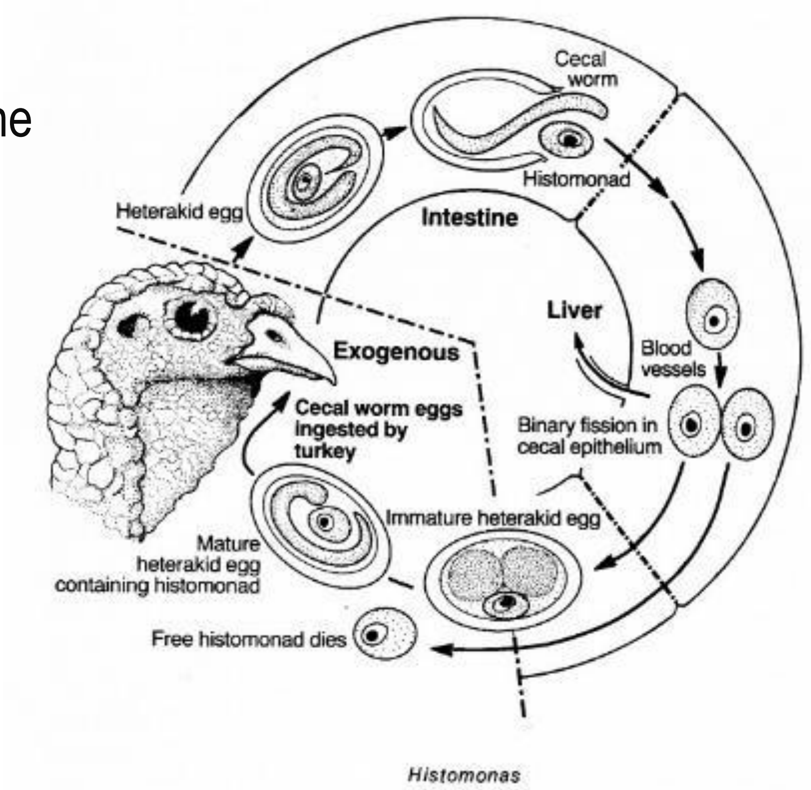


https://doi.org/10.1016/j.protis.2009.11.005

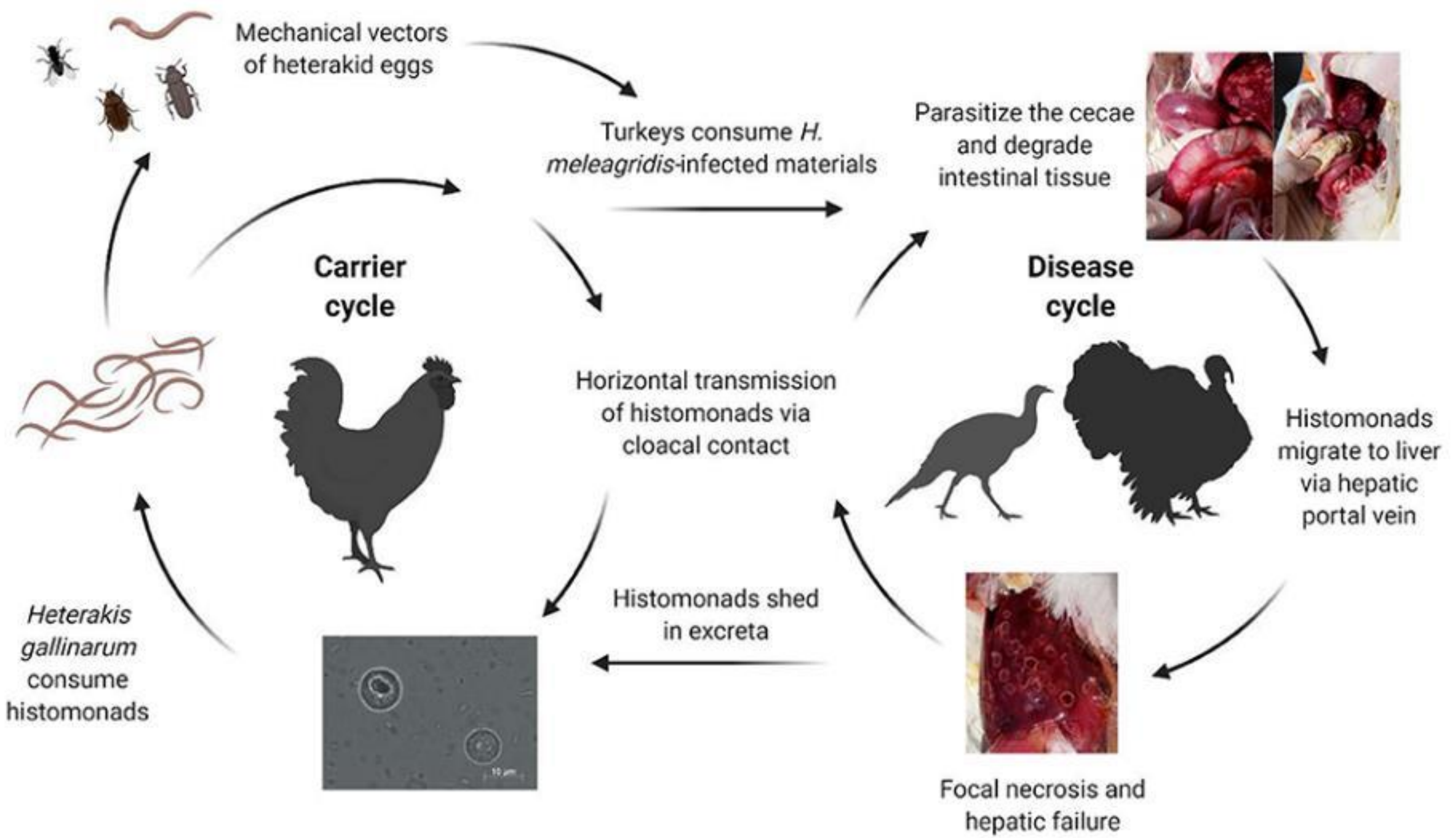
Histomonas meleagridis

- wide range of birds including chickens, turkeys, quail and pheasants
- blackhead disease, infectious enterohepatitis, or histomoniasis
- in two forms: amoeboid (8-15 μm) in tissues x flagellated (spherical, up to 30 μm) in lumen or free in the contents of caecum
- both forms are shed in faeces and survive in the external environment for only a few hours
- transmitted in the eggs of nematode *Heterakis gallinarum* (caecal parasite, eggs of which are transmitted by earthworms)





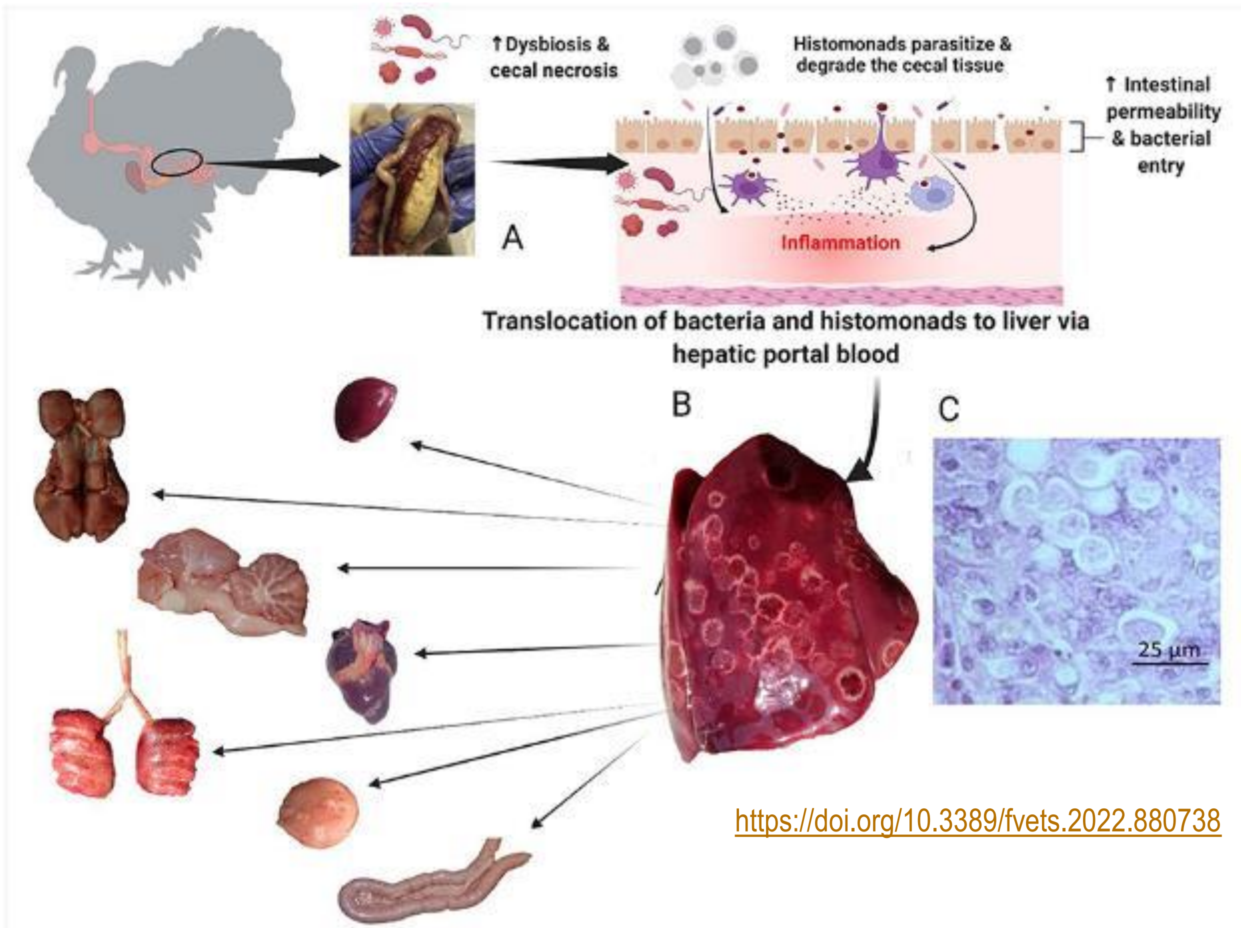
Complex life cycle of *Histomonas meleagridis*



https://doi.org/10.3389/fvets.2022.880738

Blackhead disease

- symptoms within 7-12 days after infection - depression, reduced appetite, poor growth, increased thirst, sulphur-yellow diarrhoea, listlessness, drooping wings
- symptoms highly fatal to turkey, less pathogenic in chickens; young birds most susceptible (particularly those 3-12 weeks old) - mortality up to 70 %



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- synergistic interaction with other enteropathogens (such as coccidia, *Clostridium perfingens*)

Blackhead disease

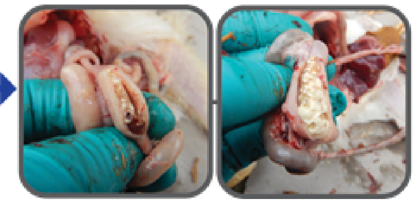
Disease Transmission and Process

Vectors (disease carriers)
- Ingestion of cecal worm eggs, larvae or adults
- Ingestion of earthworms containing eggs infected with histomonads
- Possibly by transmission from beetles/flies mechanically carrying the histomonads
- Rodents, equipment and other fomites such as clothes, footwear, etc.
- Flood water may act as a trigger for earthworm activity

Biosecurity
- Dirt floors and litter with cecal worms and eggs are a RISK
- Do not pile or spread litter nearby
- Do no re-use contaminated litter
- Do not place birds on untreated dirt floors
- Ensure adequate cleaning and disinfection procedures are in place
- Change clothing and footwear between sheds
- Do not move equipment between sheds

Histomoniasis (Blackhead)
→ **Organism ingested**
→ **Organism reaches ceca**
→ **Cecal wall thickening and damage**

→ **Cecal cores develop**

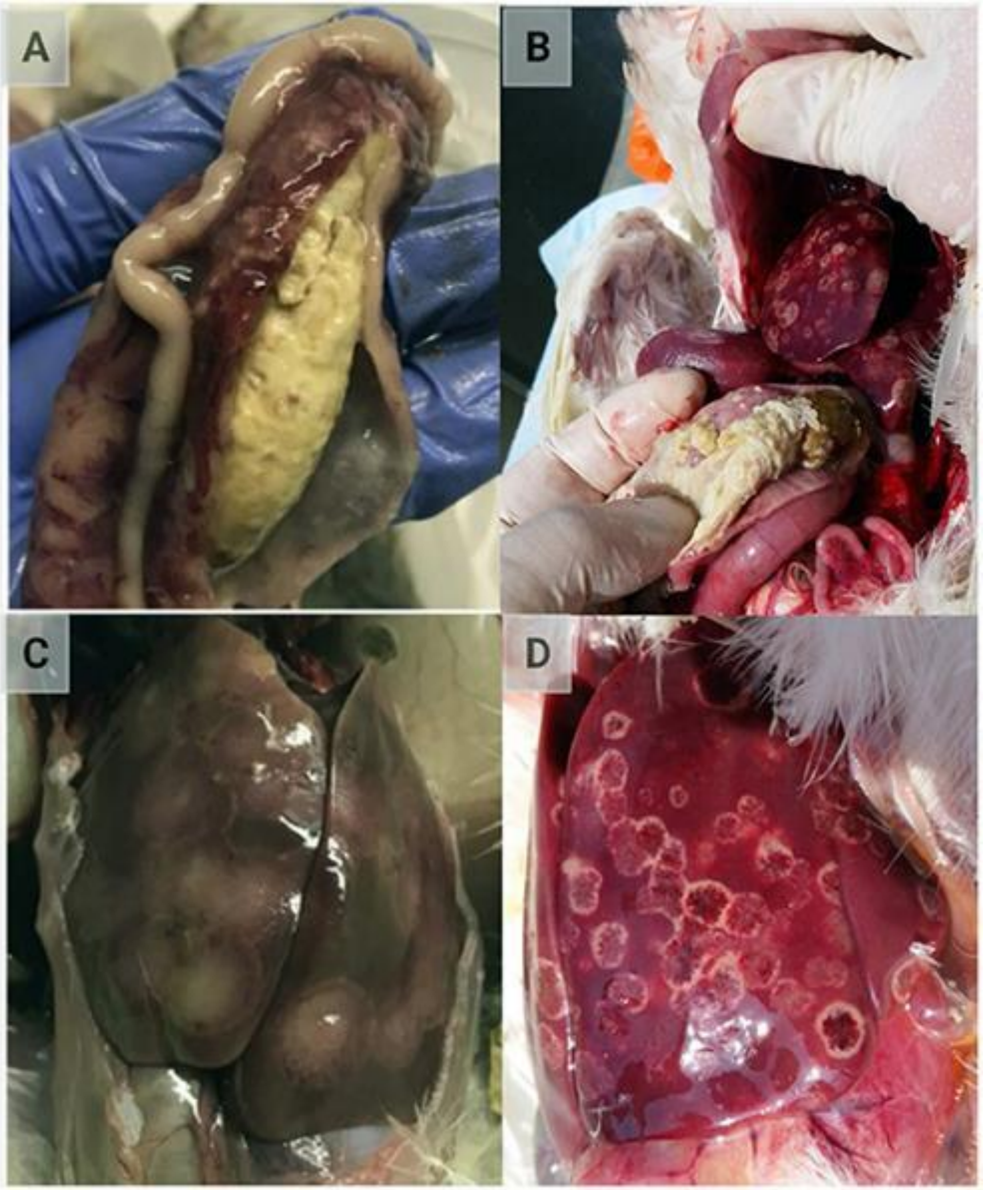


→ **Organism enters blood stream and reaches liver** (Facilitated by concurrent infection with bacteria/coccidia)
→ **Liver may present with necrotic lesions**

→ **CLINICAL SIGNS AND DEATH**

Cecal worms (*Heterakis gallinarum*)
- Eggs passed in feces

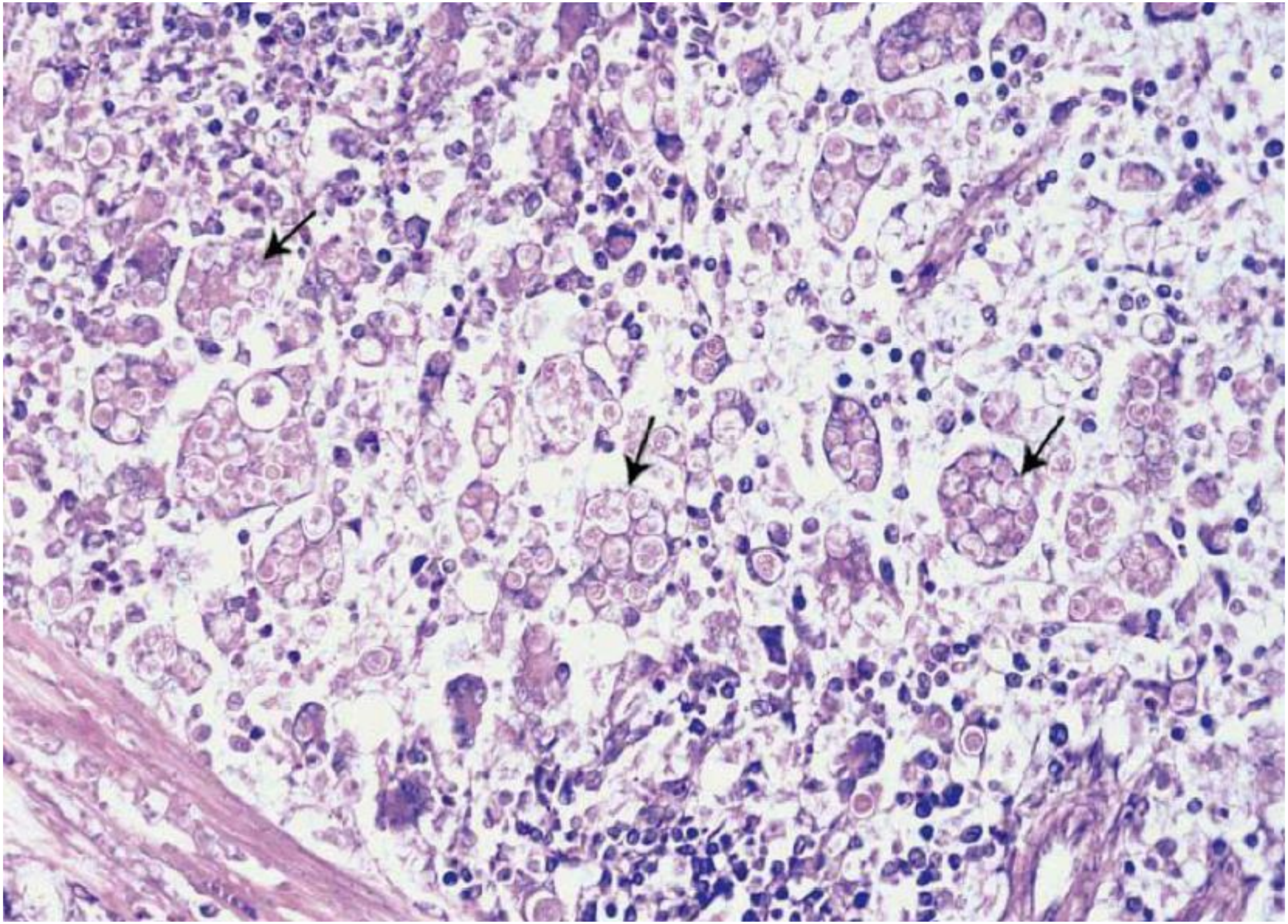
Pathology of blackhead disease



Typical lesions resulting from *Histomonas meleagridis* infection

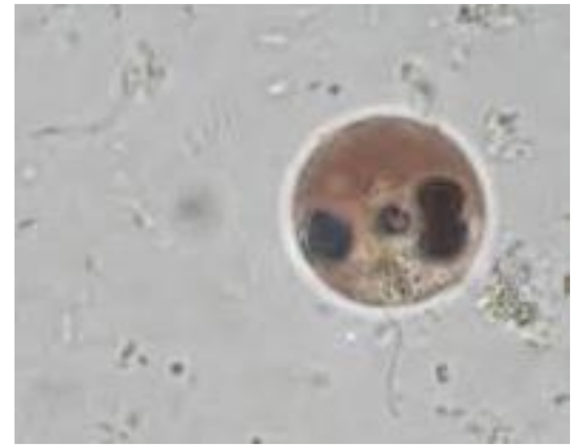
(**A**,**B**) caseous cheese-like caecal core
(**C**,**D**) focal necrosis resulting in target-like liver lesions

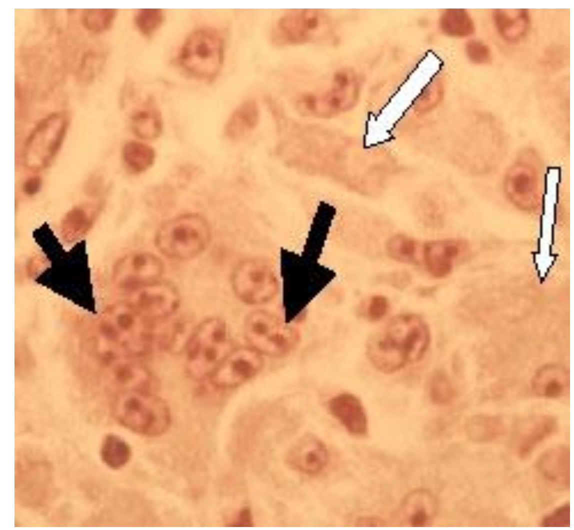
Pathology of blackhead disease



Diagnosis of blackhead disease

- ✓ clinical symptoms
- ✓ pathological findings
- ✓ trophozoites of *H. meleadridis* in tissues (edges of pathological lesions)
- ✓ cultivation
- ✓ histopathology – PAS (Periodic Acid Schiff) reaction
- ✓ PCR





Histological section stained with H&E of liver showing *H. meleagridis* (black arrows) and necrotic liver cells (white arrows).

Control of blackhead disease

Control focused on *Histomonas meleagridis*

- ✓ good management of the farm and sanitation are the only effective strategies to control the spread of infection
- ✓ ban of effective therapeutic and prophylactic medication in the EU (5-nitroimidazols: ronidazole, dimetronidazole, metronidazole, rodidazole)
- ✓ plant substances as an alternative treatment

Control focused on *Heterakis gallinarum*

- ✓ anthelmintic treatment

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Histomonas meleagridis—New insights into an old pathogen

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A R T I C L E   I N F O

Keywords:
Histomonas meleagridis
Molecular repertoire
Histomonosis
Diagnostics
Treatment and prophylaxis

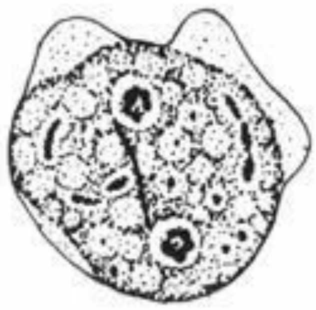
A B S T R A C T

The protozoan flagellate *Histomonas meleagridis* is the etiological agent of histomonosis, first described in 1893. It is a fastidious disease in turkeys, with pathological lesions in the caeca and liver, sometimes with high mortality. In chickens the disease is less fatal and lesions are often confined to the caeca. The disease was well controlled by applying nitroimidazoles and nitrofurans for therapy or prophylaxis. Since their introduction into the market in the middle of the previous century, research nearly ceased as outbreaks of histomonosis occurred only very rarely. With the ban of these drugs in the last two decades in North America, the European Union and elsewhere, in combination with the changes in animal husbandry, the disease re-emerged. Consequently, research programs were set up in various places focusing on different features of the parasite and the disease. For the first time studies were performed to elucidate the molecular repertoire of the parasite. In addition, research has been started to investigate the parasite's interaction with its host. New diagnostic methods and tools were developed and tested with samples obtained from field outbreaks or experimental infections. Some of these studies aimed to clarify the introduction of the protozoan parasite into a flock and the transmission between birds. Finally, a strong focus was placed on research concentrated on the development of new treatment and prophylactic strategies, urgently needed to combat the disease. This review aims to summarize recent research activities and place them into context with older literature.

https://doi.org/10.1016/j.vetpar.2014.12.018

Dientamoeba fragilis

- pleomorphic trophozoite (4–20 µm), typically binucleate
- parabasal apparatus and hydrogenosomes
- permanently lost flagella and kinetosomes
- pseudopodia in fresh material



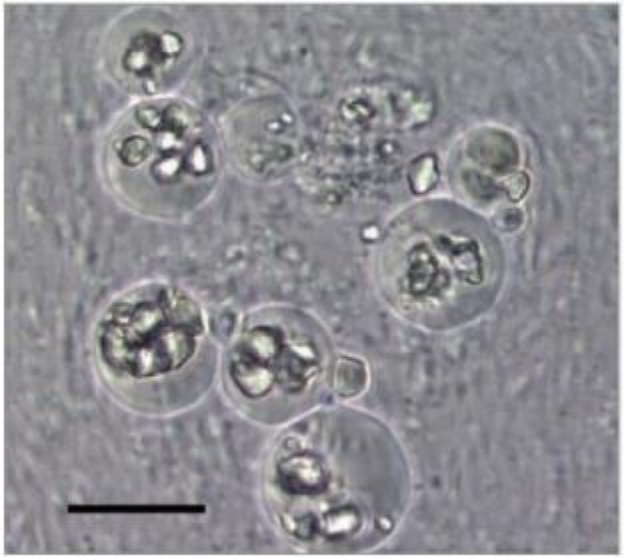
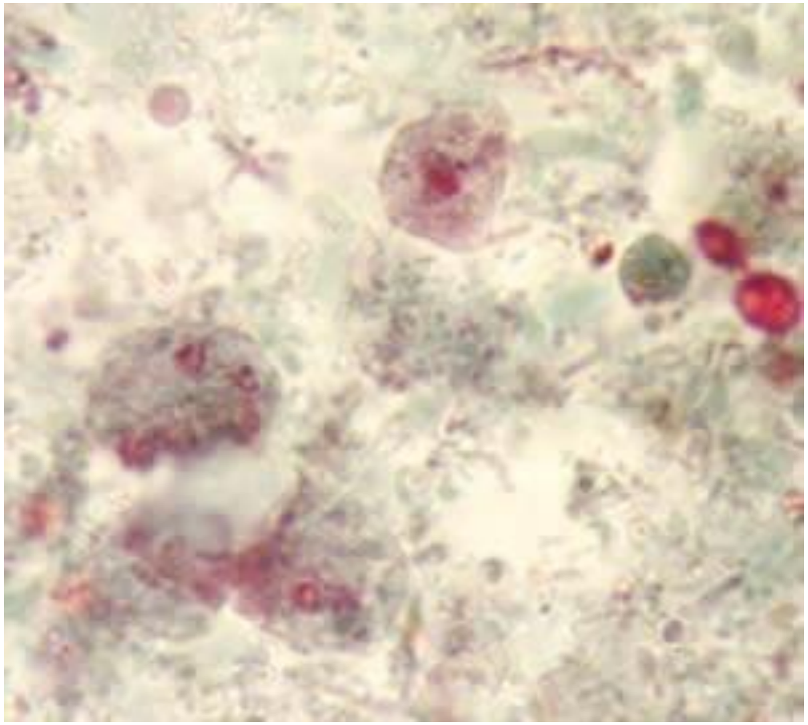
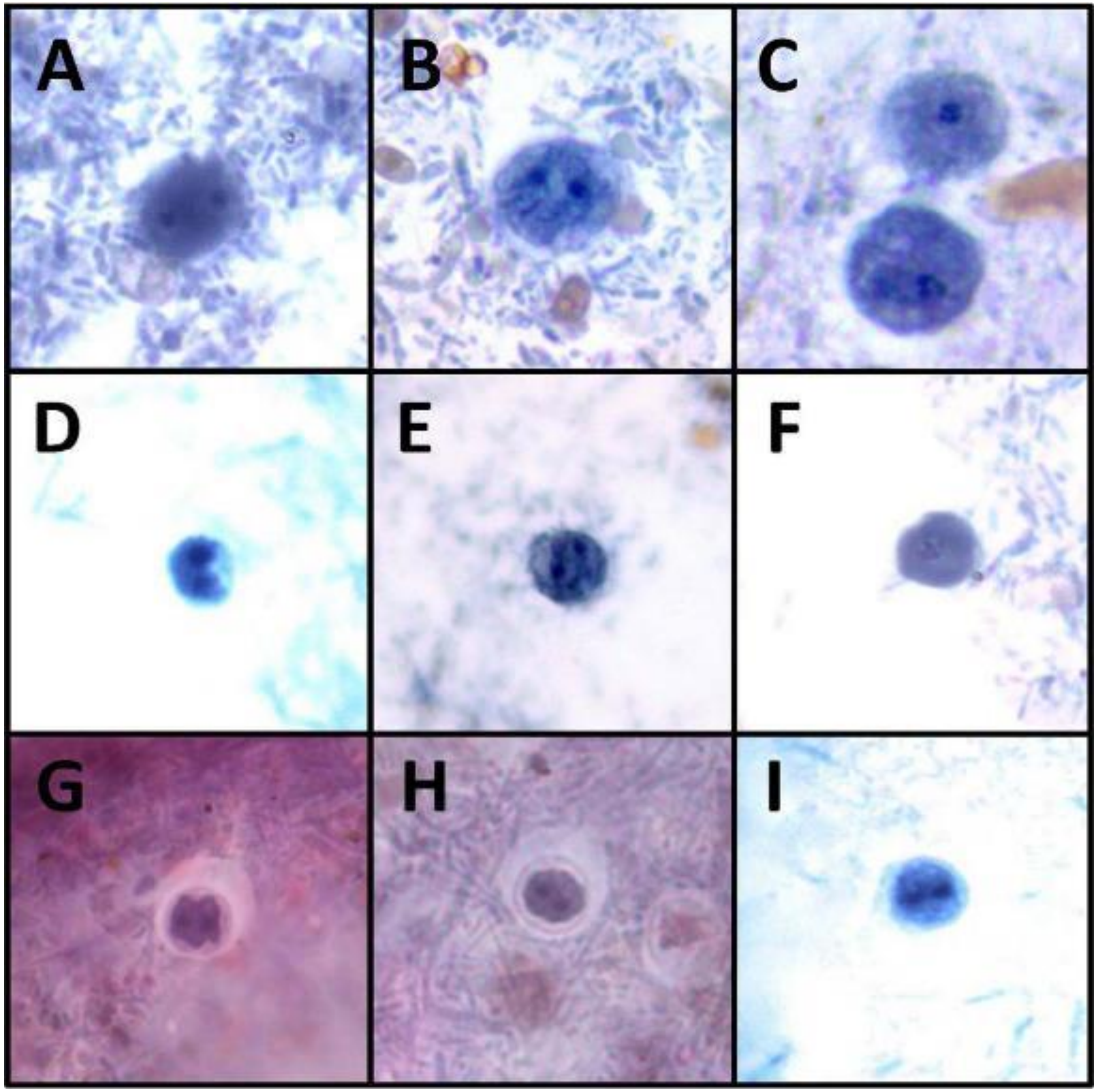


Figure 2. Light micrograph of non-flagellated trophozoite forms of *Dientamoeba fragilis* cultured in a Modified Boeck and Drbohlav's medium. Micrograph was taken under bright field microscopy. Rice starch granules can be observed as large refractile bodies within the cytoplasm. Bar represents 10 µm.



Dientamoeba fragilis

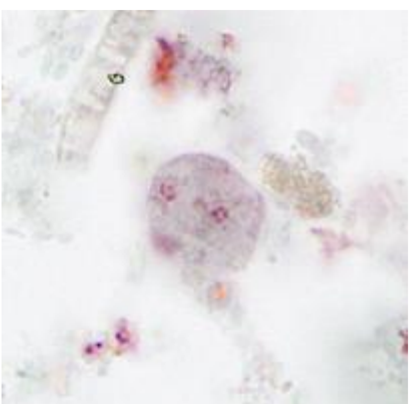
https://doi.org/10.1128/JCM.00813-14

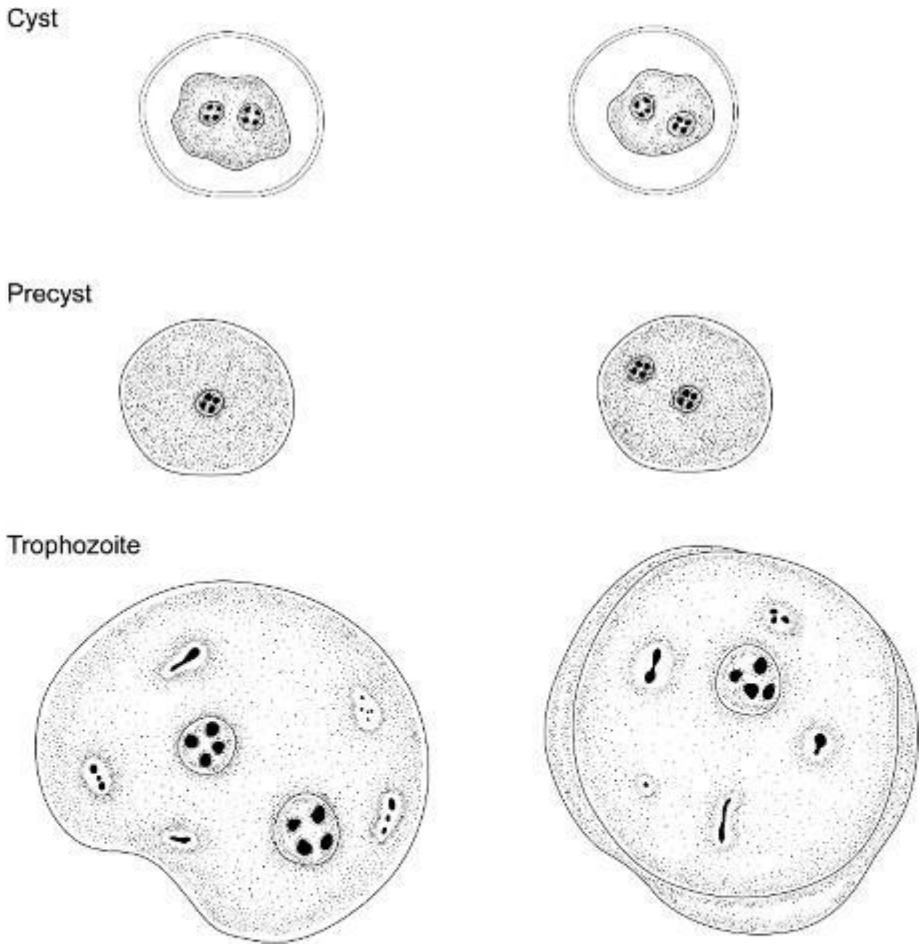


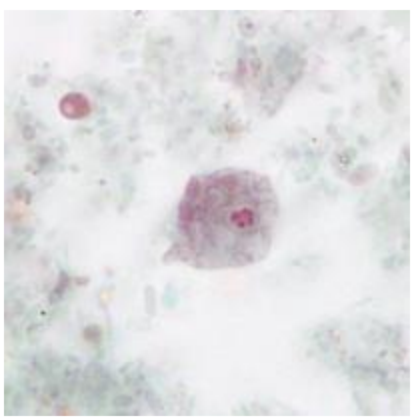
A-C) **trophozoites**, **D-F**) **precysts**, **G-I**) **cysts** stained with a modified iron haematoxyline

https://doi.org/10.1128/JCM.00813-14

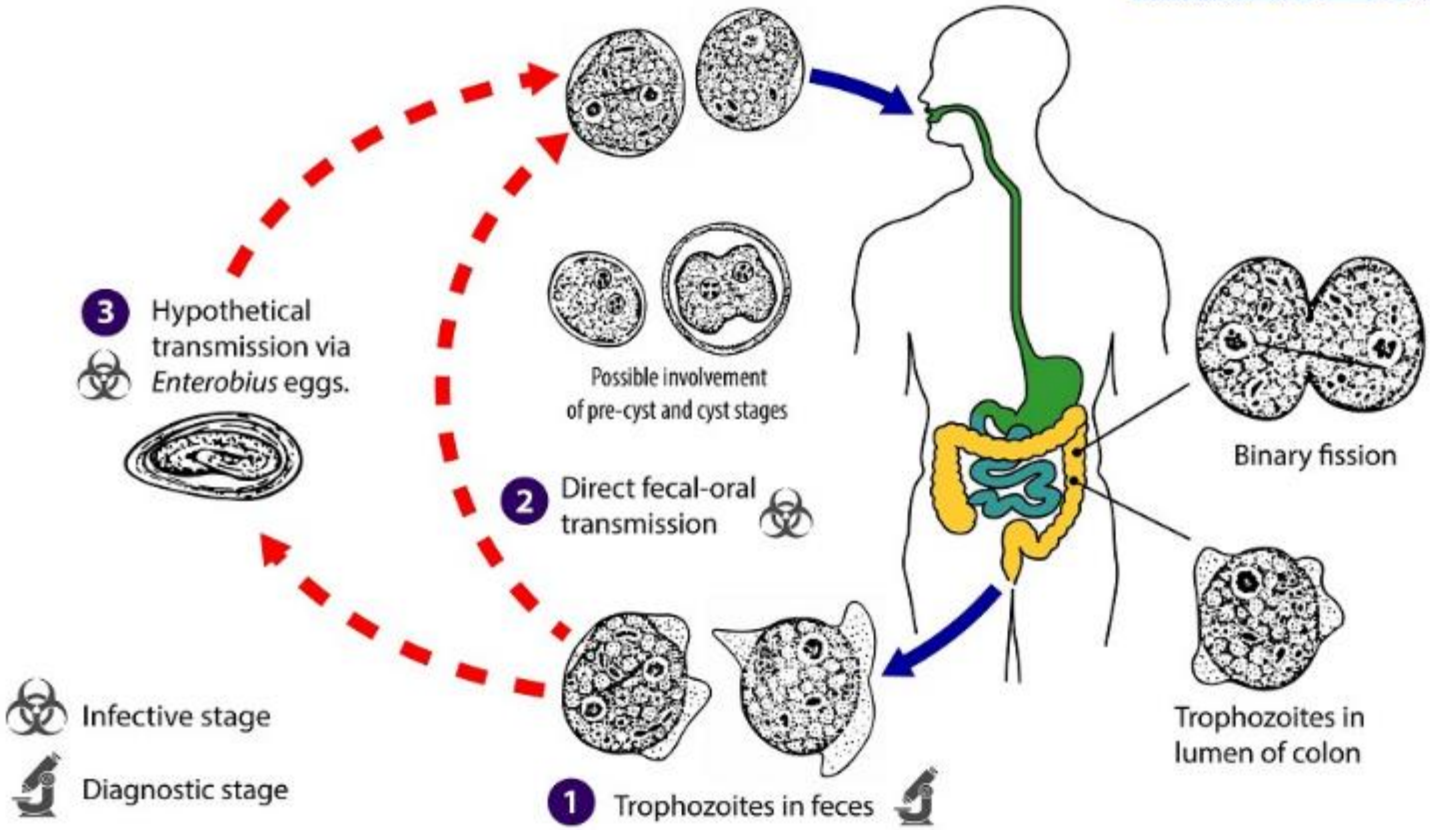
Dientamoeba fragilis





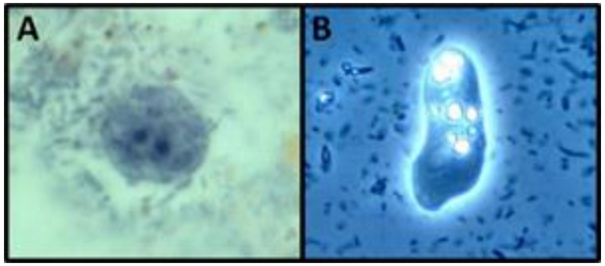


Dientamoeba fragilis

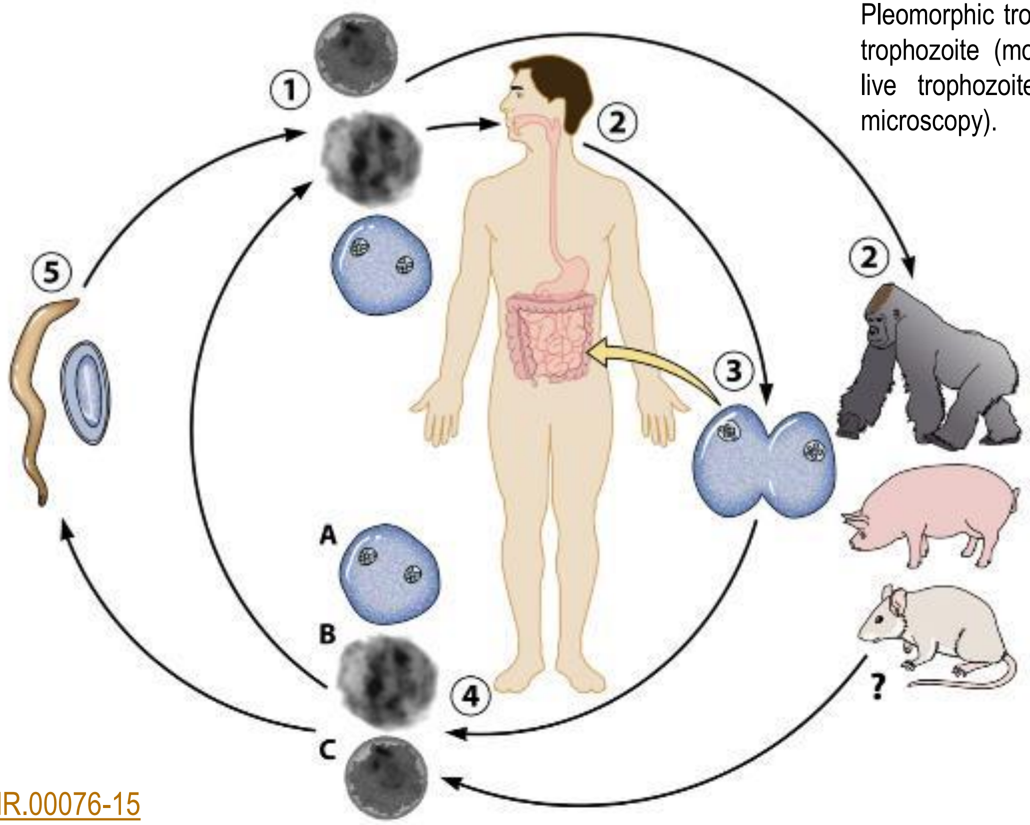


The complete life cycle of *D. fragilis* has not yet been elucidated. Assumptions have been made on the basis of clinical observations and the biology of related species (*H. meleagridis* in particular). Trophozoites that multiply via binary fission in the large intestine lumen are shed in the stool. **Only the trophozoites of *D. fragilis* have been detected so far.** Although rare putative forms of **cysts** and **precysts** have been described in human clinical specimens; it is not yet known whether and under what conditions transmission to humans occurs through their ingestion, in contrast to other faecal-oral transmission routes. Transmission via helminth eggs (e.g. *Enterobius vermicularis*) has been postulated.

Putative life cycle of *Dientamoeba fragilis*



Pleomorphic trophozoites of *D. fragilis*. (A) Binucleate trophozoite (modified iron hematoxylin staining); (B) live trophozoite displaying motility (phase-contrast microscopy).



https://doi.org/10.1128/CMR.00076-15

FIG 7 Life cycle of *D. fragilis* showing current hypotheses on transmission. *D. fragilis* is ingested from the external environment by a host species in one or more possible forms (1). The preferred transmissible form is yet to be determined. Humans are thought to be the preferred host of *D. fragilis*, although gorillas, pigs, and rodents are also considered natural hosts (2). Once ingested, *D. fragilis* travels to the large intestine, where it multiplies by binary fission (3). *D. fragilis* trophozoites (A), precysts (B), and cysts (C) are passed into the environment in the feces (4), where they contaminate food and/or water sources. *D. fragilis* parasites are then ingested by a new host, completing the cycle. While *D. fragilis* trophozoites are infectious to laboratory mice, they are noninfectious to larger mammals. The infectivity of precysts and purified cysts is yet to be demonstrated. It has been suggested that *D. fragilis* may be transmitted in the ova of the human pinworm, *Enterobius vermicularis* (5). Recent reports have confirmed the presence of *D. fragilis* DNA within *E. vermicularis* ova, although it is unknown whether viable and/or transmissible *D. fragilis* is present in these ova.

Review

Cell PRESS

Transmission of *Dientamoeba fragilis*: pinworm or cysts?

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Recently, conflicting evidence has been published on the mode of transmission of the trichomonad *Dientamoeba fragilis*. Detection of *D. fragilis* DNA inside *Enterobius vermicularis* eggs agrees with the prediction of Dobell in 1940 that the eggs of a nematode act as a vector for transmission. However, the identification of a cyst stage of *D. fragilis* in the stool of rodents infected with a human isolate has also been reported, and this implies a life cycle similar to those of most other intestinal protistan parasites. Herein we discuss the recent data, identify gaps in the experimental evidence, and propose a method for determining which view of the life cycle of this organism is correct.

Box 2. Outstanding questions

- Is *D. fragilis* transmitted by cysts, by nematode eggs, and/or by other means?
- Do multiple modes of transmission exist, and if so what circumstances determine which mode is used?
- If *D. fragilis* produces cysts, why have these never been reported in humans?
- Can *D. fragilis* cultures be obtained from *D. fragilis* DNA-containing *Enterobius* eggs or cysts from rodents?
- Can experimental *D. fragilis* infections be produced from surface-sterilized eggs or cysts?

https://doi.org/10.1016/j.pt.2014.01.005

Clinical presentation and diagnosis of dientamoebiasis

- pathogenicity and clinical importance of *D. fragilis* continue to be investigated, including whether particular genotypes, subtypes, or strains are associated with symptomatic infection in humans
- reported both the asymptomatic and symptomatic infection (e.g., with various nonspecific gastrointestinal symptoms such as intermittent diarrhoea, abdominal pain, nausea, anorexia, malaise, fatigue, poor weight gain and unexplained eosinophilia
- symptoms often reported to be like colitis, appendicitis or irritable bowel syndrome

Diagnosis

- microscopic detection in faecal smears (Heidenhain‘s iron haematoxylin, trichrome, other permanent stains)
- trophozoites not usually detectable by stool concentration methods, can easily be overlooked or misidentified due to their pale-staining nuclei that sometimes resemble those of *Endolimax nana* or *Entamoeba hartmanni*
- cultivation on egg serum media (Dobell-Laidlaw)
- PCR, antigen detection…

More details in the review: https://doi.org/10.1128/JCM.00400-16

genus ***Monocercomonas***

- <u>uninucleate</u>, <u>quadriflagellate</u>
- undulating membrane and costa absent
- axostyle of *Trichomonas* type
- non-pathogenic

Monocercomonas colubrorum

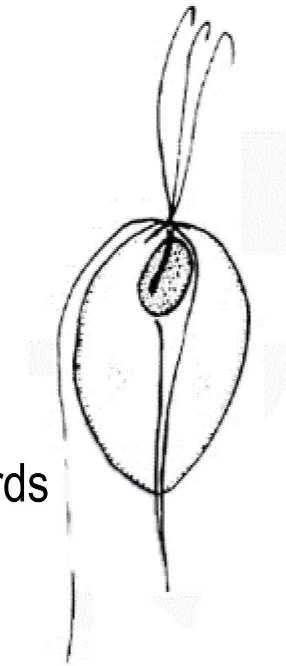
- widely distributed in cloaca of snakes and lizards

M. ruminantium

- rumen - beef cattle, sheep

M. cuniculi

- caecum of rabbits



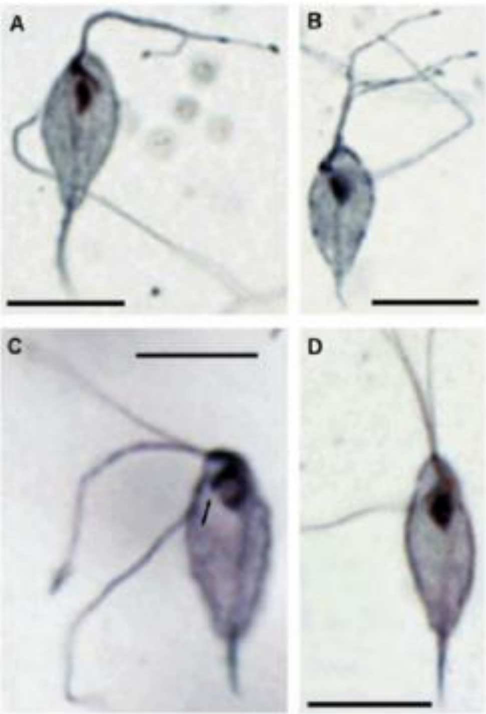
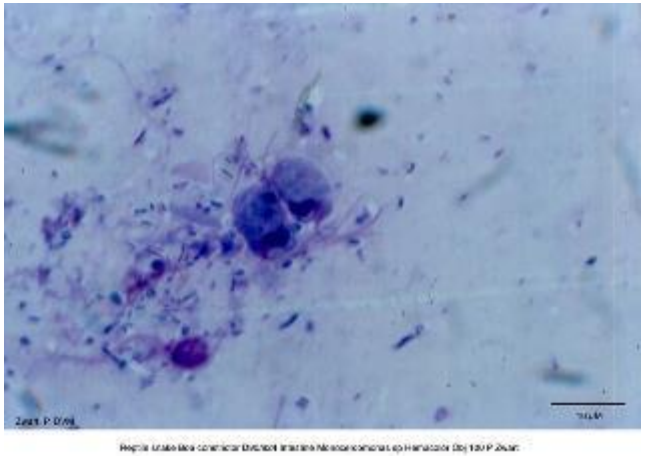
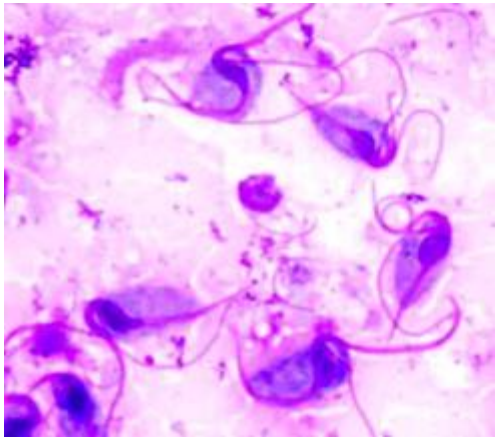


Figure 7. Protargol-stained specimens of *Monocercomonas colubrorum* strains, arrow indicates the parabasal body. **A** – La10; **B** – NS1-PR; **C** – BOA5; **D** – R183. Bars = 10 μm.

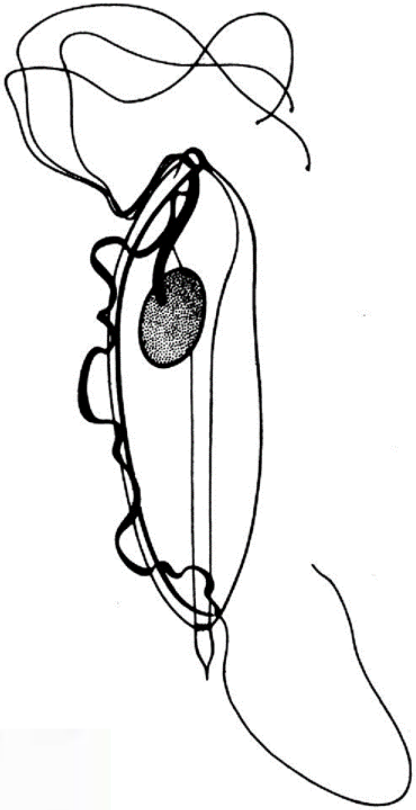
https://doi.org/10.1016/j.protis.2007.02.003





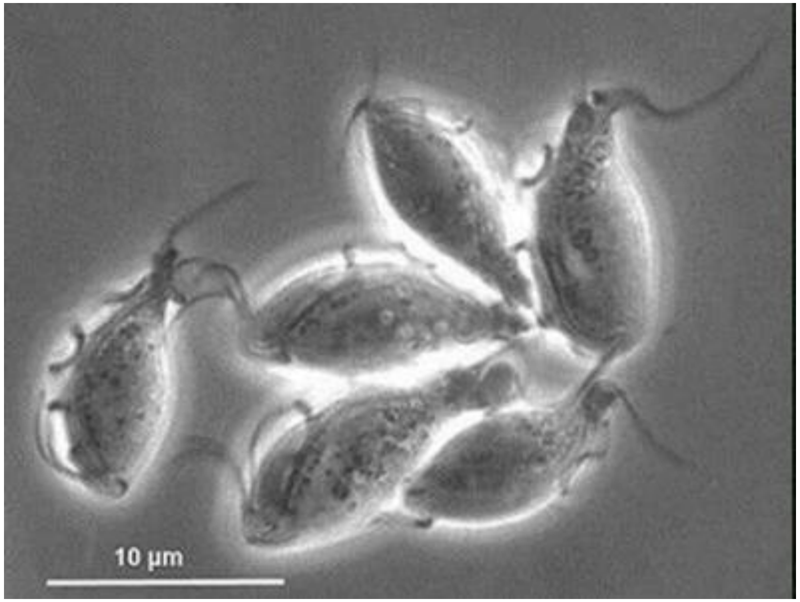
genus ***Tritrichomonas***

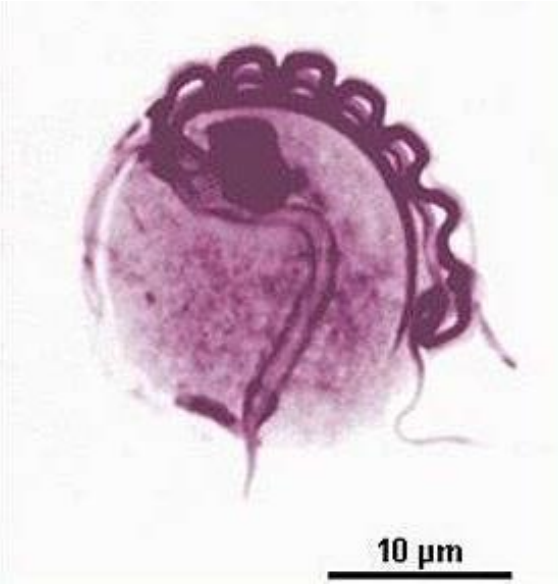
- <u>quadriflagellate</u> – 3 anterior flagella + well developed undulating membrane with a long free flagellum
- rod-shaped parabasal apparatus
- nutrition by pinocytosis



Tritrichomonas muris

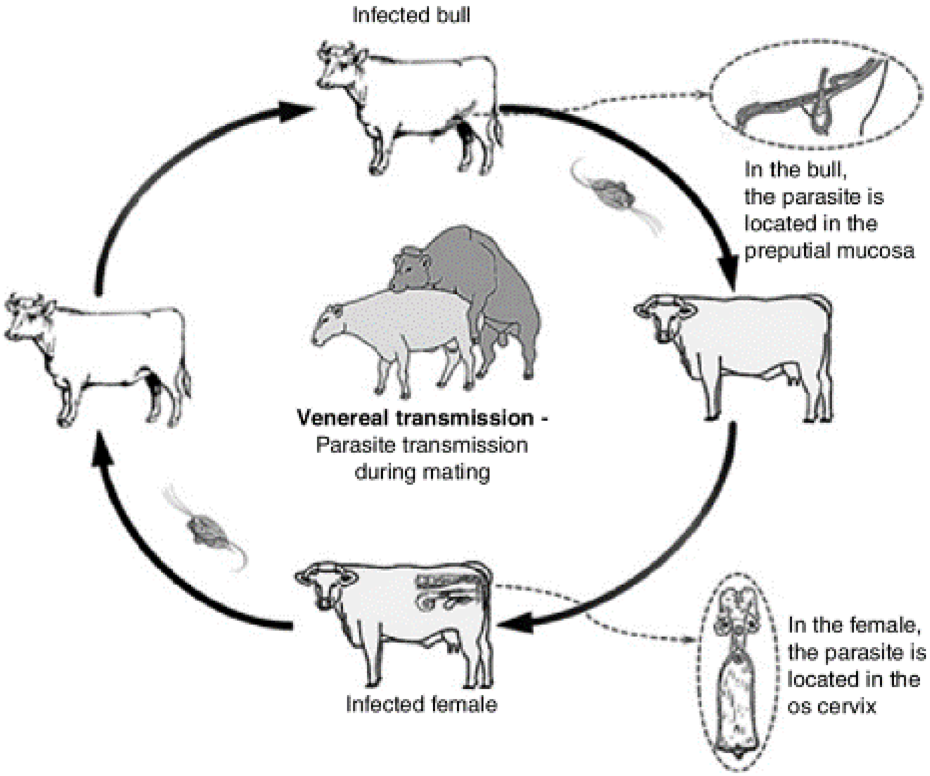
- nonpathogen in cecum, colon, and small intestine of rodents

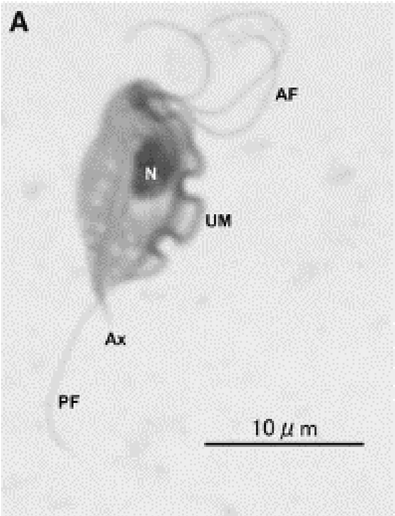


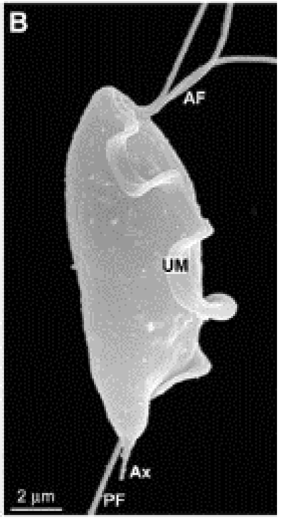


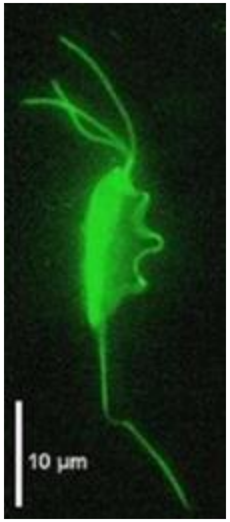
Tritrichomonas foetus

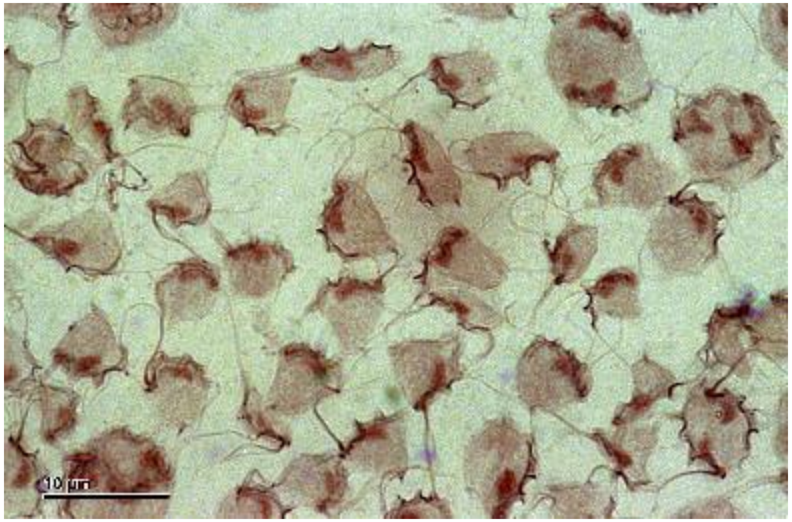
- spindle shaped (10-25 μm) cells resembling miniature tadpoles
- traditionally identified as a cause of reproductive disease in cattle
- currently important cause of diarrheal in cats











Tritrichomonas foetus

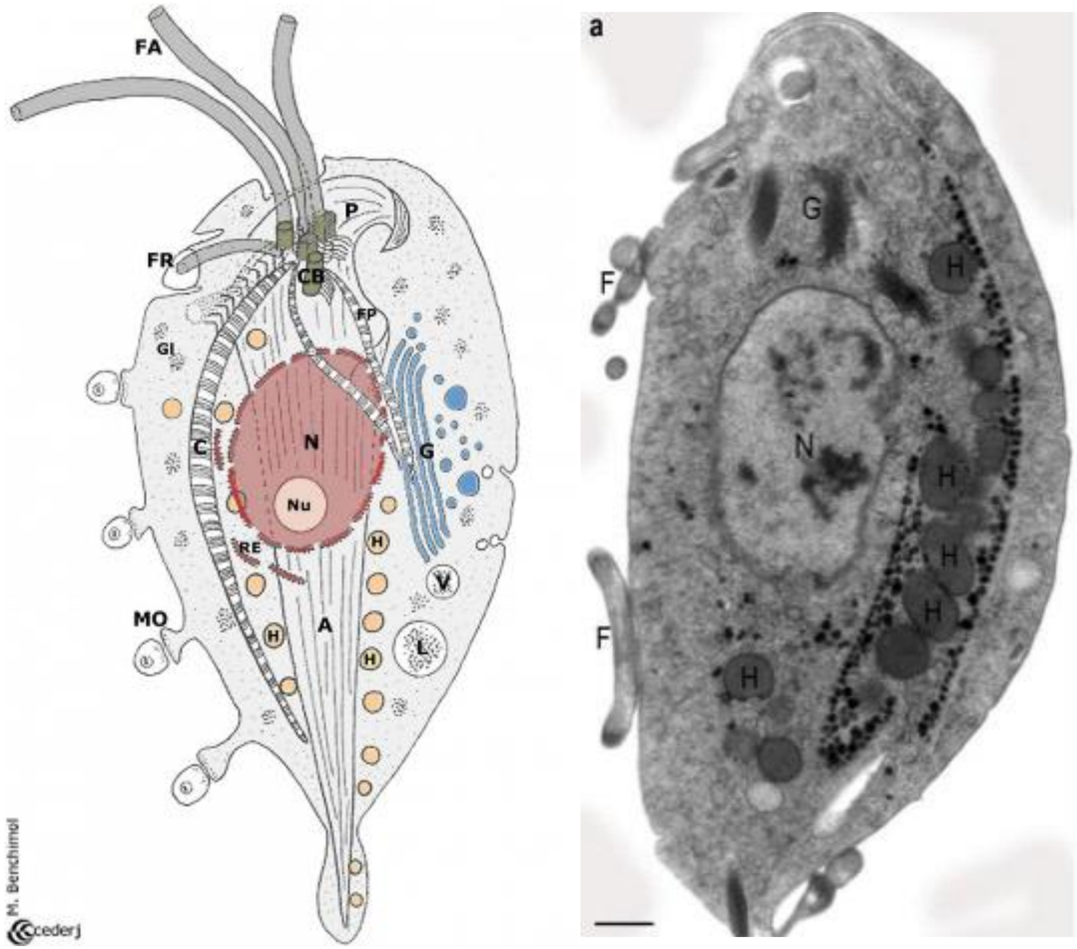


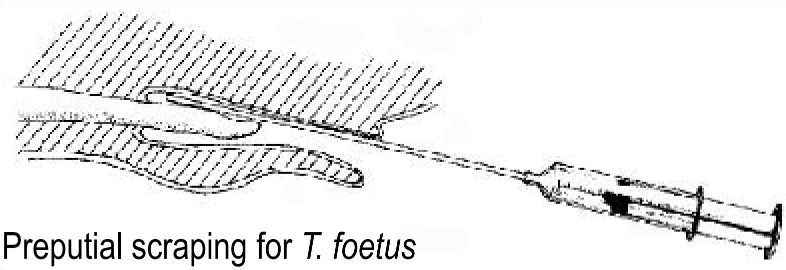
Diagram and TEM of *Tritrichomonas foetus*. Hydrogenosomes (**H**) are preferentially located along the axostyle (**Ax**) and costa (**C**). **AF**- anterior flagella, **BB** - basal bodies, **ER**- endoplasmic reticulum, **F** - parabasal filament, **G** - Golgi, **GL** - glycogen granules, **L** - lysosomes, **N** - nucleus, **Nu** - nucleolus, **P** - pelta, **R** - recurrent flagellum, **UM** - undulating membrane, **V** - vacuoles

Bovine trichomoniasis

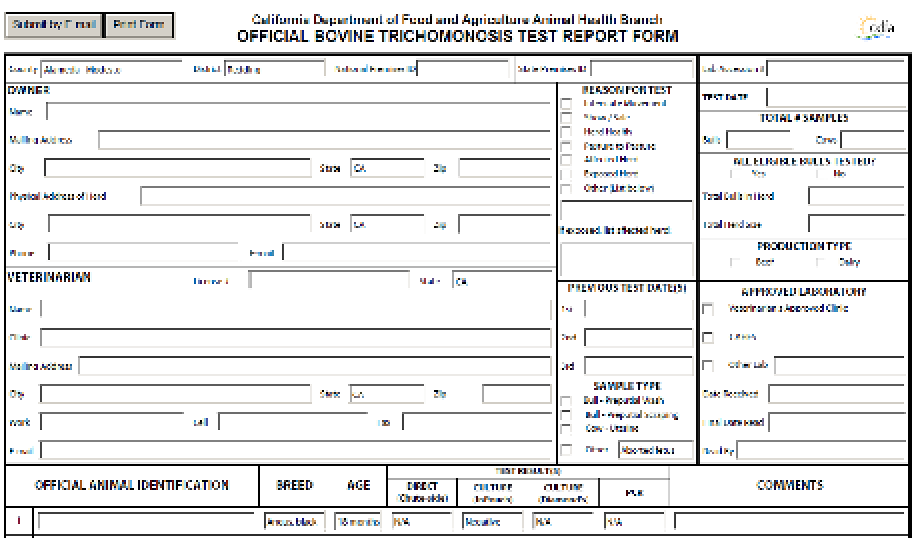
- **sexual transmission**
- bulls = asymptomatic carriers, parasite multiplication in foreskin
- in cows causing endometritis, abortion and permanent infertility
- pyometritis = death of the foetus in utero
- artificial insemination in breeding cattle to eliminate organism from the cattle population

Bovine trichomoniasis

- reportable disease with no available treatment
- bulls = primary carriers ⇨ most diagnostic sampling strategies focus on testing bulls and affected bulls and cows must be culled
- sample collection methods: preputial scraping, flushing, and sponge sampling



Preputial scraping for *T. foetus*



California Department of Food and Agriculture Animal Health Branch

OFFICIAL BOVINE TRICHOMONOSIS TEST REPORT FORM

OWNER

REASON FOR TEST

TOTAL # SAMPLES

PRODUCTION TYPE

VETERINARIAN

PREVIOUS TEST DATE(S)

APPROVED LABORATORY

SAMPLE TYPE

OFFICIAL ANIMAL IDENTIFICATION | BREED | AGE | TEST RESULT(S) | COMMENTS

- ✓ microscopic examination of cultured preputial samples
- ✓ PCR and staining techniques to differentiate from other trichomonads
- ✓ …

Journal of Medical Microbiology (2013), **62**, 1–9 **DOI** 10.1099/jmm.0.047365-0

Review

Diagnosis of *Tritrichomonas foetus*-infected bulls, an ultimate approach to eradicate bovine trichomoniasis in US cattle?

Chaoqun Yao

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Bovine trichomoniasis is a sexually transmitted protozoan disease with a worldwide distribution. It has been endemic in the USA for more than 80 years. Mississippi and all the states west of the Mississippi River, except Iowa and Minnesota, have rules/regulations to reduce the spread of the disease. The core of these regulations consists of testing bulls and prohibiting importation of non-*Tritrichomonas foetus*-free bulls. Factors such as sampling methods and intervals, shipping medium and temperature, and testing techniques are reviewed for their effect on diagnostic accuracy. Finally, a comprehensive approach for controlling and eventually eradicating the disease is presented.

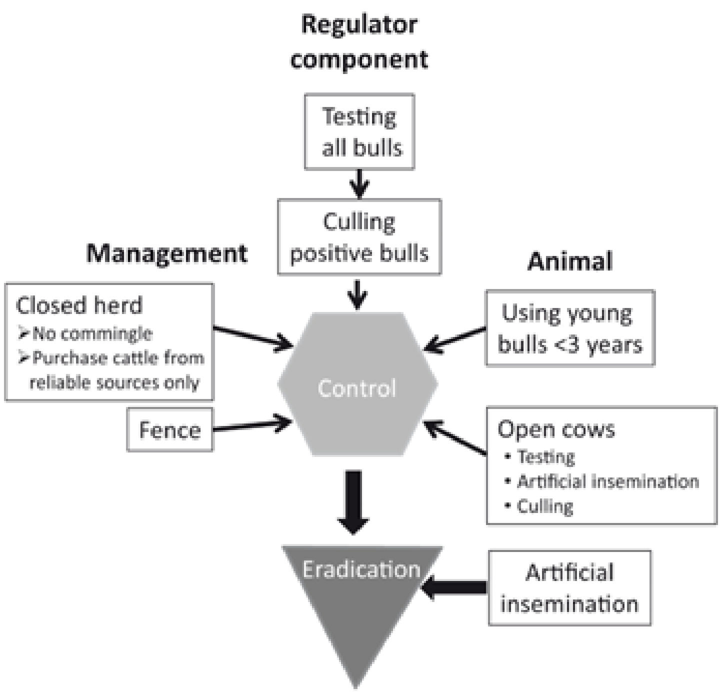

Fig. 1. A comprehensive approach to control and eradicate bovine trichomoniasis.

https://doi.org/10.1099/jmm.0.047365-0

J. Eukaryot. Microbiol., 49(2), 2002 pp. 154–163

https://doi.org/10.1111/j.1550-7408.2002.tb00360.x

Cattle Pathogen *Tritrichomonas foetus* (Riedmüller, 1928) and Pig Commensal *Tritrichomonas suis* (Gruby & Delafond, 1843) Belong to the Same Species

JAN TACHEZY,[a] RUTH TACHEZY,[a,1] VLADIMÍR HAMPL,[a] MIROSLAVA ŠEDINOVÁ,[a] ŠTĚPÁNKA VAŇÁČOVÁ,[a] MARTIN VRLÍK,[a] MARC VAN RANST,[b] JAROSLAV FLEGR[a] and JAROSLAV KULDA[a]

[a]Department of Parasitology, Faculty of Science, Charles University, Viničná 7, Prague 12844, Czech Republic, and
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Opinion TRENDS in Parasitology Vol.21 No.3 March 2005

Are *Tritrichomonas foetus* and *Tritrichomonas suis* synonyms?

Zhao-Rong Lun[1], Xiao-Guang Chen[2], Xing-Quan Zhu[3], Xiang-Rui Li[4] and Ming-Quan Xie[1]

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[2]Department of Parasitology, South Medical University, Guangzhou 510515, The People's Republic of China
[3]Department of Parasitology, College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, The People's Republic of China
[4]College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, The People's Republic of China

***Tritrichomonas suis*, a tritrichomonad of pigs, and the related species *Tritrichomonas foetus*, a tritrichomonad of cattle, are morphologically identical. The taxonomic relationship between these two tritrichomonads has been questioned ever since they were established as distinct species in 1843 and 1928, respectively. Here, we compare the similarities of morphology, ultrastructure, distribution, host specificity, characteristics of *in vitro* cultivation, immunology, biochemistry and analysis of molecular data from published sources between these two species. All data indicate that these two trichomonad species are identical. Thus, we propose that *T. foetus* and *T. suis* are synonyms.**

https://doi.org/10.1016/j.pt.2004.12.001

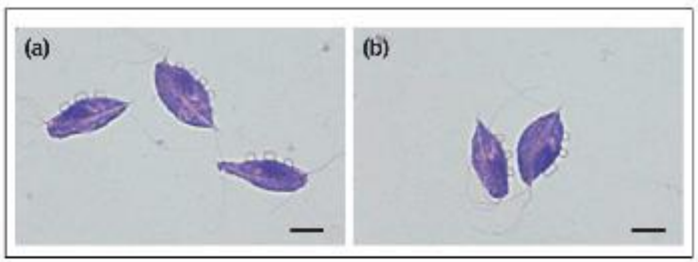

Figure 1. Photomicrographs of *T. foetus* **(a)** and *T. suis* **(b)** from culture with Diamond's medium and stained with the modified method described by Lun and Gajadhar [50]. Scale bars = 5 µm.

International Journal for Parasitology 42 (2012) 1143–1149

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology

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

Comparative analysis of *Tritrichomonas foetus* (Riedmüller, 1928) cat genotype, *T. foetus* (Riedmüller, 1928) cattle genotype and *Tritrichomonas suis* (Davaine, 1875) at 10 DNA loci

Jan Šlapeta[a,*], Norbert Müller[b], Colin M. Stack[c], Giselle Walker[d], Ala Lew-Tabor[e], Jan Tachezy[f], Caroline F. Frey[b]

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A B S T R A C T

The parasitic protists in the genus *Tritrichomonas* cause significant disease in domestic cattle and cats. To assess the genetic diversity of feline and bovine isolates of *Tritrichomonas foetus* (Riedmüller, 1928; Wenrich and Emmerson, 1933, we used 10 different genetic regions, namely the protein coding genes of cysteine proteases 1, 2 and 4–9 (CP1, 2, 4–9) involved in the pathogenesis of the disease caused by the parasite. The cytosolic malate dehydrogenase 1 (MDH1) and internal transcribed spacer region 2 of the rDNA unit (ITS2) were included as additional markers. The gene sequences were compared with those of *Tritrichomonas suis* (Davaine, 1875) Morgan and Hawkins, 1948 and *Tritrichomonas mobilensis* Culberson et al., 1986. The study revealed 100% identity for all 10 genes among all feline isolates (=*T. foetus* cat genotype), 100% identity among all bovine isolates (=*T. foetus* cattle genotype) and a genetic distinctness of 1% between the cat and cattle genotypes of *T. foetus*. The cattle genotype of *T. foetus* was 100% identical to *T. suis* at nine loci (CP1, 2, 4–8, ITS2, MDH1). At CP9, three out of four *T. suis* isolates were identical to the *T. foetus* cattle genotype, while the *T. suis* isolate SUI-H3B sequence contained a single unique nucleotide substitution. *Tritrichomonas mobilensis* was 0.4% and 0.7% distinct from the cat and cattle genotypes of *T. foetus*, respectively. The genetic differences resulted in amino acid changes in the CP genes, most pronouncedly in CP2, potentially providing a platform for elucidation of genotype-specific host-pathogen interactions of *T. foetus*. On the basis of this data we judge *T. suis* and *T. foetus* to be subjective synonyms. For the first time, on objective nomenclatural grounds, the authority of *T. suis* is given to Davaine, 1875, rather than the commonly cited Gruby and Delafond, 1843. To maintain prevailing usage of *T. foetus*, we are suppressing the senior synonym *T. suis* Davaine, 1875 according to Article 23.9, because it has never been used as a valid name after 1899 and *T. foetus* is widely discussed as the cause of bovine trichomonosis. Thus bovine, feline and porcine isolates should all be given the name *T. foetus*. This promotes the stability of *T. foetus* for the veterinary and economically significant venereal parasite causing bovine trichomonosis.

https://doi.org/10.1016/j.ijpara.2012.10.004

Cat trichomoniasis

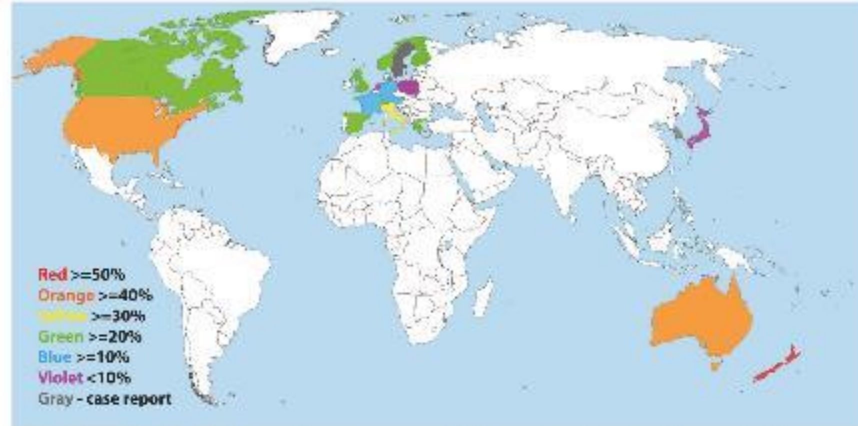


Figure 4 Geographical distribution of surveys for and case report of *Tritrichomonas foetus* detected positive cats worldwide.

Yao and Köster *Veterinary Research* (2015) 46:35
DOI 10.1186/s13567-015-0169-0

https://doi.org/10.1186/s13567-015-0169-0

REVIEW

Tritrichomonas foetus infection, a cause of chronic diarrhea in the domestic cat

Chaoqun Yao[1,3*] and Liza S Köster[2,3]

Abstract

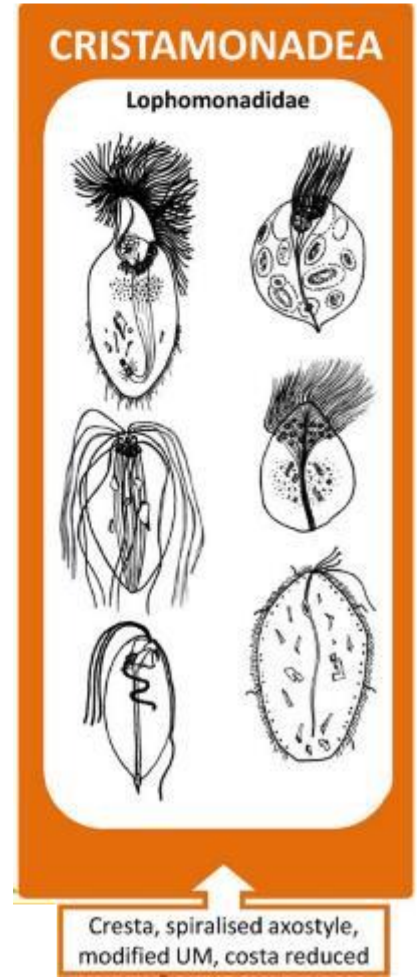
Tritrichomonas foetus is a very intriguing trichomonad protozoan with respect to its varied choice of residence in the different host species. It is an obligate parasite of the reproductive and the gastrointestinal tract of bovine and feline host respectively, leading to trichomonosis. Bovine trichomonosis is a sexually transmitted disease whereas feline trichomonosis is a disease with a purported fecal-oral route of spread. Further, the trichomonad is a commensal in the nasal passages, stomach, cecum and colon of swine host. Advances have been exponential in understanding the trichomonad biology and specifically feline trichomonosis since late 1990s and early 2000s when *T. foetus* was soundly determined to be a causative agent of chronic diarrhea in the domestic cat. It is a challenging task, even for a skilled investigator not to mention the busy clinical veterinarian, to keep up with the vast volume of information. Here we comprehensively reviewed the trichomonad biology, clinical manifestations, pathogenesis, host immunity, world map of distribution, risk factors, diagnosis and treatment. Risk factors associated with *T. foetus*-positive status in the domestic cat include young age, purebred, history of diarrhea, co-infections with other enteral pathogens. In addition, molecular similarity of bovine and feline isolates of *T. foetus* in DNA sequence was concisely discussed. The data presented serve as an information source for veterinarians, and investigators who are interested in biology of *T. foetus* and feline trichomonosis.

Parabasalia

Cristamonadea

- uninucleate to multinucleate
- two to thousands of flagella/cilia per mastigont
- kinetosomes, except for ‘privileged kinetosomes’, often discarded during cell division in highly flagellated/ciliated taxa
- axostyle ancestrally of “*Tritrichomonas* type”
- secondarily thin or reduced in some; multiple axostyles in multinuclear forms
- parabasal body single or multiple, ellipsoid or rod-shaped, often spiralled or ramified
- symbionts inhabiting the hindgut of the lower termites
- ***Coronympha***, ***Deltotrichonympha***, ***Devescovina***, ***Foaina***, ***Joenia***, ***Mixotricha, Calonympha***

Čepička et al. 2010: Protist 161



https://doi.org/10.1016/j.protis.2009.11.005

Parabasalia

Cristamonadea

Calonympha grassii

- termite *Cryptotermes brevis*

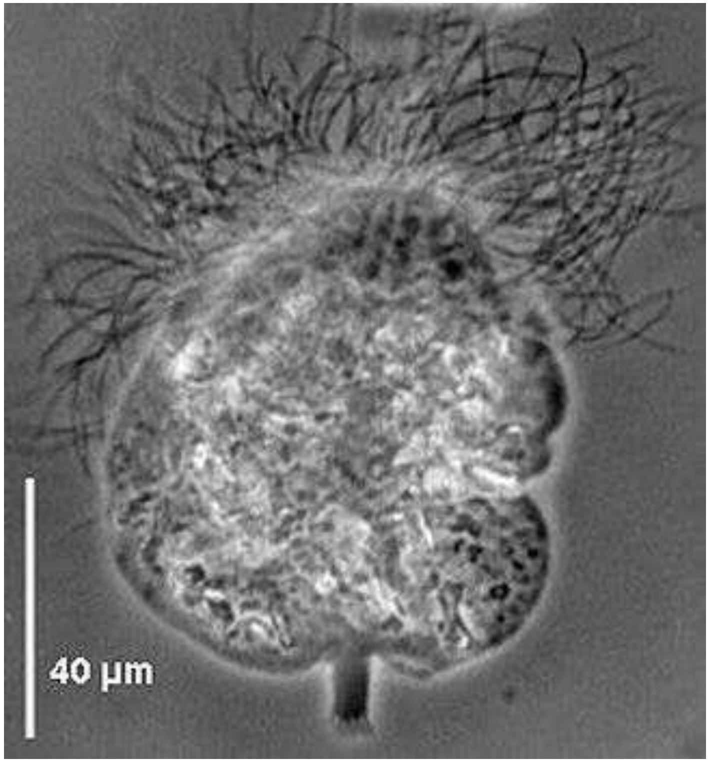


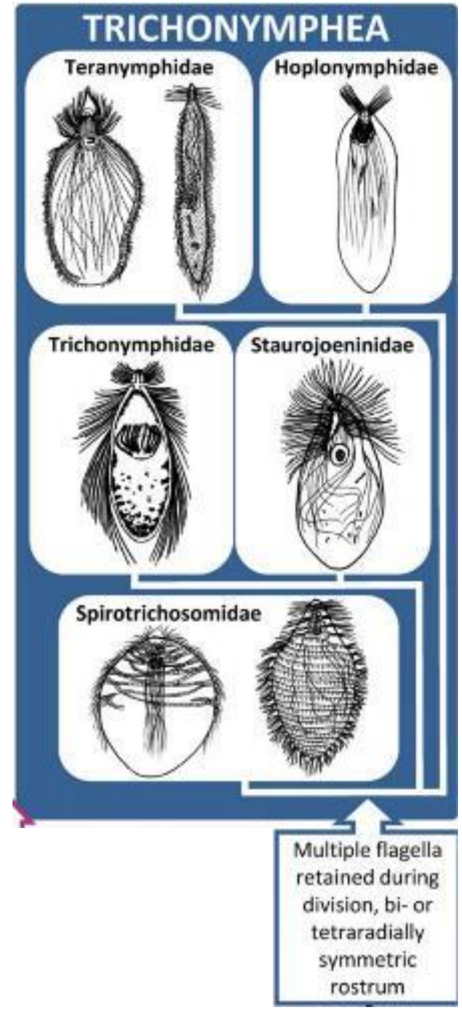
Fig. 637. *Calonympha grassii*. Totalansicht. Vergr. 1800. (Nach JANICKI.)

Parabasalia

Trichonymphea

- bilaterally or tetraradially symmetrical, with anterior rostrum divided into 2 hemirostra
- each hemirostrum bears 1-2 areas with hundreds to thousands of flagella/cilia
- flagella/cilia usually retained during cell division; one hemirostrum goes to each daughter cell; numerous parabasal fibres
- numerous thin axostyles do not protrude outside the cell
- symbionts in hindguts of lower termites and the wood-feeding cockroach
- ***Barbulanympha***, ***Hoplonympha***, ***Staurojoenia***, ***Trichonympha***

Čepička et al. 2010: Protist 161

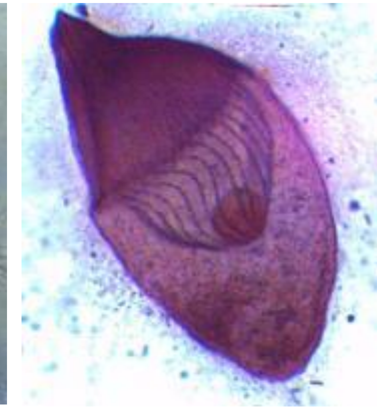


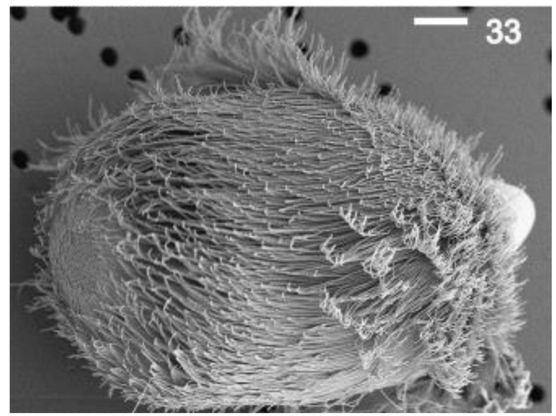
https://doi.org/10.1016/j.protis.2009.11.005

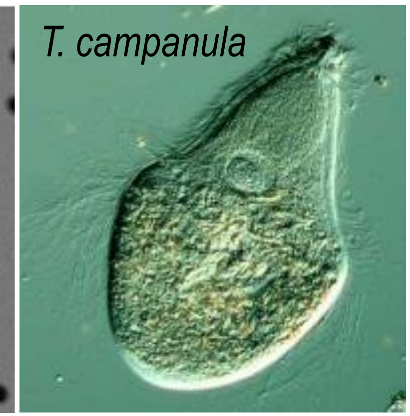
Parabasalia

Trichonymphea

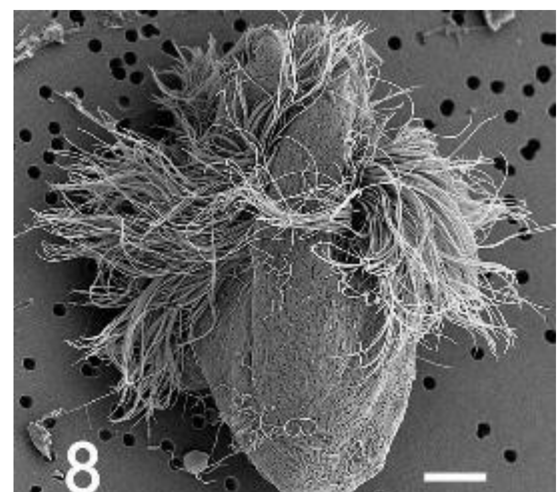
Trichonympha spp.







Staurojoenina mulleri



Parabasalia

Spirotrichonymphea

- kinetosomes in counter clockwise spiral rows
- flagella/cilia retained during cell division with the ciliary rows dividing between daughter cells
- axostyle single of "*Tritrichomona*s type", or multiple in thin bands, or reduced
- symbionts in hindguts of lower termites
- ***Holomastigotes***, ***Holomastigotoides***, ***Microjoenia***, ***Spirotrichonympha***

Čepička et al. 2010: Protist 161

https://doi.org/10.1016/j.protis.2009.11.005

Parabasalia

Spirotrichonymphea

A) protargol-stained juvenile ***Microjoenia*** sp. from *Reticulitermes lucifugus*

B) protargol-stained adult ***Microjoenia*** sp. from *R. lucifugus*

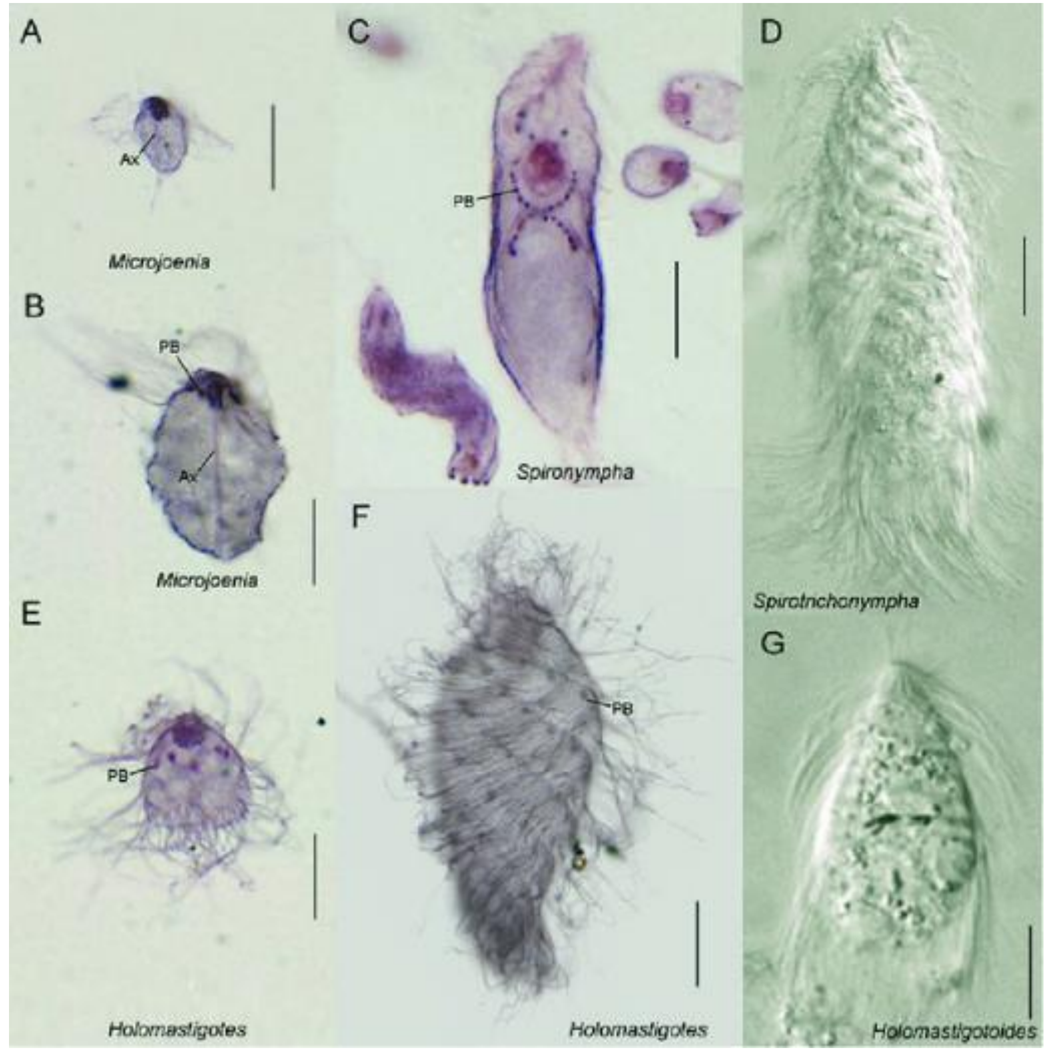
C) protargol-stained ***Spironympha*** sp. from *Reticulitermes flaviceps*

D) living ***Spirotrichonympha flagellata*** from *Reticulitermes hesperus*, DIC

E) protargol-stained juvenile ***Holomastigotes elongatum*** from *Reticulitermes lucifugus*

F) protargol-stained adult ***H. elongatum*** from *R. lucifugus*

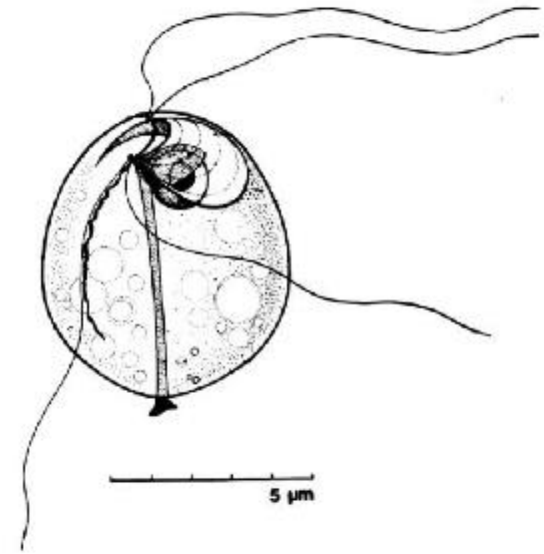
G) living ***Holomastigotoides*** sp. from *Coptotermes testaceus,* DIC



Scale bars = 10 μm for A-C, E and F; 20 μm for D, and 50 μm for G

Preaxostyla

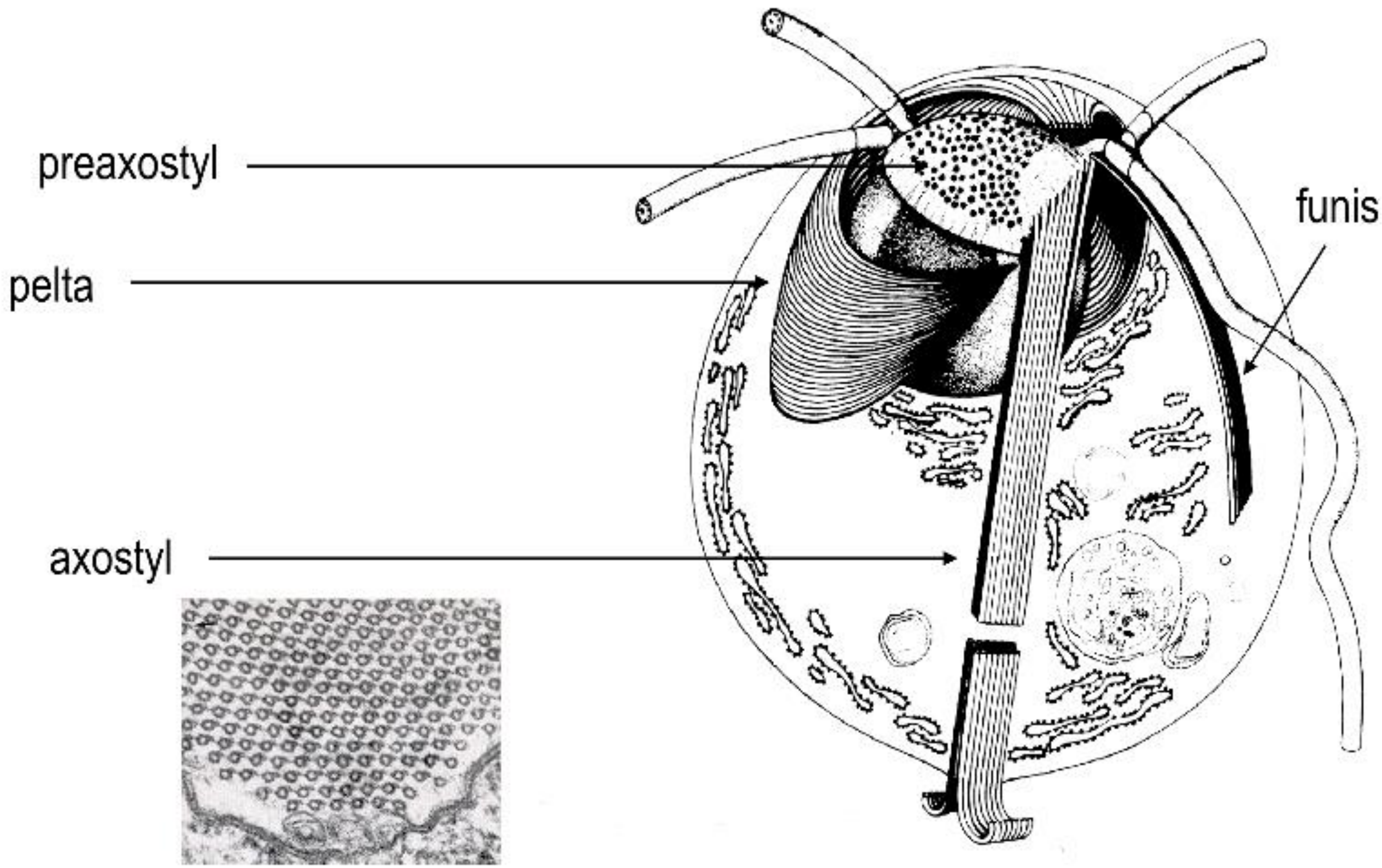
Oxymonadida



- single kinetid (occasionally multiple kinetids) consisting of 2 pairs of ciliated kinetosomes distantly
- separated by preaxostyle (microtubular root R2, with paracrystalline lamina), from which arises a microtubular axostyle
- axostyle is contractile or motile in some taxa, microtubular pelta usually present
- many taxa attach to host using an anterior holdfast
- closed mitosis with internal spindle
- lacking Golgi and mitochondria
- intestinal endosymbionts, mostly in lower termites and *Cryptocercus* cockroaches

Preaxostyla

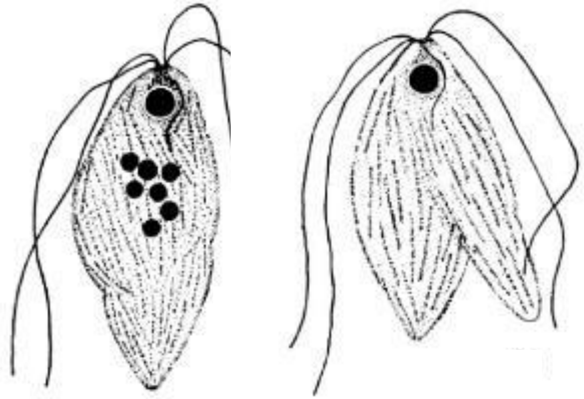
Oxymonadida



Preaxostyla

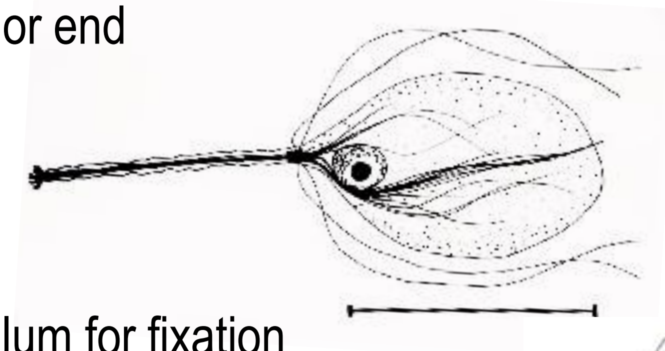
Oxymonadida

Polymastix melolonthae



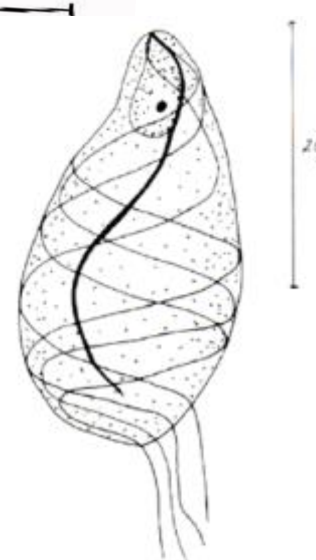
- 4 free anterior flagella + 1 recurrent
- slender axostyle and a row of microtubules or pelta covering the anterior end
- larvae of beetles

Oxymonas spp.



- 40-165 µm
- 4 flagella, anterior rostellum for fixation
- gut of termites

Pyrsonympha spp.



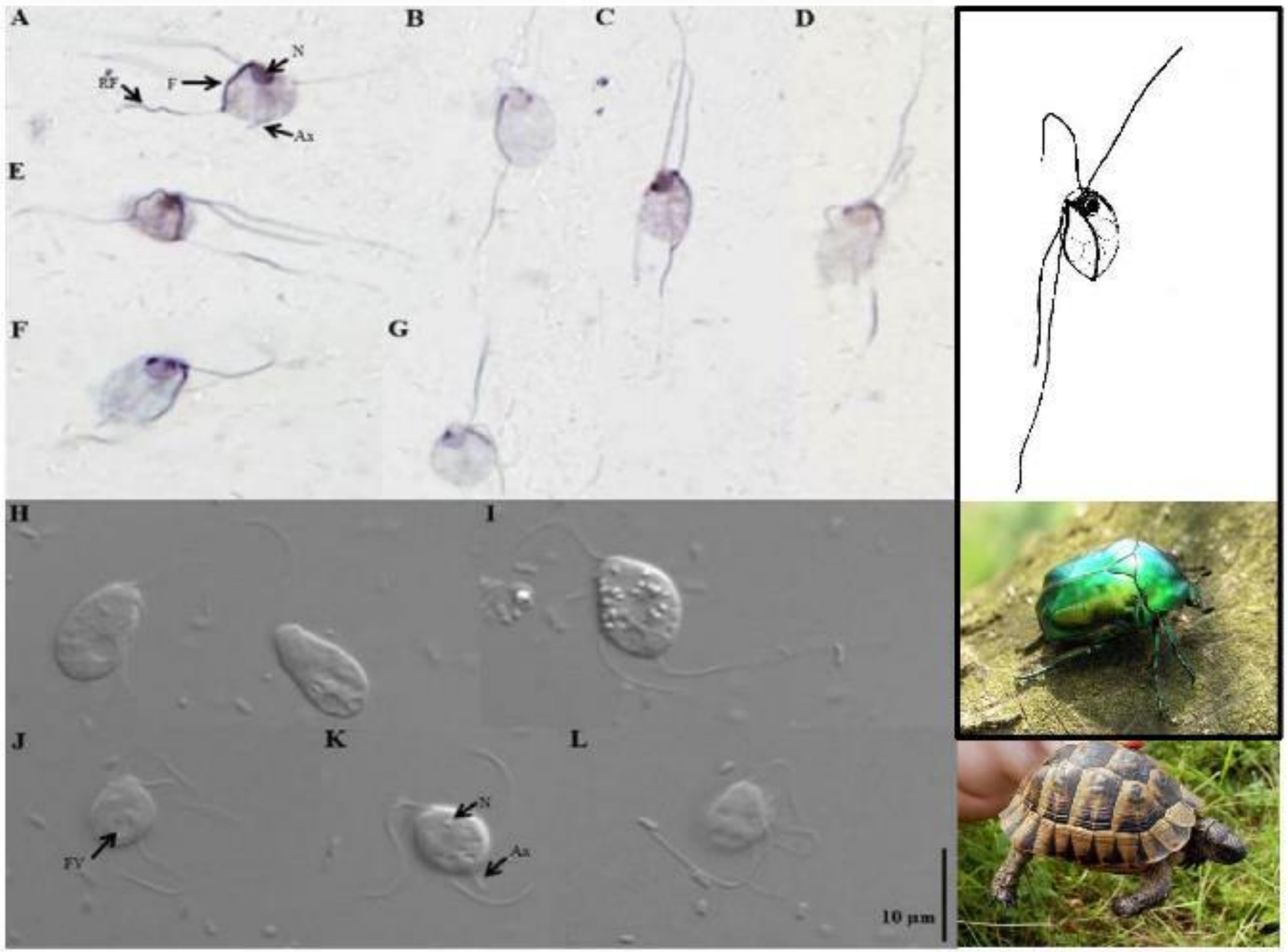
- spirally twisted and contractile body, 20-150 µm
- 4 or 8 flagella wrapped around the cell body and having a posterior trailing portion
- symbionts of gut of termites

Preaxostyla

Oxymonadida

Monocercomonoides sp.

- intestine of reptiles and intestine of beetles



From marginated tortoise (*Testudo marginata*) (Vlasáková 2014, Master thesis)

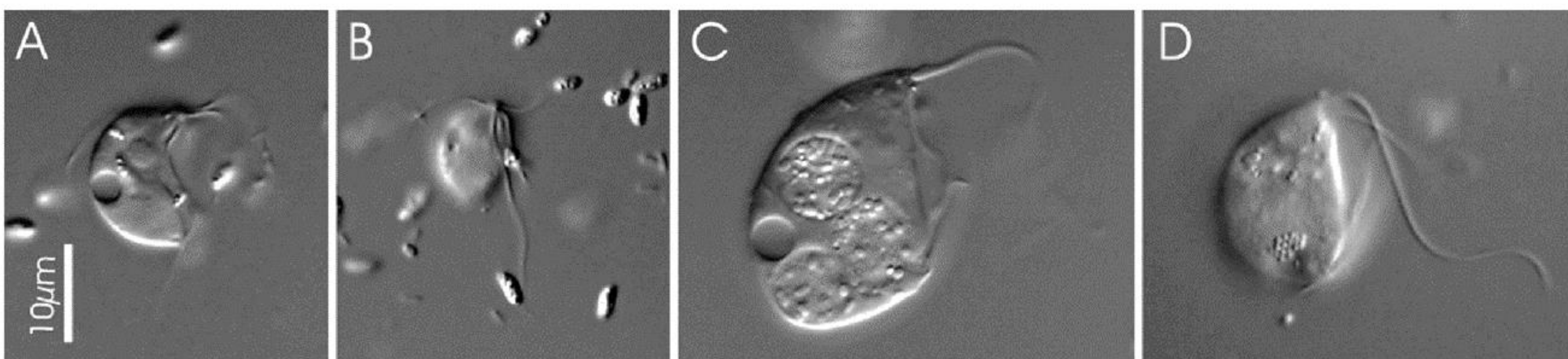
Preaxostyla

Trimastigida

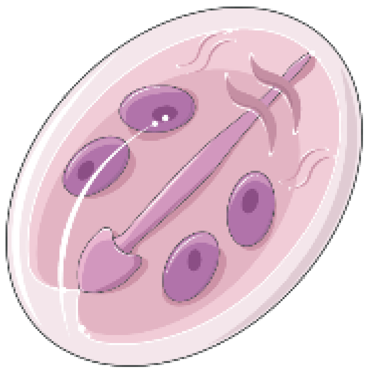
- sister group to oxymonads

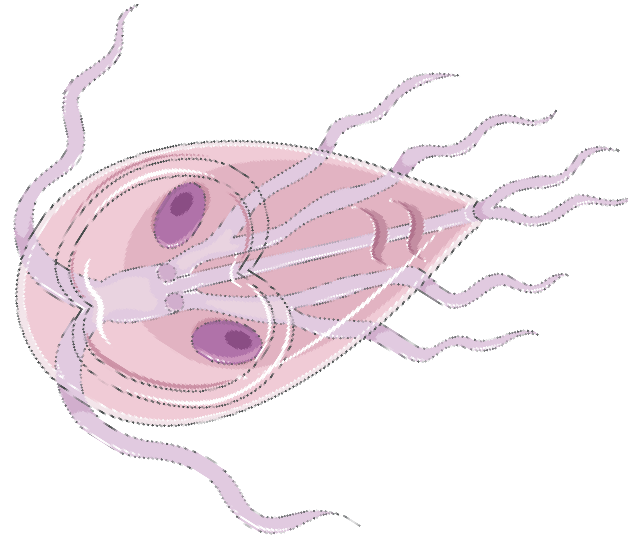
genus ***Trimastix***

- heterotrophic free-living flagellates with 4 flagella
- broad ventral feeding groove, in which beats the posteriorly directed flagellum (with 2 broad vanes)
- small dense organelles bounded by two membranes in place of mitochondria

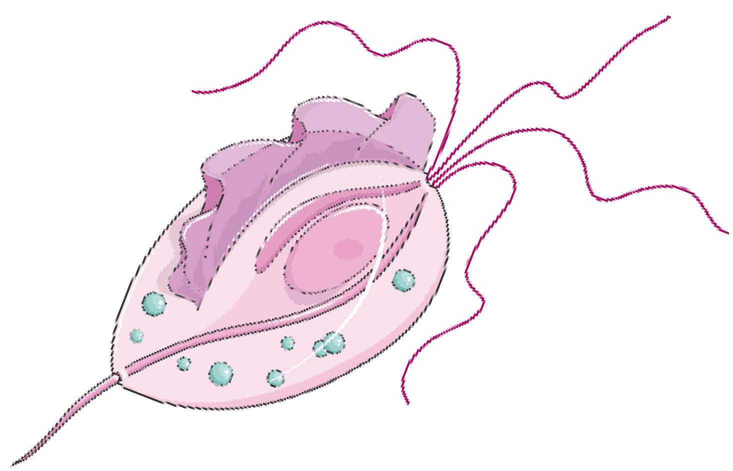


Light microscopy (DIC) images of *Trimastix pyriformis* (A,B) and *Trimastix marina* (C,D). © Vladimir Hampl





Thank you for your attention ☺



Lectures

- ✓ Introduction: BPP 2022 I
- ✓ Euglenozoa (Excavata): BPP 2022 II
- ✓ Fornicata / Preaxostyla / Parabasala (Excavata): BPP 2022 III
- ⇨ **Apicomplexa I (SAR): BPP 2022 IV**
- Apicomplexa II (SAR): BPP 2022 V
- Amoebae (Excavata, Amoebozoa): BPP 2022 VI
- Ciliophora, Opalinata (SAR): BPP 2022 VII

- *Pneumocystis* (Opisthokonta, Fungi): BPP 2022 VIII
- Microsporidia (Opisthokonta, Fungi): BPP 2022 IX
- Myxozoa (Opisthokonta, Animalia): BPP 2022 X