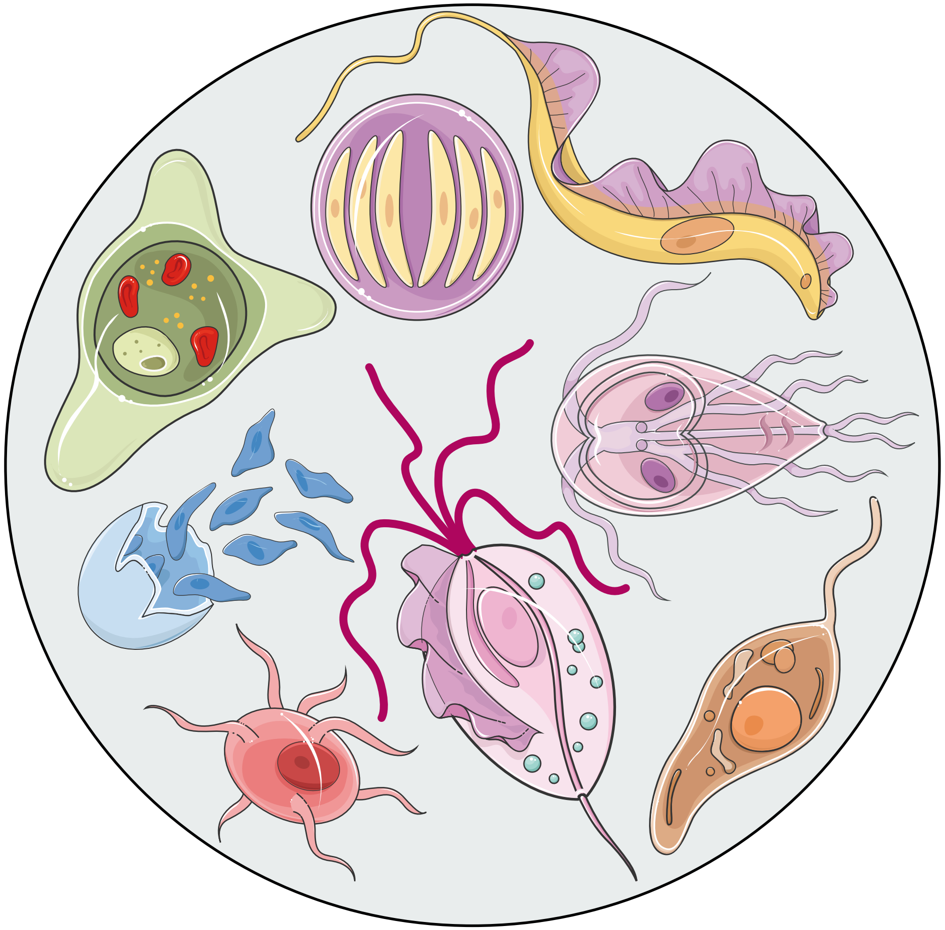
Biology of parasitic protozoa

II. Euglenozoa (Excavata, Discoba)



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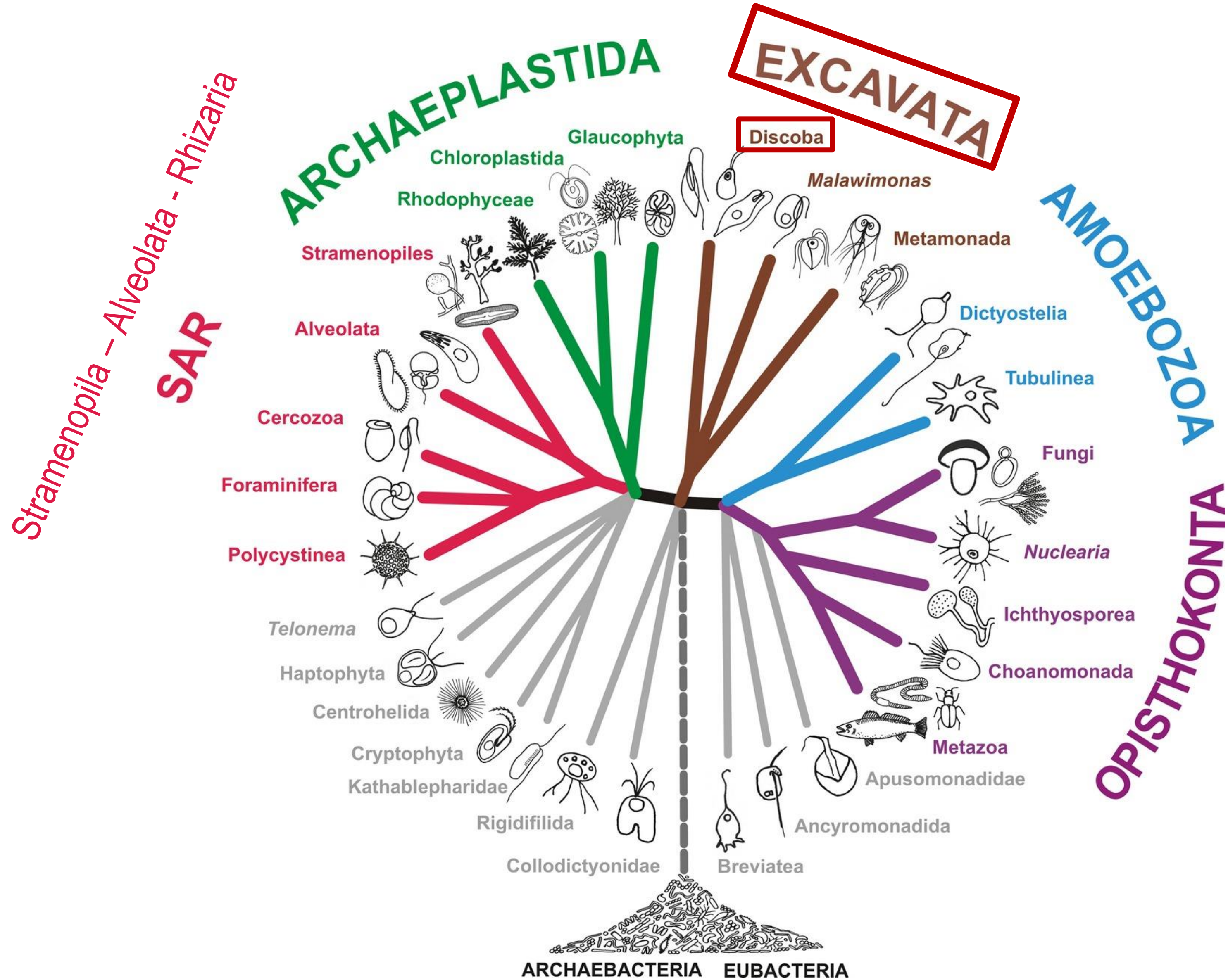
Notice

5 supergroups = megagroups

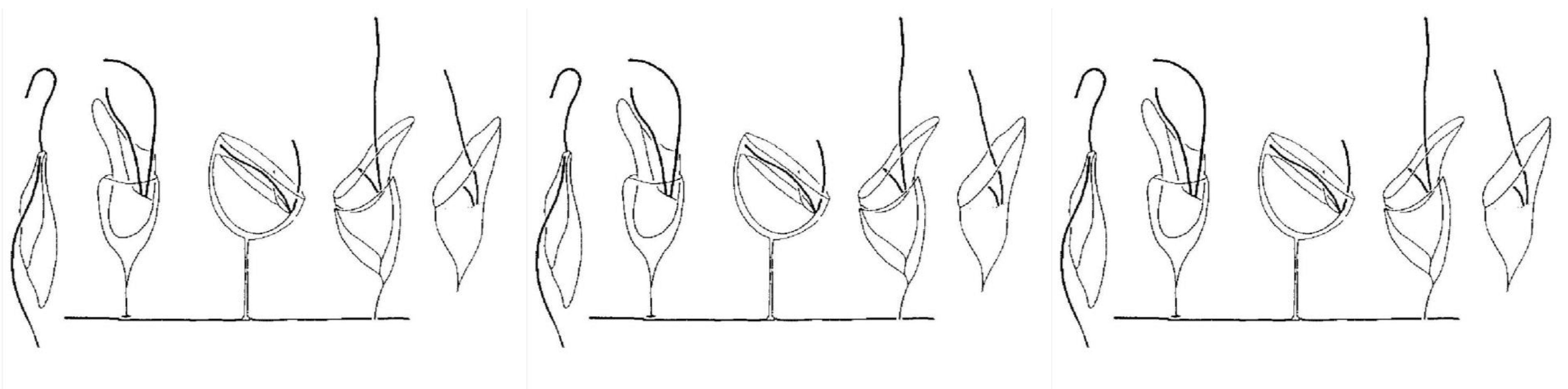


J. EUKARYOT. MICROBIOL., 59, NO. 5, SEPTEMBER–OCTOBER 2012

Journal of Eukaryotic Microbiology 2019, **66**, 4–119

Excavata

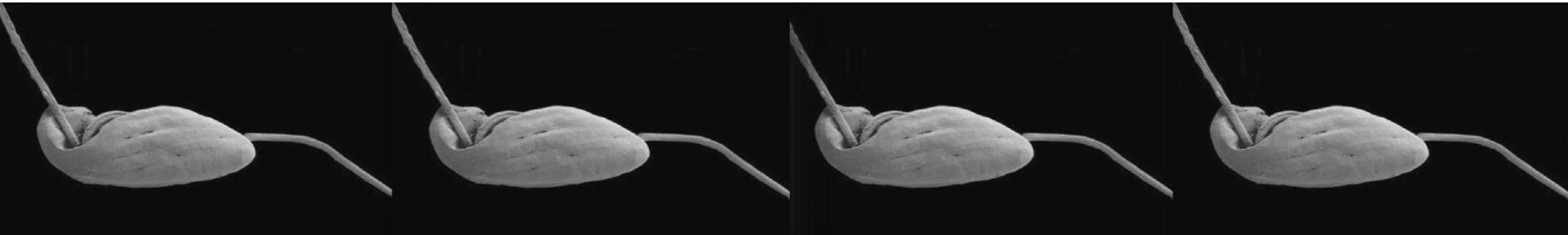
- have a conspicuous **ventral feeding groove** that is “excavated” from one side and through which pass one or more recurrent flagella; the ventral groove has characteristic ultrastructure and is supported by microtubules
- originally 2 flagellated state - many changes: multiplication of flagella, reduction 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 **Discoba**•: **Euglenozoa**•••



Journal of Eukaryotic Microbiology 2019, **66**, 4–119

Euglenozoa

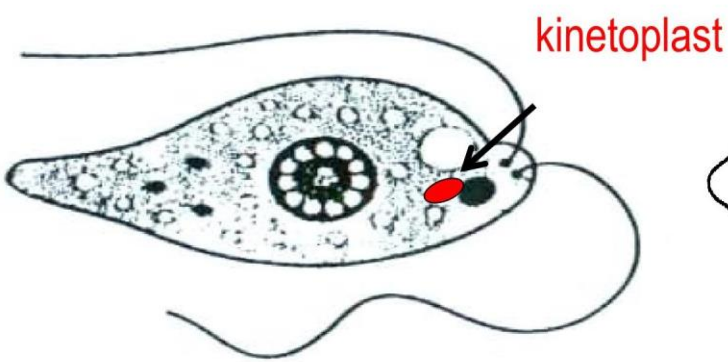
- monophyletic (non-monophyletic*) group consisting of flagellates with very different modes of nutrition, including predation, osmotrophy, parasitism and photoautotrophy
- paddle-shaped, discoidal mitochondrial cristae
- 2 flagella: an anteriorly directed dorsal flagellum and a posteriorly directed ventral flagellum
- flagellar apparatus consisting of 3 microtubular roots: dorsal root, intermediate root and ventral root
- microtubule-reinforced ventral or anterior feeding apparatus (MtR pocket)
- mostly anaerobic
- 4 groups in **Discoba•**: Euglenidida, Diplonemea, Symbiontida and Kinetoplastea

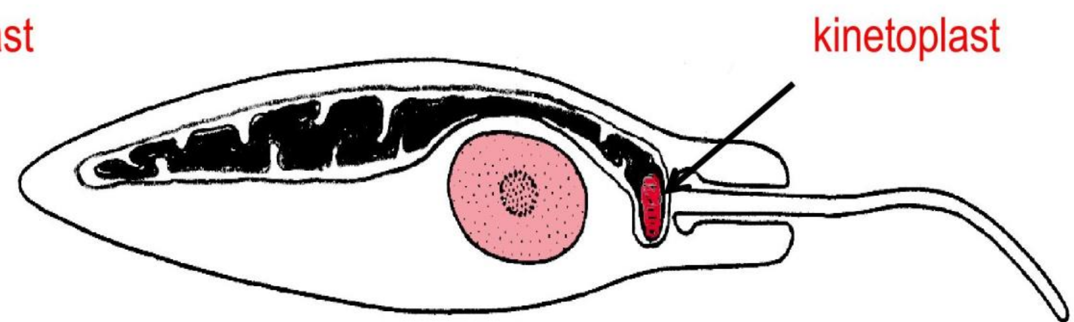


Journal of Eukaryotic Microbiology 2019, **66**, 4–119

Kinetoplastea (Kinetoplastida)

- at least one stage in the life cycle equipped with one or two flagella, arising from a prominent flagellar pocket
- presence of extensive mitochondrial DNA, termed kinetoplast DNA
- **kinetoplast** - DNA-containing granule within the cell's single mitochondrion
- **kinetoplast** - modified mitochondrion,10-100 x 20 μm
- based on morphology: biflagellate bodonids and uniflagellate trypanosomatids
- free-living or parasitic
- **Bodonida** (Eubodonida, Parabodonida, Neobodonida), **Prokinetoplastida**, **Trypanosomatida**





ORIGINAL ARTICLE

Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes

Journal of Eukaryotic Microbiology 2019, **66**, 4–119

EXCAVATES [Excavata Cavalier-Smith 2002, emend. Simpson 2003] (P)
Typically with suspension-feeding groove of the "excavate" type, secondarily lost in many taxa; feeding groove used for capture and ingestion of small particles from feeding current generated by a posteriorly directed cilium (F1); right margin and floor of groove are supported by parts of the R2 microtubular root, usually also supported by microtubular fibres (B fibre, composite fibre), and the left margin by the R1 microtubular root and C fibre. Grouping of Metamonada and Discoba and Malawimonads is somewhat controversial, although recent multigene phylogenies have markedly increased support for monophyly of Metamonada, and of Discoba, separately. Apomorphy: Suspension-feeding groove, homologous to that in *Jakoba libera*. Recent phylogenies indicate Metamonada and Dicoba probably do not share the same node.

●● Euglenozoa Cavalier-Smith 1981, emend. Simpson 1997
Cells with two cilia, occasionally one, rarely more, inserted into an apical/subapical ciliary pocket; with rare exceptions, emergent cilia with heteromorphic paraxonemal rods; usually with tubular feeding apparatus associated with ciliary apparatus; basic ciliary apparatus pattern consisting of two functional kinetosomes and three asymmetrically arranged microtubular roots; single mitochondrion mostly with discoidal cristae. Apomorphy: heteromorphic paraxonemal rods, tubular/whorled in anterior cilium F2 and a parallel lattice in posterior cilium F1.

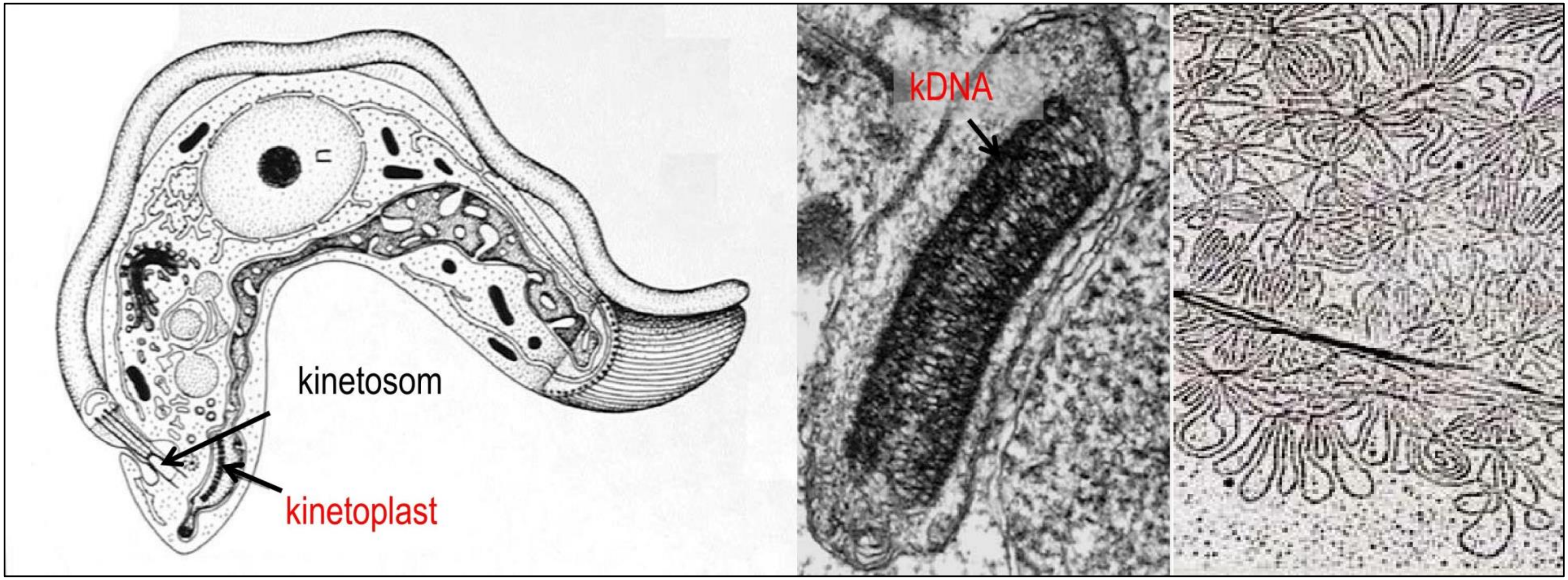
●●● Kinetoplastea Honigberg 1963
Cells with a kinetoplast, which is a large mass(es) of mitochondrial (=kinetoplast; k) DNA; Apomorphy: kinetoplast; mitochondrial RNA editing; *trans*-splicing of splice leader RNA; polycistronic transcription.

●●●● Metakinetoplastina Vickerman in Moreira et al. 2004 (R)
Group identified by SSU rRNA phylogenies. Node-based definition: clade stemming from the most recent common ancestor of Neobodonida, Parabodonida, Eubodonida, and Trypanosomatida.

(R) robust clades recovered in phylogenetic analysis that do not have morphological diagnosis are indicated by R (ribo-group);

Kinetoplast

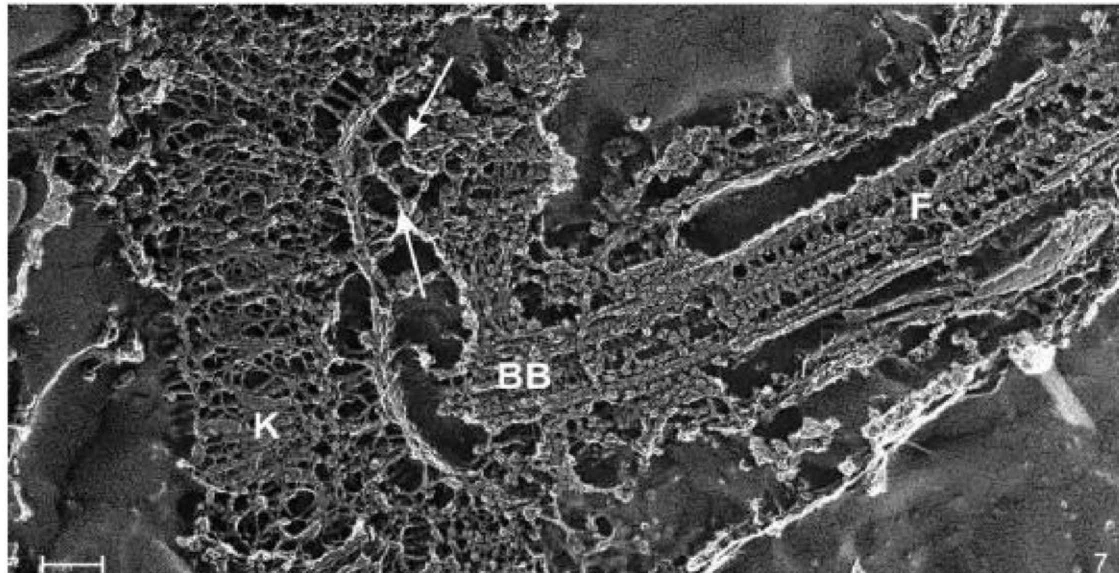
- network of concatenated circular DNA molecules and their associated structural proteins along with DNA and RNA polymerases
- found at the base of a cell's flagella and associated to the flagellum basal body by a cytoskeletal structure
- about 40 % of a total DNA, maxicircles 20-28 kb (RNA editing), minicircles 0.5-10 kb (encoding the guide RNA genes)
- maxicircles and minicircles catenated to form a planar network chainmail

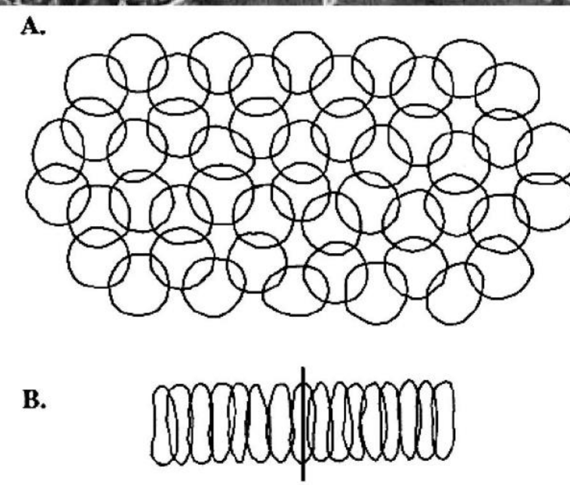


Variations of kinetoplast networks

Catenated kinetoplasts

- **eukinetoplast**
- circles are catenated to form a planar network that has a topology resembling that of chain mail in medieval armour
- genera *Trypanosoma*, *Crithidia* and *Leishmania*

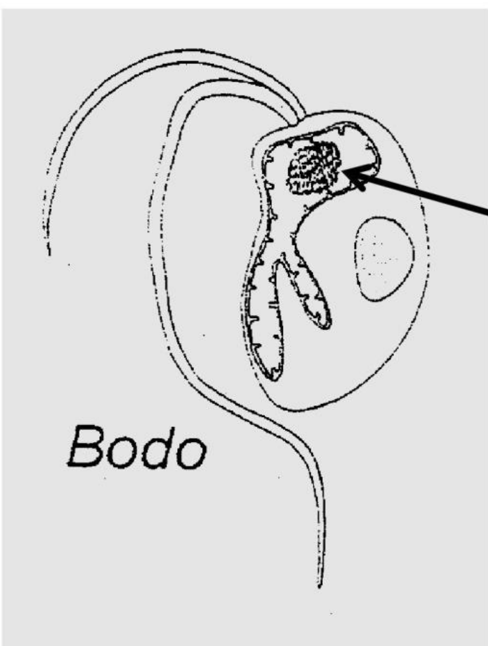


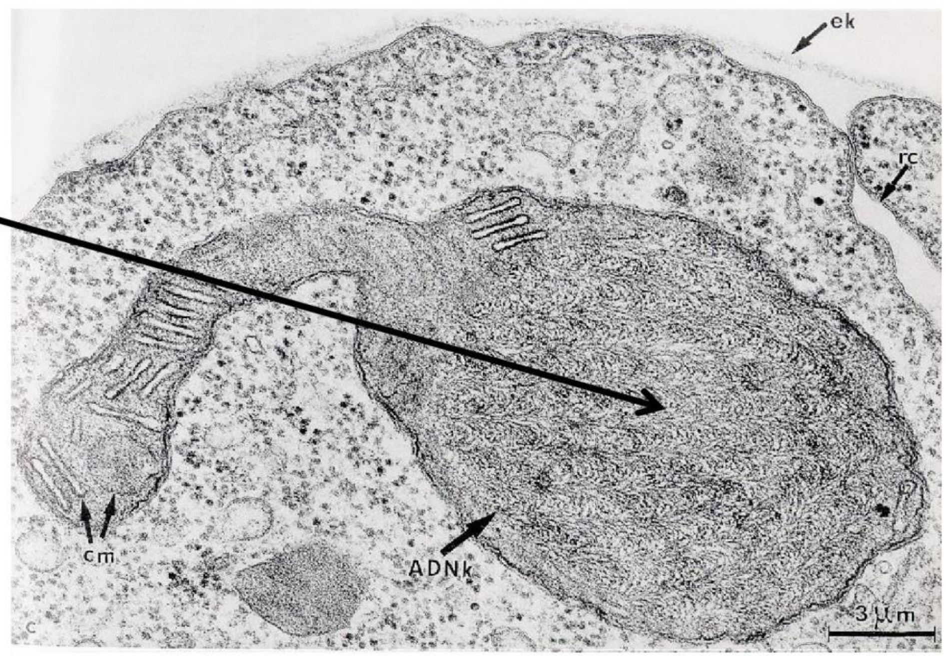


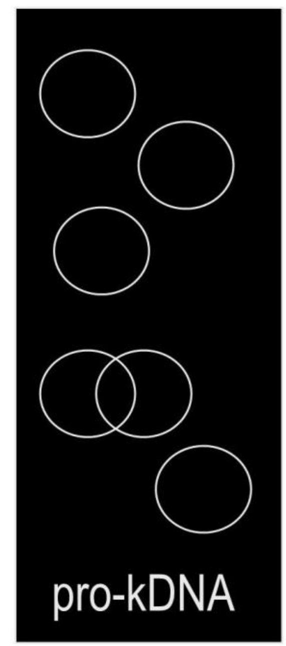
Variations of kinetoplast networks

Noncatenated kinetoplasts

- **prokinetoplast** (pro-kDNA kinetoplast)
- a bundle-like structure in the mitochondrial matrix that superficially resembles a kDNA disk
- contains very little catenation, maxicircles and minicircles are relaxed instead of supercoiled



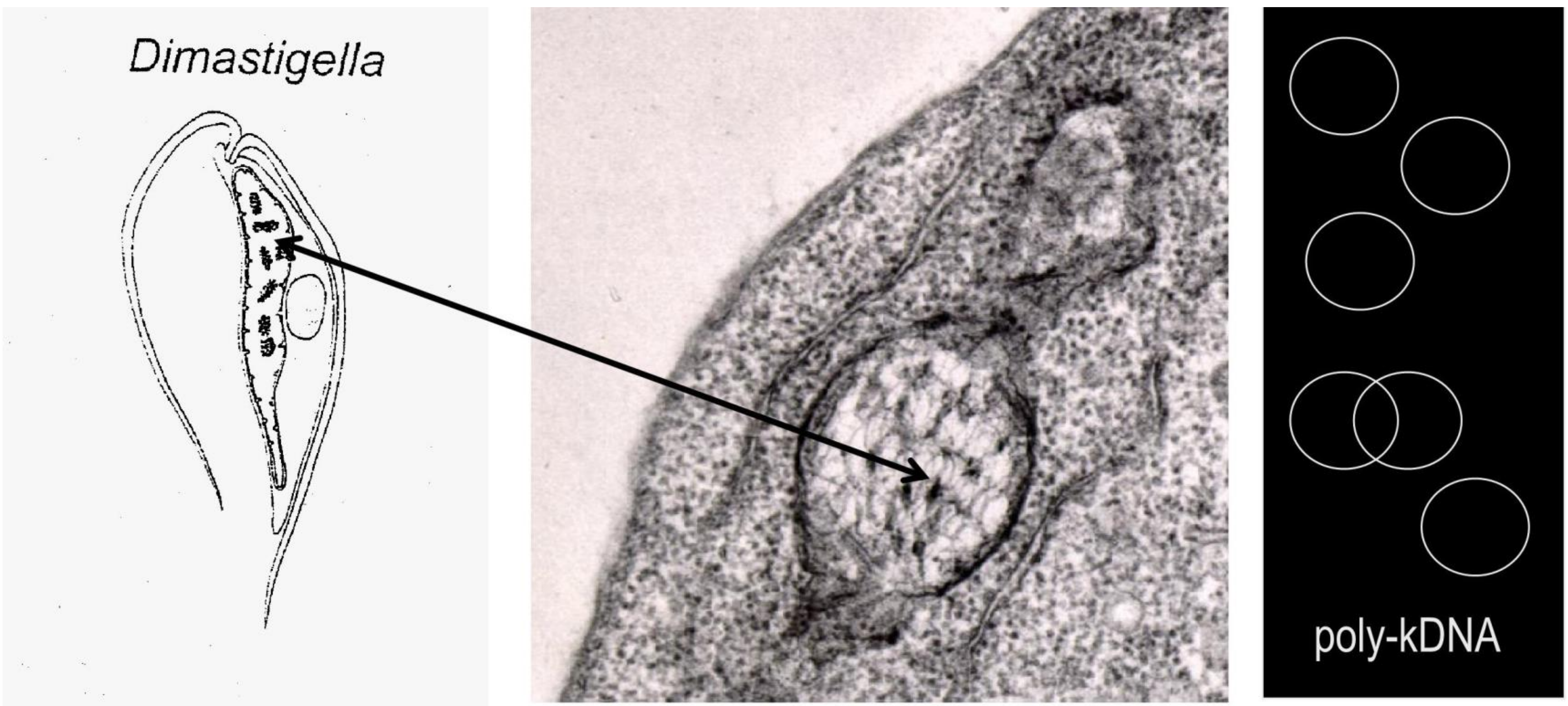




Variations of kinetoplast networks

Noncatenated kinetoplasts

- **polykinetoplast (**poly-kDNA kinetoplast)
- kDNA is distributed among various discrete foci throughout the mitochondrial lumen
- little catenation and no supercoiling

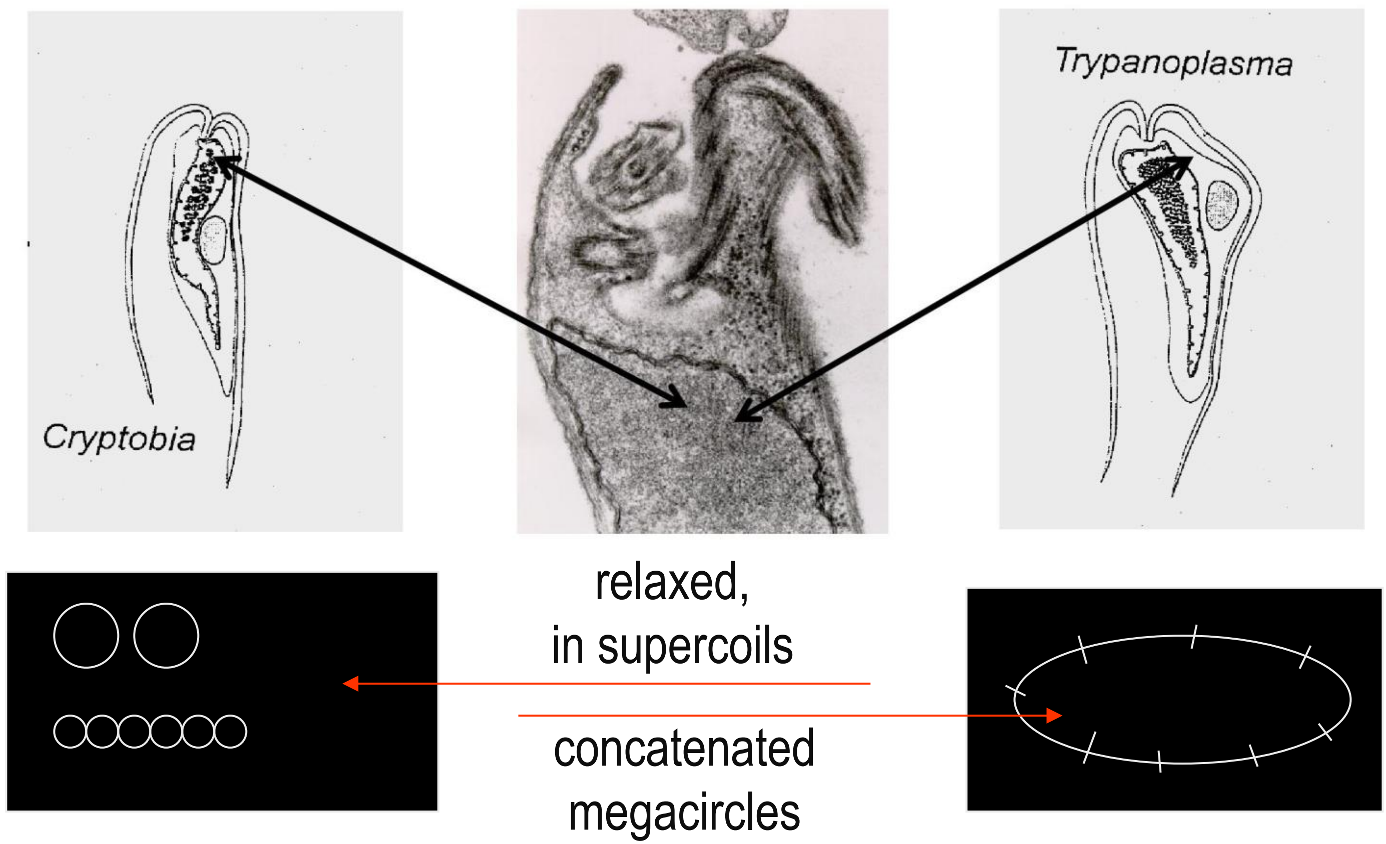


Dimastigella, *Cruzella*, *Ichtyobodo*

Variations of kinetoplast networks

Noncatenated kinetoplasts

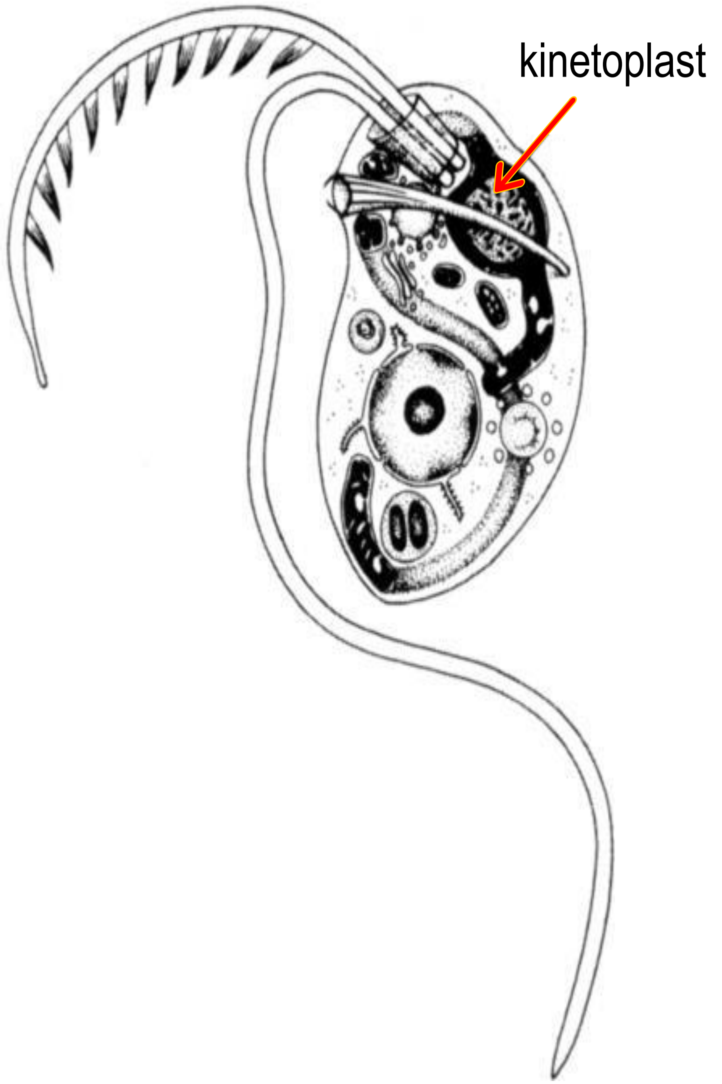
- **pankinetoplast** (pan-kDNA kinetoplast)
- fills most of the mitochondrial matrix, not limited to discrete foci like poly-kDNA
- also a lesser degree of catenation, minicircles are not relaxed but are supercoiled



Eubodonida

genus *Bodo*

- free-living and parasitic, 3-15 µm
- two heterodynamic flagella: a short anterior projecting flagellum and a longer posterior-projecting flagellum without hairs (acronematic) that extends beyond the length of the cell
- free-living bacteriotrophs



Bodo urinarius

- contaminated water, cysts

Bodo saltans

- free-living, distributed throughout the world in both freshwater and marine environments
- a key species to study the origin of the parasitic trypanosomatids; complete genome sequencing

Bodo saltans

The Kinetoplastida (Euglenozoa) are unicellular flagellates that include the trypanosomatid parasites, most notably *Trypanosoma brucei*, *T. cruzi* and *Leishmania spp.* These organisms cause substantial mortality and morbidity in humans and their livestock worldwide as the causative agents of African sleeping sickness, Chagas disease and leishmaniasis respectively. Draft genome sequences are available for several species of both *Trypanosoma* and *Leishmania*, many of which are described elsewhere in these pages. *Bodo saltans* is a free-living heterotroph found worldwide in freshwater and marine habitats. It is a kinetoplastid, but not a trypanosomatid, and possesses the diagnostic kinetoplastid features, such as flagella sited within a specialised flagellar pocket, glycolytic processes confined to a dedicated organelle (the 'glycosome'), and the characteristic concentration of mitochondrial DNA at the base of the flagellum (the 'kinetoplast').

The purpose of a *B. saltans* genome sequence is to provide an 'outgroup' for comparative genomic studies. As it is among the closest bodonid relatives of the trypanosomatids, it will provide a model of the ancestral trypanosomatid to distinguish those derived parts of the parasite genomes (i.e., unique trypanosomatid adaptations) from those which are a legacy of the free-living ancestor. This objective can be resolved into three principal comparative issues:

1. Trypanosomatid disease; understanding how human trypanosomatid parasites acquired their distinct pathological strategies;

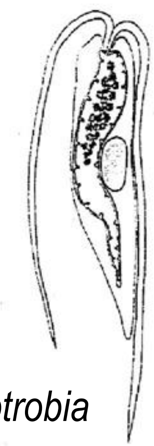
2. Evolution of parasitism; understanding how the ancestral trypanosomatid became parasitic in terms of derived innovations (e.g., cell surfaces) and loss of genomic repertoire;

3. Eukaryotic evolution; understanding how typical kinetoplastid features (e.g., glycosomes) evolved and how these might have been modified for parasitism.

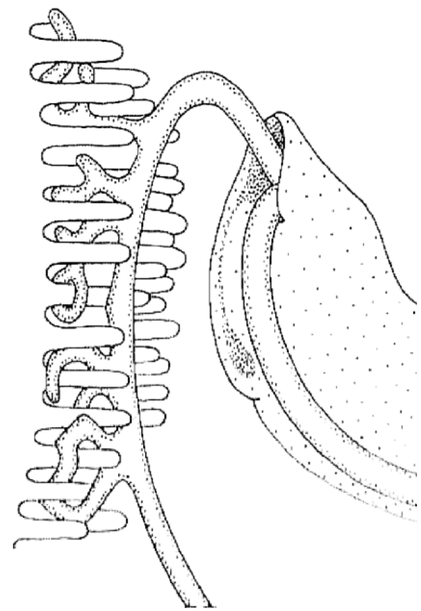
Parabodonida

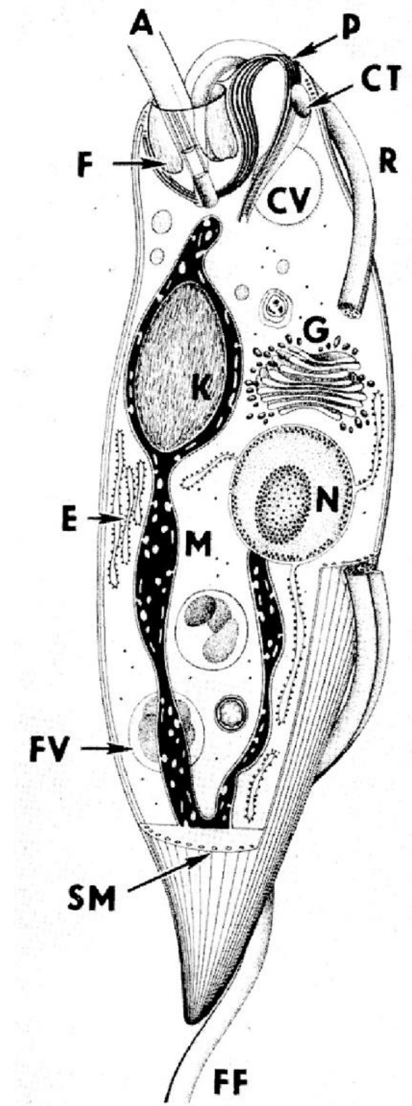
genus ***Cryptobia***

- no cysts
- ectocommensals or ectoparasites of fish and amphibians - gills and skin; endocommensals or endoparasites of invertebrates and poikilothermal vertebrates (blood, digestive tract)
- two flagella: free anterior flagellum with folds and a recurrent posterior flagellum marking the outer margin of undulating membrane
- attached by recurrent flagellum
- ventral and dorsal stripes of microtubules



Cryptrobia





Parabodonida

genus ***Cryptobia***

Cryptobia branchialis

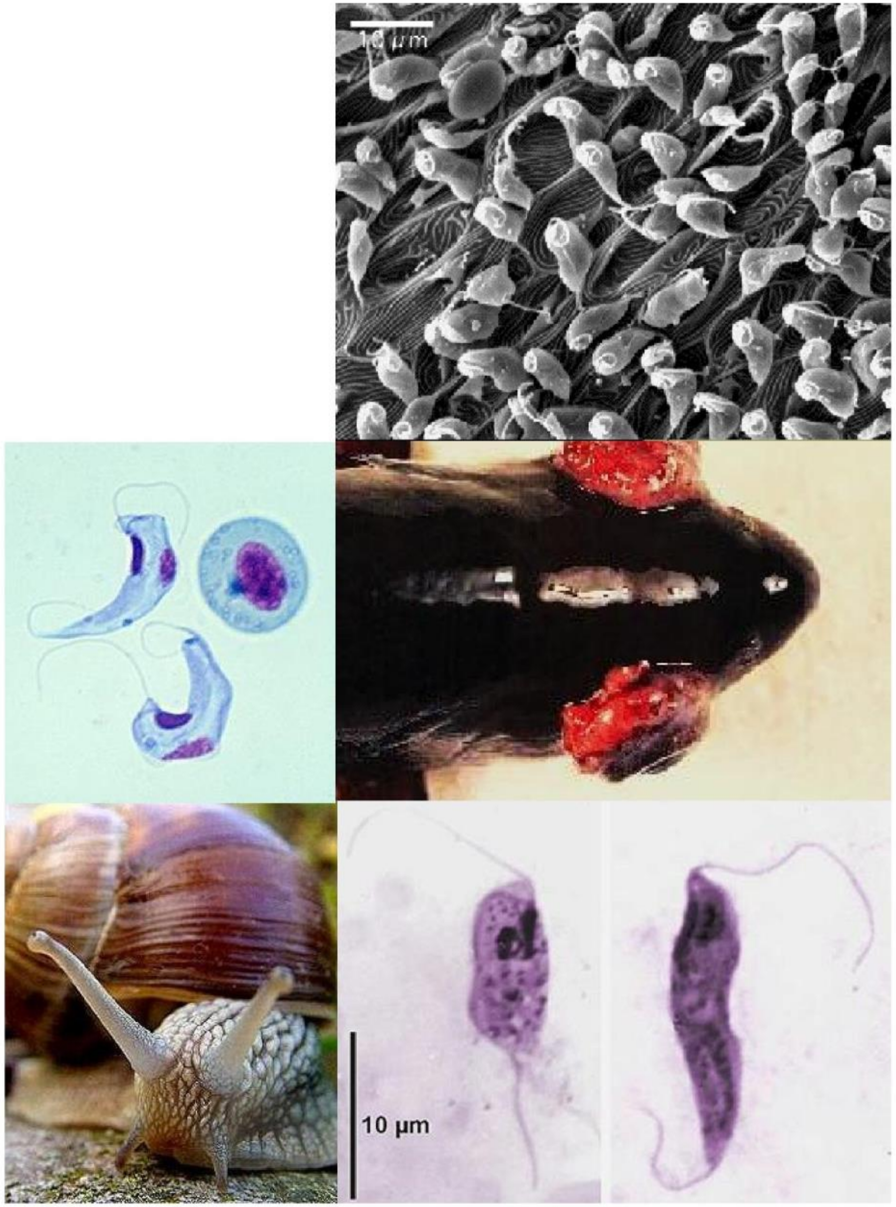
- ectoparasites on fish skin or gills
- fish are anorexic and swim close to a water surface

Cryptobia salmositica

- blood parasites causing anemia and lesions in the hematopoietic tissues of salmonids
- transmission by blood-feeding leeches

Cryptobia helicis

- receptaculum seminis of snails



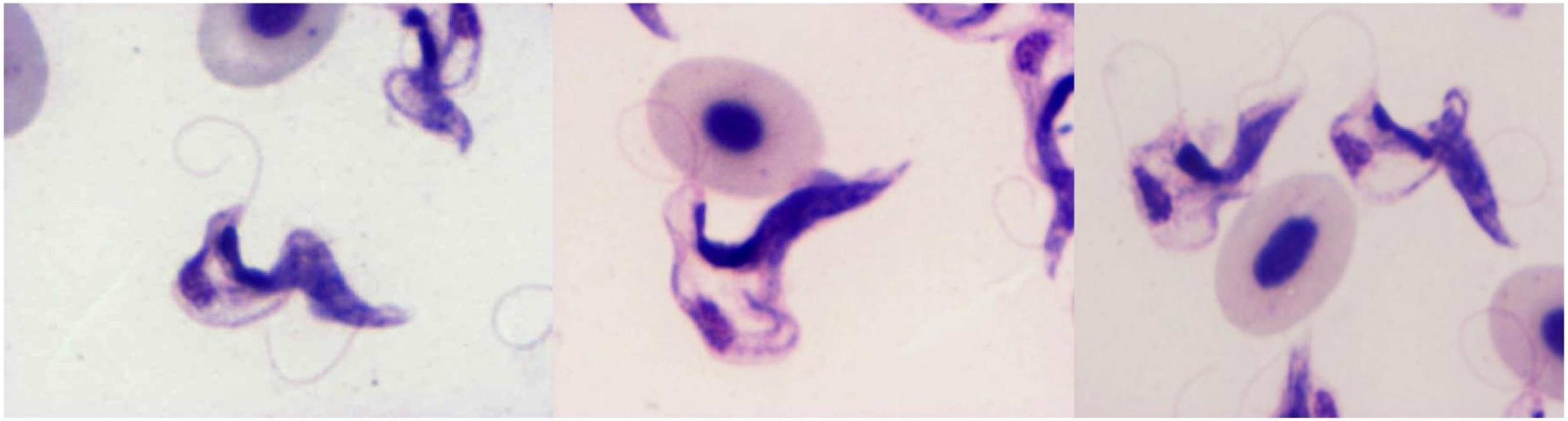
Parabodonida

genus ***Trypanoplasma***

- haematozoic, digenetic endoparasites transmitted by leeches (*Piscicola* sp.)
- undulating membrane and short anterior flagellum
- large kinetoplast, surface glycocalyx – thinner in stages from leeches
- non-sterile immunity

Trypanoplasma borreli

- 18-30 x 3-5 µm, pathogenic in cyprinid fish ⇨ anaemia and splenomegaly
- affecting wild fish and commercial fisheries



Trypanoplasma borreli

Trypanoplasma (Cryptobia) borreli is a haematozoic endoparasite transmitted by leeches (*Piscicola* sp.). As a species of *Cryptobia*, *T. borreli* causes cryptobiosis in cyprinid fish, characterized severe anemia and splenomegaly, affecting both wild fish and commerical fisheries. *T. borreli* is often found co-infecting fish with *Trypanosoma* spp.; both *Cryptobia* spp. (Parabodonidae) and *Trypanosoma* spp. (Trypanosomatidae) are members of the Kinetoplastida and represent independent origins of blood parasitism.

As part of our efforts to understand the evolution of trypanosomatid genomes, we have produced a draft genome sequence for *T. borreli* K-100 (ATCC 50432) using the Illumina HiSeq platform. 400bp and 3kb-insert libraries were created from whole genomic DNA isolated from an axenic *T. borreli* culture. The primary purpose of the genome sequence is to provide an outgroup for the comparison of trypanosomatids with *Bodo saltans*, a free-living kinetoplastid more closely related to trypanosomatids than *T. borreli*. When compariing the free-living *B. saltans* with parasitic trypanosomatids, we need to distinguish losses of conserved kinetoplastid genes in trypanosomatids (present in *T. borreli*) from Bodo-specific gene gains (absent in *T. borreli*). Secondarily, we will compare the *T. borreli* and *Trypanosoma brucei* genomes to explore any similarities associated with the convergent evolution of blood parasitism.

Published Genome Data

The *T. borreli* genome sequence was assembled from 100bp paired-end Illumina reads from a 400bp-insert library using *Velvet*. This assembly was then corrected for misassembly and subsequently expanded using reads from a 3kb-insert library using custom scripts and *IMAGE*.

The final assembly contains 25,816,007bp in 23,265 contigs (N50 = 12100bp). The average contig length is 1109.6 bp and the largest contig is 133333bp.

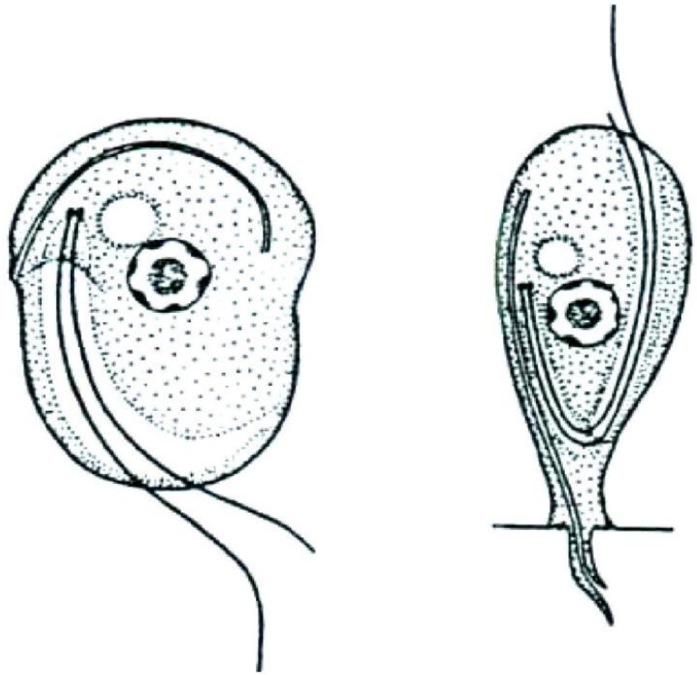
Prokinetoplastida

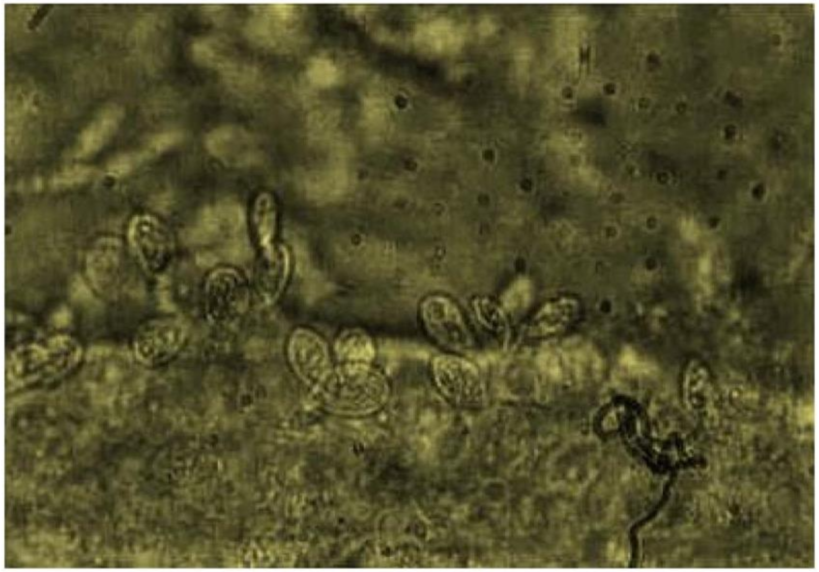
genus ***Ichtyobodo*** (syn. *Costia*)

Ichthyobodo necator (syn. *Costia necatrix*)

Ichthyobodo hippoglossi

- complex of species
- does not form cysts and lasts several hours without host
- 10 x 5 µm, feeding via cytostome and cytopharyngeal canal protruding into the host cell
- gills and skin of fish + occasionally amphibian tadpoles, attached by an attachment plate ⇨ prominent mucus production
- heavy infected fish exhibit anorexia and petechial haemorrhagic lesions in the skin





Ichtyobodo necator ⇨ cytopharyngeal canal protruding into the host cell

Trypanosomatida (trypanosomatids)

lower trypanosomatids

monoxenous

intestine of insects
transmission to plants

genera *Leptomonas*
Phytomonas
Crithidia
Blastocrithidia
Herpetomonas

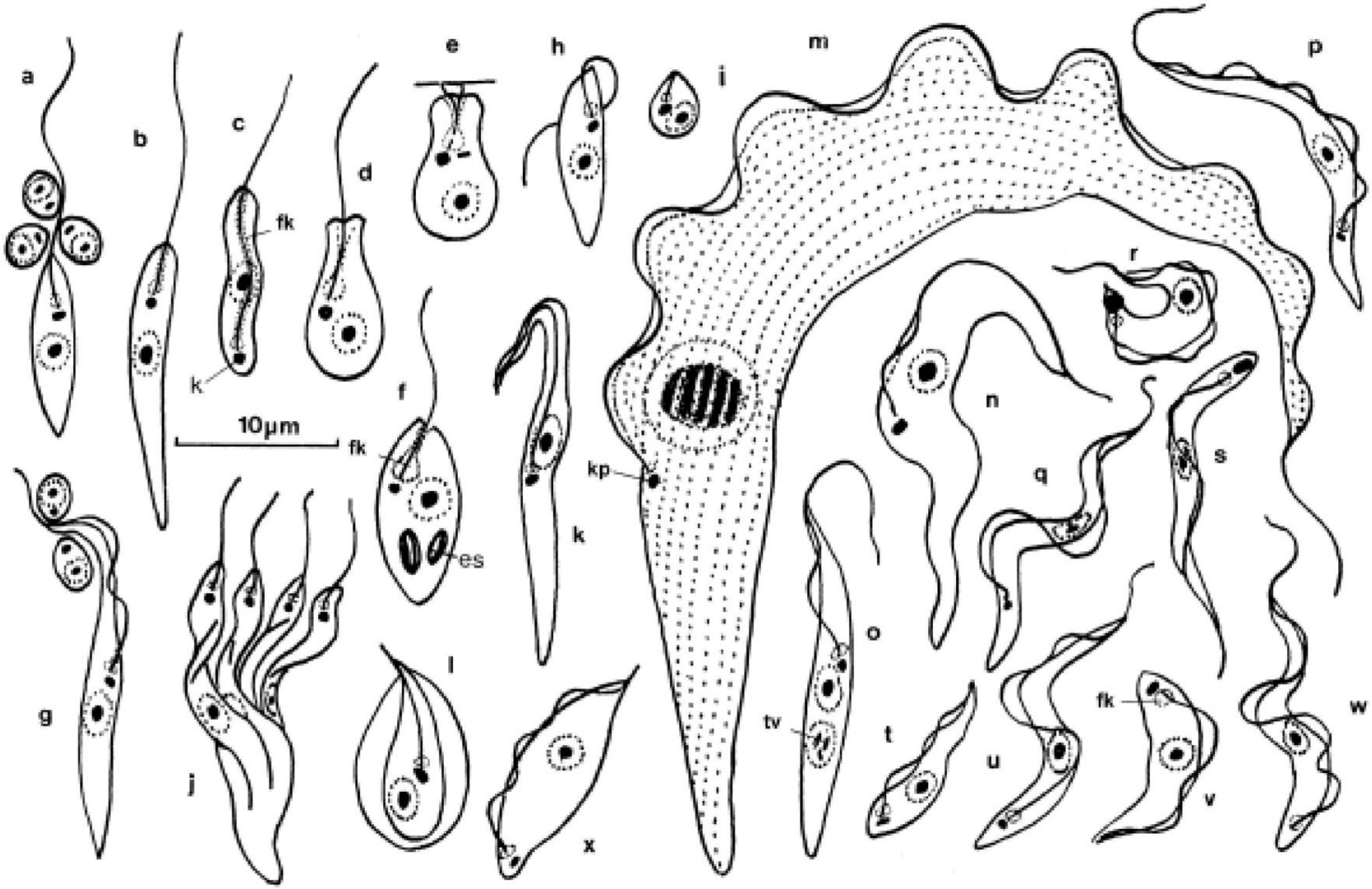
higher trypanosomatids

dixenous

invertebrate vector
vertebrate host

Trypanosoma
Endotrypanum
Leishmania

Morphology of trypanosomatids



Major morphological classes of trypanosomatids

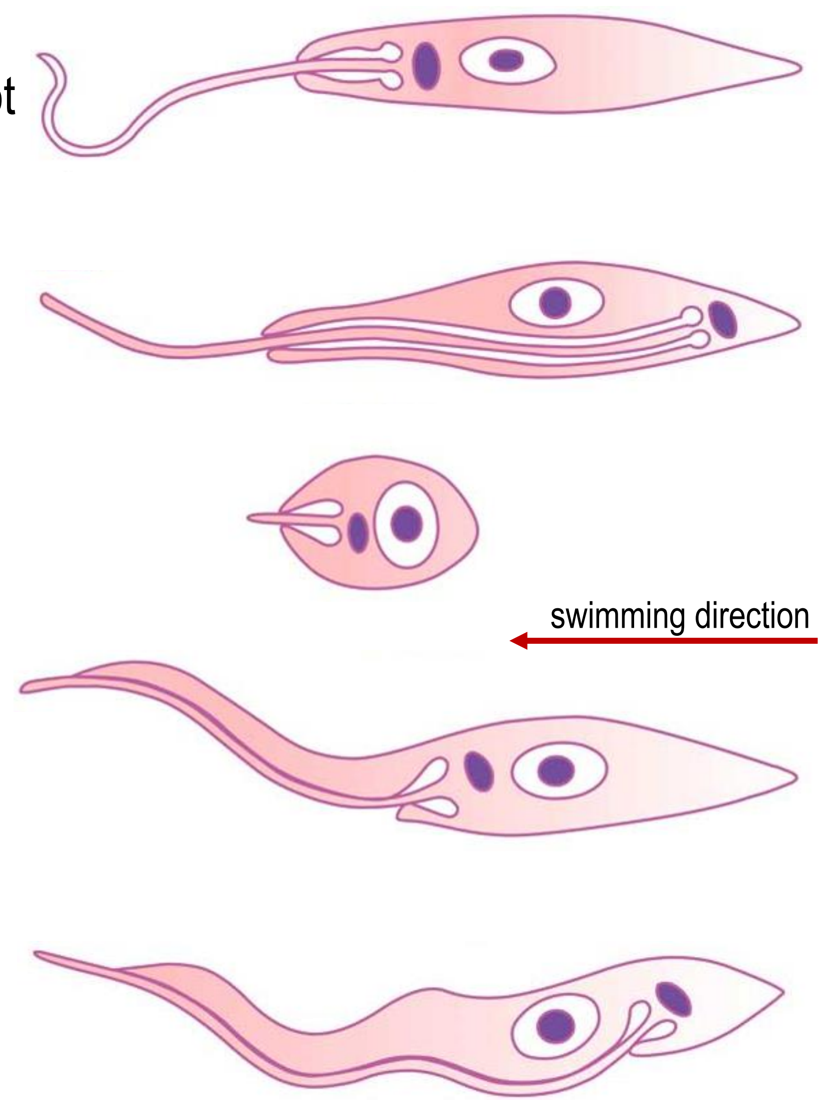
Promastigote (leptomonad)* – flagellum and kinetoplast anterior to the nucleus and flagellum not attached to the cell

Opisthomastigote (herpetomonad)* - flagellum posterior to the nucleus, passing through a long groove in the cell

Amastigote (leishmanial)* - very short flagellum, projecting only slightly beyond the flagellar pocket

Epimastigote (crithidial)* - flagellum exits the cell anterior to the nucleus and is connected to the cell for a part of its length by undulating membrane, kinetoplast located between the nucleus and the anterior cell end

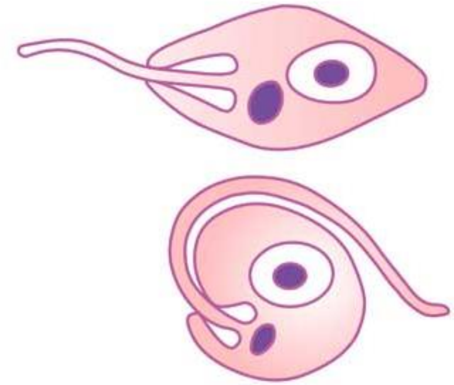
Trypomastigote (trypanosomal)* - flagellum lies attached to the cell for most of its length by undulating membrane, kinetoplast located near the posterior cell end

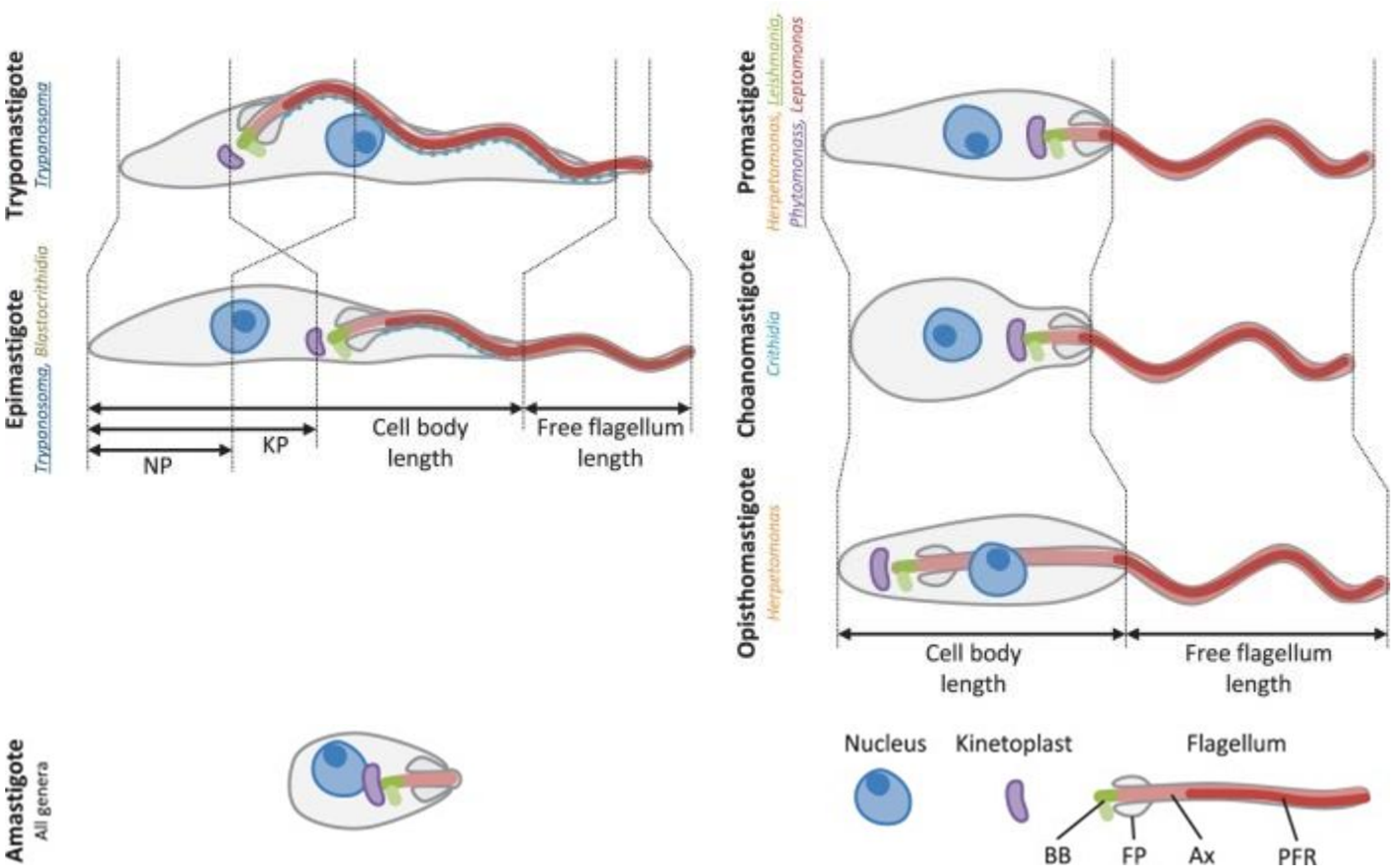


* in Czech: leptomonádové, herpetomonádové, leishmaniové, crithidiové a trypanosomové stádium

Choanomastigote – flagellum emerges through a collar-like extension surrounding the anterior cell end

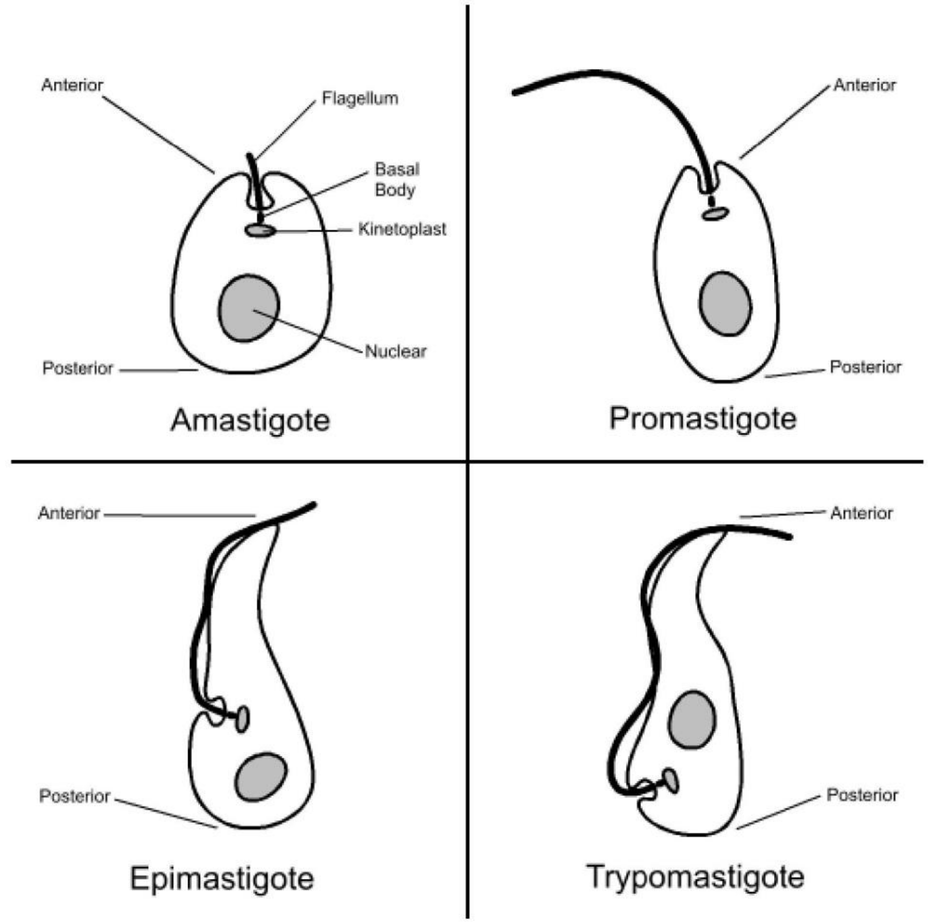
Spheromastigote - flagellum develops and begins to function



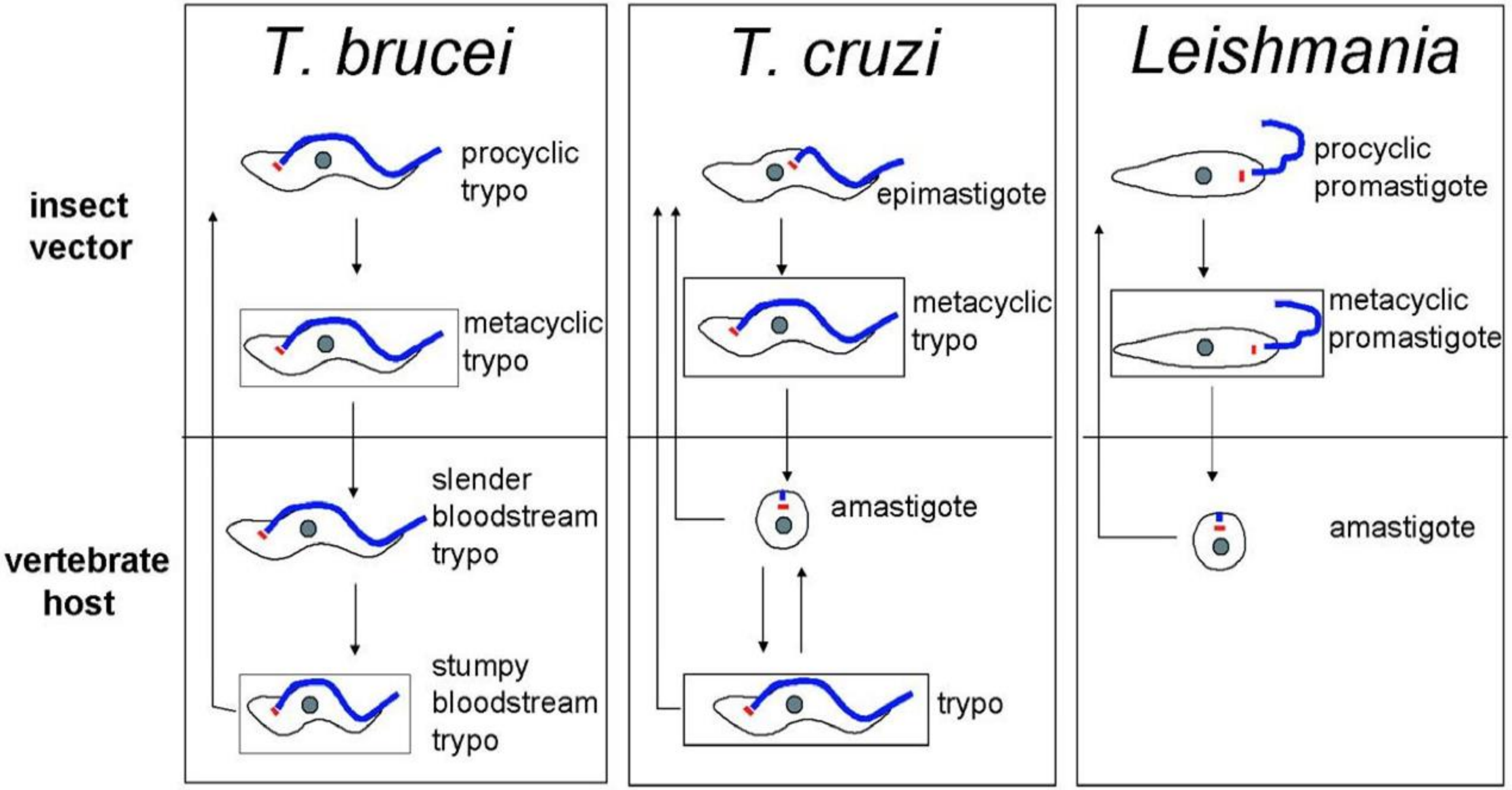


Czech etymology of morphological forms

- „a“ - řecky = bez
- „pro“ - řecky = před
- „epi“ - řecky = nad
- „trypanon“ - řecky = vrták
- „choane“ - řecky = nálevka
- „opisthe“ - řecky = vzadu

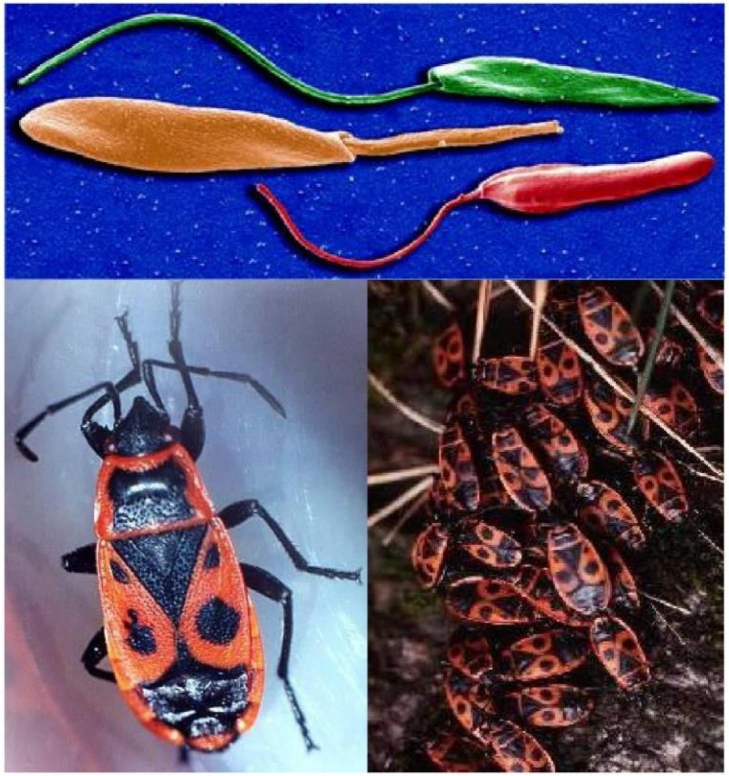


Morphological forms of higher trypanosomatids



Malcolm J. McConville et al. Microbiol. Mol. Biol. Rev. 2002;66:122-154

Lower trypanosomatids



genus ***Leptomonas***

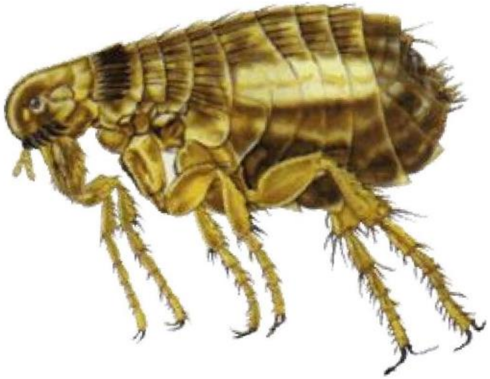
- promastigotes
- cyst-like stages (amastigotes in the form of pseudocyst)

Leptomonas pyrrhocoris

- intestine of *Pyrrhocoris apterus*

L. ctenocephali

- intestine of *Ctenocephalides canis*



Lower trypanosomatids

genus ***Phytomonas***

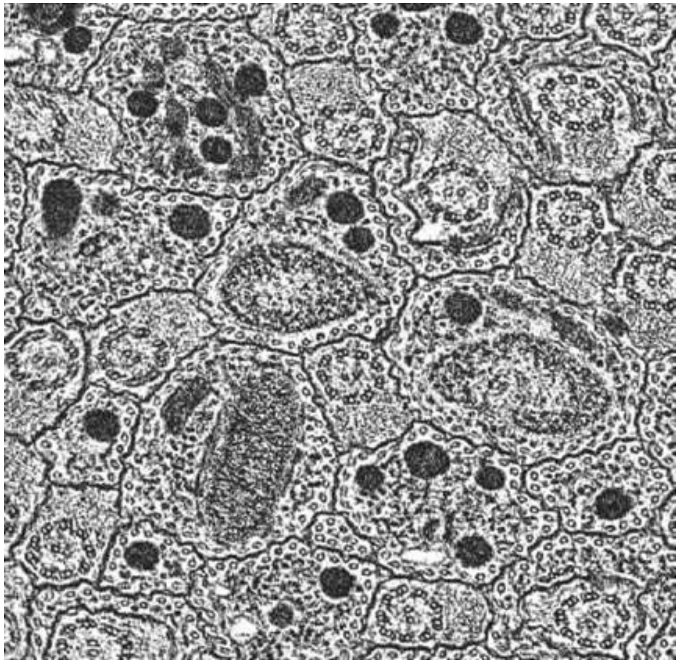
- promastigotes
- most identified species have not been associated with any plant pathology
- only two species spread by different insects cause plant disease

Phytomonas staheli

- “hartrot“ (fatal wilt of palms) of coconut palm (*Cocos nucifera*)

P. leptovasorum

- coffee phloem necrosis



genus ***Crithidia***

- epimastigotes
- common parasites of insect gut

Crithidia oncopeli

- true bug *Oncopeltus fasciatus*

genus ***Blastocrithidia***

- epimastigotes, cysts

Blastocrithidia culicis

- *Aedes* and *Culex* mosquitos

B. triatomae

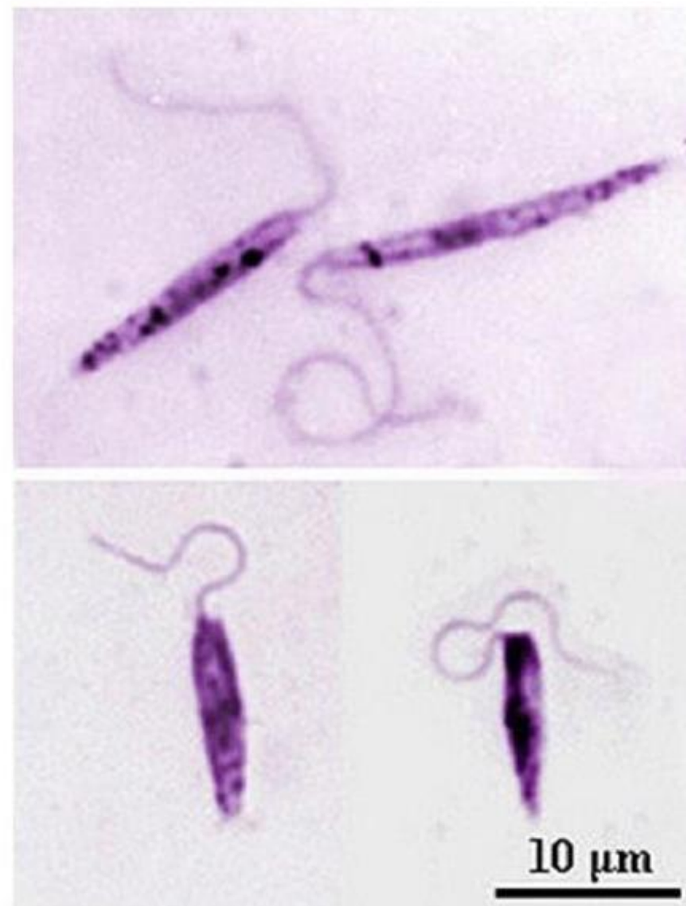
- true bugs (*Triatoma infestans*)

genus ***Herpetomonas***

- dominant promastigote stage in the life cycle but producing also opisthomastigotes
- parasite of Diptera and possibly other insects

Herpetomonas muscarum

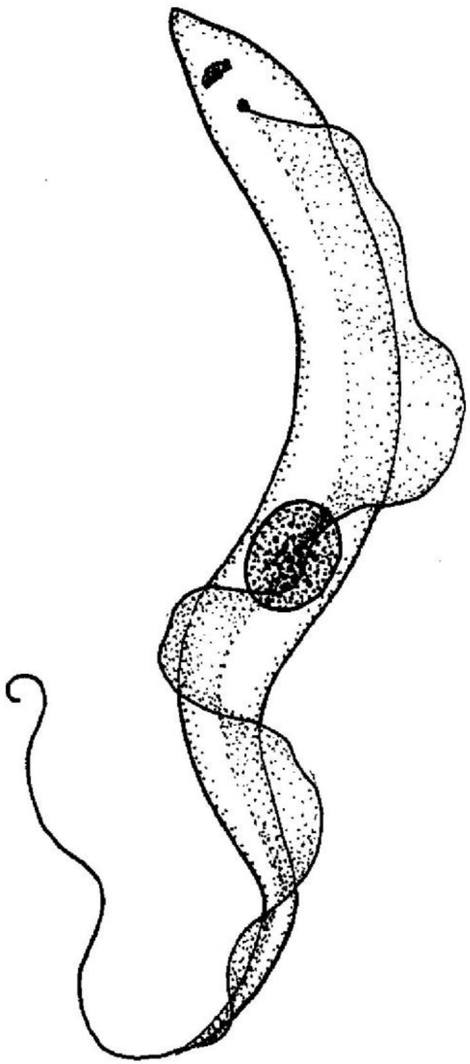
H. ztiplika



Higher trypanosomatids

genus ***Trypanosoma***

- more than 300 species in all vertebrates families



Stercoraria (stercus = faeces)

Stercorarian trypanosomes infect the insect when taking a blood meal, most often a triatomid kissing bug, develop in its posterior gut and infective stages are released in the faeces and deposited on the skin of host vertebrate. Parasites then penetrate and can disseminate throughout the host body.

Salivaria (saliva)

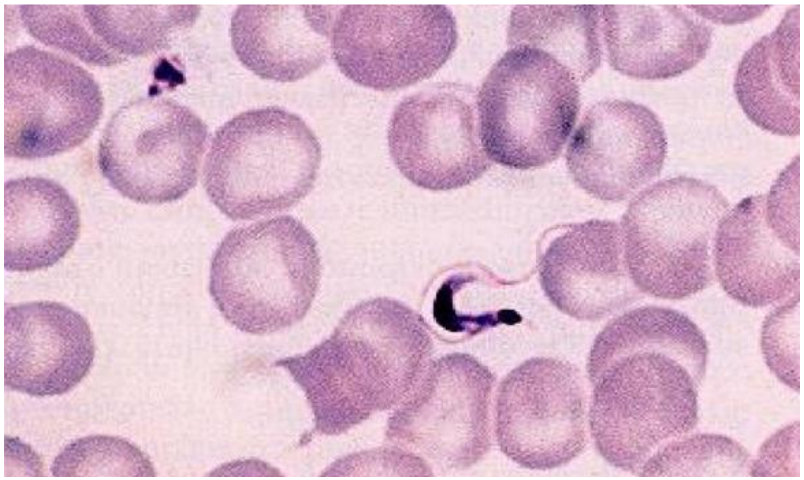
Salivarian trypanosomes develop in the anterior gut of insects, most importantly the Tsetse fly, and infective stages are inoculated into the vertebrate host via the insect bite prior to feeding.

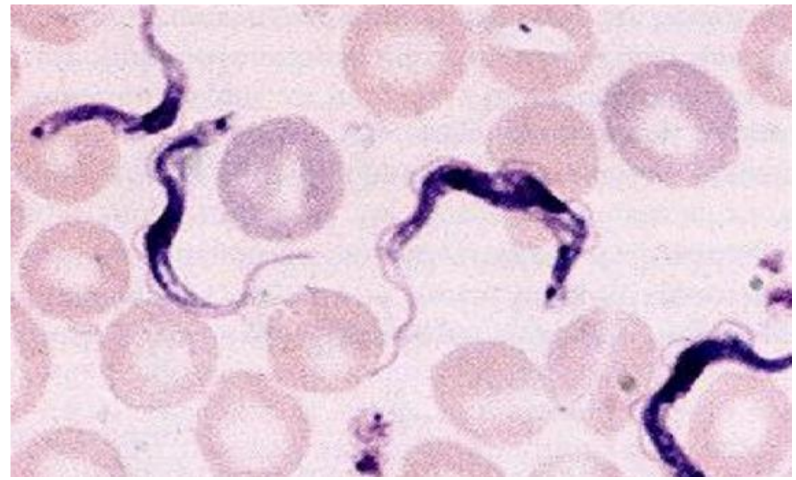
Stercoraria

Trypanosoma cruzi

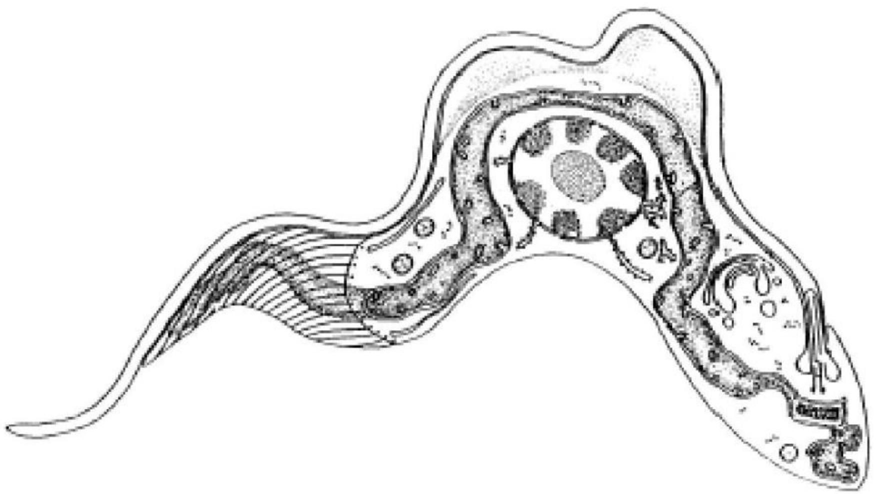
Salivaria

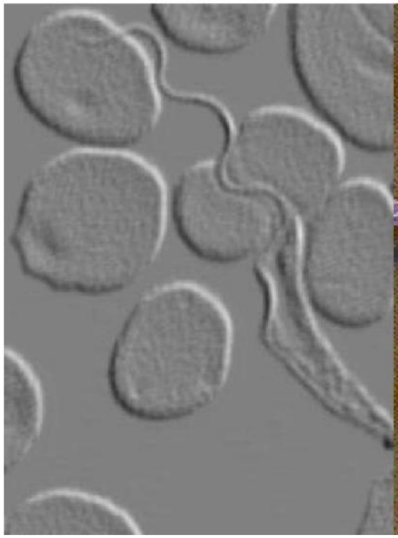
Trypanosoma brucei

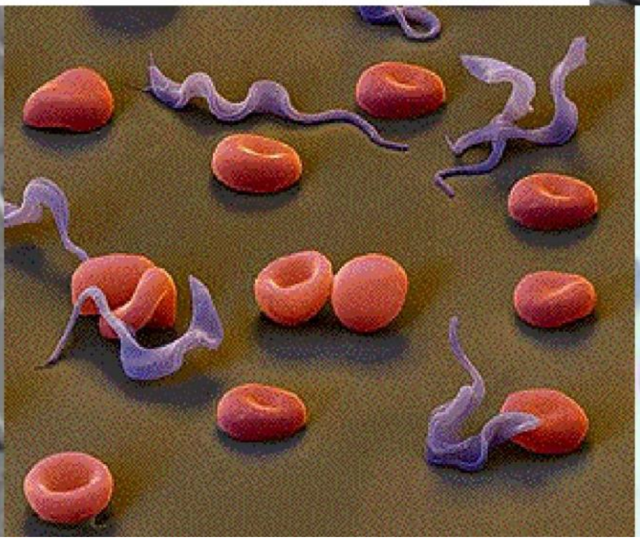


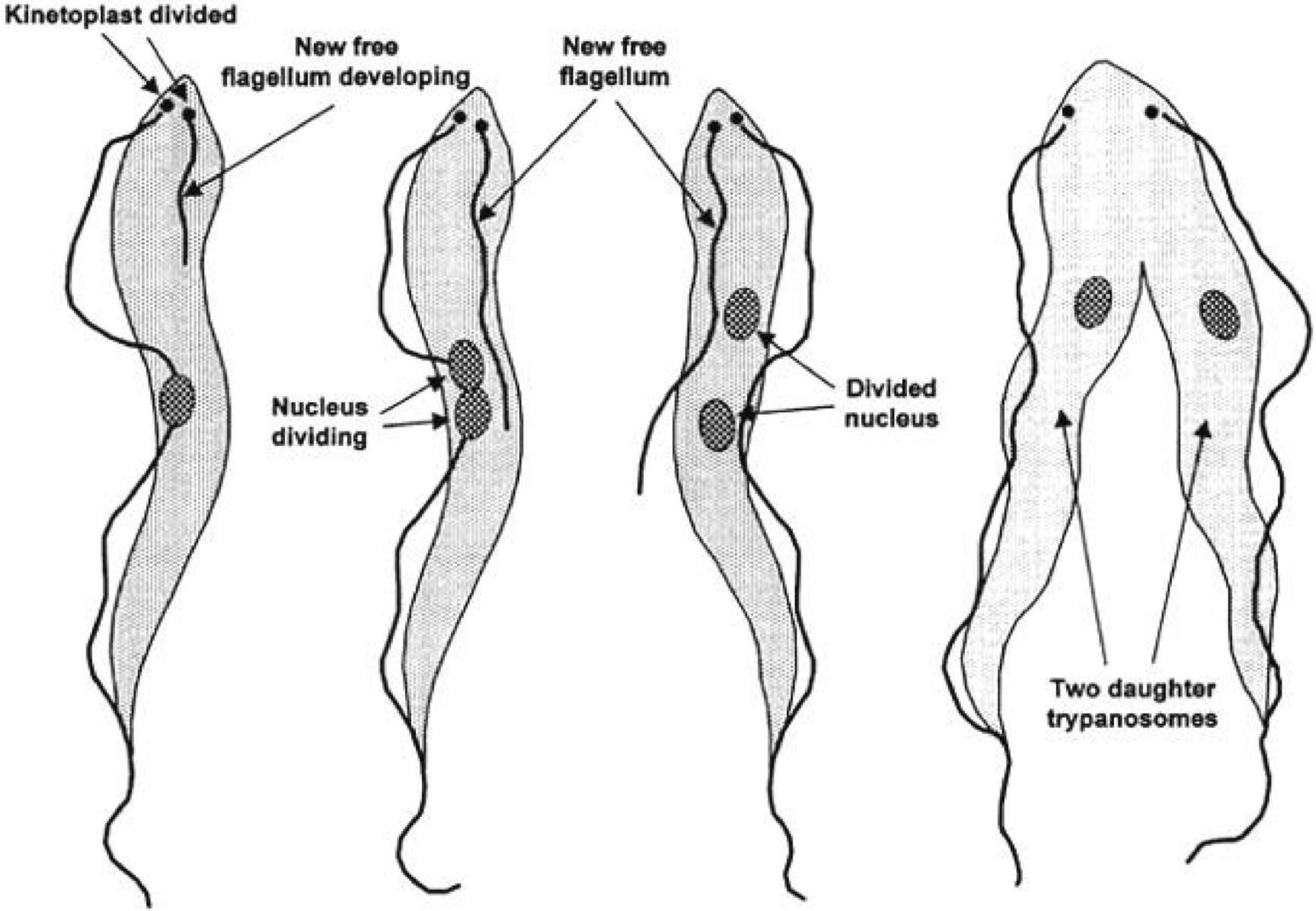


Morphology of ***Trypanosoma*** spp.







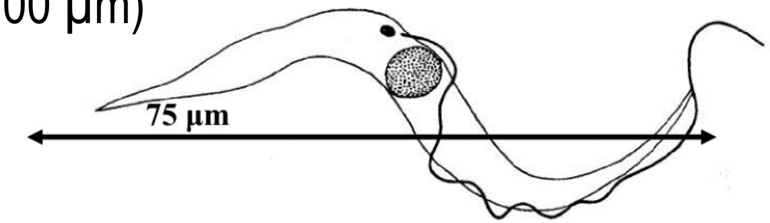

Kinetoplast divided
New free
flagellum developing
New free
flagellum
Nucleus
dividing
Divided
nucleus
Two daughter
trypanosomes

Stercoraria

- multiplication in vertebrate blood and tissues – epimastigotes or amastigotes
- intracellular in visceral tissues
- originally classified in subgenera ***Megatrypanum***, ***Herpetosoma***, ***Schizotrypanum***

subgenus ***Megatrypanum***

- largest mammalian blood trypanosomatids (40-100 µm)
- ruminant hosts
- epimastigotes multiplying in vertebrates
- small kinetoplast situated very close to the nucleus

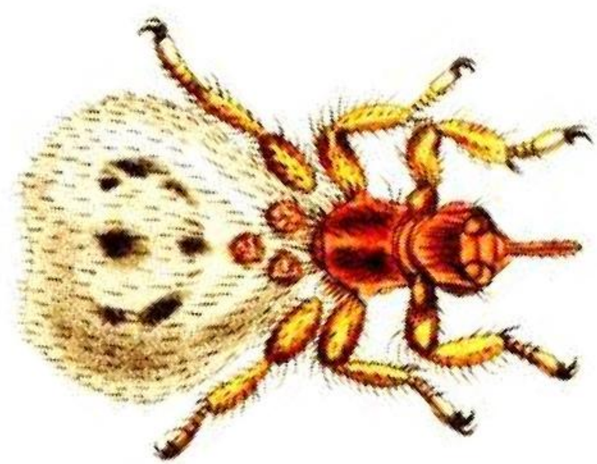


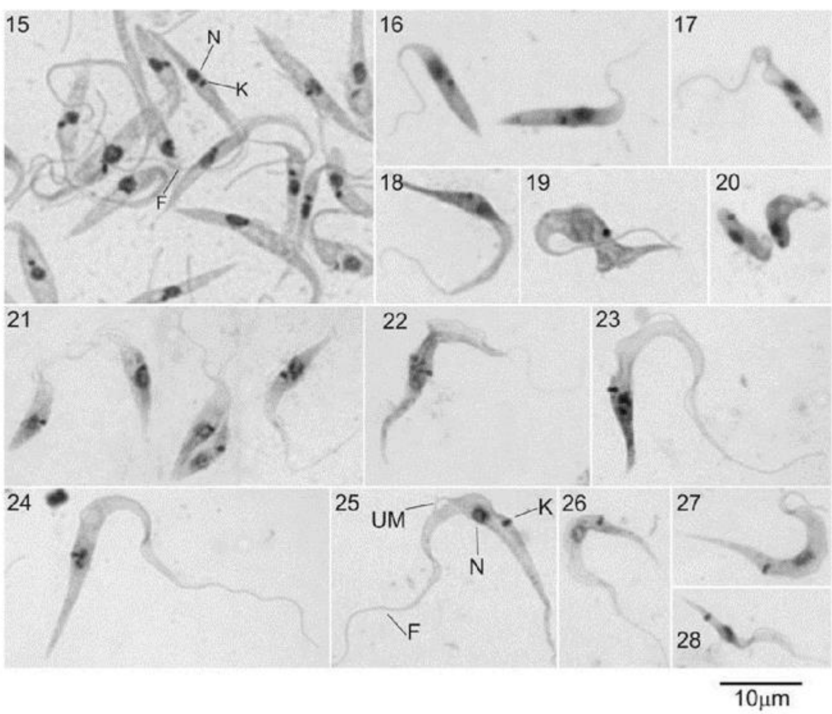
Trypanosoma (Megatrypanum) theileri

- host: cattle, other ruminants
- vector: tabanid flies
- distributed worldwide from the tropics to near the Arctic Circle, with higher prevalence in tropical and neotropical areas
- considered non-pathogenic

Trypanosoma (Megatrypanum) melophagium

- sheep parasite transmitted by louse flies, the sheep restricted ectoparasite *Melophagus ovinus* (Diptera: Hippoboscidae)
- non-pathogenic
- in about 90 % of sheep louse flies





subgenus ***Herpetosoma***

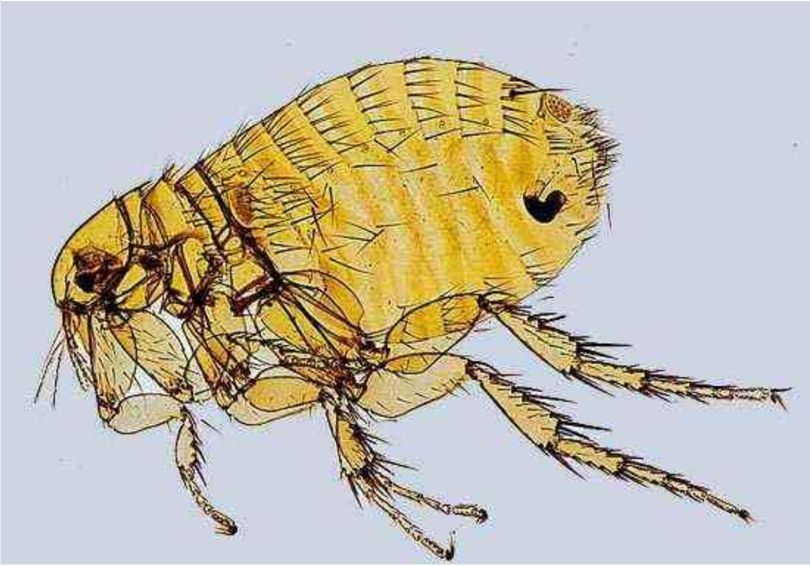
- medium sized trypanosomatids (20-40 µm)
- mostly rodent hosts
- epimastigotes multiplying in vertebrates
- non-pathogenic

Trypanosoma (Herpetosoma) lewisi

- host: rats, but found also in primates including man
- vector: fleas (*Nosopsyllus fasciatus*, *Xenopsylla cheopis*)

Trypanosoma (Herpetosoma) musculi

- host: house mouse (*Mus musculus*)
- vector: fleas



subgenus ***Trypanosoma*** (*Schizotrypanum*)

- small trypanosomes (15-24 µm)
- multiplication in vertebrates – amastigotes

Trypanosoma (*Schizotrypanum*) *cruzi*

- Chagas disease
- host: > 100 mammal species (rodents, opossums, armadillos, dogs..) including man
- vector: "barbieros“ or „kissing bugs„ triatomine of the family Reduviidae (*Triatoma*, *Rhodnius*, *Panstrongylus* + other 12 genera); aggregating in refuges during day and searching for blood during night when the host is asleep, and the air is cooler

- transmission can occur through blood transfusions, organ transplantation, transplacentally, and in laboratory accidents

History of Chagas disease

- the oldest record of Chagas disease (*T. cruzi* DNA) has been found in almost 9,000 years old mummies from northern Chile and southern Peru

- evidence of Chagas disease vectors (*Triatoma infestans*) in human dwellings in pre-Columbian Inca and Chinchorro cultures, suggesting progressive introduction of domestic transmission

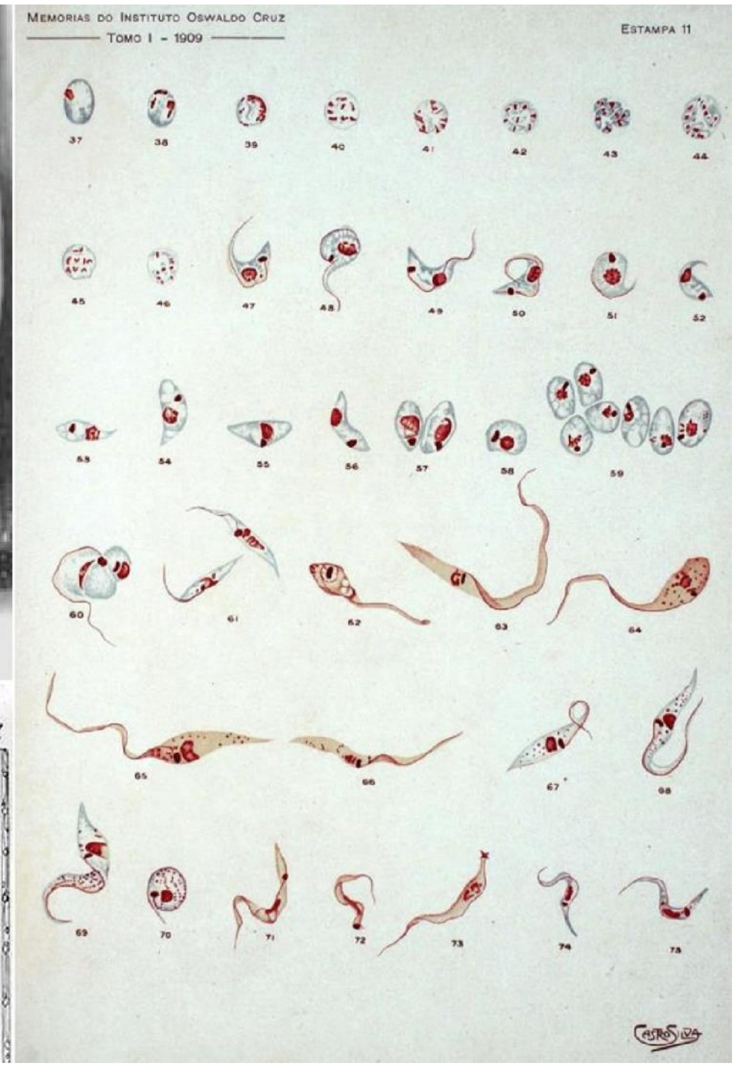
- over the past 200-300 years, with progressive deforestation for agriculture and livestock ranching and construction of transport routes (highways and railways), triatomine bugs increasingly lost their primary food source of wild-animal blood ⇨ more opportunities to spread

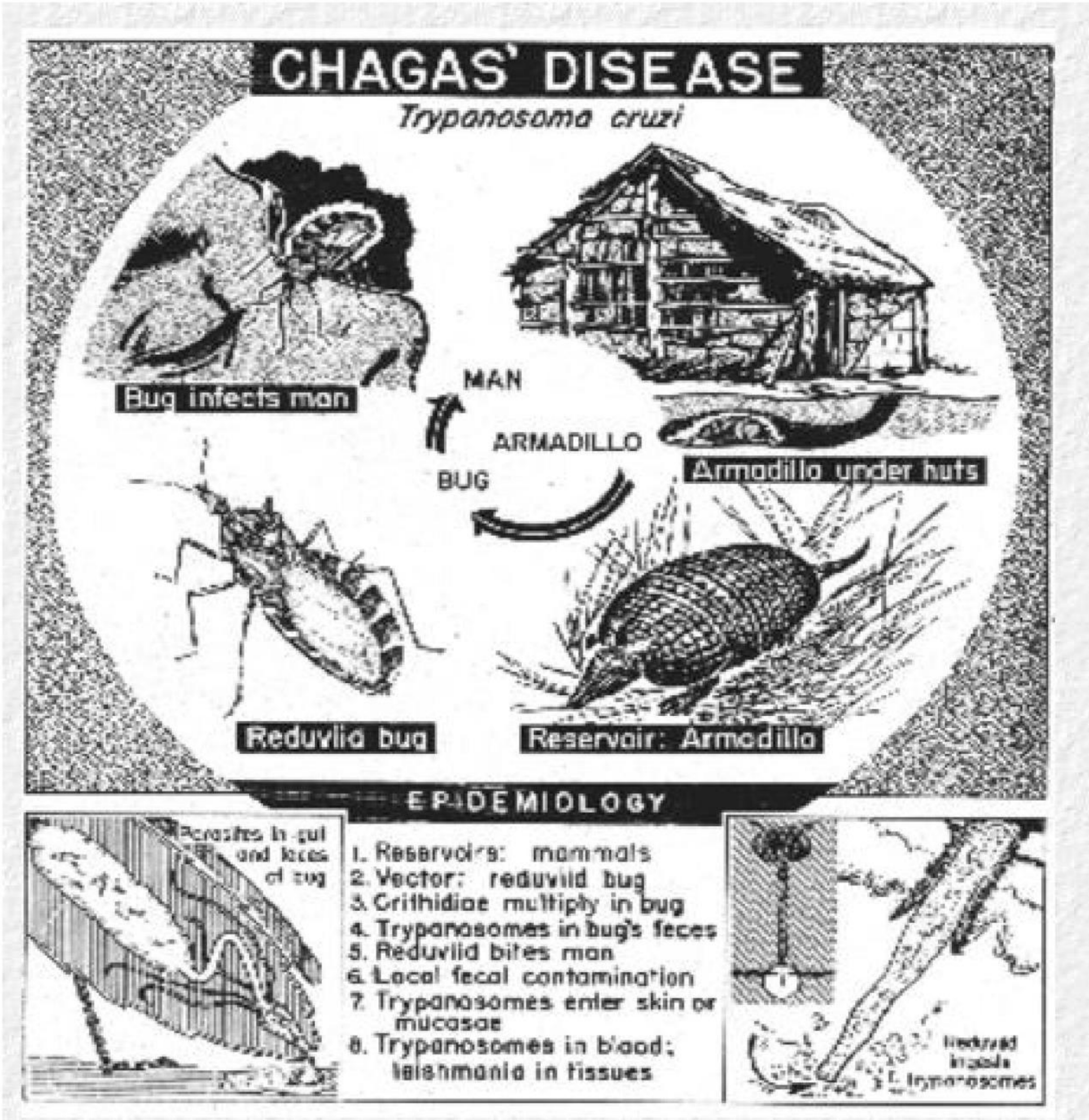
- 1907: dr. Carlos Chagas first becomes aware of the barbiero

- 1909: first publications on newly discovered trypanosome

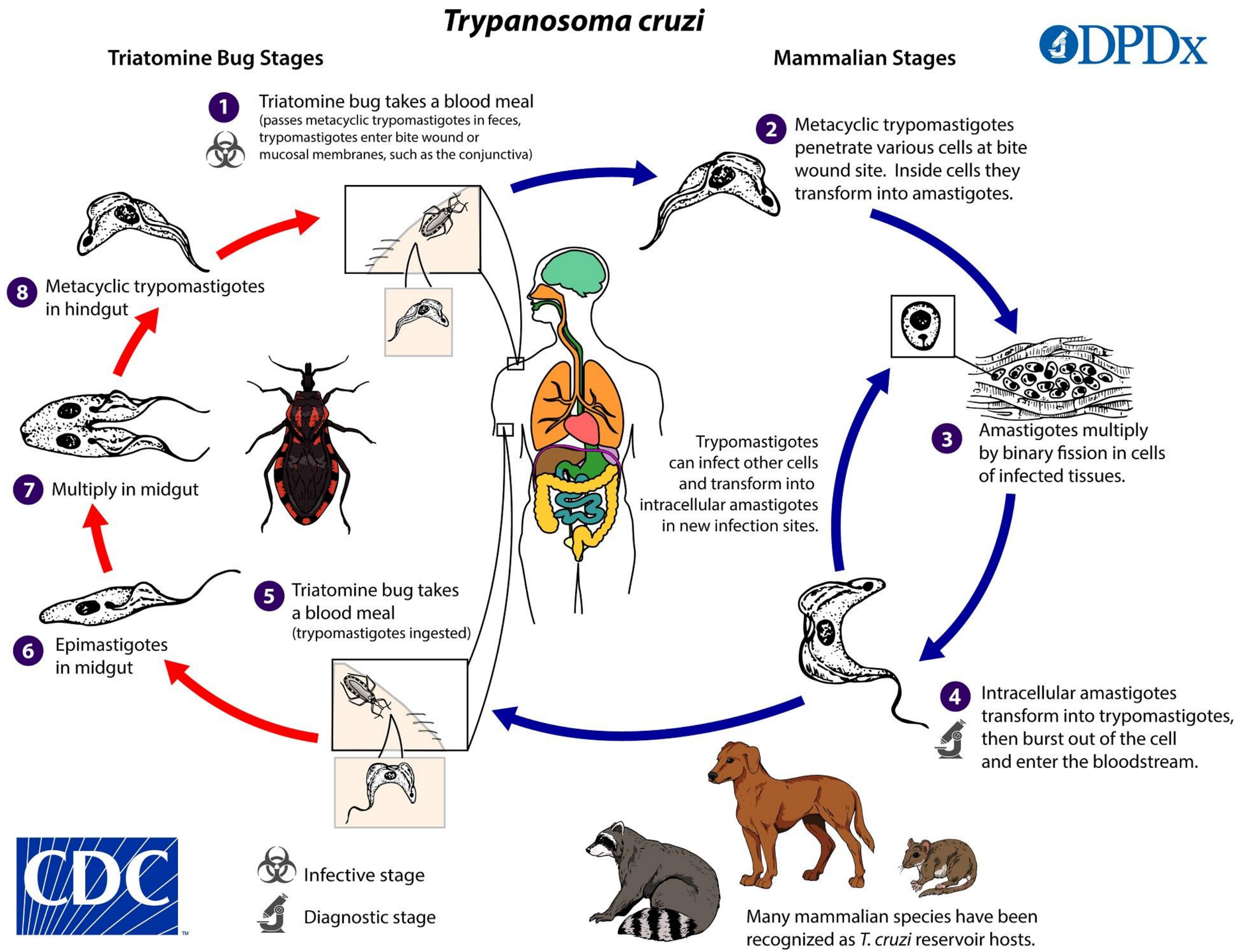
- 1930s: public health importance becomes known

Carlos Justiniano Ribeiro Chagas (1879-1934)



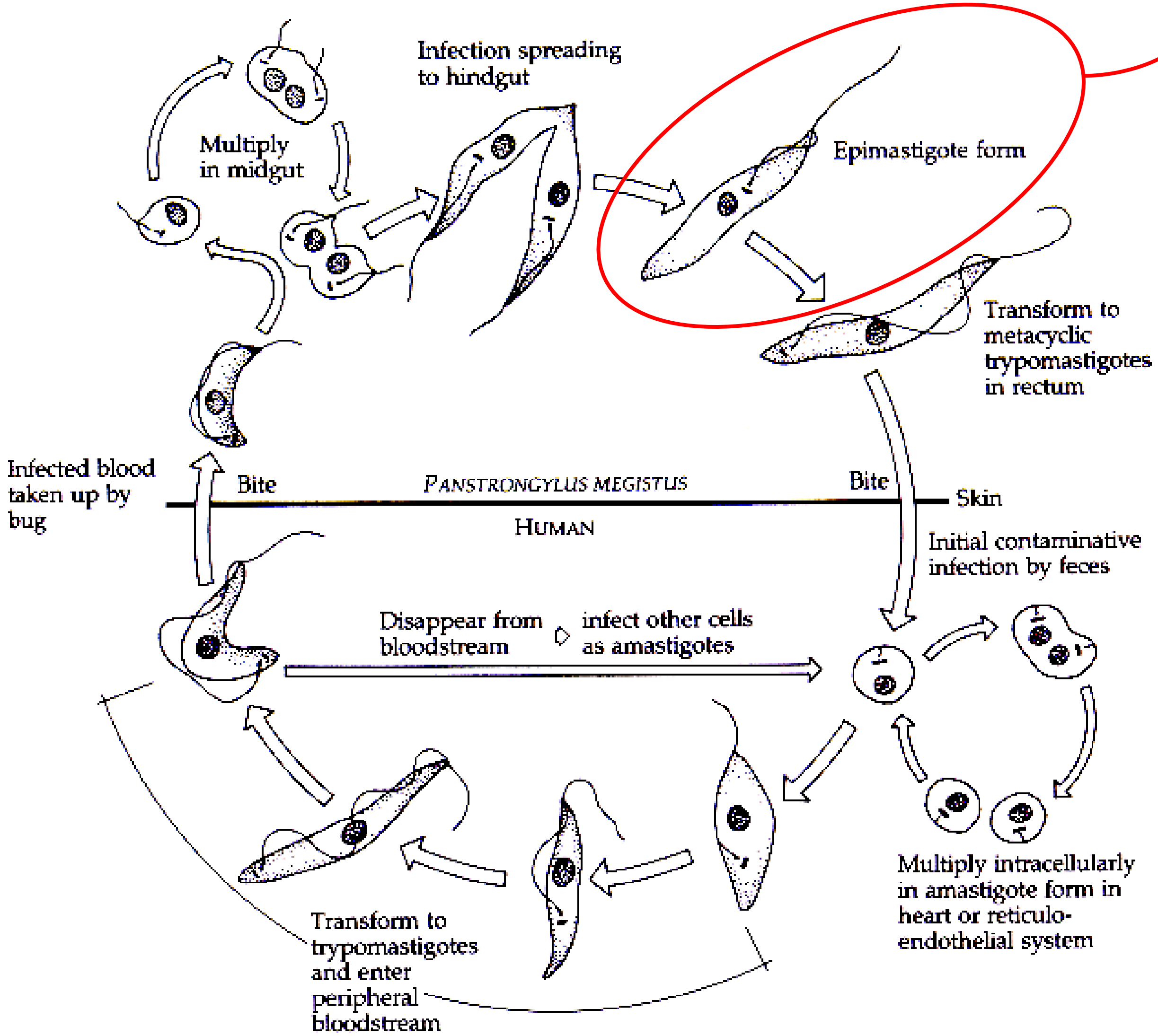

CHAGAS' DISEASE
Trypanosoma cruzi
Bug infects man
MAN
ARMADILLO
BUG
Armadillo under huts
Reduviia bug
Reservoir: Armadillo
EPIDEMIOLOGY
1. Reservoirs: mammals
2. Vector: reduviid bug
3. Crithidiae multiply in bug
4. Trypanosomes in bug's feces
5. Reduviid bites man
6. Local fecal contamination
7. Trypanosomes enter skin or mucosae
8. Trypanosomes in blood; leishmania in tissues

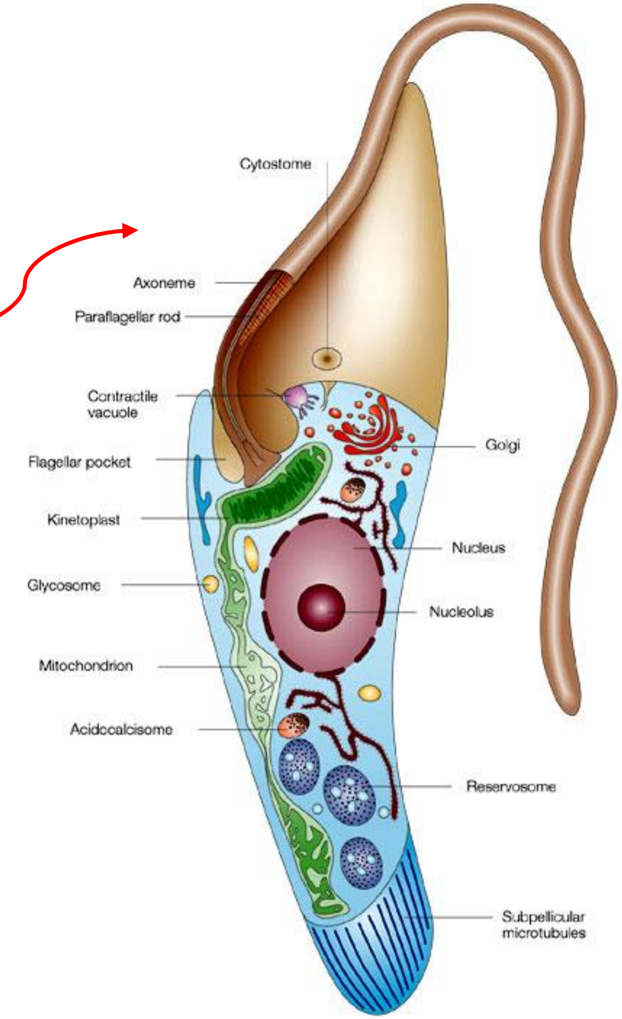
Chagas disease



https://www.youtube.com/watch?v=1ais69H0li8
https://www.youtube.com/watch?v=di72_yCsUVY

Life cycle of *Trypanosoma cruzi*





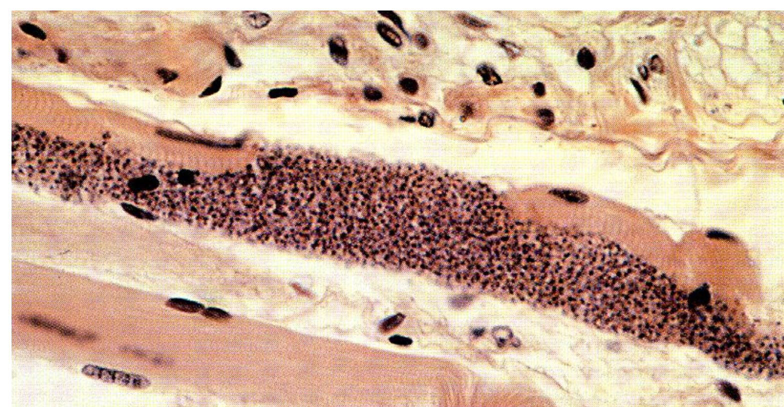
Chagas disease

Incubation period

- 5-4 days after exposure to triatomine insect faeces
- 20-40 days after blood transfusion

Acute phase

- restricted to the inoculation site
- most adults asymptomatic
- parasites found in blood
- Romaña's sign, a chagoma (=localised painless induration) over the eyelid - marker of acute Chagas disease infection
- oedema of eyes, conjunctivitis
- usually resolves in weeks to months



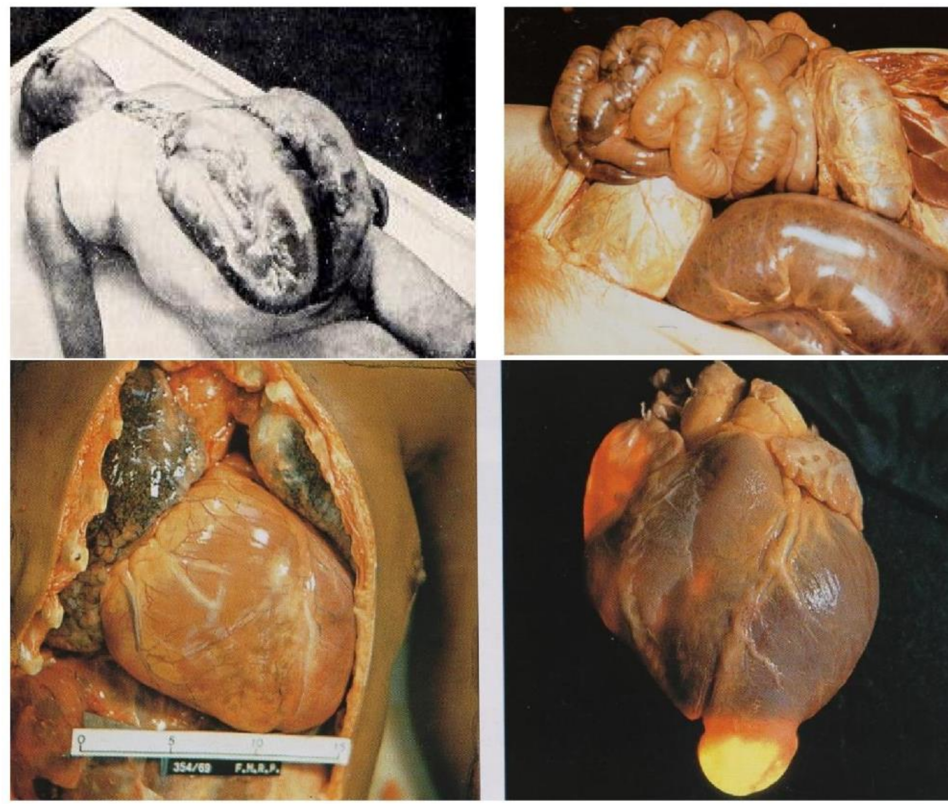
Chagas disease

Intermediate phase

- asymptomatic phase of varying length
- parasites disappear from blood ⇨ most patients enter chronic phase within 5 -15 years
- indeterminate phase can last as long as 40 years

Chronic phase

- degenerative disease of hollow organs and organ failures
- heart disease – most common chronic form
- digestive organs abnormalities - megaoesophagus, megacolon
- up to 10% of deaths in endemic areas
- successful treatment only in the acute phase (benznidazole, nifurtimox)



Chagas disease

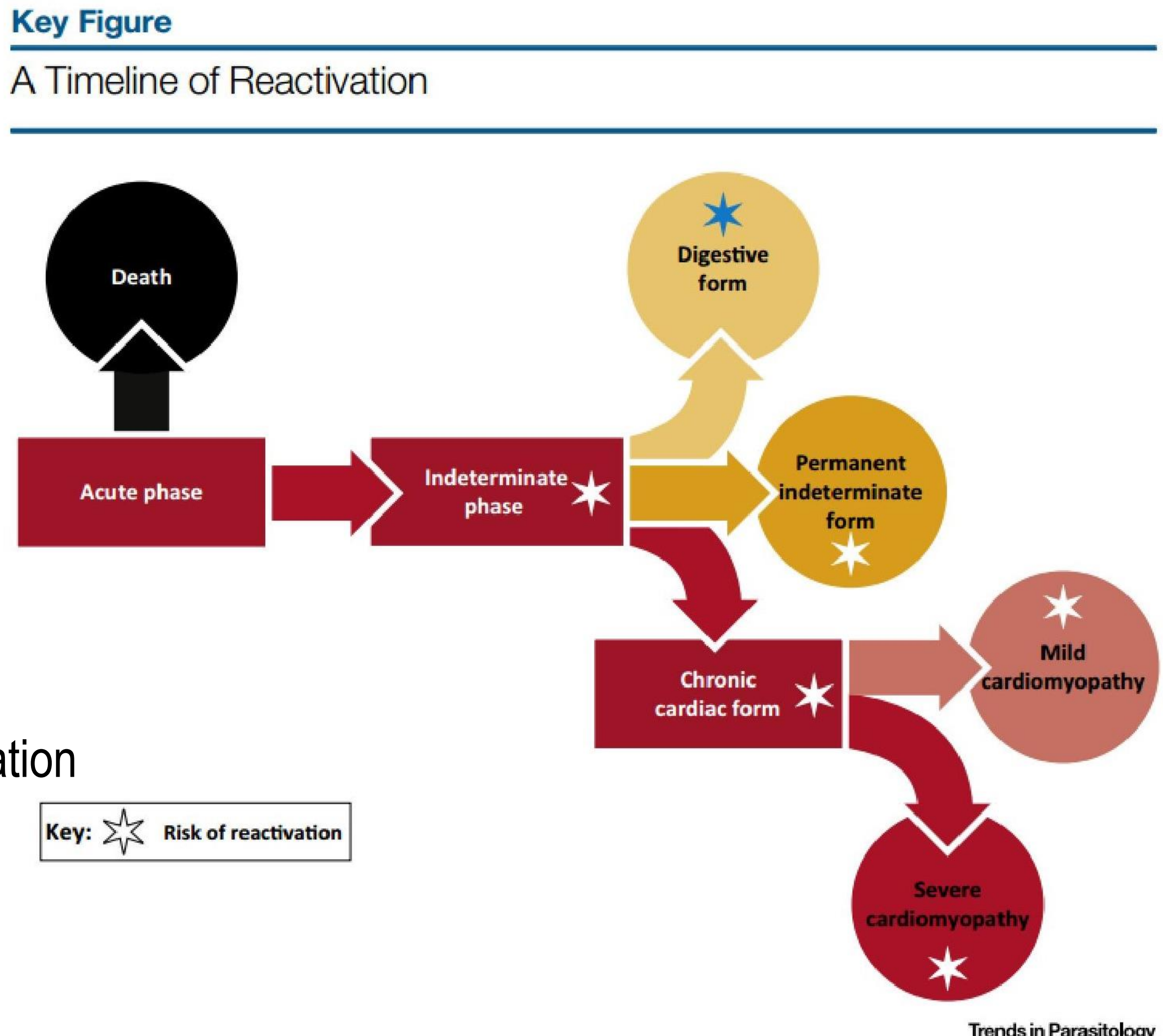
Immunocompromised people can be severely affected

Pregnant women

- congenital infection
- premature birth

AIDS patients

- brain abscesses
- higher likelihood of reactivation



https://www.sciencedirect.com/science/article/pii/S1471492215001439

Diagnosis of Chagas disease

Microscopy – acute stage

- ✓ blood
- ✓ CSF
- ✓ tissues

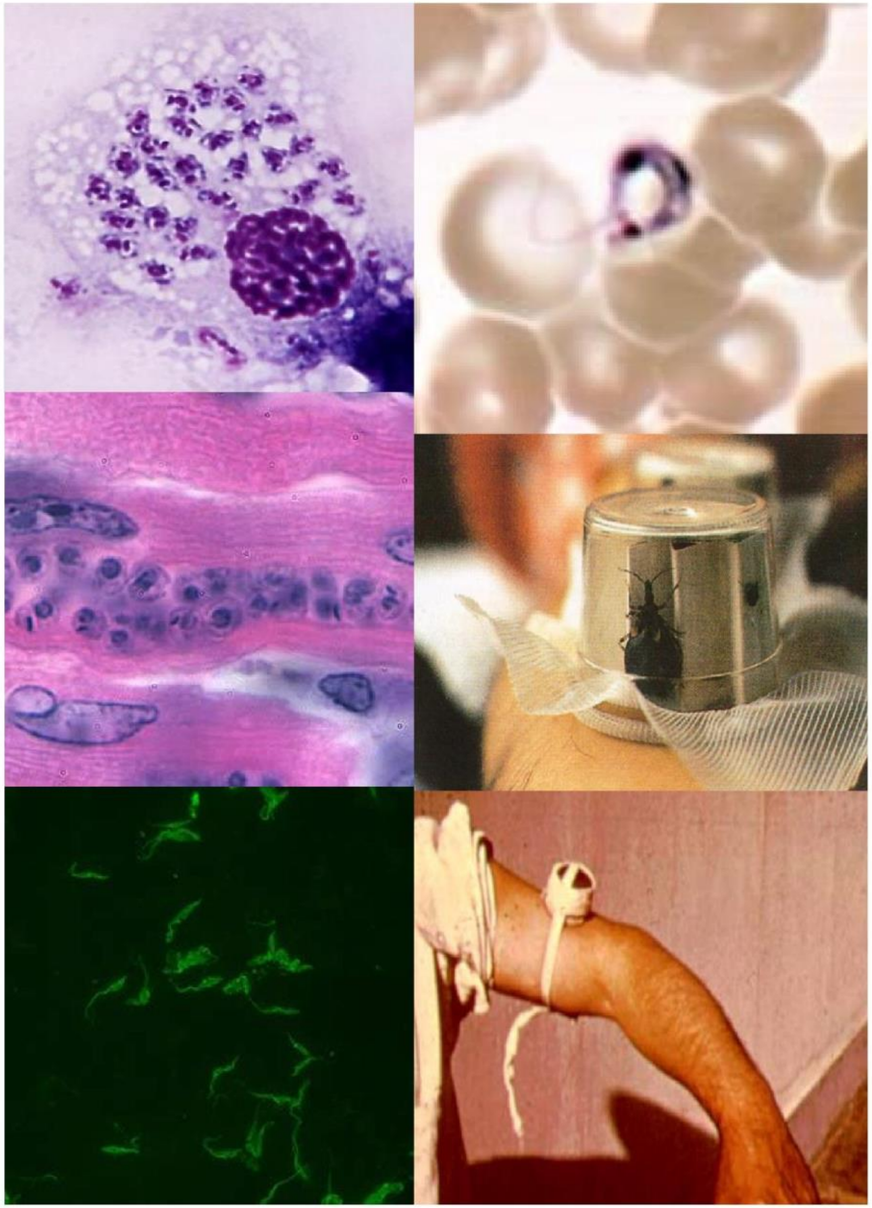
Parasite isolation

- ✓ xenodiagnosis (exposition to and examination of kissing bug for parasites)
- ✓ cultivation

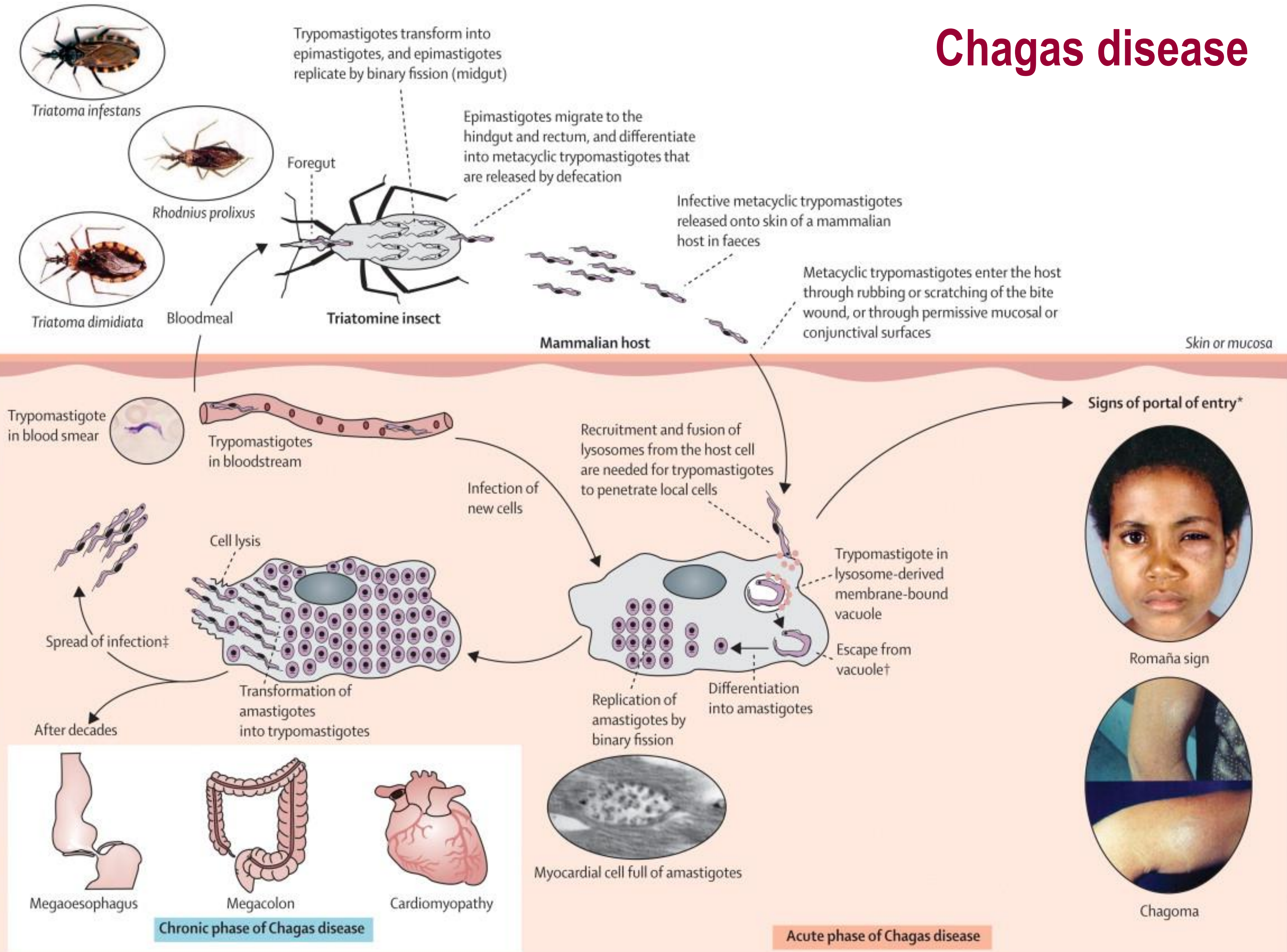
- ✓ **Serology – chronic stage**
- ✓ IFAT
- ✓ ELISA

Molecular techniques

- ✓ PCR



Chagas disease



Transmission of *T. cruzi*

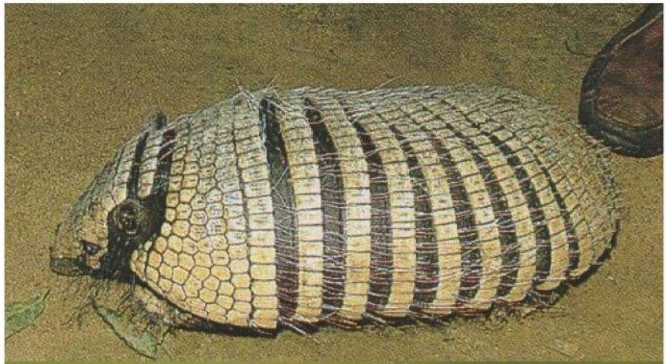
Three basic transmission cycles

1. **sylvatic (wild)**
    - wildlife-insect transmission
    - human infections rare
2. **domestic**
    - human-insect transmission
3. **peridomestic**

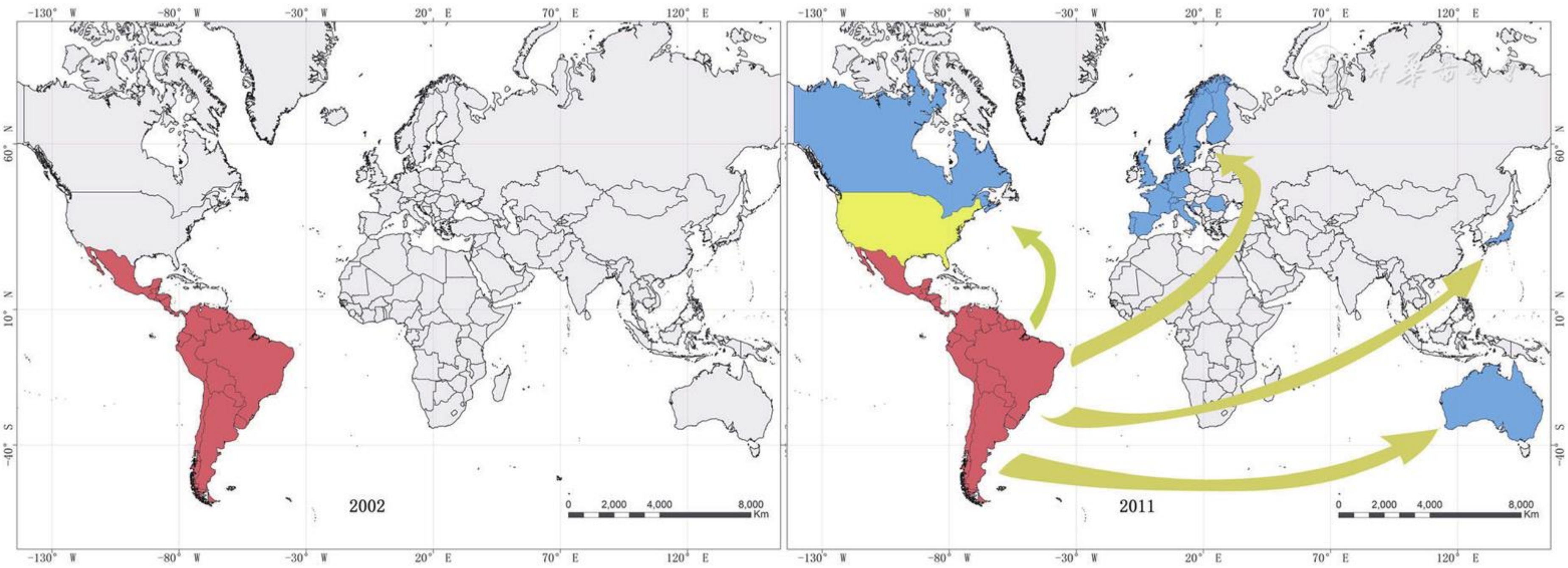
Transmission:

- blood, organs, ingestion, *in utero*, milk

Increased global population mobility increased the possibility of establishing vector transmission to areas where Chagas disease was previously non-endemic (Asia, Australia).



Global spreading patterns of Chagas disease

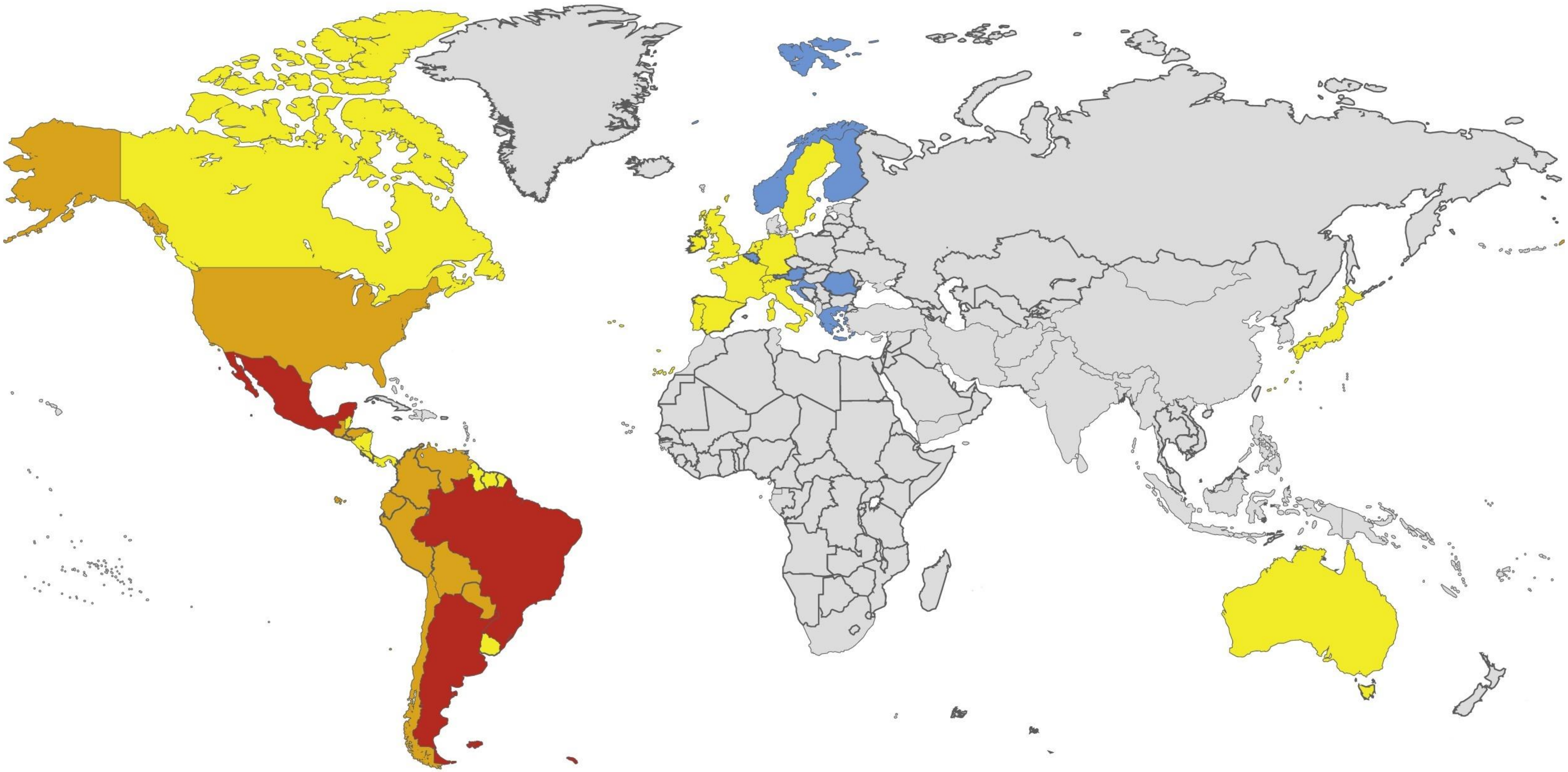


Red Endemic area of Chagas disease transmitted by local vectors.

Yellow Endemic area of Chagas diseases transmitted by local vector occasionally.

Blue Non-endemic areas of Chagas disease introduced by imported cases with non-vectorial transmission

Global distribution of cases of chagas disease, based on official estimates, 2006–2010



Estimated number of *T. cruzi*-infected cases

Key:	<900	900–89 999	90 000–899 999	≥900 000	Officially no cases reported

Trends in Parasitology

Chagas disease

2 April 2020

Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi (T. cruzi)*.

About 6 million to 7 million people worldwide are estimated to be infected with *Trypansosoma cruzi*, the parasite that causes Chagas disease. Chagas disease is found mainly in endemic areas of 21 continental Latin American countries, where it has been mostly transmitted to humans by contact with faeces or urine of triatomine bugs (vector-borne), known as 'kissing bugs', among many other popular names, depending on the geographical area.

Chagas disease is named after Carlos Ribeiro Justiniano Chagas, a Brazilian physician and researcher who discovered the disease in 1909. In May 2019, following up on decision of the 72 World Health Assembly, the World Chagas Disease Day was established to be celebrated on 14 April (the date of the year 1909 when Carlos Chagas diagnosed the first human case of the disease, a two-year old girl called Berenice).
T. cruzi parasites are mainly transmitted to human by the infected feces of blood-sucking triatomine bugs, known as the "kissing bug". *T. cruzi* can infect several species of the triatomine bug, the majority of which are found in the Americas. A person becomes exposed when the infected insect deposit its feces in the person's skin when he or she is sleeping during the night. The person will scratch the infected area, unintentionally introducing the insect's feces in in the wounds of the skin, the eyes, or the mouth.

Chagas disease control

- ✗ no vaccine available
- ✓ vector control remains the most effective method of preventing transmission in Latin America
- ✓ blood screening has become increasingly more important to prevent infection through transfusion and organ transplantation

Level of prevention	Primary prevention	Secondary prevention	Tertiary prevention
Target population	At risk	Early/asymptomatic disease	Established disease
Strategies	• Vector control • Prevention of transmission by transfusion • Prevention of transmission by organ transplantation • Prevention of accidental transmission	• Screening • Case finding • Early intervention • Antiparasitic treatment • Periodic health examination	• Antiparasitic treatment? • Symptomatic treatment • Pharmacological interventions • Non-pharmacological interventions • Management of complications • Continuing care
Responsible sectors	• Public health • Primary health care • Other sectors	• Primary health care • Public health	• Specialist services • Hospital care
Intervention objectives	• Prevent the transmission of the disease	• Prevent or inhibit disease in cases where infection has occurred	• Prevent morbidity and mortality once disease is established
	No Chagas' disease	Indeterminate form	Cardiac/digestive forms

Disease progression →

Drugs: nifurtimoxand, benznidazole

Common side effects of ***benznidazole*** *treatment:* allergic dermatitis, peripheral neuropathy, anorexia and weight loss, insomnia

Common side effects of ***nifurtimox*** *treatment:* anorexia and weight loss, polyneuropathy, nausea, vomiting, headache, dizziness or vertigo

Chagas disease prevention

- prevent contact with triatomine bugs and their faeces
- improve substandard housing
- use screens/bed nets when sleeping
- spray homes with insecticides
- cook contaminated foods
- screen blood and organ donors
- **travellers** should wear thick clothing and avoid substandard housing

Chagas disease prevention

La transmisión oral de Chagas se da mediante:

1. El consumo de alimentos o jugos que en su preparación no hayan tenido los cuidados higiénicos, y que fueron contaminados con heces o vinchucas accidentalmente.
2. La contaminación de los utensilios usados para la preparación de los alimentos.
3. El consumo de alimentos contaminados con secreciones de la glándula anal de comadrejas que contenga el parasito.
4. El consuno de carne cruda o mal cocida de animales infectados.
5. La contaminación con sangre que contenga el parásitos en el faeneo de animales silvestres.

Cuidados e higiene de los alimentos

1. **Mantener la limpieza de las manos y utensilios** porque los microbios son transportados en las manos y utensilios.
2. **Separar alimentos crudos y cocidos** Porque los alimentos crudos pueden estar contaminados con microbios que provocan enfermendades peligrosas y pueden pasar a otros alimentos.
3. **Los alimentos deben estar bien cocidos** Porque la correcta cocción mata casi todo los microorganismos. Cocinar los alimentos en todas sus partes a 70° C, garantiza la seguridad de los alimentos para consumo.
4. **Mantener los alimentos refrigerados** Porque algunos microorganismos pueden multiplicarse rápidamente en temperatura ambiente.
5. **Los granos y alimentos deben estar bien almacenados y conservados** Porque los productos pueden tener microorganismos y productos químicos que hacen daño a la salud, lavar bien los productos o también pelarlos

Entre todos lograremos eliminar la enfermedad de Chagas de nuestras familias

¿Que es la enfermedad de Chagas?

Es una enfermedad producida por un parásito llamado *"Trypanosoma cruzi"* que puede afectar algunos órganos del cuerpo, principalmente al corazón e intestinos

¿Cómo se transmite la enfermedad de Chagas?

Mediante las heces de las vinchucas
La vinchuca pica a las personas o animales para alimentarse de sangre, deja sus heces en la piel. En las heces es donde se encuentra los parasitos, y al momento de rascarse la herida introducimos los parásitos a nuestro cuerpo.

Por transfusión de Sangre
Si una persona recibe sangre contaminada de otra persona que tiene Chagas y que no haya sido controlada puede contraer la enfermedad.

De la madre al niño (congénito)
Una madre que esta embarazada puede transmitir la enfermedad al hijo.

Por trasmisión oral
Por el consumo de alimentos o jugos contaminados con heces de vinchuca o secreciones de comadrejas contaminadas con el parasito.

DATOS

Departamento: .... Numero C.V.. ....
Municipio: .... Nombre de el/la Jefe de familia: ....
Comunidad: .... Nombre del Alumno: ....
Fecha: .... Unidad Educativa: ....
Nº de habitantes en la vivienda: .... Nº de habitaciones en la vivienda: ....

ministerio de salud y deportes · Programa Nacional de Chagas · CEADES · Plataforma Chagas · FUNDACIÓ CLÍNIC BARCELONA · Facultad de Medicina U.M.S.S. · aecid Agencia Española de Cooperación Internacional para el Desarrollo

Chagas disease prevention

Salivaria

- originally parasites of tsetse flies
- complex of species
- parasites of vertebrates - trypomastigotes in blood, lymph and cerebrospinal fluid
- taxonomy based on morphology and location of trypomastigotes

subgenus ***Trypanozoon*** - *Brucei* group

- small kinetoplast, undulating membrane

subgenus ***Nannomonas*** - *Congolense* group

- middle sized kinetoplast, without free flagellum

subgenus ***Dutonella*** - *Vivax* group

- large terminally located kinetoplast, free flagellum

Salivaria

Classification of the pathogenic African trypanosomes[11]

Subgenus	Species/group	Development/transmission[*]
Duttonella	*Vivax* group: *T. vivax* *T. uniforme*	In tsetse: proboscis only Can also persist by mechanical transmission
Nannomonas	*Congolense* group: *T. congolense* *T. simiae* *T. godfreyi*	In tsetse: midgut and proboscis Not known to maintain itself exclusively by mechanical transmission
Trypanozoon	*Brucei* group: *T. brucei brucei* *T. brucei rhodesiense* [**] *T. brucei gambiense* [**] *T. evansi* *T. equiperdum*	In tsetse: midgut and salivary glands Oral transmission in carnivores Mechanical transmission[12] Venereal transmission

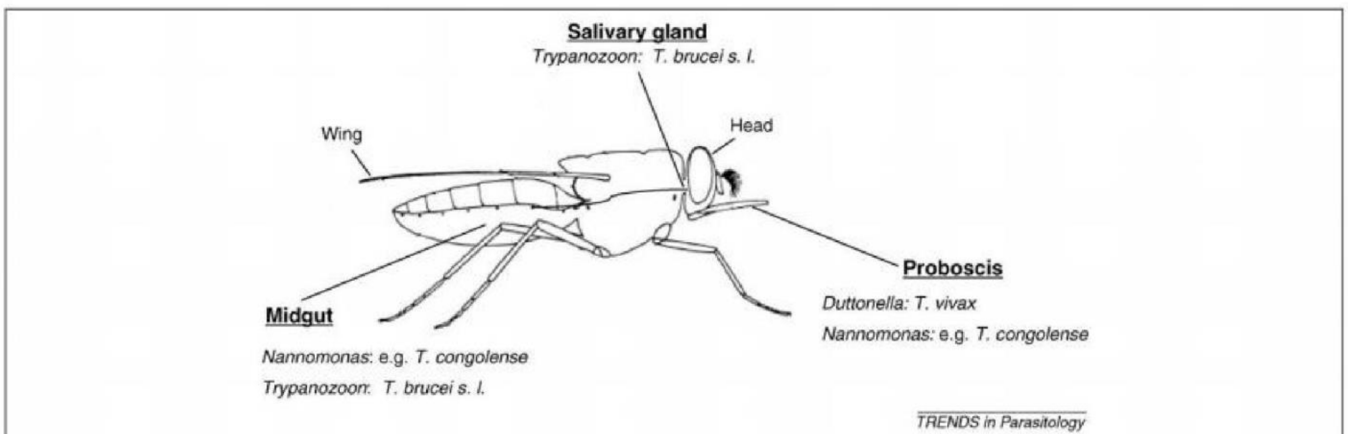


Figure 1. Development sites of trypanosomes of medical and veterinary importance in tsetse flies. When trypanosomes are found in the proboscis only, they are designated as belonging to subgenus *Duttonella* (*T. vivax*). When they are found in the proboscis and midgut, they are listed in the subgenus *Nannomonas* (e.g. *T. congolense*), and when they are found in the midgut, proboscis and salivary glands, they are designated to the subgenus *Trypanozoon* (*T. brucei* group). When trypanosomes are only found in the midgut, they are said to be immature and not classified.

African trypanosomes in domestic animals

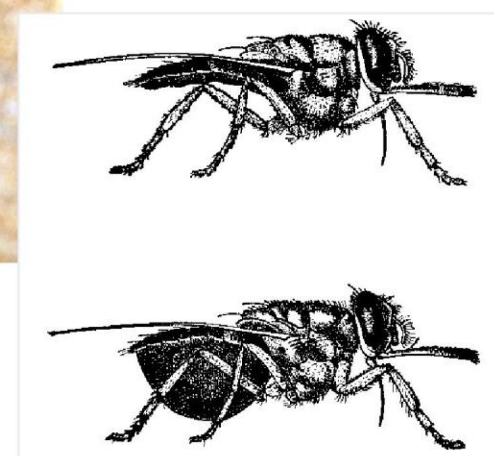
Trypanosoma species	Domestic animals affected	Reservoir hosts
T. congolense	Cattle, camels*, horses, dogs, sheep, goats, pigs	Several groups of wild mammals
T. simiae	Pigs	Wart hog, bush pig
T. godfreyi	Pigs	Wart hog
T. vivax	Cattle, sheep, goats, domestic buffalo, horses	Several groups of wild mammals
T. uniforme	Cattle, sheep, goats	Various wild ruminants
T. brucei brucei	Horses, camels*, dogs, sheep, goats, cattle, pigs	Several groups of wild mammals
T. brucei gambiense *T. brucei rhodesiense*	Human sleeping sickness; affect domestic animals as *T. brucei brucei***	Several groups of wild mammals (particularly *T. brucei rhodesiense*)
T. brucei evansi	Camels, horses, dogs, domestic buffalo, cattle	Several wild mammals in Latin America
T. brucei equiperdum	Horses, donkeys, mules	None known
T. theileri and *T. ingens*	Cattle, domestic buffalo***(not pathogenic)	Various wild ruminants

* Camels are highly susceptible to *T. congolense* and to *T. brucei*, but do not usually penetrate into tsetse country.

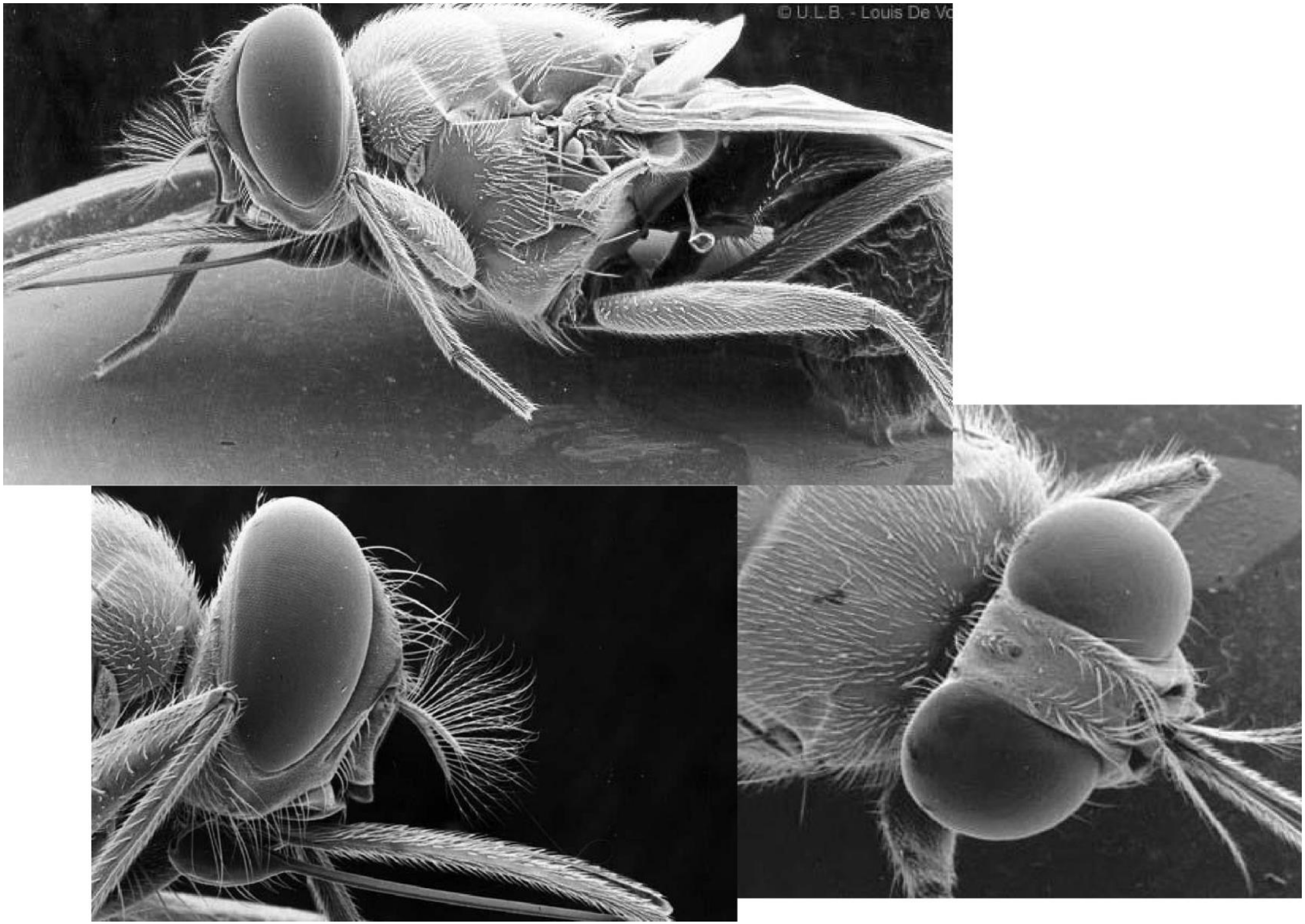
** In particular, the behaviour of *T. brucei rhodesiense* in domestic animals is quite similar to that of *T. b. brucei*, whereas *T. brucei gambiense* is on the average more chronic (as it is in humans).

*** Of the two only *T. theileri* has been reported from domestic buffalo.

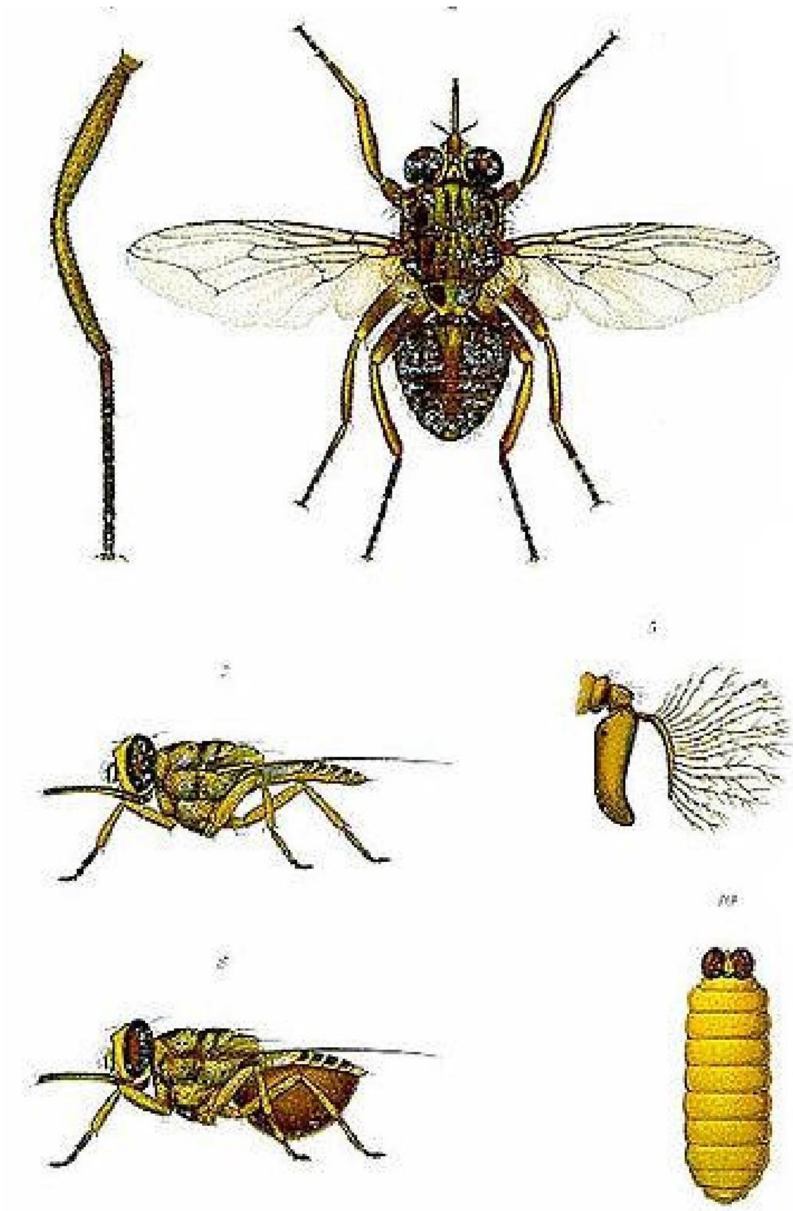
Glossina sp.



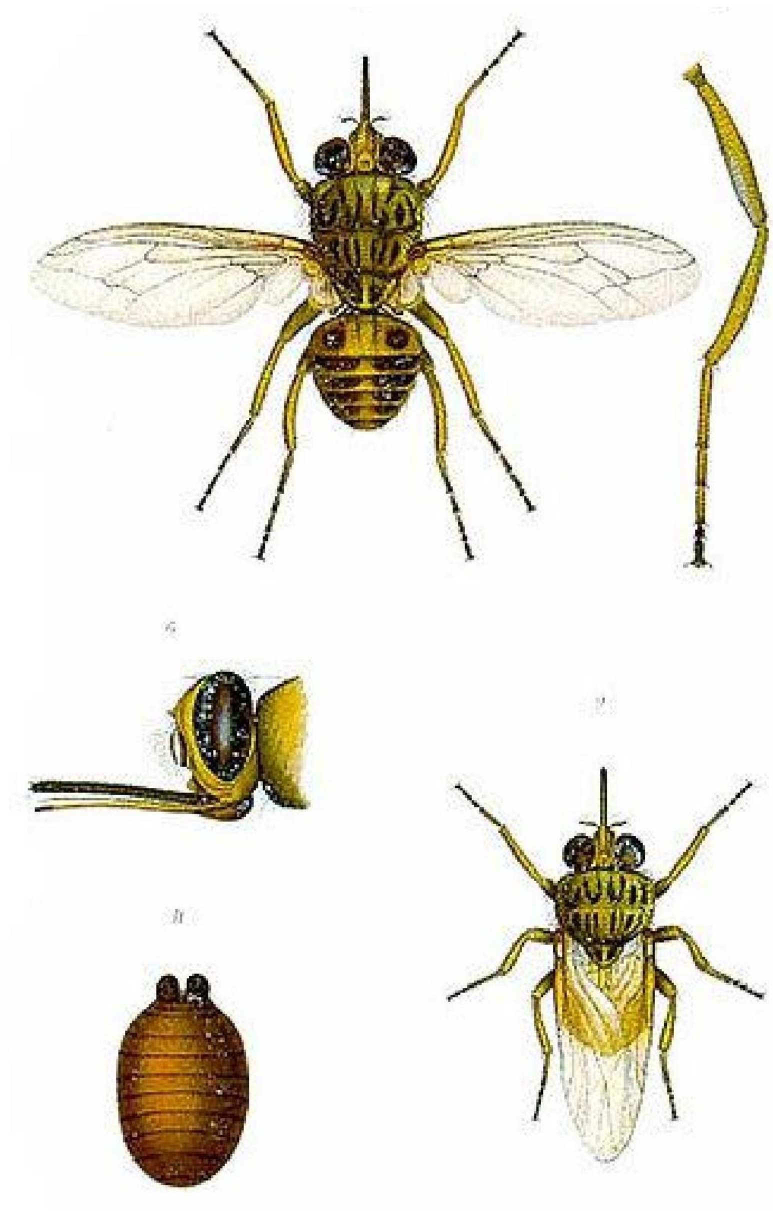
Glossina sp. under scanning electron microscopy



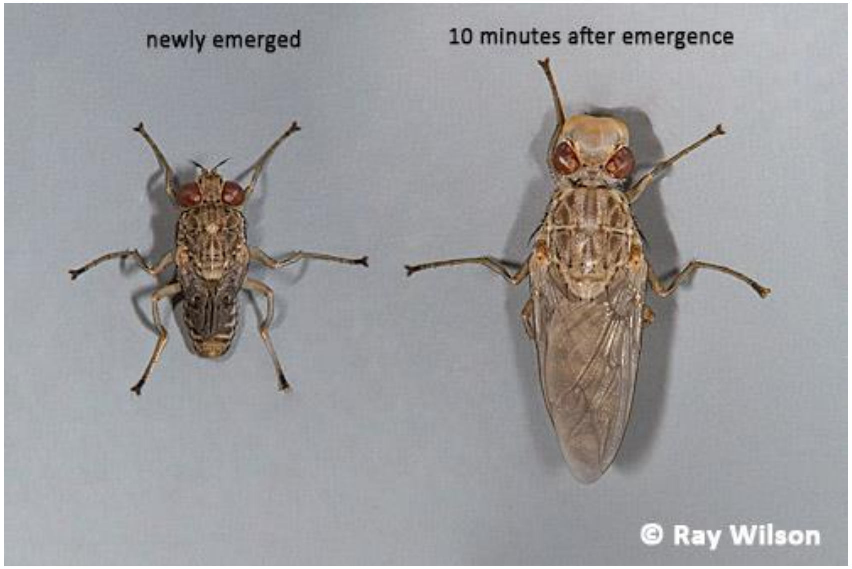
Glossina palpalis



Glossina morsitans



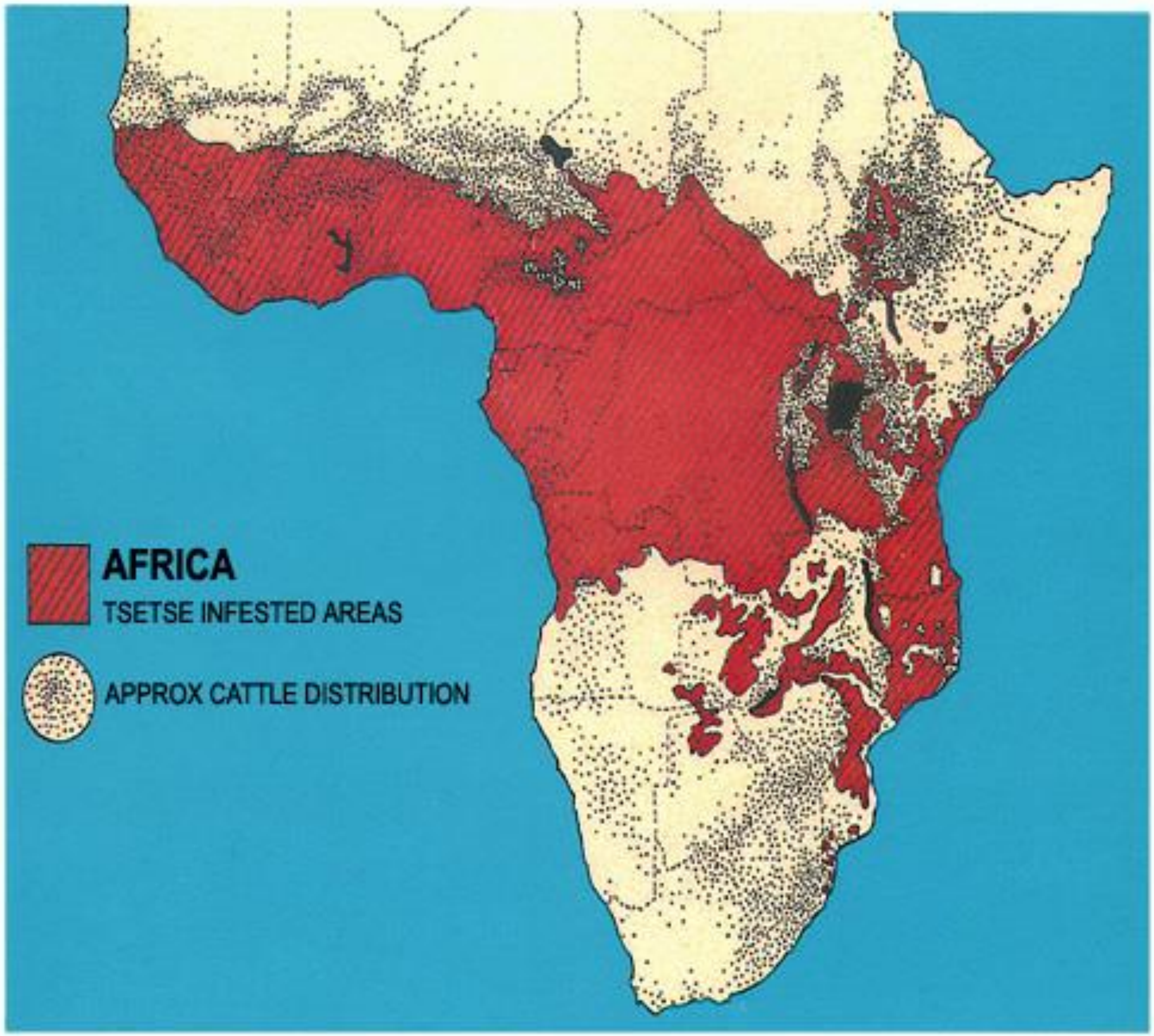
Glossina morsitans



https://www.youtube.com/watch?v=odCtCote9U0

http://www.raywilsonbirdphotography.co.uk/Galleries/Invertebrates/vectors/Tsetse_Fly.html

Distribution of tsetse fly

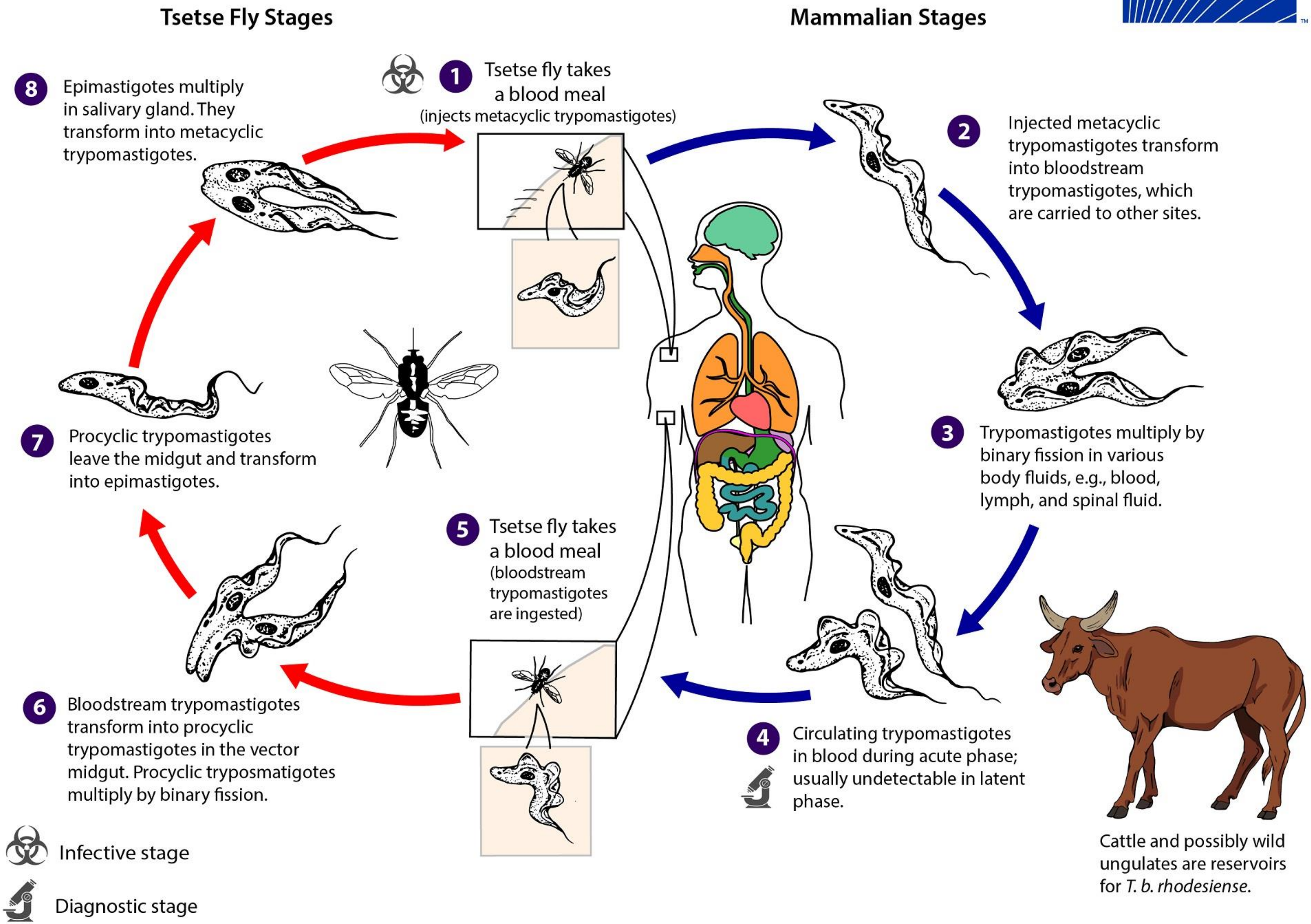


Sleeping sickness in humans

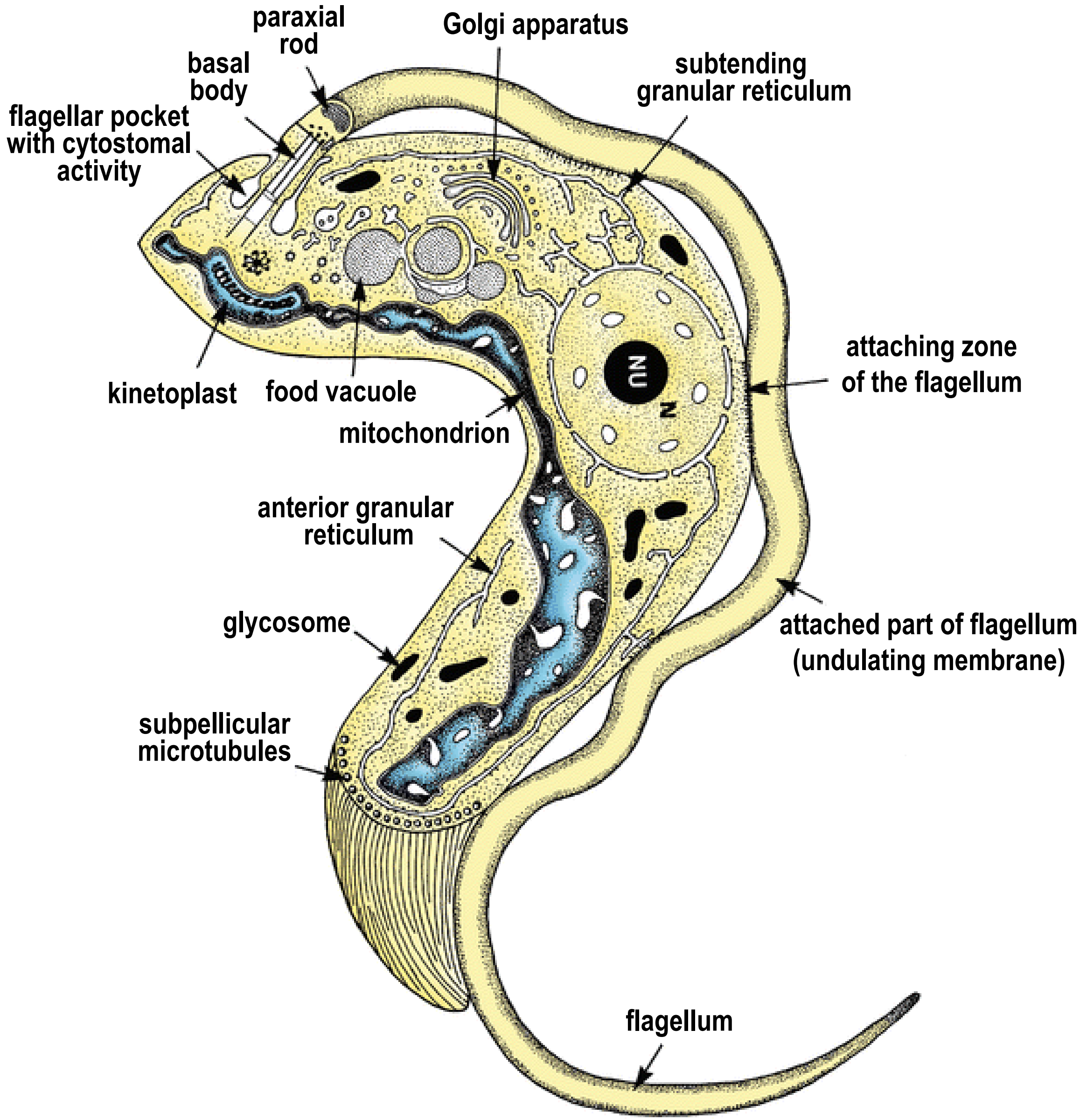
Trypanosoma species	*T. b. rhodesiense*	*T. b. gambiense*
tsetse vector	*G. morsitans*	*G. palpalis*
ecology	dry bush, woodland	rainforest, riverine, lakes
transmission cycle	ungulate-fly-human	human-fly-human
non-human reservoir	wild animals	domestic animals
epidemiology	sporadic, safaris	endemic, some epidemics
disease progression	rapid, often fatal	slow (~1 yr.), chronic
asymptomatic carriers	rare	common

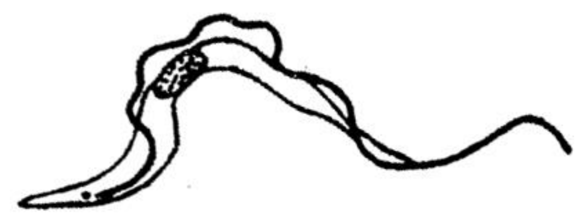
African Trypanosomiasis

Trypanosoma brucei gambiense & *Trypanosoma brucei rhodesiense*

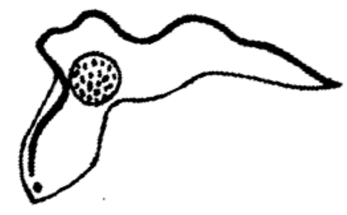


Morphology of *Trypanosoma brucei brucei*





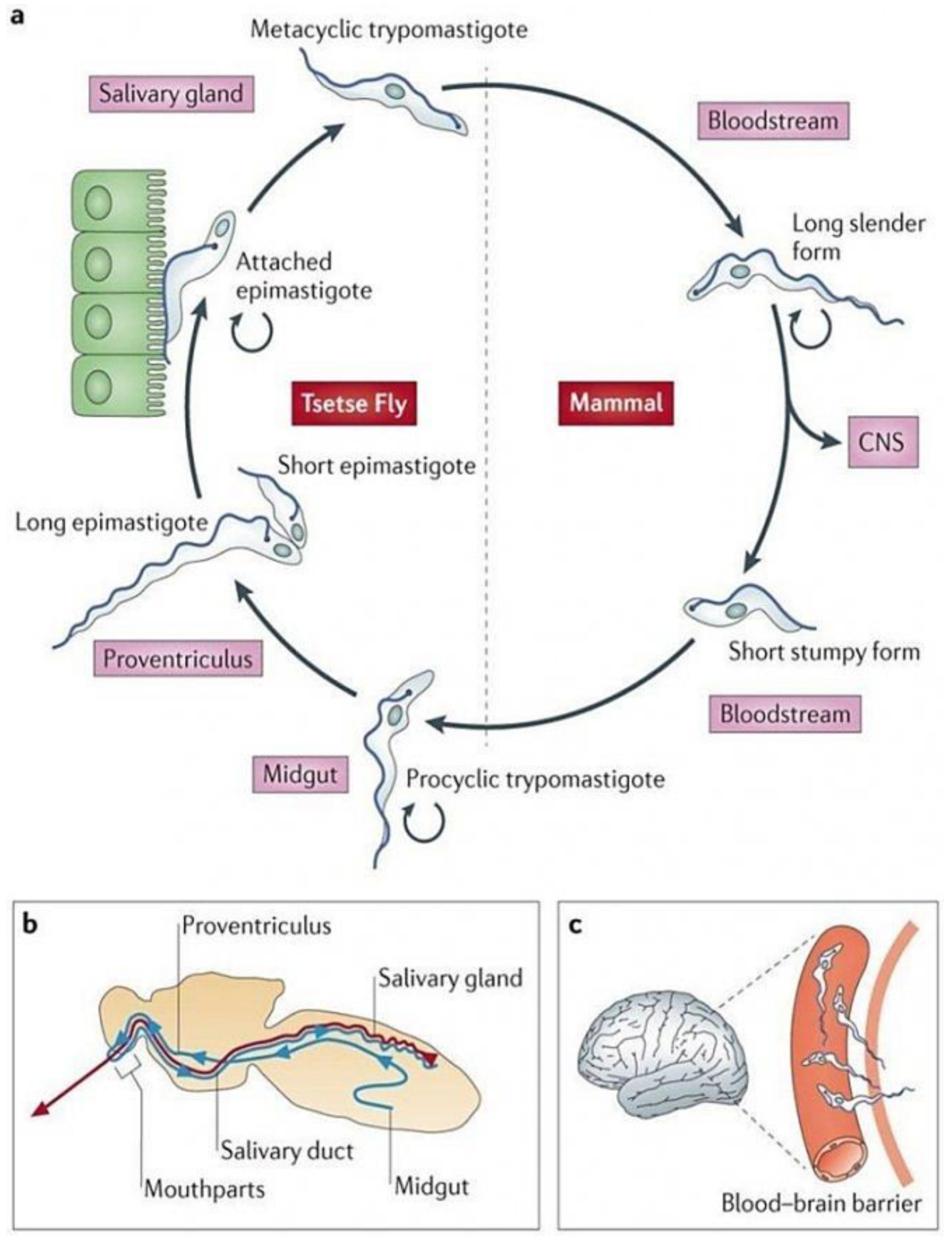
slender forms



stumpy form
(without free flagellum)

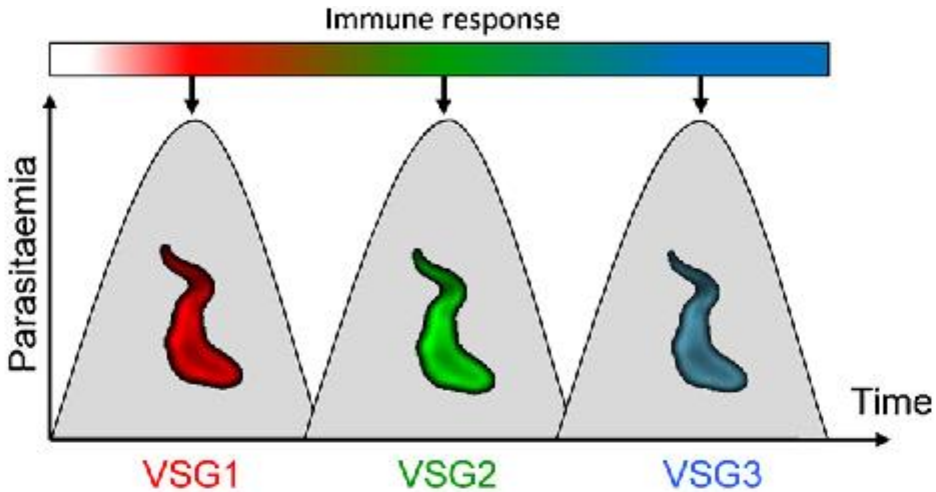
Life cycle of *Trypanosoma brucei*

- 20-30 days
- metacyclic trypomastigotes ⇨ mammalian bloodstream
- differentiation into proliferating long slender forms
- differentiation into short stumpy forms (pre-adapted to survive in the tsetse fly)
- differentiation into procyclic trypomastigotes in tsetse fly midgut
- migration through the peritrophic matrix into the salivary gland, to generate one long epimastigote and one short epimastigote
- attached short epimastigotes generate free metacyclic trypomastigotes in the salivary gland lumen



Antigenic variation in African trypanosomes

- „*T. brucei* parasite has evolved an elegant mechanism to display a completely new coat of VSG antigen, rendering it once again invisible to the host's immune system. The parasite's genome has over 1,000 genes that code for different variants of the VSG protein.“
- around 10^7 **V**ariant **S**urface **G**lycoprotein (~60kDa protein densely coating the cell surface) molecules expressed on the parasite's cell surface,
- 6-10% of the total genome is coding for VSGs (over 1,000 genes)
- only one is expressed at a given time, others are „silent“
- this 12-15 nm thick coat is doffed periodically (internalised via the flagellar pocket) and replaced with an antigenically distinct version of VSG ⇨ this antigenic variation causes cyclical waves (5-8 days) of parasitemia



History

1902 Ford and Dutton, two English physicians working in The Gambia, identified the culprit, a parasite which they named Trypanosoma brucei gambiense.

1903 Doctor Castellani, working in Uganda for Her Majesty, The Queen of England, observed the parasite in the cerebrospinal fluid of one of his patients.

In the same year, the tsetse fly was recognized by David Bruce as being the vector of the parasite.

1905 Doctor Ayres Kopke introduced an arsenic compound, Atoxyl, for the treatment of the disease.

1920 Doctor Jamot, a colonel in the French army working on trypanosomiasis control, observed that in the Ubangi river loop more than half of all deaths were due to sleeping sickness. The major epidemics early in the century claimed hundreds of thousands of lives. Entire populations were affected, and indeed Jamot reported that a whole ethnic group had been wiped out in northern Congo.

1924 Tryparsamide, a drug still based on arsenic but less toxic than Atoxyl, was used on a wide scale in Belgian Congo and Cameroon.

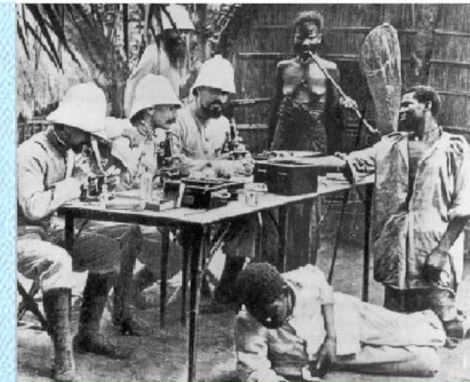
1930 A headline in the famous French magazine "L'illustration" stated: 'Our doctors have vanquished the tsetse fly!'

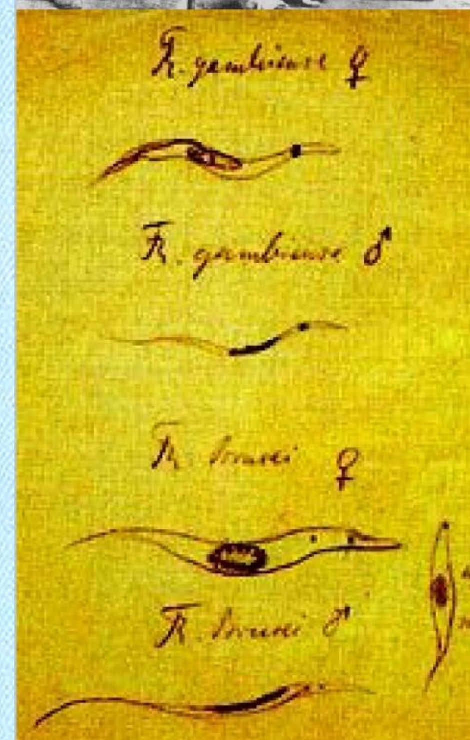
1932 A terrible setback - 700 patients became blind after receiving the wrong dose of Atoxyl. The medical community was thunderstruck. In response to this disaster, Professor Friedheim, a Swiss physician and chemist, developed the drug melarsoprol, the bold concept of which was a single product containing a highly toxic arsenic-based molecule and its antidote.

1950 This new drug, melarsoprol, was used systematically in cases where there was involvement of the central nervous system.

1984 WHO launched a programme entitled 'The primary health care approach to the control and prevention of Sleeping Sickness'.

1993 WHO develops the central African initiative, a project entitled 'Prevention and Control of human trypanosomiasis in central african and neighbouring foci', a regional approach to Sleeping Sickness control in ten countries, namely: Angola, Cameroon, Central African Republic, Congo, Gabon, Equatorial Guinea, Uganda, Sudan, Chad and Zaire.

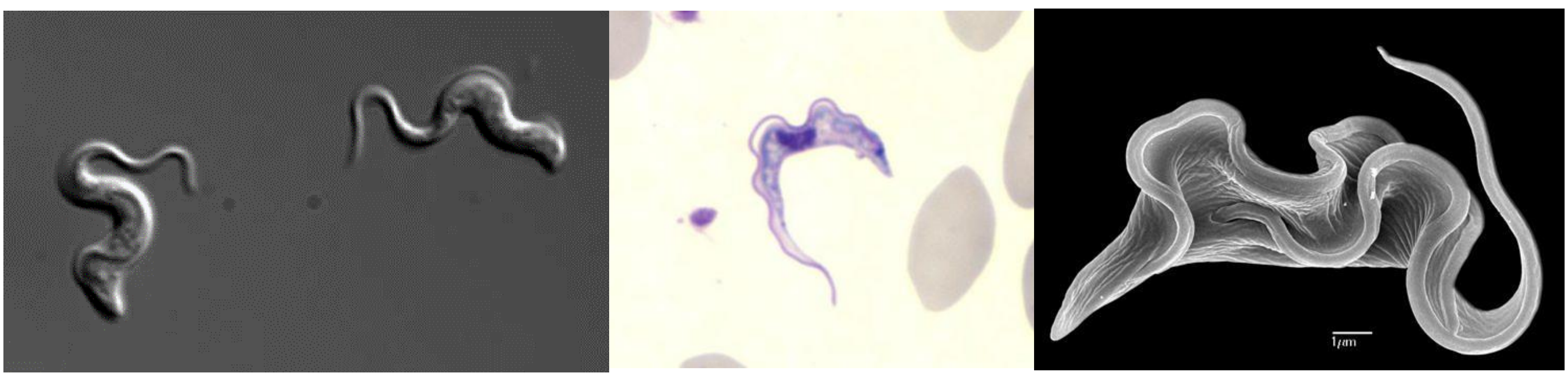




Clinical features of African trypanosomiasis

Pathogenesis – general

- trypanosomes live in blood, lymph nodes, spleen (= not intracellular)
- they are particularly abundant in intercellular spaces in brain
- clinical course depends on host susceptibility
- *T. b. brucei*-vertebrate hosts (Equidae, dogs, some ruminants) exhibit acute disease with death in ~ 2 weeks
- if the host survives, blindness develops (especially common in dogs)



https://youtu.be/EnsydwITLYk

Pathogenesis of African trypanosomiasis

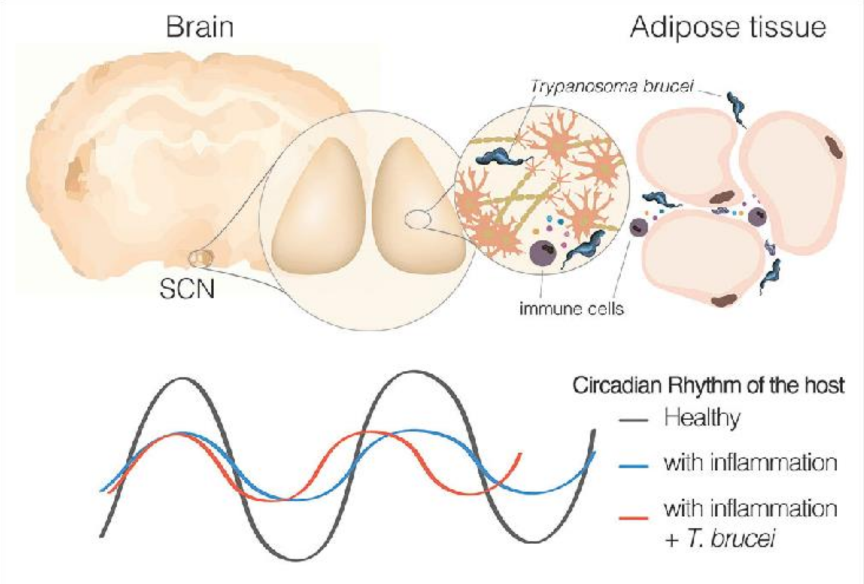
Humans

- **local reaction**: painful sore at site of bite - disappears after a couple of weeks
- trypanosomes reproduce rapidly after entering the blood and lymph system ⇨ lymphadenopathy, generalized invasion of all organs
- **Winterbottom's sign** – swollen lymph nodes at skull base (a sign of certain death according to slave traders)
- binding of specific antibody to adsorbed trypanosome on host cell, coupled with complement, leads to lysis – cause of anaemia

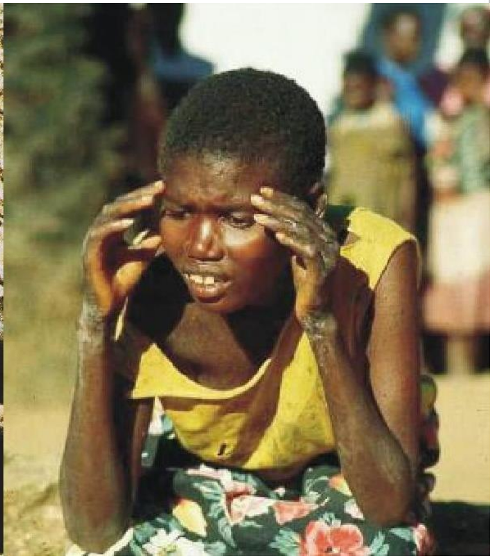
Pathogenesis of African trypanosomiasis

Mechanisms under investigation

- circadian rhythms - alterations in endogenous rhythms correlate with clinical symptoms
- suprachiasmatic nucleus (SCN) – “biological clock” – regulating the hormonal, sleep, body thermostat activity
- spontaneous rhythm of SCN is altered with trypanosome infection

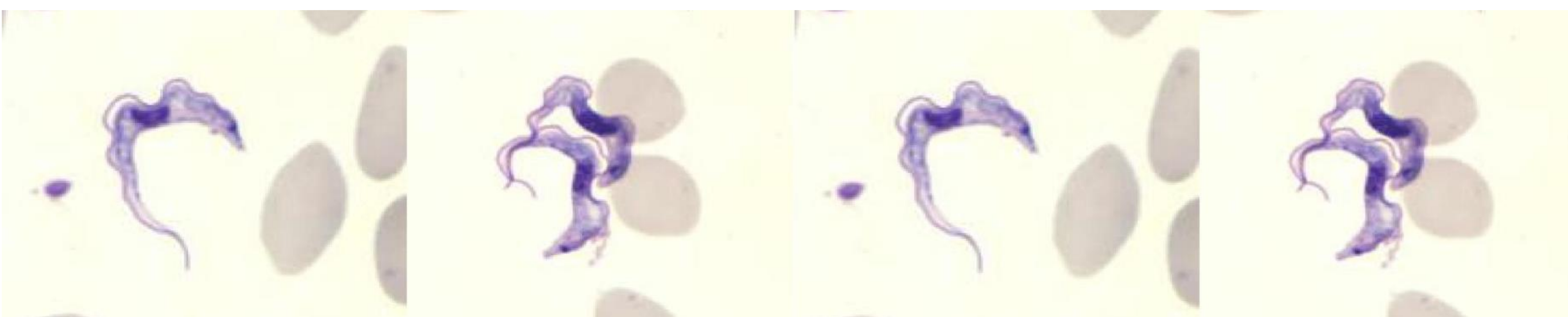


T. brucei accelerates the clock of the host: the clock of both neurons in the SCN and adipocytes

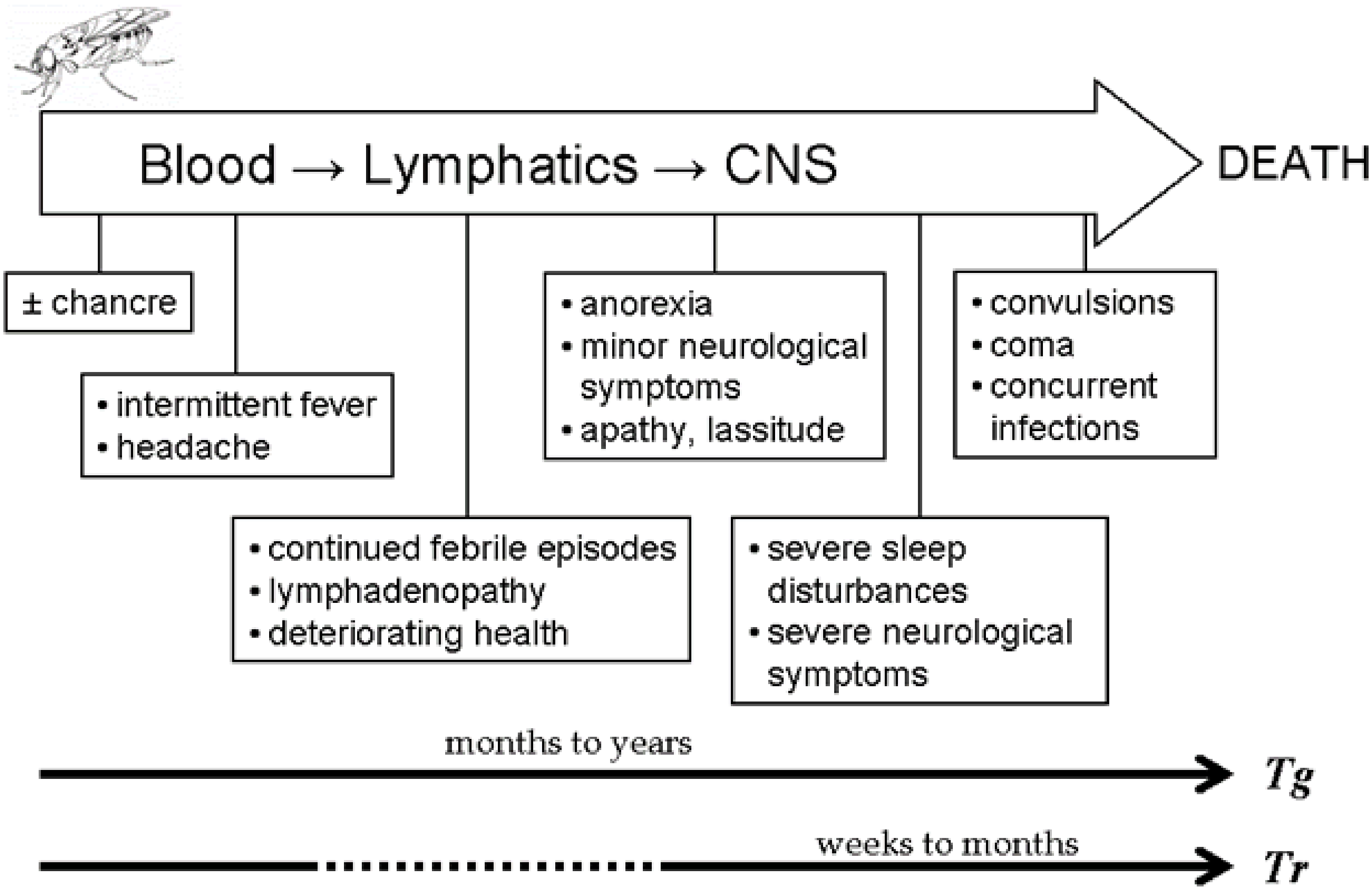


Diagnosis of African trypanosomiasis

- clinical features of infection are not sufficiently specific
- based on **finding the parasite in body fluid or tissue** by microscopy
- parasite load in *T. b. rhodesiense* infection is substantially higher than in *T. b. gambiense*
- *T. b. rhodesiense* parasites are easily found in the blood, but it is difficult to detect *T. b. gambiense* there
- parasites may also be found in lymph node fluid or in fluid or biopsy of a chancre
- classic method of diagnosing *T. b. gambiense* infection is a microscopic examination of a lymph node aspirate, usually from a posterior cervical node

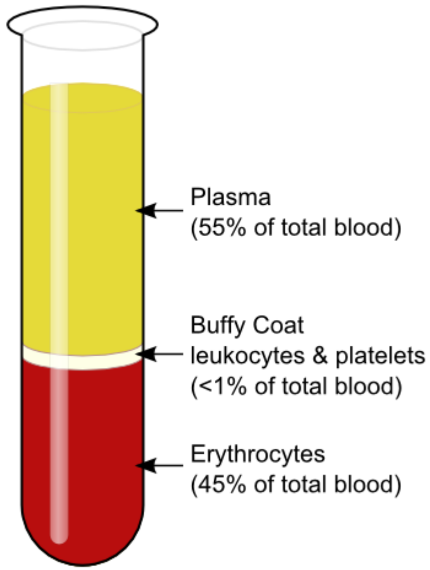


Progression of African trypanosomiasis



Diagnosis of African trypanosomiasis

✓ examination of cerebrospinal fluid obtained by lumbar puncture



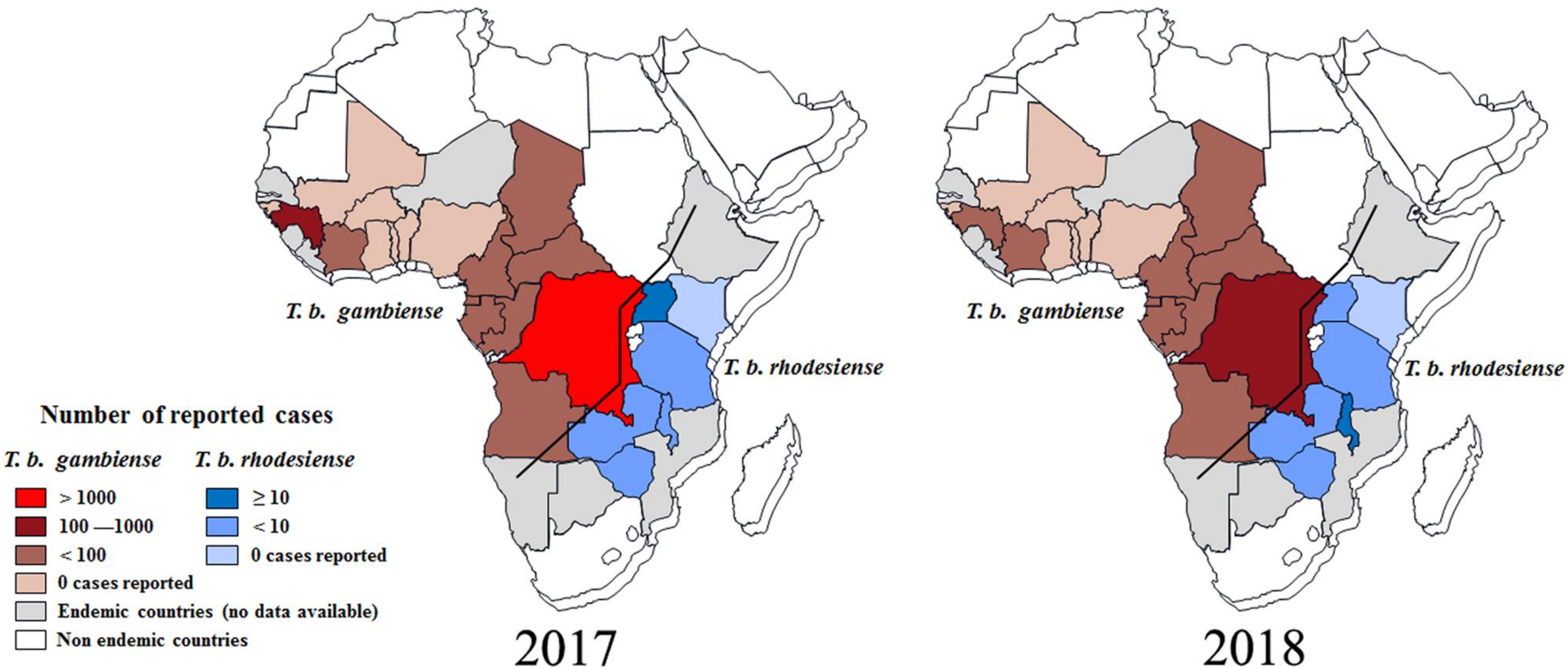
✓ “buffy coat” (haematocrit centrifugation)

✓ serology

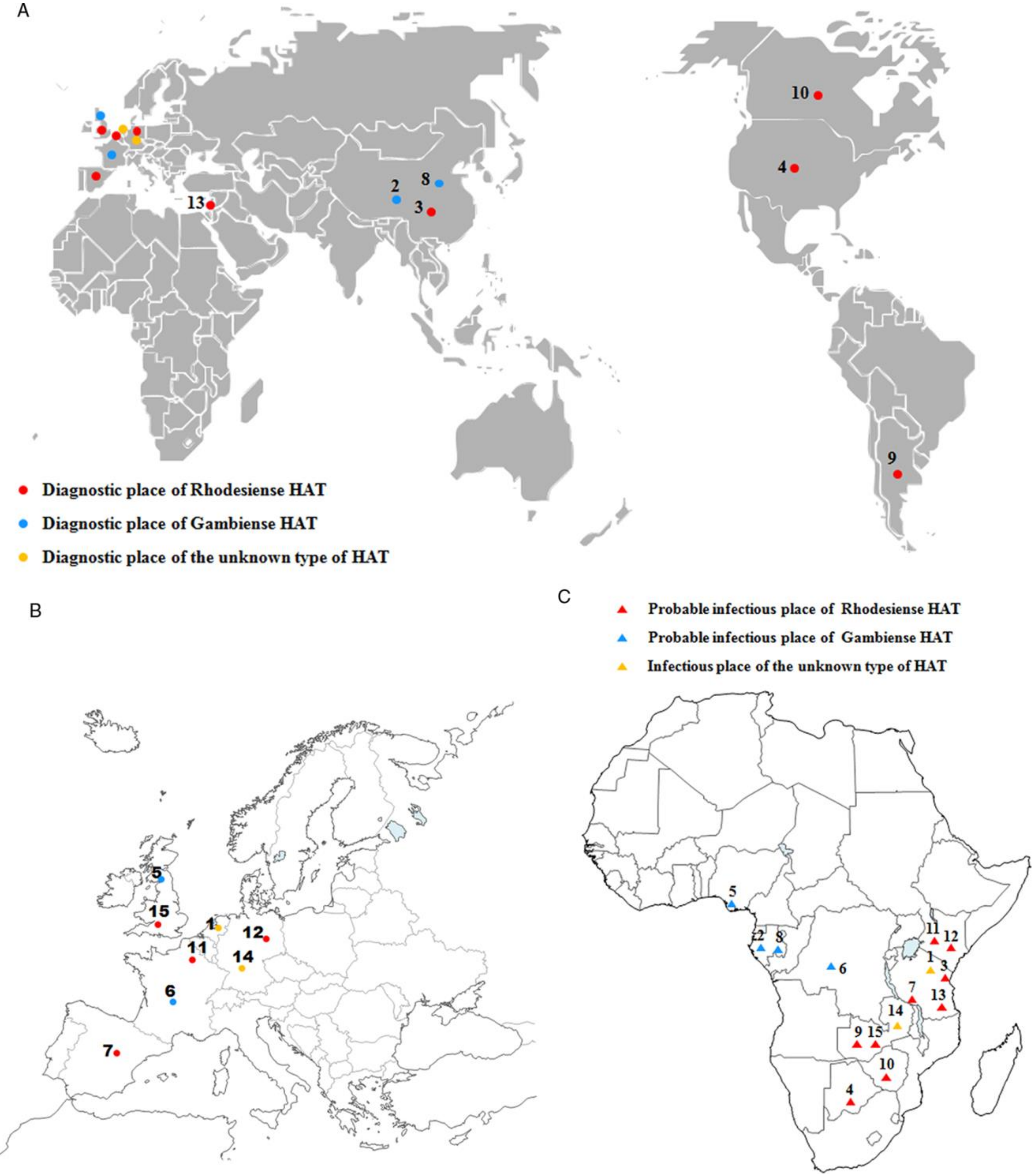
✓ CATT “card agglutination test for trypanosomes”

✓ PCR

Distribution of human African trypanosomiasis in endemic countries



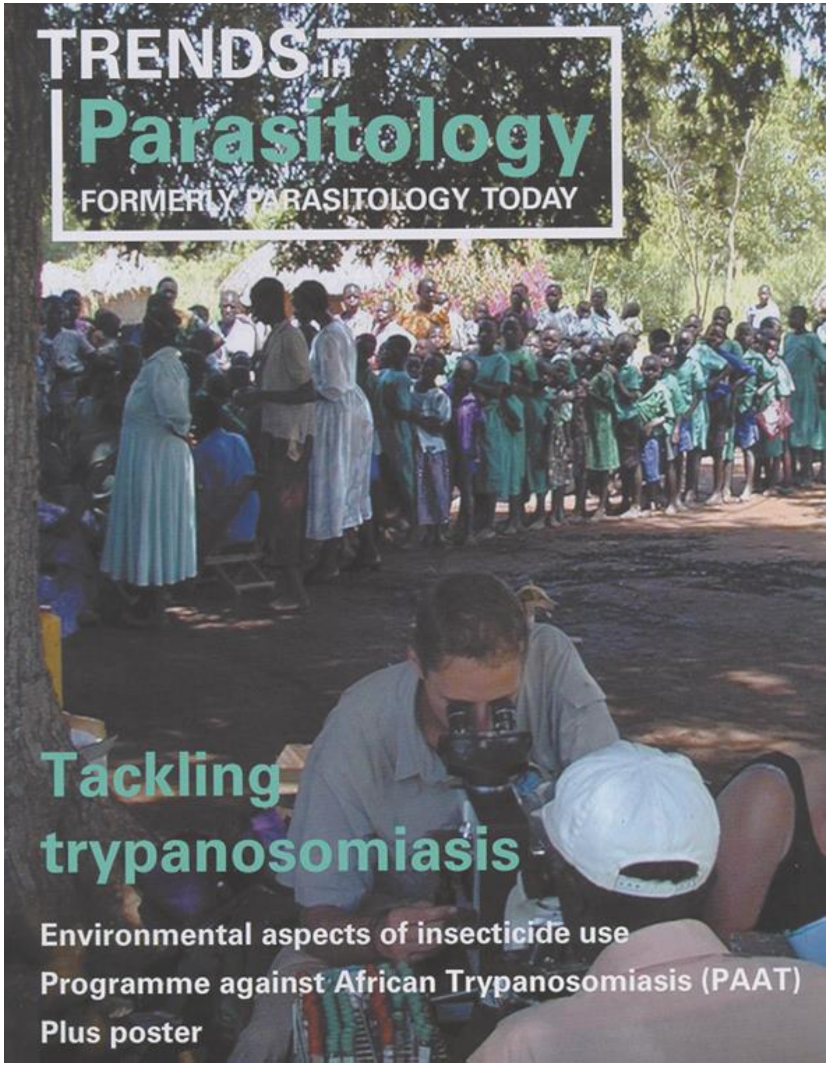
HAT cases that were diagnosed and confirmed in non-endemic countries from 2011 to 2018



Epidemiology of African trypanosomiasis

Risk factors

- ✓ geographic region
- ✓ occupation
- ✓ socioeconomic status
- ✓ host susceptibility – genetics?



Trypanosomiasis, human African (sleeping sickness)

10 January 2022

Key facts

- African trypanosomiasis is caused by parasites of genus Trypanosoma and transmitted by infected tsetse flies and is endemic in 36 sub-Saharan African countries where there are tsetse flies that transmit the disease. Without treatment, the disease is considered fatal.
- The people most exposed to the tsetse fly and to the disease live in rural areas and depend on agriculture, fishing, animal husbandry or hunting.
- Human African trypanosomiasis takes 2 forms, depending on the subspecies of the parasite involved: Trypanosoma brucei gambiense accounts for more than 95% of reported cases.
- Sustained control efforts have reduced the number of new cases. In 2009 the number reported dropped below 10 000 for the first time in 50 years, and in 2019 there were with 992 and 663 cases reported in 2019 and 2020 cases recorded respectively.
- Diagnosis and treatment of the disease is complex and requires specifically skilled staff.

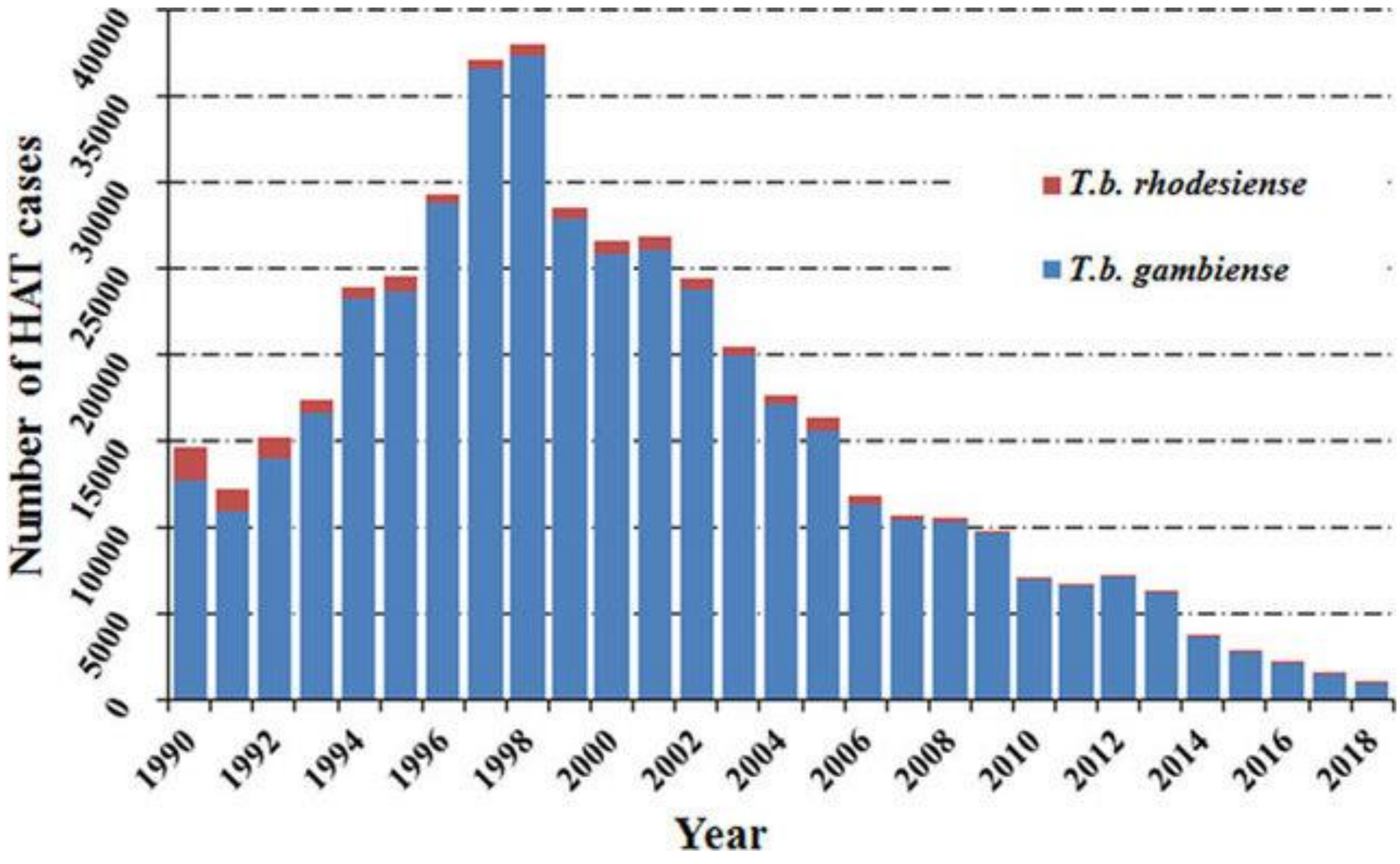
Comparison of the biology of African trypanosomes subspecies: *T. b. gambiense* and *T. b. rhodesiense*

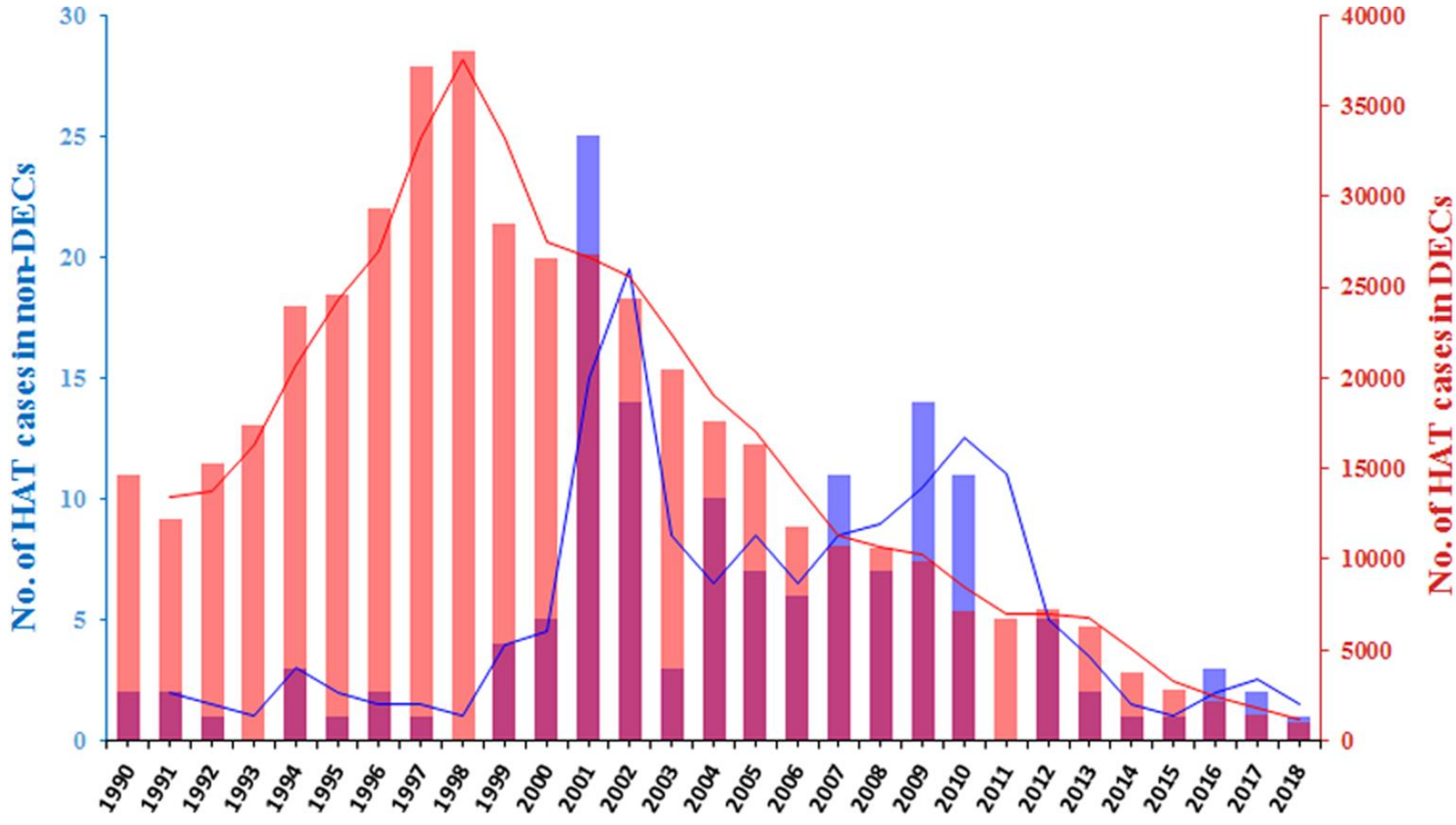
Characteristic	*T. b. gambiense*	*T. b. rhodesiense*
Disease	Chronic	Acute
	Low parasitemia	High parasitemia
	Incubation period is months to years	Incubation period is days to weeks
Main tsetse vector	*G. palpalis* group	*G. morsitans* group
	G. palpalis	*G. morsitans*
	G. fuscipes	*G. pallidipas*
	G. tachinoides	*G. swynnertoni*
Transmission	Human reservoir (primary)	Animal reservoir (primary)
	Riverine tsetse (secondary)	Savanna and woodland tsetse (secondary)
	Animal reservoir	Human reservoir
Reservoir hosts	Possibly kob, hartebeest, domestic pigs, dogs	Bushbuck, other antelope, hartebeest, hyena, lion, domestic cattle; possibly warthog and giraffe
Geographical range	West Africa, western, and northern Central Africa	East Africa and north Central Africa

a) Reviewed in Seed and Hall (1992)

https://doi.org/10.1002/9780470688618.taw0183

Epidemiology of African trypanosomiasis





The dynamic trend changes in HAT cases diagnosed in non-DECs and DECs

DEC - disease-endemic countries

Treatment of African trypanosomiasis

First stage treatments

- **Pentamidine**: discovered in 1941, used for the treatment of the first stage of *T. b. gambiense* sleeping sickness. Despite a few undesirable effects, it is well tolerated by patients.
- **Suramin**: discovered in 1921, used for the treatment of the first stage of *T. b. rhodesiense* sickness. It provokes certain undesirable effects in the urinary tract and allergic reactions.

Second stage treatments

- **Melarsoprol**: discovered in 1949, used in both forms of infection. It derives from arsenic and has many undesired side effects - the most dramatic is reactive encephalopathy (encephalopathic syndrome) that may be fatal (3-10 %). An increase of resistance to the drug in several foci - particularly in central Africa.
- **Eflornithine**: registered in 1990, a less toxic alternative to melarsoprol treatment. Effective only against *T. b. gambiense*. The regime is strict and difficult to apply.

Trypanosoma species in domestic animals

***Trypanosoma* species**	**Domestic animals affected**	**Reservoir hosts**
T. congolense	Cattle, camels*, horses, dogs, sheep, goats, pigs	Several groups of wild mammals
T. simiae	Pigs	Wart hog, bush pig
T. godfreyi	Pigs	Wart hog
T. vivax	Cattle, sheep, goats, domestic buffalo, horses	Several groups of wild mammals
T. uniforme	Cattle, sheep, goats	Various wild ruminants
T. brucei brucei	Horses, camels*, dogs, sheep, goats, cattle, pigs	Several groups of wild mammals
T. brucei gambiense *T. brucei rhodesiense*	Human sleeping sickness; affect domestic animals as *T. brucei brucei***	Several groups of wild mammals (particularly *T. brucei rhodesiense*)
T. brucei evansi	Camels, horses, dogs, domestic buffalo, cattle	Several wild mammals in Latin America
T. brucei equiperdum	Horses, donkeys, mules	None known
T. theileri and *T. ingens*	Cattle, domestic buffalo***(not pathogenic)	Various wild ruminants

* Camels are highly susceptible to *T. congolense* and to *T. brucei*, but do not usually penetrate into tsetse country.

** In particular, the behaviour of *T. brucei rhodesiense* in domestic animals is quite similar to that of *T. b. brucei*, whereas *T. brucei gambiense* is on the average more chronic (as it is in humans).

*** Of the two only *T. theileri* has been reported from domestic buffalo.

Salivaria

African *Trypanosoma* spp. in domestic animals

Economic impact on agriculture

- >3 million deaths per year, major reduction in food production
- 50% reduction in herd size
- 25% reduction in milk production
- 20% loss in calving

zebu cattle

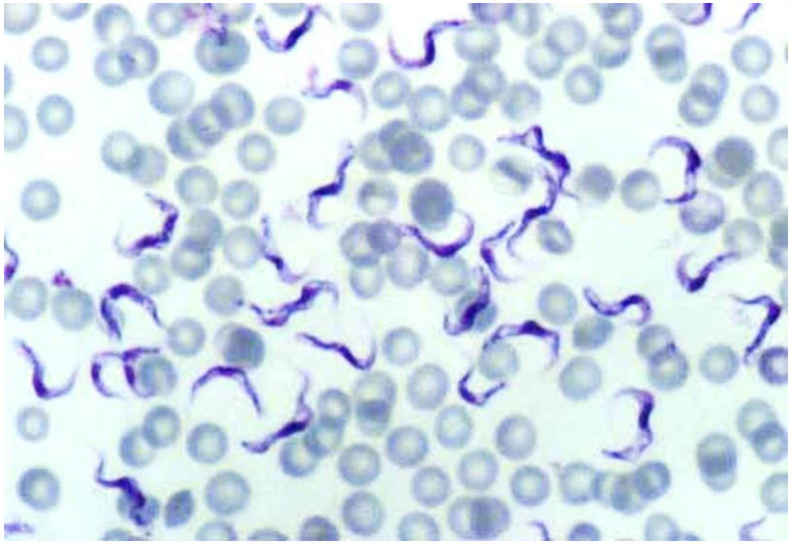
×

N'Dama – trypanotolerant population

Trypanosoma b. equiperdum

Dourine = syphilis equorum

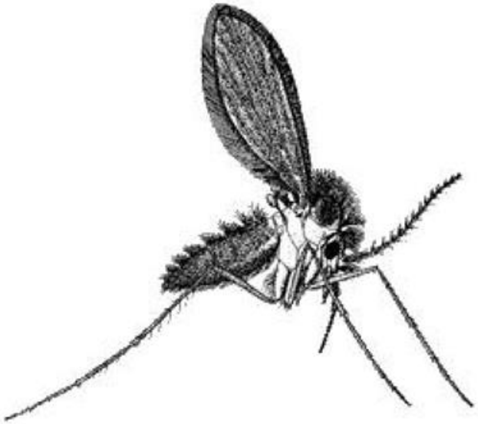
- cosmopolitan distribution
- in horses and other equids
- in genital organs and secretions
- genetic analyses ⇨ derivative of T. brucei
- spreading primarily via sexual transmission = adaptation that has allowed the parasite to escape beyond the range of the tsetse fly
- mother-foal transmission possible
- donkeys are carriers but show no symptoms



Other higher trypanosomatids

Endotrypanum schaudinni

- dixenous
- unique among the Kinetoplastida in that they infect erythrocytes of their mammalian host (forest-dwelling two-toed sloths of the genus *Choloepus*)
- probably transmitted by the bite of infected phlebotomine sandflies (Diptera: Psychodidae)
- trypomastigotes in mammalian erythrocytes, promastigotes in vectors (sandflies)



⚠ according to phylogenetic data, the genus **_Endotrypanum_ set inside the _Leishmania_ group**

THE ENDOTRYPANUM OF HOFFMAN'S SLOTH.*

S. T. DARLING.

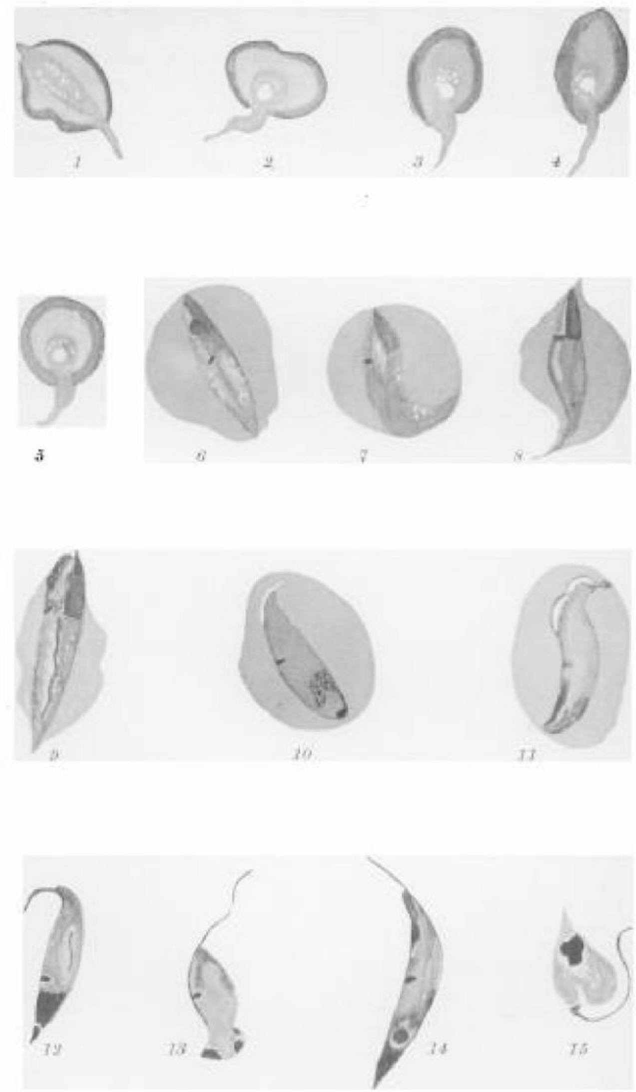
(From Board of Health Laboratory, Ancon, Canal Zone.)

This little known parasite, Endotrypanum Schaudinni Mesnil and Brimont, is of peculiar interest on account of its being an example of a hemoflagellate of "crithidia" type invading the erythrocytes of a mammal. It was encountered first in Guiana by Brimont in stained films taken from the blood of the Edentate Choloepus didactylus (L.), and a short account of its appearance in stained films was presented by Mesnil and Brimont before the Société de Biologie, Paris, in 1908.[1]

An opportunity occurred during the past year for a brief but unfortunately an interrupted study of the living parasite, and while little has been learned about its life cycle some new information on the morphology of the fresh and stained organism has been obtained. The host, Choloepus didactylus (L), an edentate not uncommon in Panama, is sometimes known as Hoffman's, or two-toed sloth, from the fact that each hand is supplied with two digits. No ectoparasites were detected on this sloth though carefully searched for; the arboreal habits of the animal would expose it to attacks from mosquitoes, biting flies, etc., but one would not expect to find it infested with ticks. The animal is well covered with coarse hair, but not so thickly covered as another sloth from this region, Bradypus castaneiceps. After being well fed in the laboratory for nine months, the growth of hair has become much thicker. Possibly the animal is more exposed to attack from biting pests when it is in a half-starved condition during the dry season, or has lost its hair from other cause.

The animal, while very sluggish, has powerful claws and may inflict severe scratches or tears during handling. It was found that by holding the animal with "supple knots," blood could be obtained from the heart under anesthesia

* Received for publication July 18, 1914.



Trypanosomatids of fish and amphibians

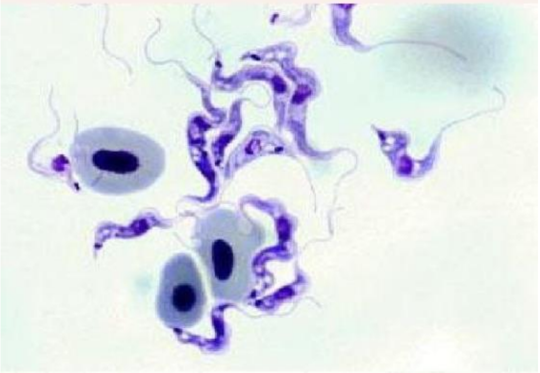
- transmission via blood sucking leeches – epimastigotes in gut, trypomastigotes in proboscis
- transformation to metacyclic trypomastigotes

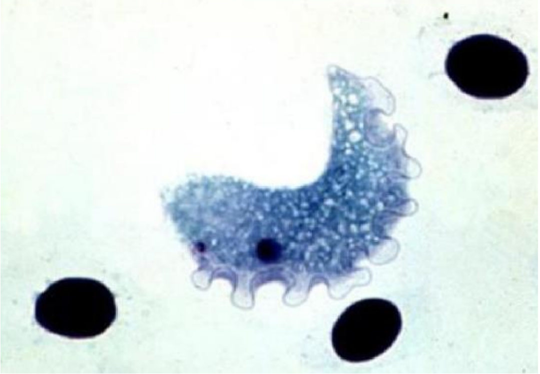
Trypanosoma carassii

- thought to be a non-pathogenic trypanosome offish in natural population
- vascular system of many economically important fish such as carp (*Cyprinus carpio*), eel (*Anguilla* spp.) or tench (*Tinca tinca*)

Trypanosoma rotatorium

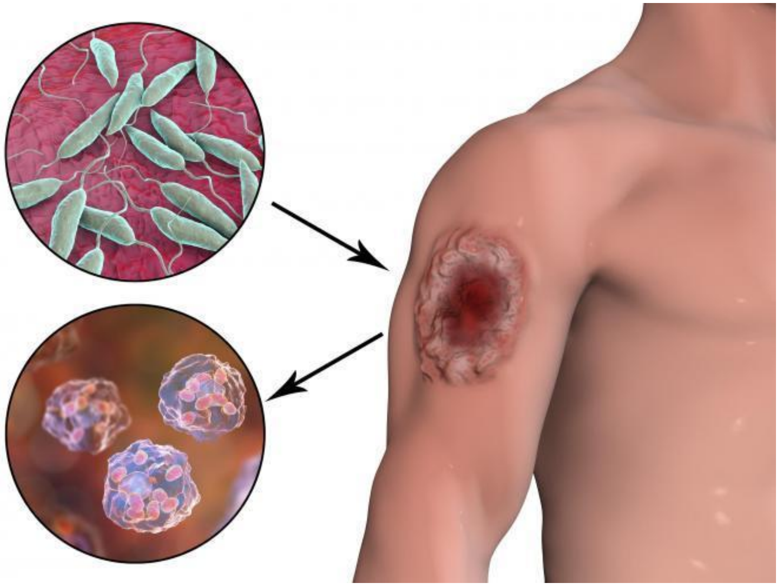
- trypomastigotes reaching up to 70 µm
- host: frogs (*Pelophylax esculentus*, *Rana temporaria*)
- vector: freshwater leeches *Hemiclepsis marginata*





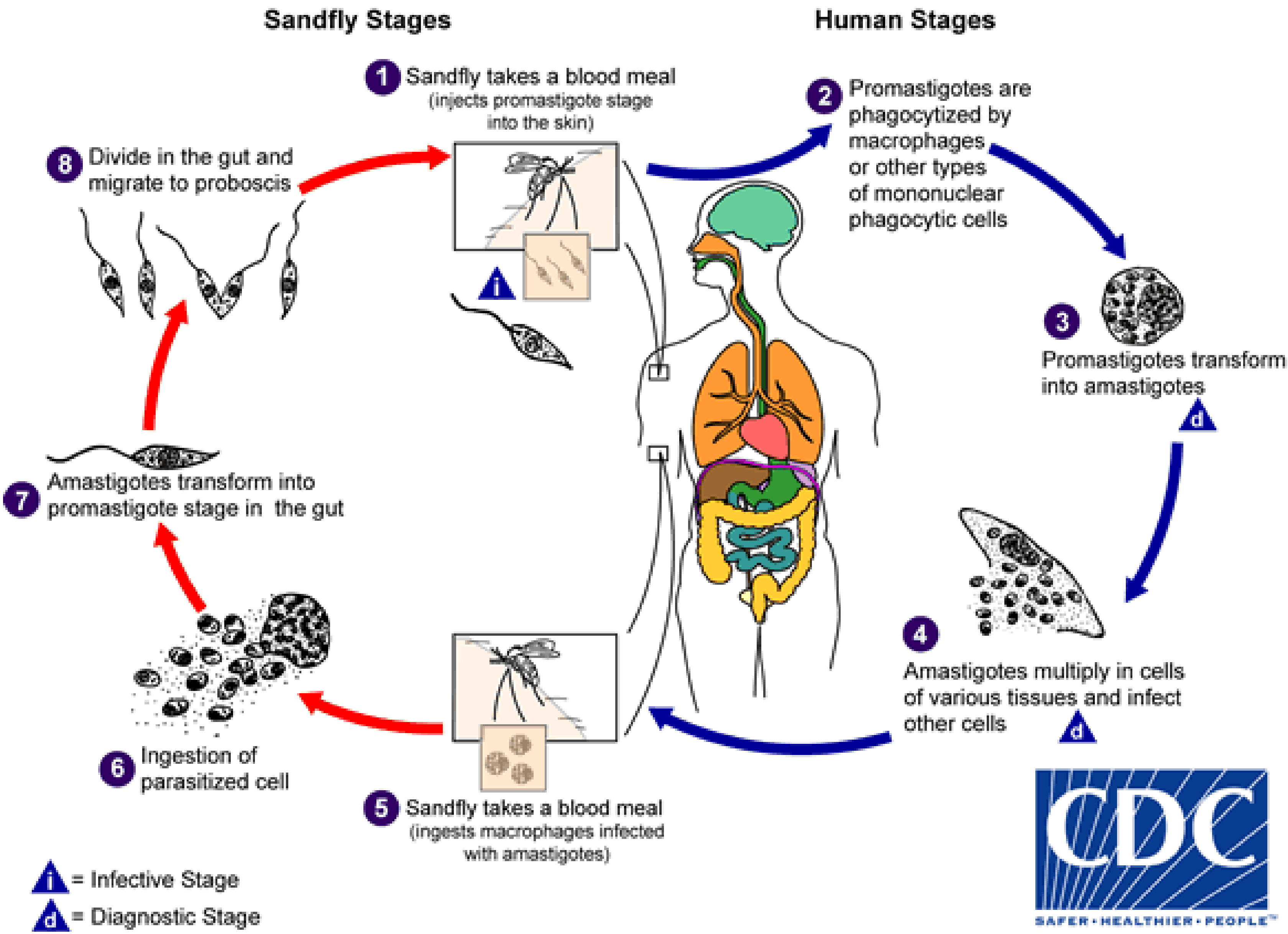
genus ***Leishmania***

- host: vertebrates (mostly hyraxes, canids, rodents) including human
- vector: phlebotomine sand flies
- obligate intracellular parasite – amastigotes in mononuclear phagocytes and circulatory systems of vertebrate host
- motile and extracellular promastigotes in alimentary tract of sandflies
- leishmaniasis classified as a neglected tropical disease (NTD), zoonosis, human infection is caused by more than 20 species
- affecting 6 million people in 98 countries – cca 0.9-1.6 million new cases occur each year, and 21 species are known to cause disease in humans



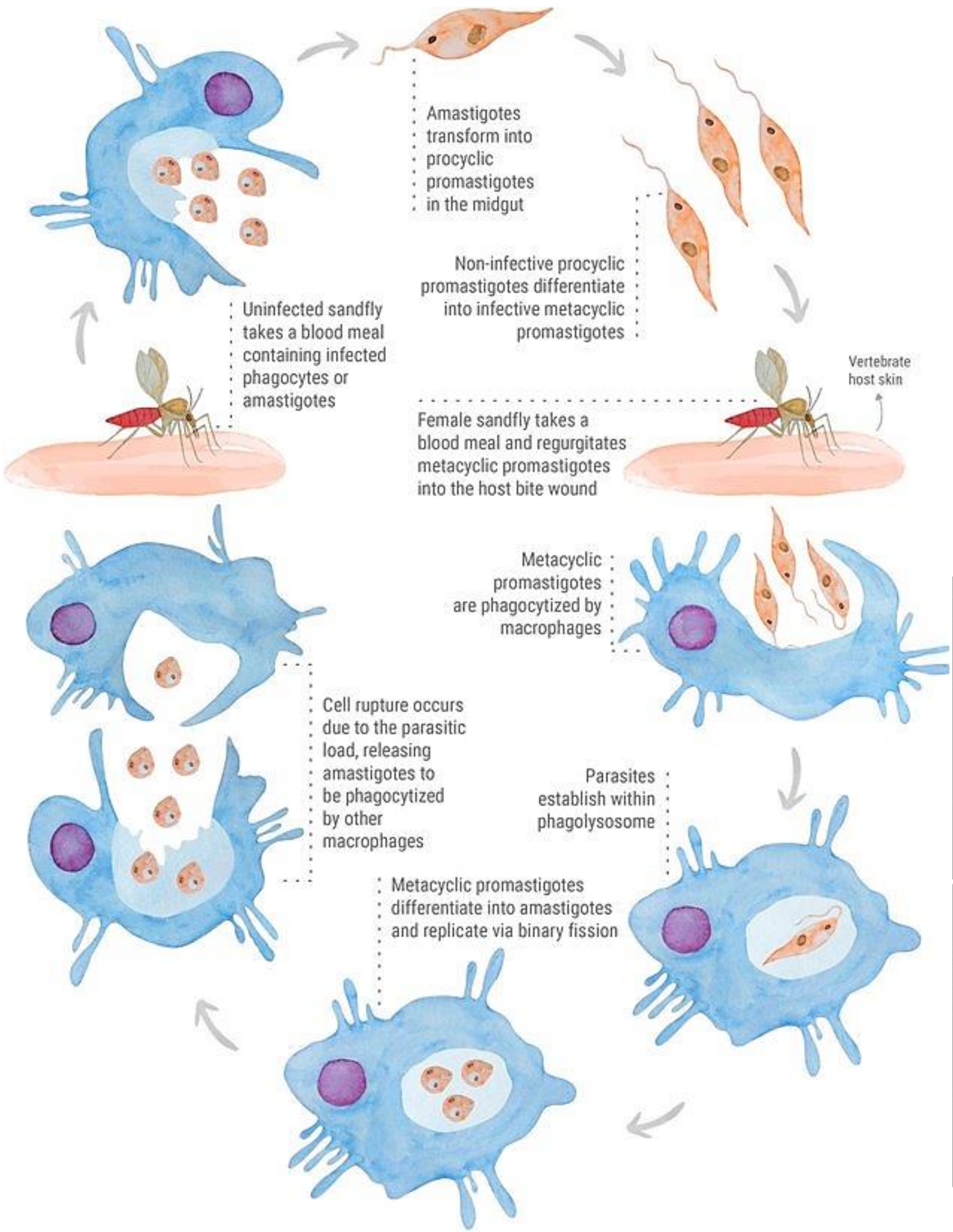
Life cycle of *Leishmania*

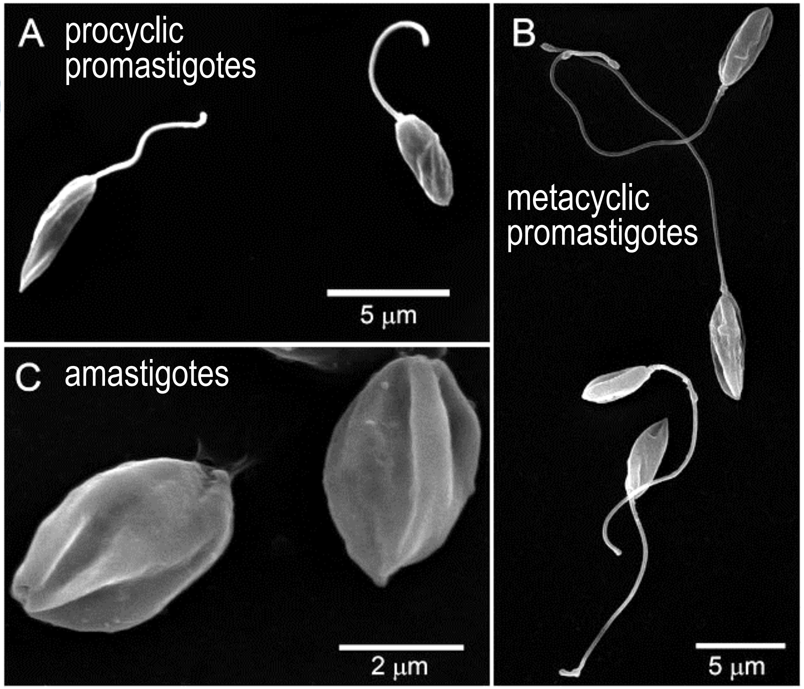
https://www.youtube.com/watch?v=wzjSmxThgJ0
https://www.youtube.com/watch?v=d-ROuaznkSM



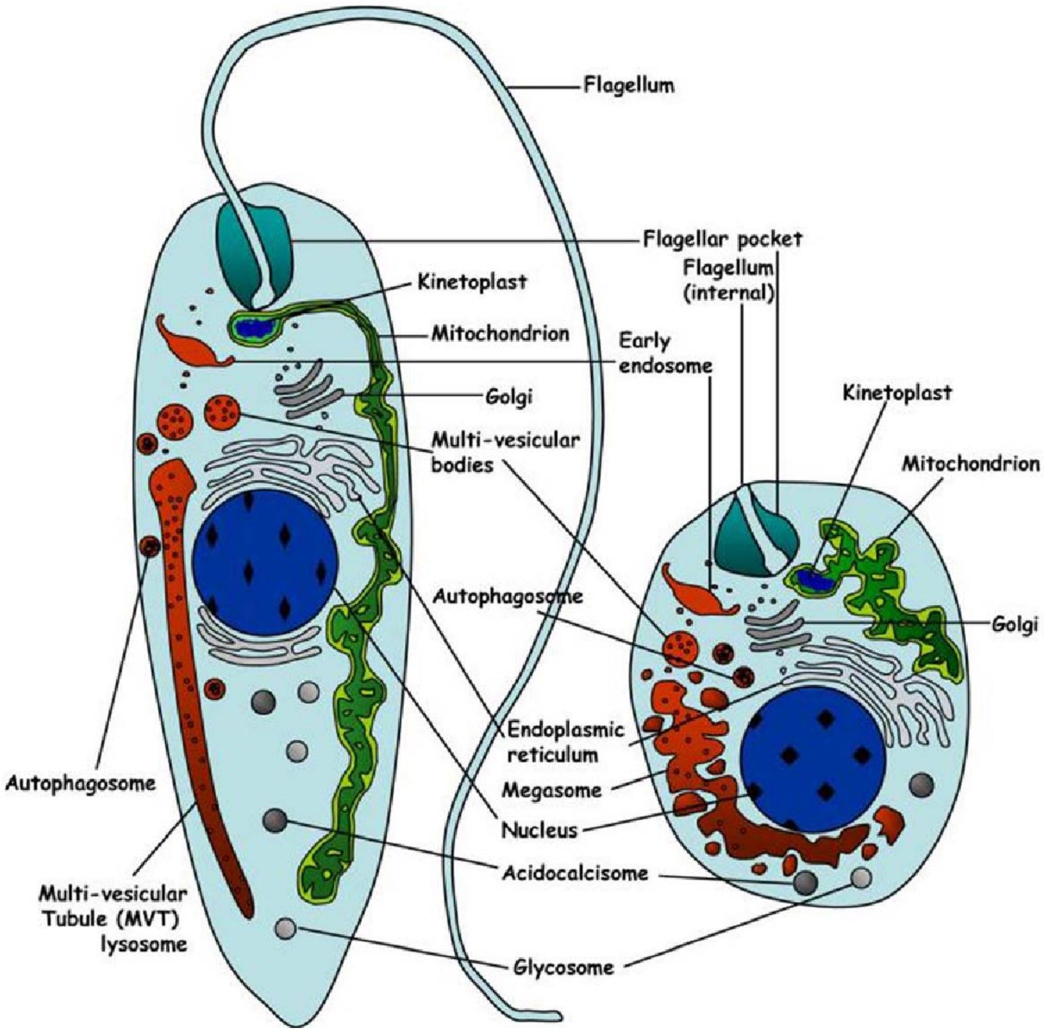
CDC
SAFER · HEALTHIER · PEOPLE™

Leishmania life cycle stages



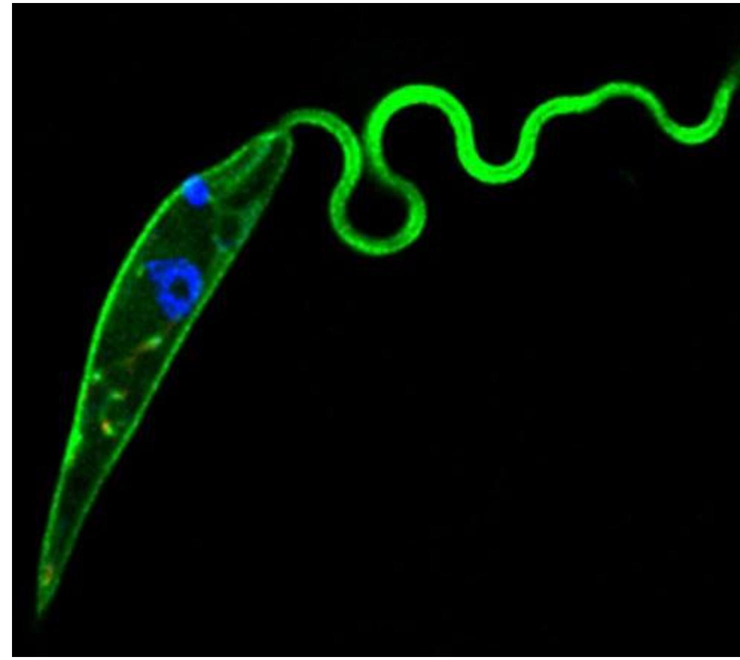


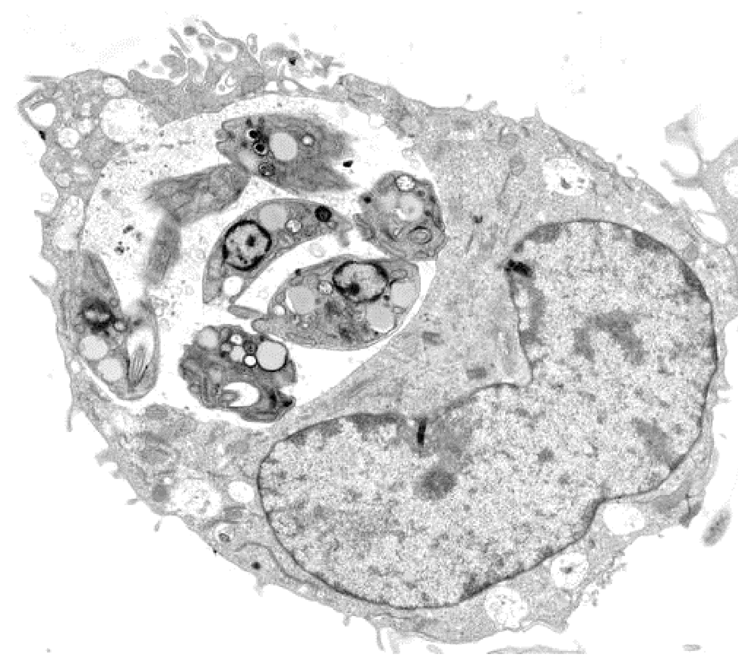
Morphology of *Leishmania*



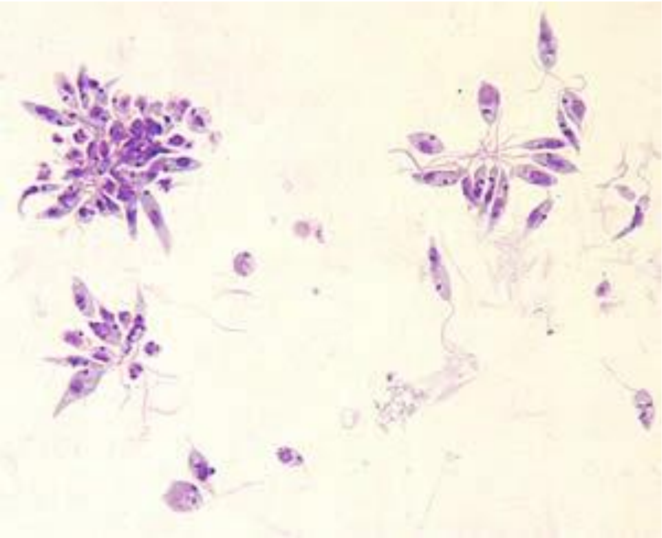
promastigote
14-20 x 1.5-3.5 µm

amastigote
2.5-5 x 1.5-2 µm

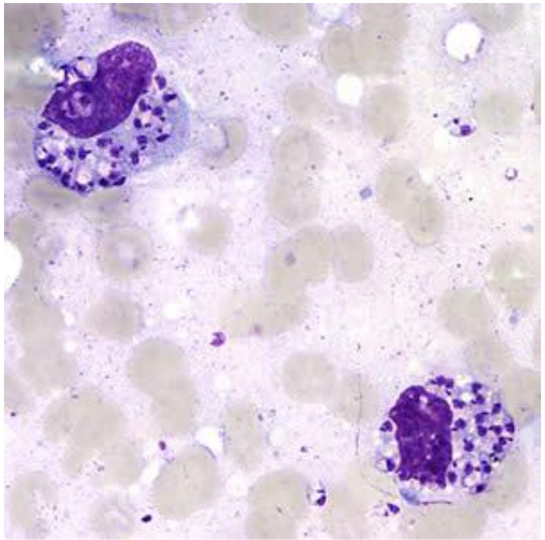




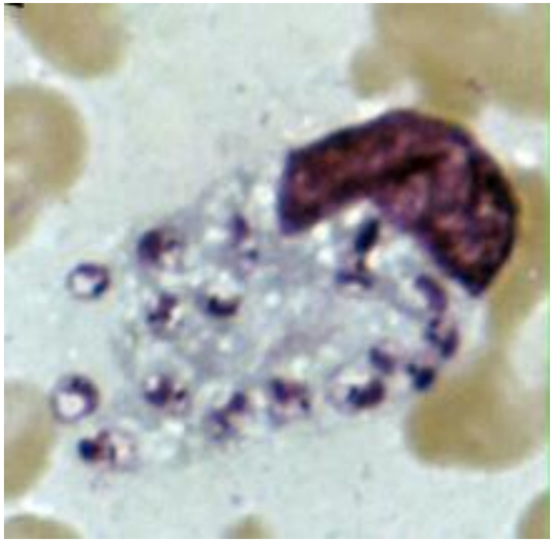
Morphology of *Leishmania*



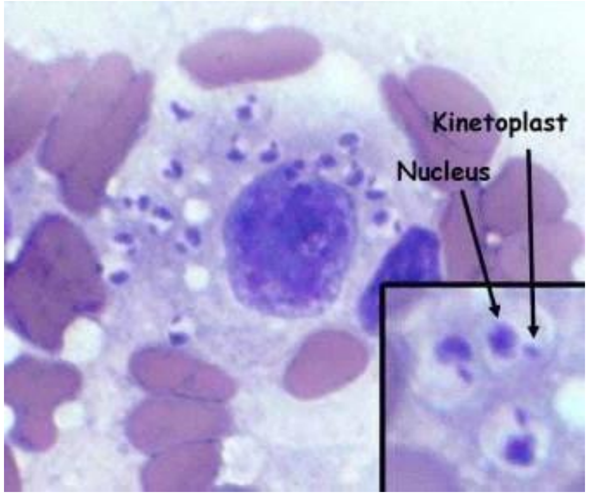
promastigotes from culture

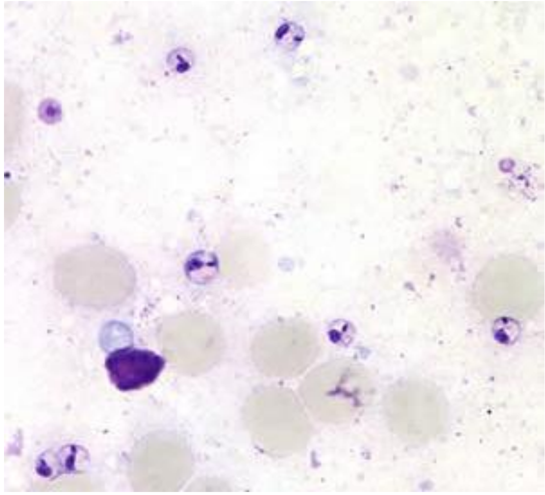


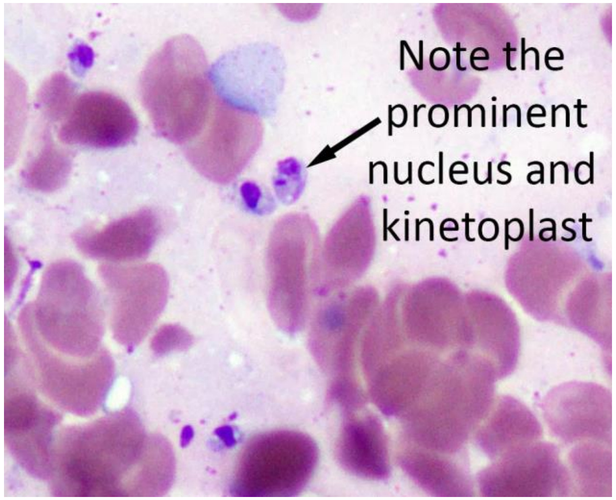
macrophage practically filled with amastigotes



amastigotes are being freed from a bursting macrophage







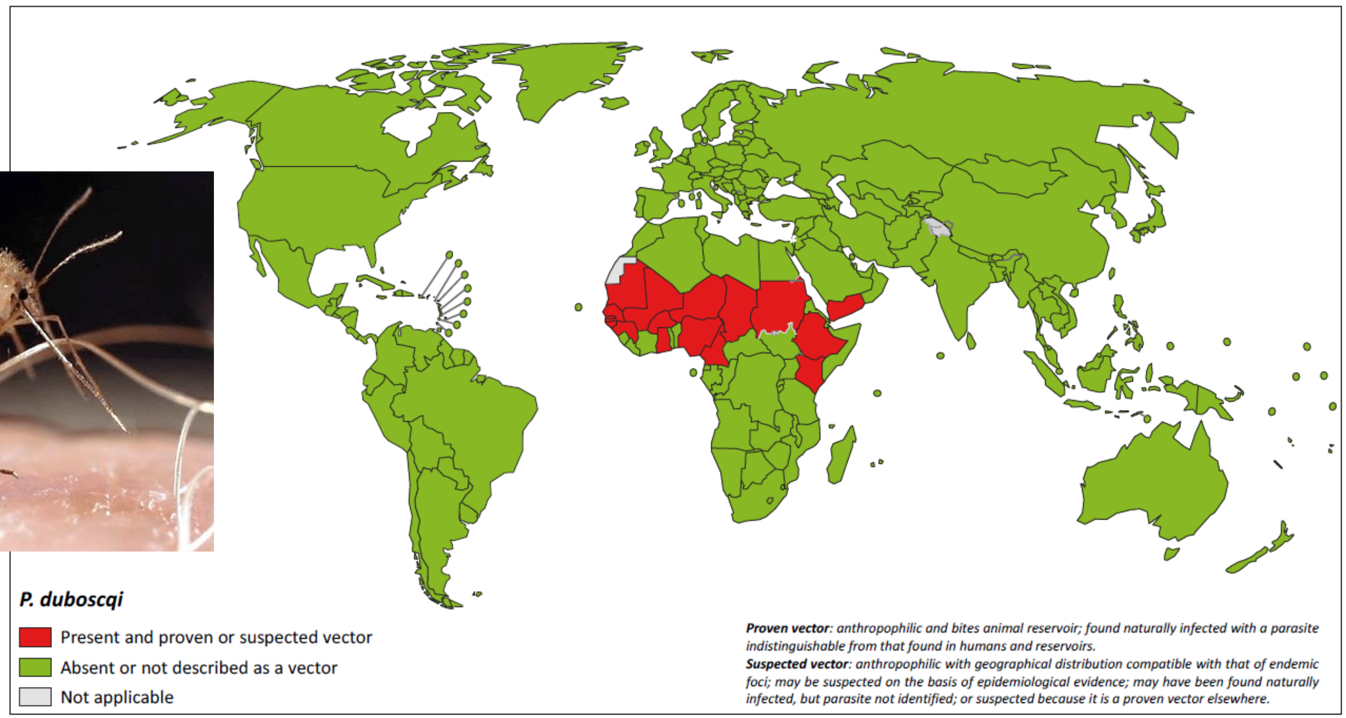
amastigotes with visible nuclei and kinetoplasts

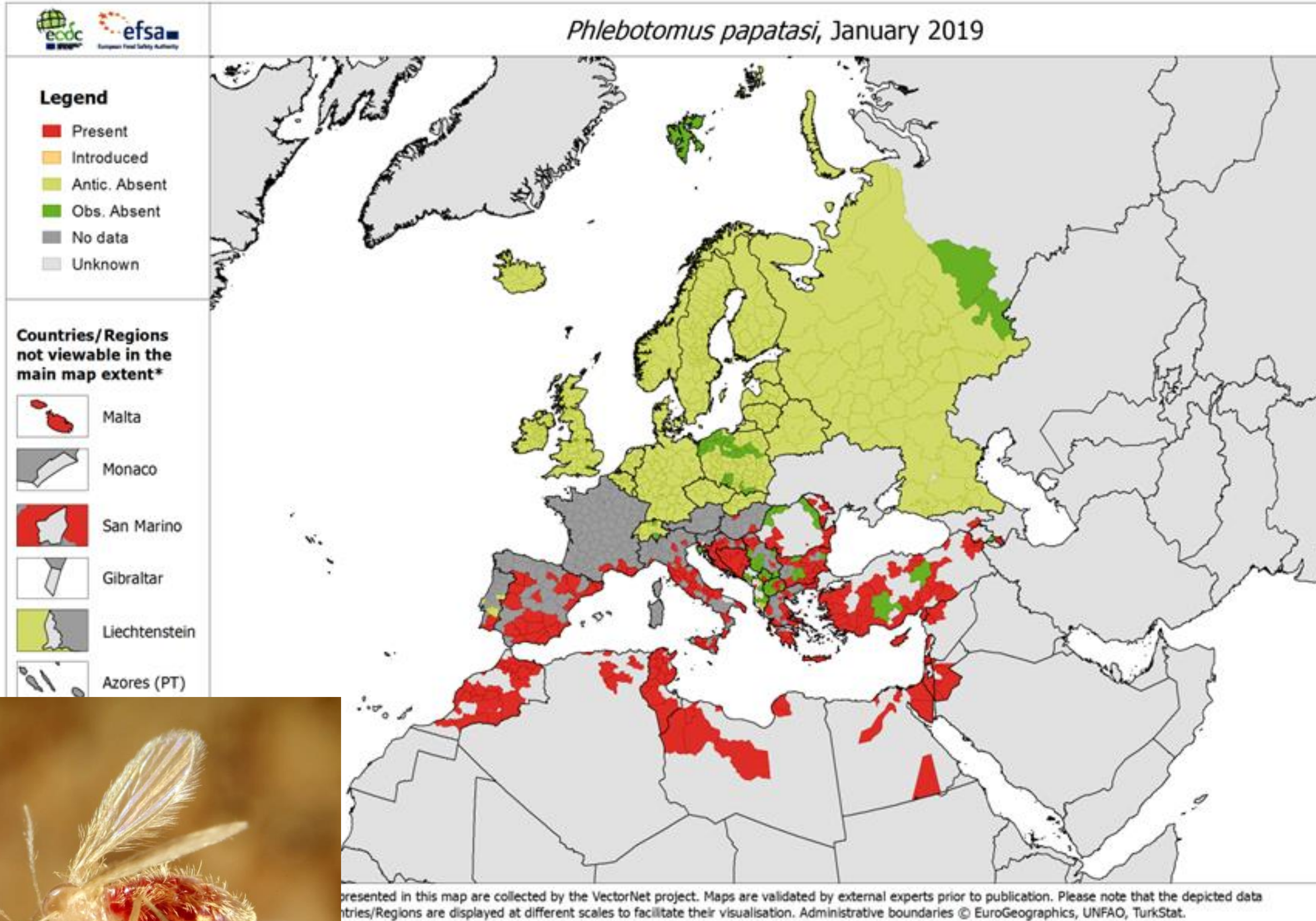
genus *Phlebotomus*

- phlebotomine sand about 1.5–3.0 mm long with hairy bodies, wings, and and legs
- yellowish in colour with conspicuous black eyes
- found only in the Old World
- number of Phlebotomus species occur in Europe, their range has increased in recent years
- only females are blood-feeding

P. duboscqi

Geographical distribution of leishmaniasis vectors – *Phlebotomus duboscqi*



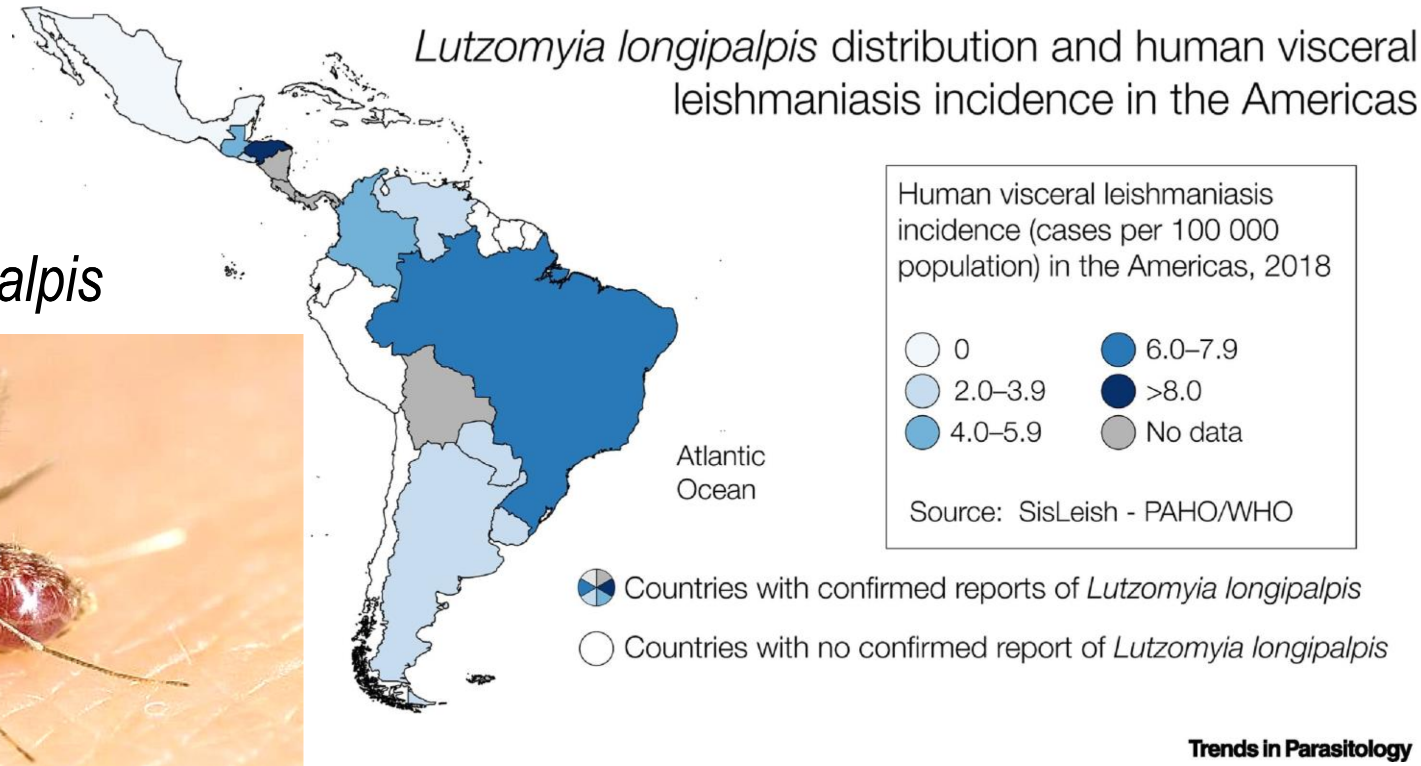


Phlebotomus papatasi

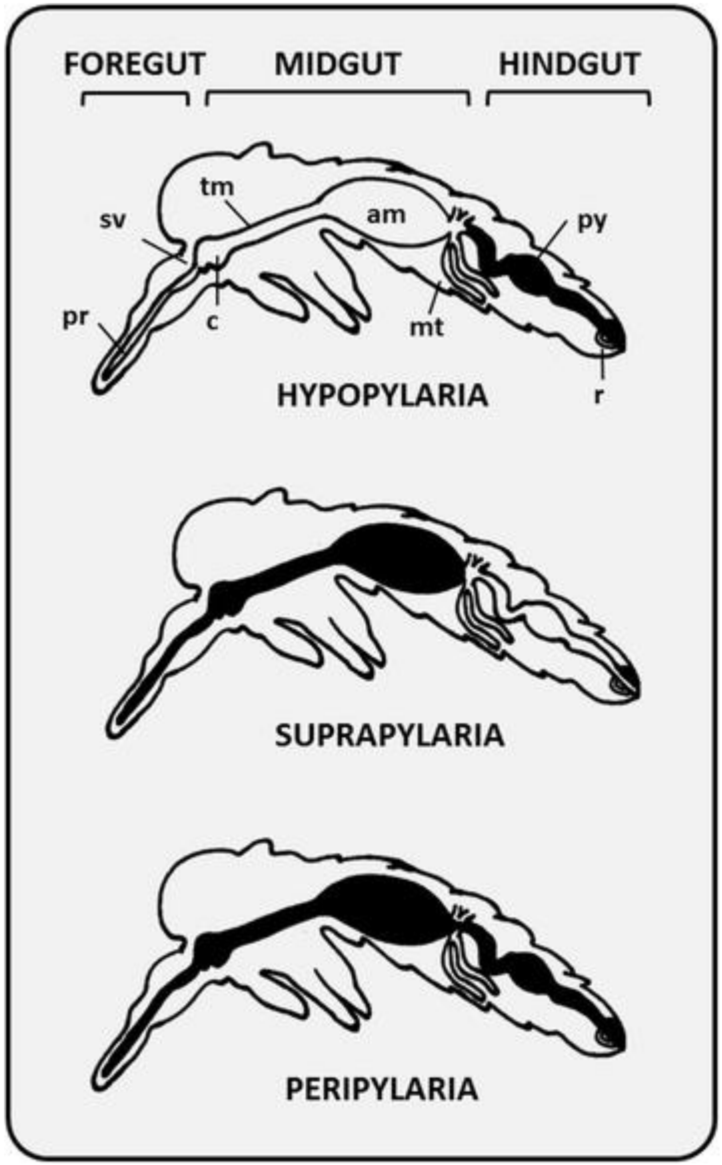
genus *Lutzomia*

- phlebotomine sand flies with a hairy body, length of up to only 3 mm
- nearly 400 species (at least 33 species are medically important vectors)
- found only in the New World
- only females are blood-feeding

Lutzomia longipalpis



Development of *Leishmania* in insect vector



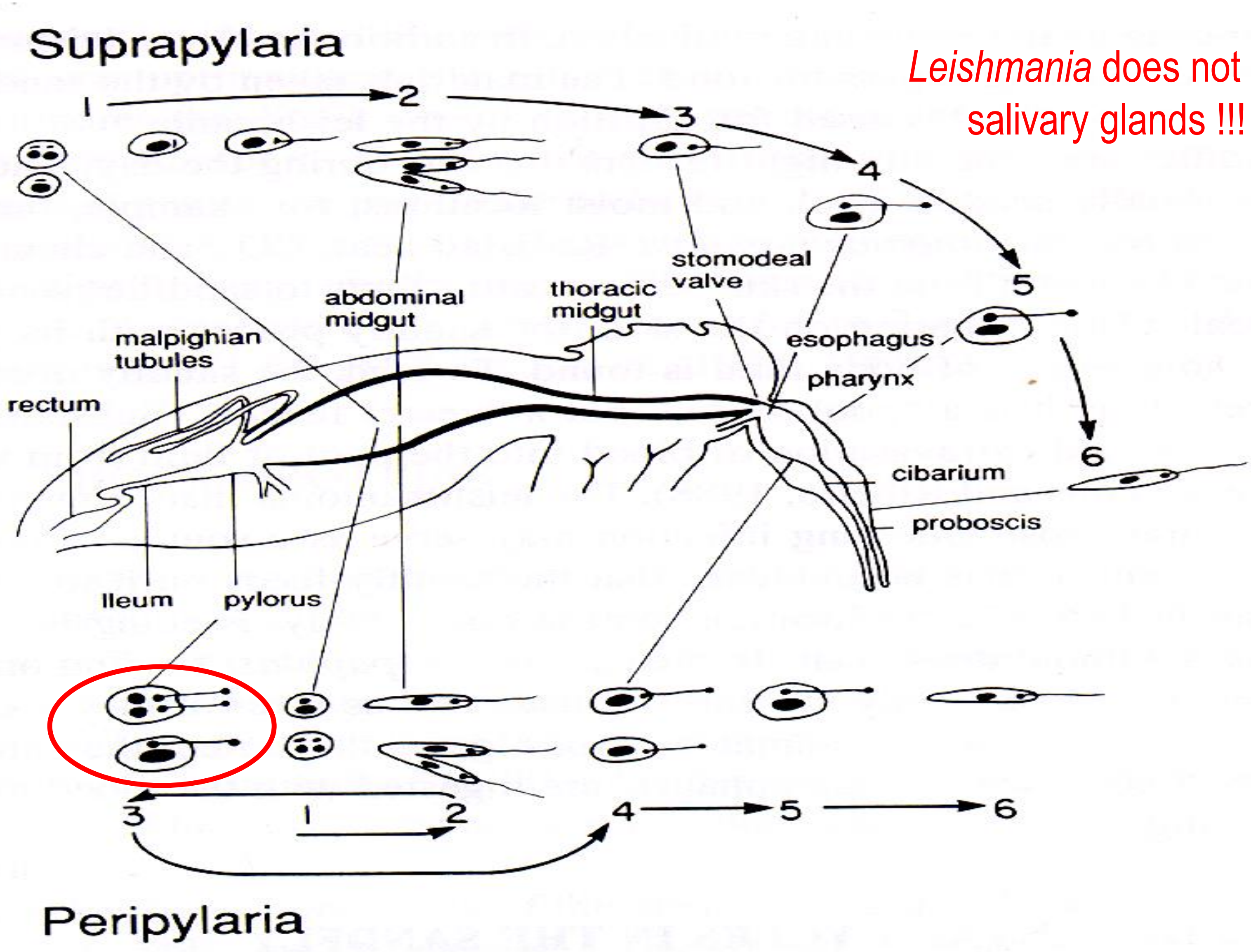
Hypopylaria - in lizards that ingest the sandfly intermediate host. Development occurs in the hindgut of the fly.

Suprapylaria - only in mammals transmitted by the bite of a sandfly, development occurs in the fore- and midgut of the fly.

Peripylaria - in mammals and lizards, development occurs in the foregut and hindgut of the fly.

Development of *Leishmania* in insect vector

Leishmania does not infect salivary glands !!!



Development of *Leishmania* in insect vector

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International Journal for Parasitology 34 (2004) 1221–1227

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Blocked stomodeal valve of the insect vector: similar mechanism of transmission in two trypanosomatid models

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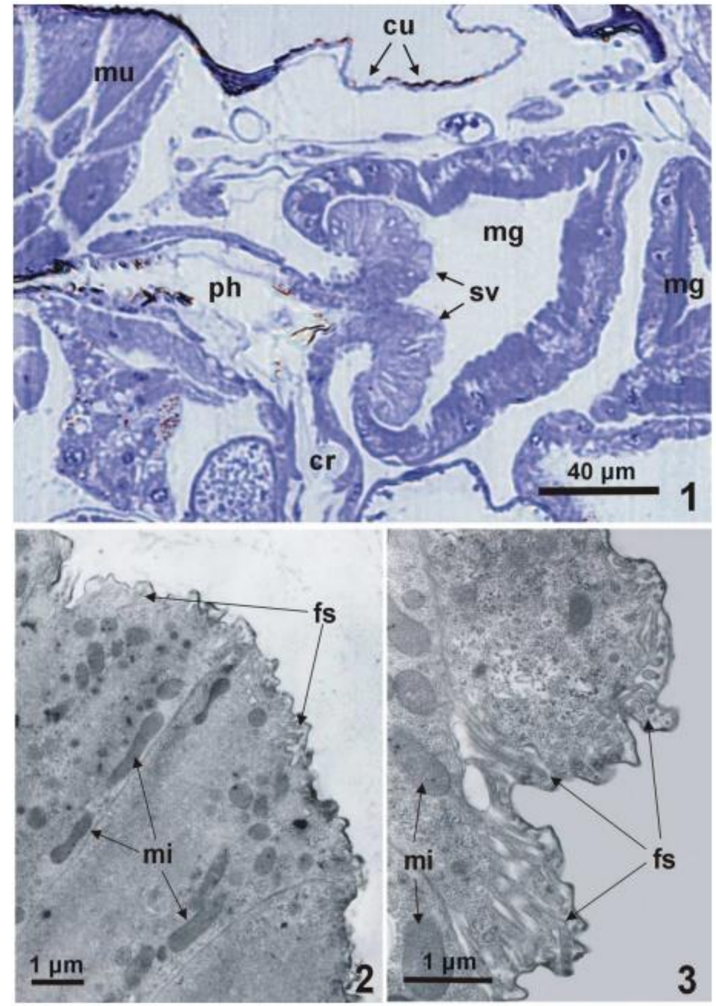
Abstract

The regurgitation of metacyclic stages from the sand fly cardia is thought to be the prevailing mechanism of *Leishmania* transmission. This regurgitation may result through damage of the stomodeal valve and its mechanical block by the parasites. We found this phenomenon in three sand fly–*Leishmania* models and also in avian trypanosomes transmitted by *Culex* mosquitoes. *Phlebotomus duboscqi*, *Phlebotomus papatasi*, *Lutzomyia longipalpis*, and *Culex pipiens* were membrane-fed on blood containing *Leishmania major*, *Leishmania chagasi* (syn. *infantum*) and an unidentified avian *Trypanosoma* from *Trypanosoma corvi* clade, respectively. Females with the late-stage infections were processed for the optical and transmission electron microscopy. Localization of the parasites and changes to the stomodeal valve were in some aspects similar in all vector–parasite pairs studied: (i) a large plug of flagellates was observed in cardia region, (ii) parasites were attached to the chitin lining of the stomodeal valve by the formation of zonal hemidesmosome-like plaques. *Leishmania* promastigotes were found both attached to the valve as well as unattached in the lumen of midgut. The stomodeal valve of infected sand flies was opened, its chitin lining was destroyed and the unique filamentous structures on the apical end of cylindrical cells were degraded. In the *Culex*–*Trypanosoma* model, the whole population of epimastigotes was found in close contact with the chitin lining, and degenerative changes of the valve were less pronounced. We suggest that the phenomenon involving a blocked valve facilitating the regurgitation of parasites into the vertebrate host may occur generally in heteroxenous trypanosomatids transmitted by the bite of nematoceran Diptera.

Keywords: Stomodeal valve; Midgut; Sand fly; Mosquito; *Leishmania*; *Trypanosoma*



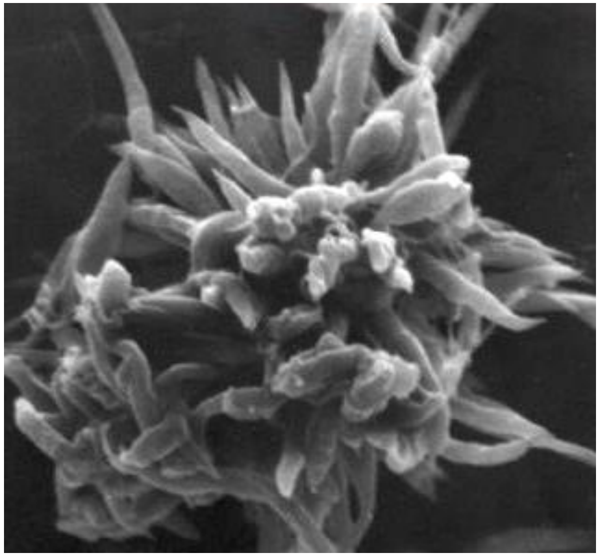
Figs. 1–3. Stomodeal valve of uninfected sand fly females. Semithin section of *Phlebotomus duboscqi* embedded in LR White resin and stained by toluidin blue. The position of filamentous structures in the stomodeal valve is showed by arrows (Fig. 1). Electron microscopy of the cylindrical cells in the inner part of the stomodeal valve of *P. duboscqi* (Fig. 2) and *Phlebotomus papatasi* (Fig. 3). Abbreviations: cr, crop; cu, cuticle; fs, filametous structures; mi, mitochondria; mg, midgut; mg + l, midgut containing Leishmania; mu, muscles; mv, microvilli of the thoracic midgut; n, nucleus of epithelial cells of the stomodeal valve; fl, Leishmania flagellum; lk, Leishmania kinetoplast; ln, Leishmania nucleus; oe, oesophagus; ph, pharynx; sg, salivary glands; sv, stomodeal valve; t, Trypanosoma; arrowheads, hemidesome-like plaques on parasite flagellum; *, degradation of the chitin layer.

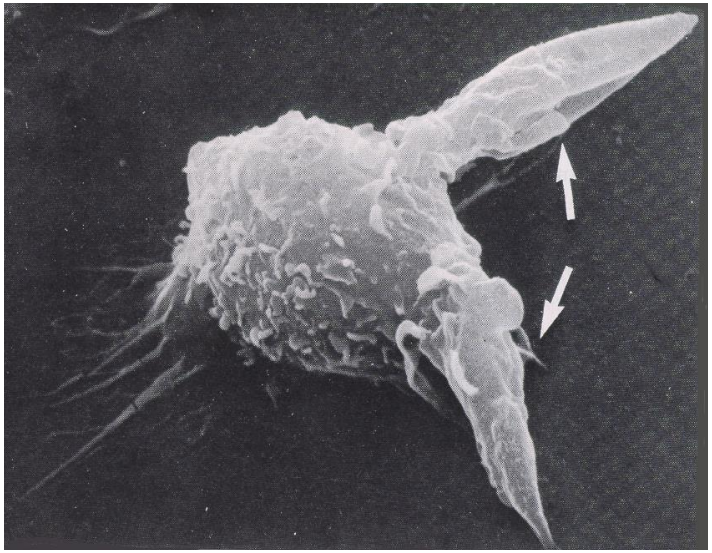
https://doi.org/10.1016/j.ijpara.2004.07.010

Interactions of *Leishmania* with host macrophages

- promastigote attach to the macrophage surface and after being phagocytosed they reside within phagolysosomal vacuoles and transform into amastigotes
- survival and multiplication of amastigotes in phagolysosome
    - ✓ inhibition of the production of oxygen radicals
    - ✓ inhibition of hydrolases

https://www.youtube.com/watch?v=0J6TMd-x6o0





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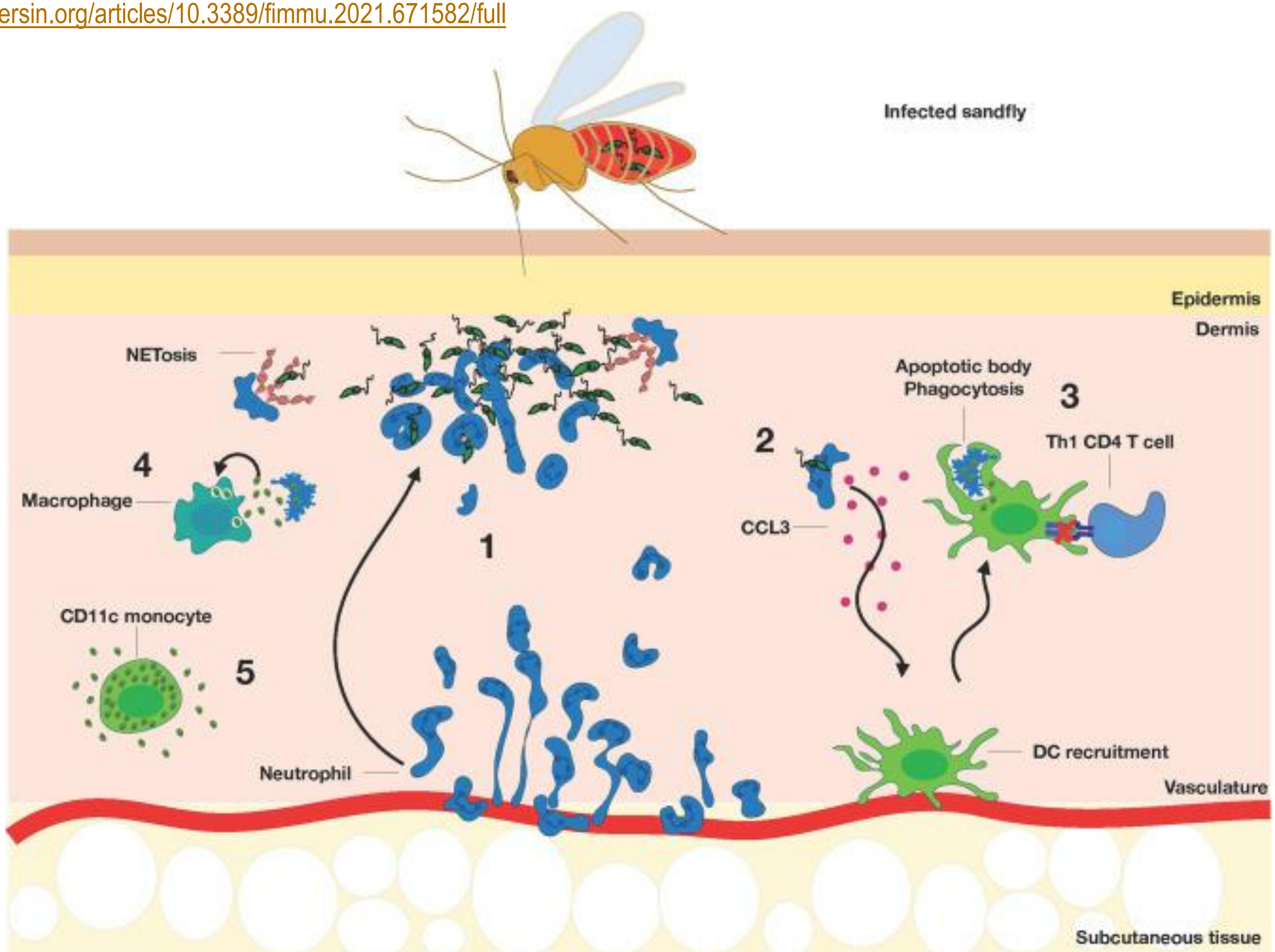

Acute response to *Leishmania* infection by a sandfly bite: (1) **Neutrophils**, recruited from the blood to the infection site, undergo NETosis and phagocytose the promastigotes. (2) Infected neutrophils recruit **dendritic cells** by producing CCL3, which subsequently engulf the apoptotic bodies of infected neutrophils and (3) lose their ability to effectively activate **Th1 response**. (4) **Macrophages** become infected by the parasites released by the dying neutrophils. (5) **CD11c+ monocytes** are highly permissive to parasite replication and further promote infection.

„Silent“ invasion and host-parasite interactions in *Leishmania*

microorganisms MDPI

Review

Hide-and-Seek: A Game Played between Parasitic Protists and Their Hosts

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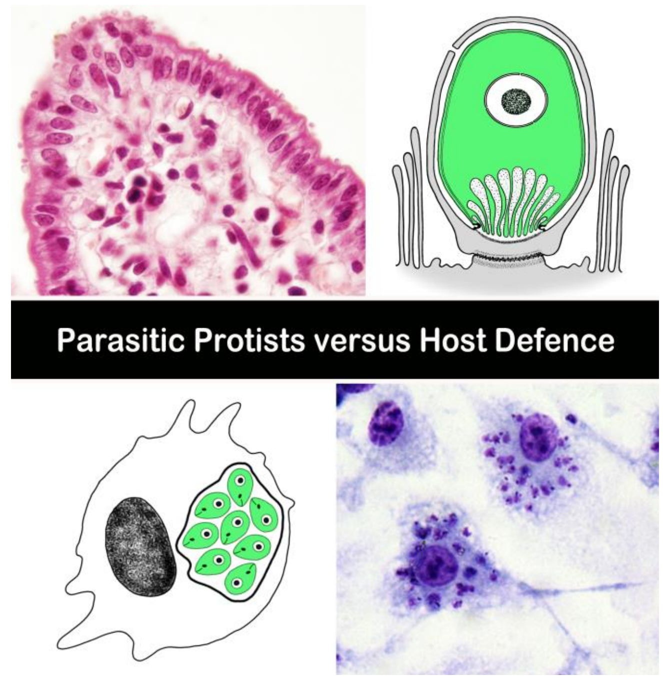
† These authors contributed equally to the work.

Abstract: After invading the host organism, a battle occurs between the parasitic protists and the host's immune system, the result of which determines not only whether and how well the host survives and recovers, but also the fate of the parasite itself. The exact weaponry of this battle depends, among others, on the parasite localisation. While some parasitic protists do not invade the host cell at all (extracellular parasites), others have developed successful intracellular lifestyles (intracellular parasites) or attack only the surface of the host cell (epicellular parasites). Epicellular and intracellular protist parasites have developed various mechanisms to hijack host cell functions to escape cellular defences and immune responses, and, finally, to gain access to host nutrients. They use various evasion tactics to secure the tight contact with the host cell and the direct nutrient supply. This review focuses on the adaptations and evasion strategies of parasitic protists on the example of two very successful parasites of medical significance, *Cryptosporidium* and *Leishmania*, while discussing different localisation (epicellular vs. intracellular) with respect to the host cell.

Keywords: unicellular parasite; parasitic protist; *Cryptosporidium*; *Leishmania*; intracellular; epicellular; extracellular; parasitophorous sac; parasitophorous vacuole; adaptation to parasitism; evasion strategies; host defence

check for updates

Citation: Kolářová, I.; Valigurová, A. Hide-and-Seek: A Game Played between Parasitic Protists and Their Hosts. *Microorganisms* **2021**, *9*, 2434. https://doi.org/10.3390/microorganisms9122434



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„Silent“ invasion and host-parasite interactions in *Leishmania*

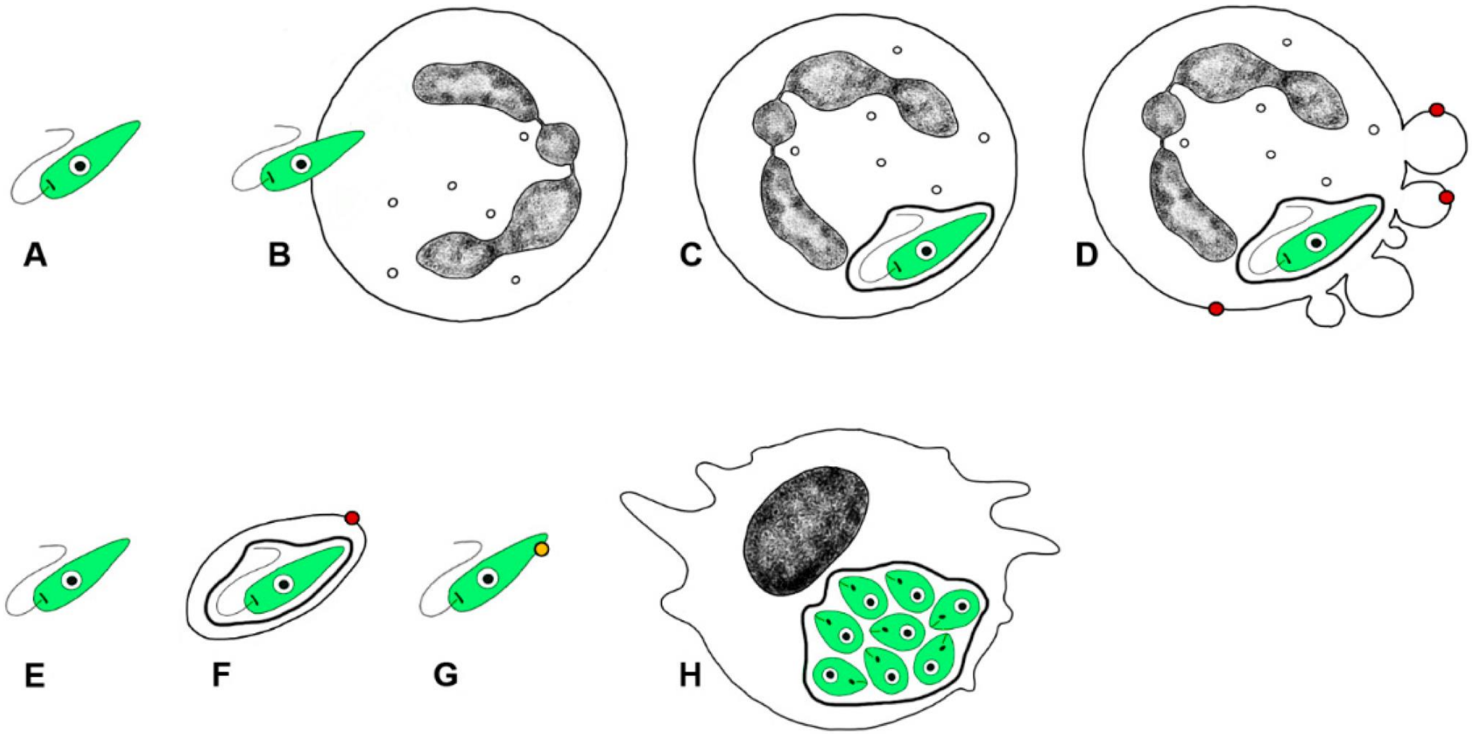
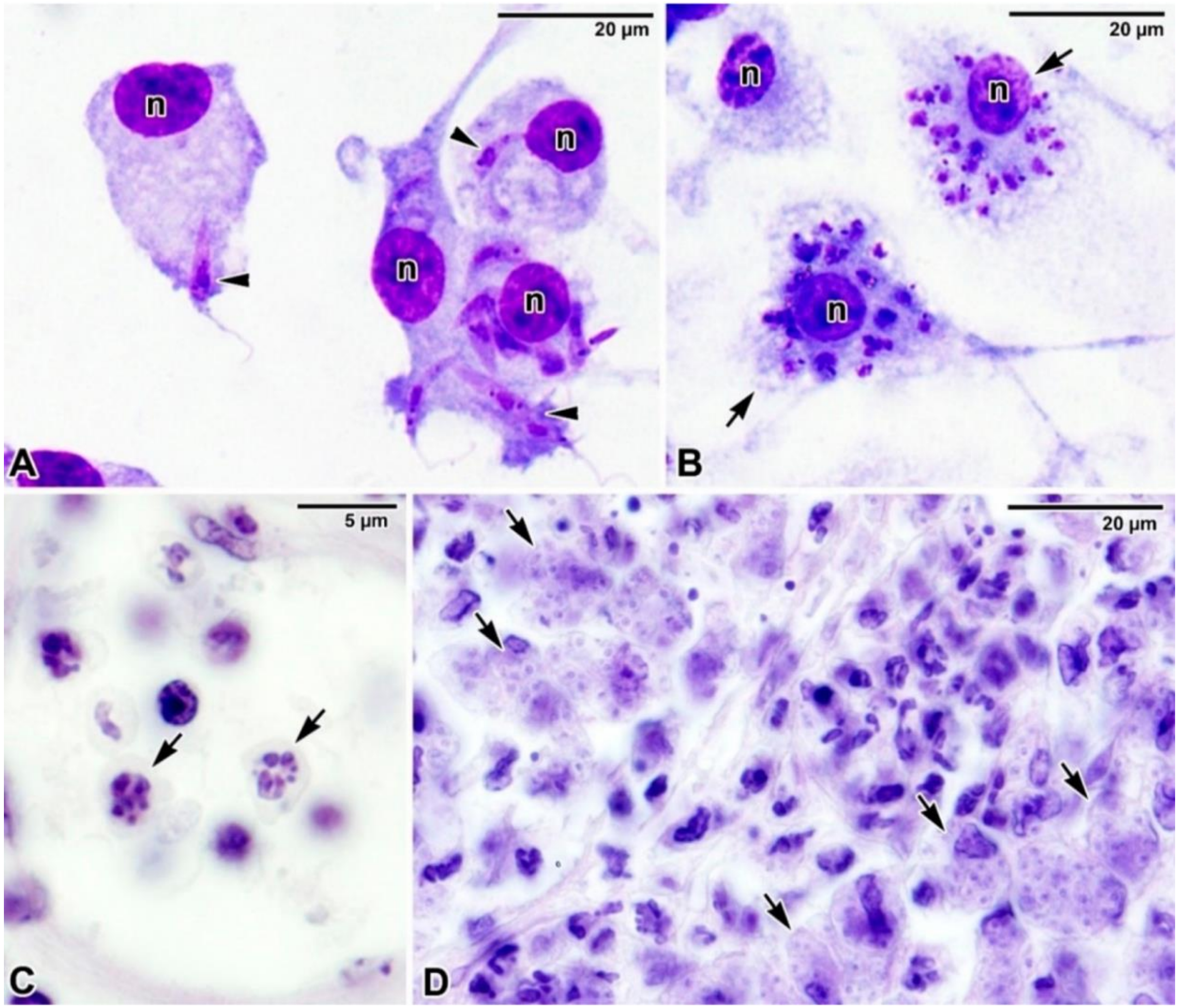


Figure 4. What is behind a silent entry? The infective inoculum, besides other factors, contains viable promastigotes represented as green cells (**A**). Neutrophils engulf promastigotes (**B**), which survive intracellularly in a parasitophorous vacuole represented by a thicker black line around the green parasite (**C**). Eventually, infected neutrophils become apoptotic, showing the phosphatidylserine on the outer side of the plasma membrane, represented as a red dot (**D**). *Leishmania* promastigotes can be phagocyted by their host cells, typically monocytes and macrophages, as free promastigotes (**E**), in neutrophil apoptotic bodies (**F**), or as apoptotic promastigotes expressing *Leishmania* phosphatidylserine analogue, represented as a yellow dot (**G**). Macrophages engulf *Leishmania* in the presence of apoptotic signals (either of neutrophil or *Leishmania* origin), which makes the microenvironment anti-inflammatory, thus supporting *Leishmania* survival and the establishment of infection (**H**).

https://doi.org/10.3390/microorganisms9122434

„Silent“ invasion and host-parasite interactions in *Leishmania*



igure 5. Intracellular localisation of *Leishmania*. (**A**,**B**) Giemsa-stained smear preparation showing (**A**) macrophages vith invading *Leishmania donovani* promastigotes (arrowheads) and (**B**) three macrophages, two of which are heavily parasitised by *L. donovani* amastigotes (arrows). *n*—macrophage nucleus. Light microscopy. (**C**,**D**) Haematoxylin-eosin stained histological sections of the BALB/c mice ear showing numerous macrophages parasitised by *Leishmania major* amastigotes (arrows). Light microscopy. Micrographs (**A**,**B**) courtesy of Dr. Tereza Leštinová.

https://doi.org/10.3390/microorganisms9122434

Leishmaniasis

8 January 2022

Key facts

- There are 3 main forms of leishmaniases – visceral (also known as kala-azar, which is and the most serious form of the disease), cutaneous (the most common), and mucocutaneous.
- Leishmaniasis is caused by protozoan parasites which are transmitted by the bite of infected female phlebotomine sandflies.
- The disease affects some of the poorest people and is associated with malnutrition, population displacement, poor housing, a weak immune system and lack of financial resources.
- Leishmaniasis is also linked to environmental changes such as deforestation, building of dams, irrigation schemes and urbanization.
- An estimated 700 000 to 1 million new cases occur annually.
- Only a small fraction of those infected by parasites causing leishmaniasis will eventually develop the disease.

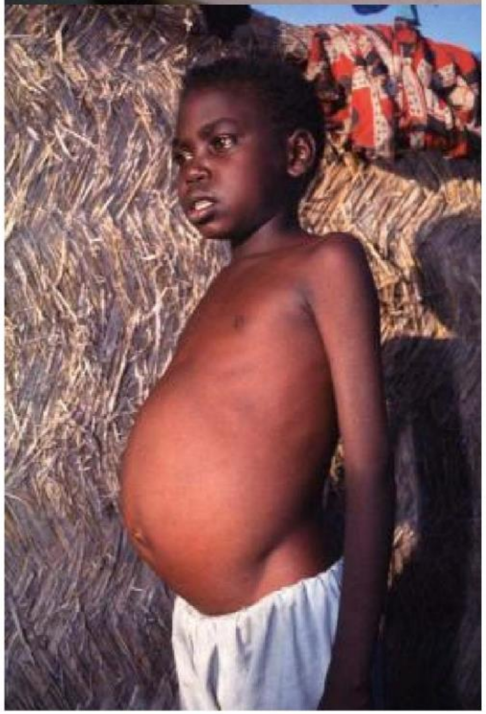
Human *Leishmania* species

- human infection caused by about 21 of 30 *Leishmania* species infecting mammals
- different species are morphologically indistinguishable
- antropozoonoses vs. antroponoses

  - *L. tropica*, *L. major*, *L. aethiopica*; Old World cutaneous leishmaniasis
  - ***L. mexicana* complex** with 3 main species: *L. mexicana*, *L. amazonensis*, and *L. venezuelensis*; New World cutaneous leishmaniasis
  - **subgenus Viannia** with 4 main species: *L. (V.) braziliensis*, *L. (V.) guyanensis*, L. *(V.) panamensis*, *L. (V.) peruviana*; New World leishmaniasis
  - ***L. donovani* complex** with 2 species: *L. donovani*, *L. infantum* (*a*lso known as *L. chagasi* in the New World); Old World visceral leishmaniasis

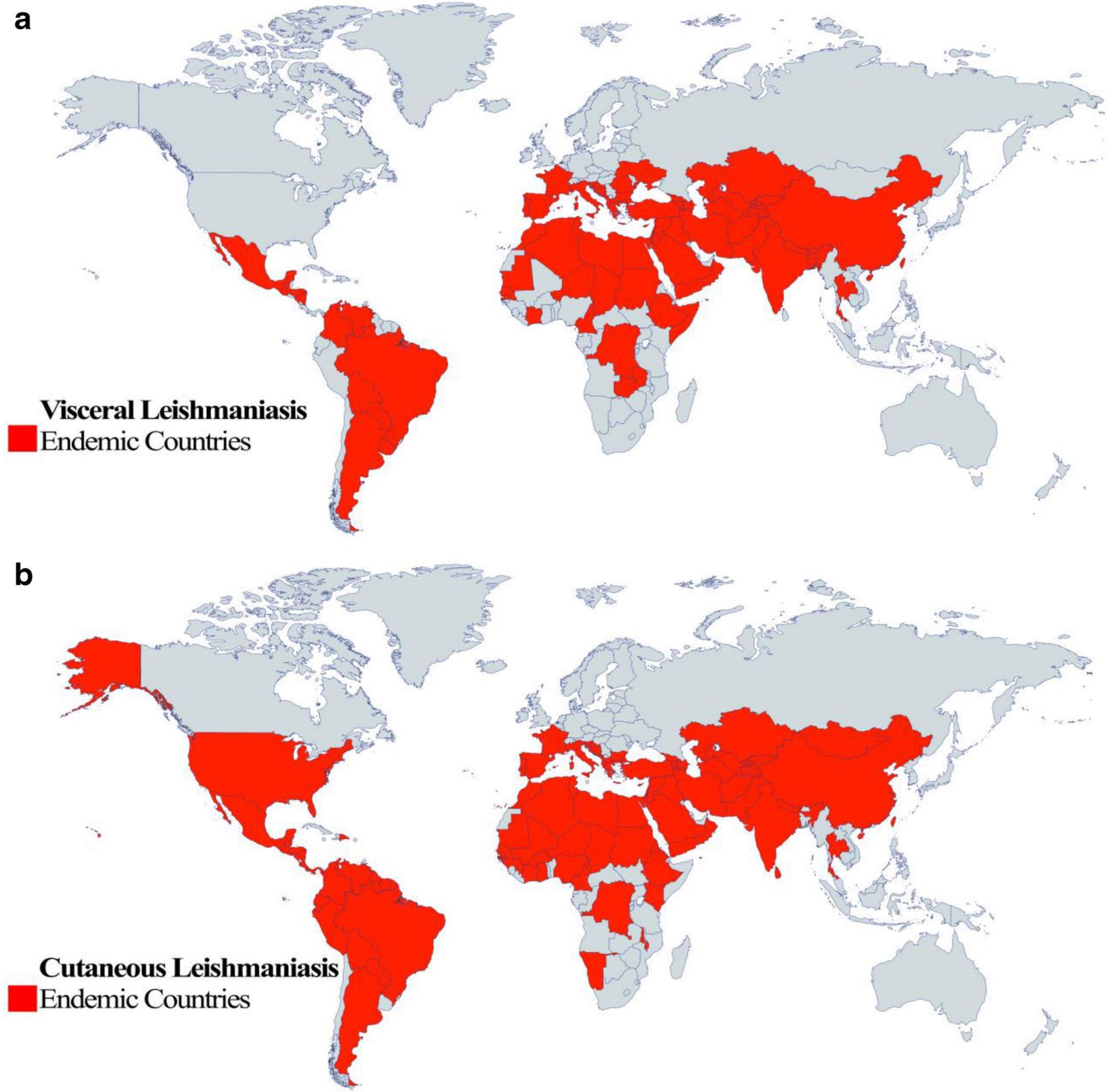
Human leishmaniosis

- different forms of human leishmaniasis
- **cutaneous leishmaniosis** - most common form
  - ➢ skin sores
  - ➢ sores usually are painless but can be painful
- **visceral leishmaniasis**
  - ➢ affects several internal organs (usually spleen, liver, and bone marrow)
  - ➢ can be life threatening
  - ➢ illness typically develops within months (sometimes years) after sand fly bite
- **mucosal leishmaniasis**
  - ➢ can be a sequela (consequence) of cutaneous leishmaniasis in parts of Latin America

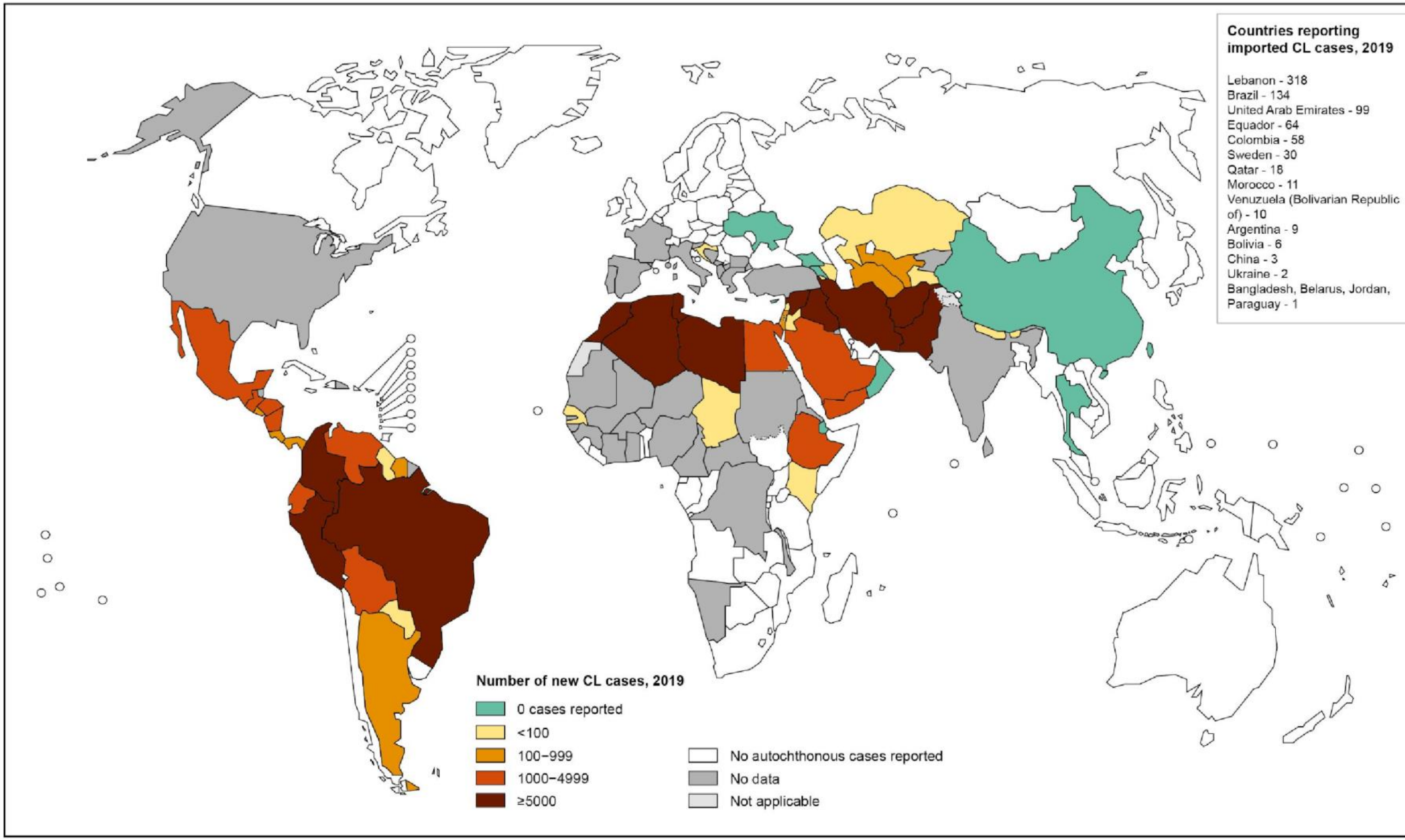


Species	Disease	Hosts	Distribution
L. major	cutaneous leishmaniasis (wet form)	humans, rodents	Africa, Asia
L. tropica	cutaneous leishmaniasis (dry form)	humans, hyrax	Africa, Asia, Europe
L. braziliensis	espundia, mucocutaneous leishmaniasis	humans, rodents	South America
L. mexicana	localised & diffuse cutaneous leishmaniasis	humans, rodents	Central America
L. infantum	visceral leishmaniasis (mostly)	humans, dogs	Africa, Europe, South America
L. donovani	kala azar, visceral leishmaniasis	humans, dogs	Africa, Asia, Europe

L. chagasi = synonymised recently with *L. infantum*


a
Visceral Leishmaniasis
Endemic Countries
b
Cutaneous Leishmaniasis
Endemic Countries

Status of endemicity of cutaneous leishmaniasis worldwide, 2019

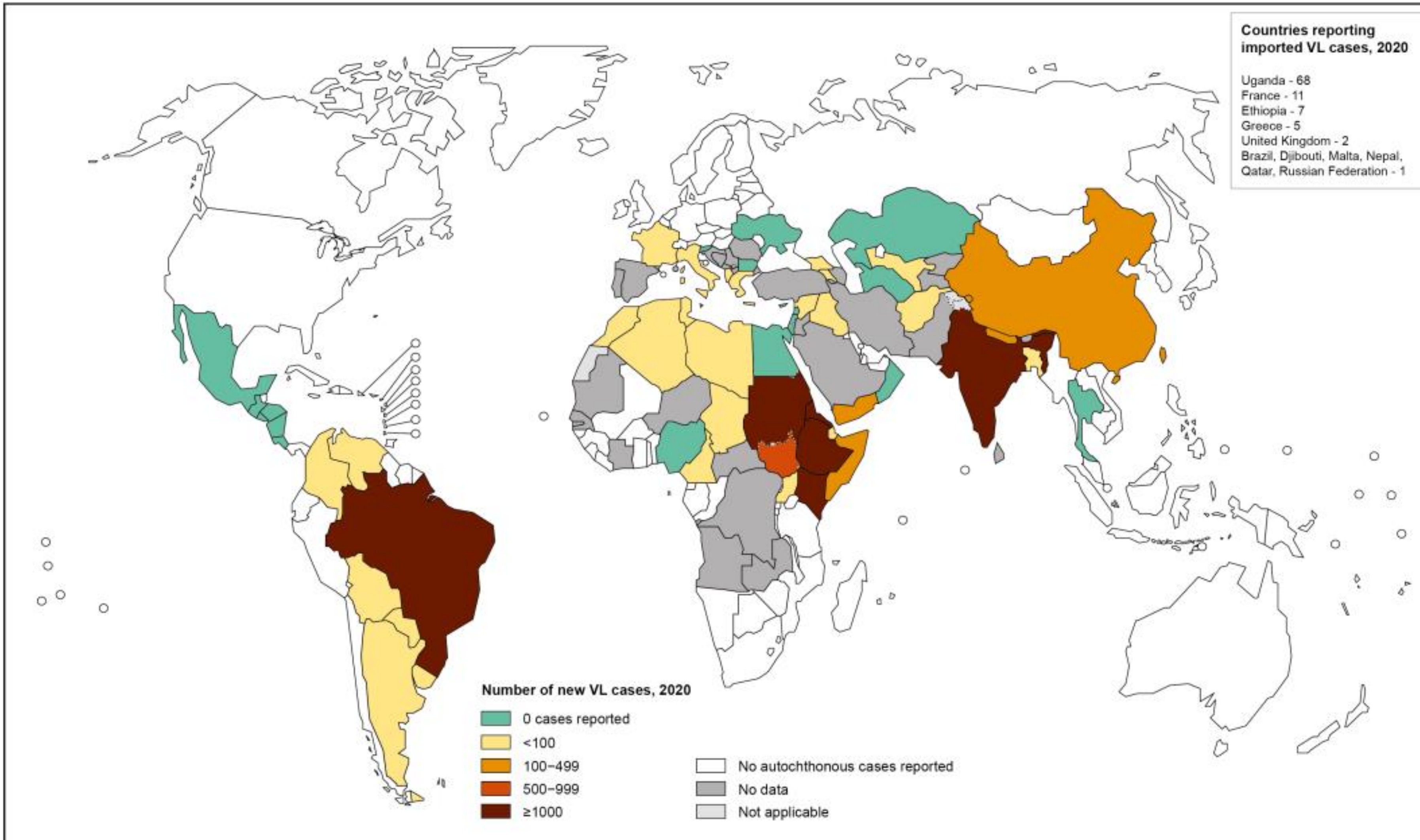


The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

Status of endemicity of visceral leishmaniasis worldwide, 2020



Data Source: World Health Organization
Map Production: Control of Neglected Tropical Diseases (NTD)
World Health Organization

World Health Organization

Leishmania tropica

- urban type
- **chronic dry sore**, “oriental sore“
- vector: *Phlebotomus sergenti*
- Mediterranean, Middle East, Central Asia, India
- anthroponosis

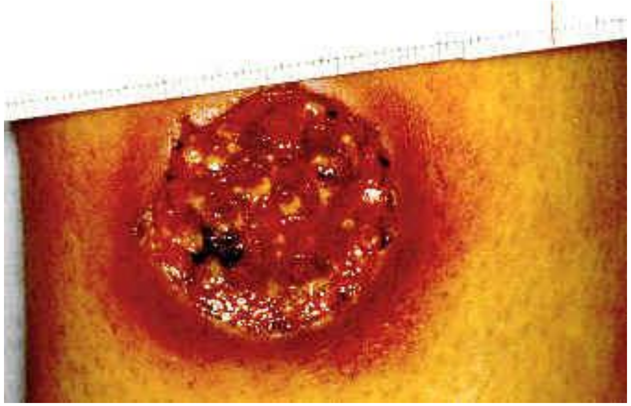
- possible visceralisation – Persian Gulf War syndrome???

Leishmania tropica

- strong immune response and few parasites during relapse

Leishmania major

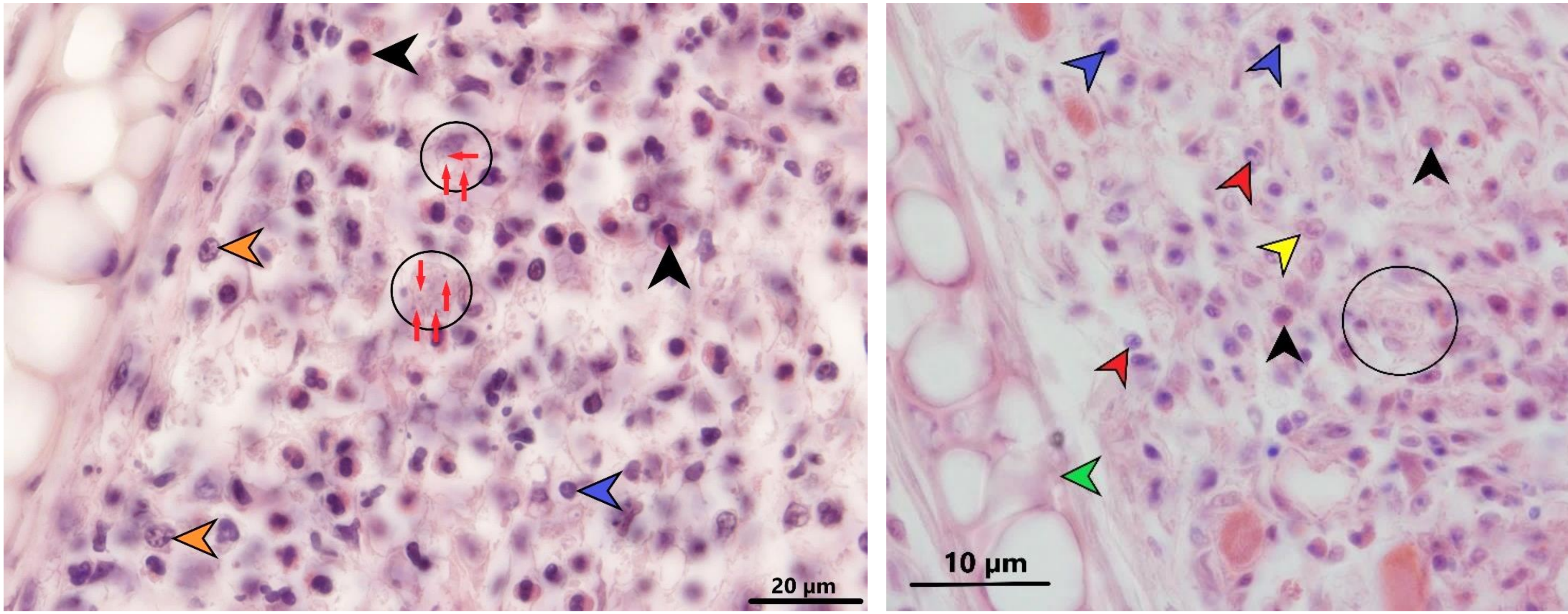
- rustic type, **acute wet sore**
- incubation period lasts 1-4 weeks, healing in 3-6 months
- vector: *Phlebotomus duboscqi*, *P. papatasi*
- arid regions, semi-deserts - Africa, Middle East, Asia
- reservoir: gerbils (*Rhombomys opimus, Meriones* spp. *Arvicanthis niloticus, Psammomys obescus*)
- vaccination through leishmanisation with live *L. major* has been used successfully but is no longer practiced because it resulted in occasional skin lesions



Pathology of cutaneous leishmaniasis caused by *Leishmania major*

Histological section of ear pinna of BALB/c mouse with **advanced leishmaniasis** **(left)** and a **healthy** mouse ear pinna **(right)**. Stained with Masson's green trichrome; ellipse indicates the lesion.

Pathology of cutaneous leishmaniasis caused by *Leishmania major*

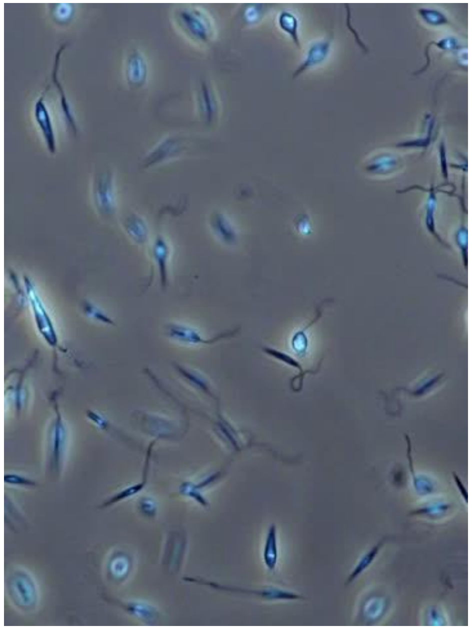


Detail of the edge of a skin lesion in the ear pinna of a mouse infected with *L. major.* Haematoxylin-eosin (left) and Giemsa (right) stained histological sections.

Circle - macrophage with *Leishmania* amastigotes (→), ➢ / ➢ - neutrophils, ➢ - lymphocytes, ➢ - eosinophils, ➢ - mast cell, ➢ - cartilage.

Leishmania aethiopica

- cutaneous - **dry sore**, oedematous non-ulcerating lesions
- often mucosal leishmaniasis or diffuse leishmaniasis
- chronic slow disease course (3 years)
- Ethiopia, Kenya
- reservoir: hyrax

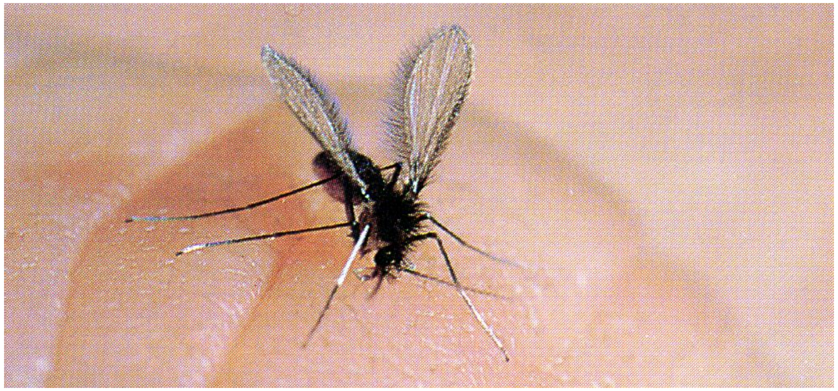


Diagnosis of cutaneous leishmaniosis

Diagnosis of cutaneous leishmaniasis. (upper left) An incision along the raised border is made and dermal scrapings are examined by Giemsa staining and microscopy. (lower left) Sterile saline is injected into the raised border and fluid is aspirated. The aspirated can be examined microscopically or preferentially used to inoculate in vitro cultures or hamsters. Note the scar of the poorly positioned biopsy punch. Biopsies should be taken at the raised border of the lesion. (right) Montenegro delayed-type hypersensitivity reaction.

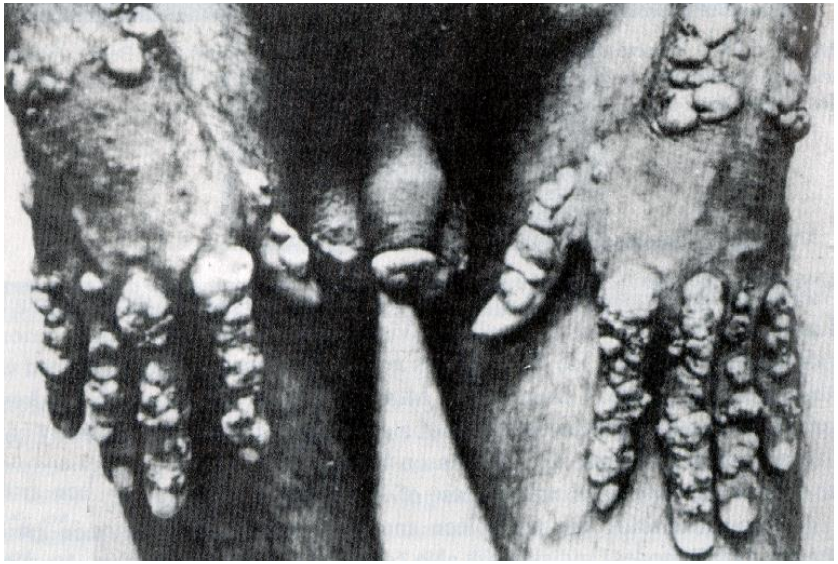
Leishmania mexicana complex

- vector: *Lutzomiya longipalpis*, etc.
- reservoir: forest rodents
- Central America



Leishmania m. amazonensis

- diffuse cutaneous leishmaniasis



Leishmania m. mexicana

- “ulcera des chicleros”

- on auricle, metastasis in the adjacent cartilages

Achras zapota

Leishmania (V.) braziliensis

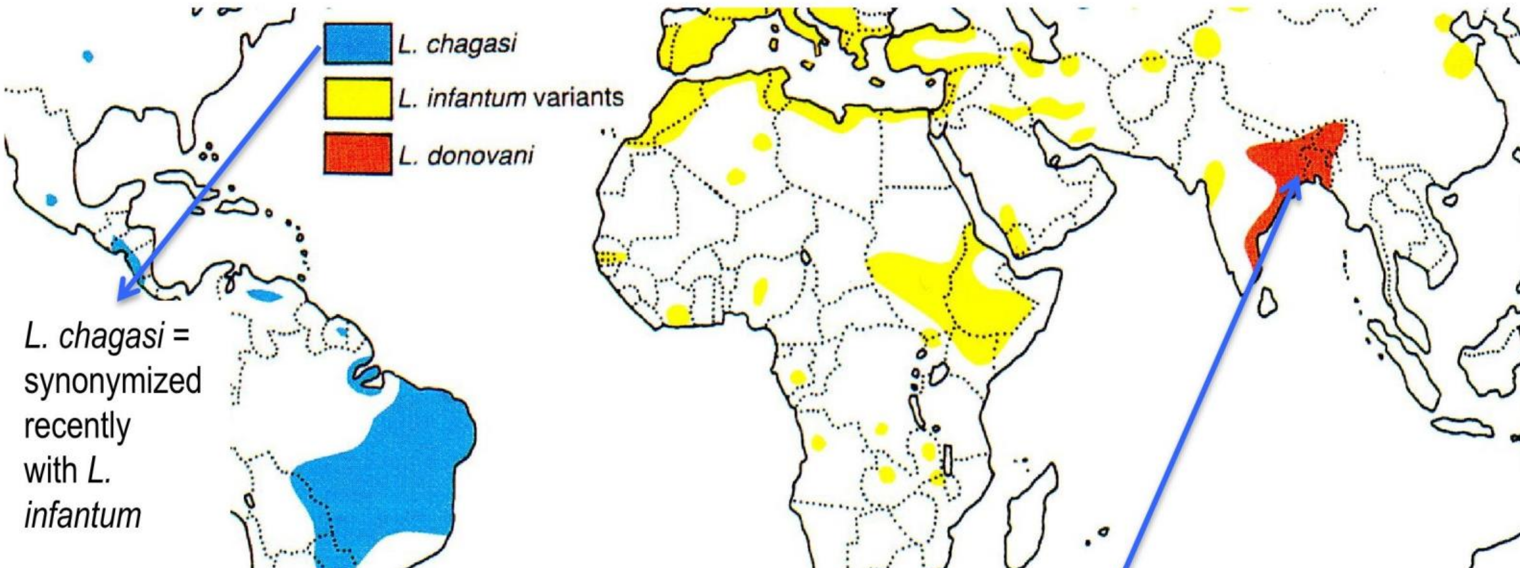
- “Espundia“
- forests in the Amazon basin
- reservoir: small forest rodents
- metastasizes to the nasopharyngeal mucosa

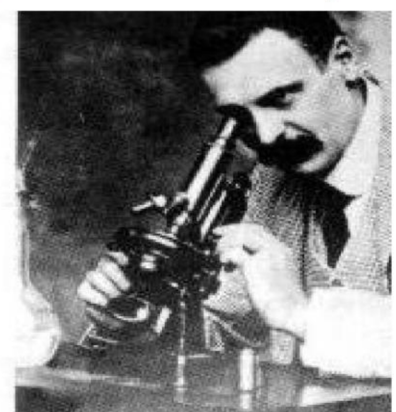
Leishmania (V.) quyanensis

- “pian bois”
- vector: *Lutzomyia umbratilis*, etc.
- reservoir: sloths, dogs
- secondary lesions, spreading along the lymphatics - diffuse leishmaniasis
- weak induction of immune responses (high parasite burden in the lesion, low antibody

Leishmania donovani complex

- visceral leishmaniosis - complex of two species: *L. d. donovani*, *L. d. infantum*





William Leishman and Charles Donovan detected amastigotes within macrophages of people with Kala-azar

Leishmania d. infantum

- Old World infant visceral leishmaniasis
- reservoir: canids; vector: *Phlebotomus papatasi*, *P. perniciosus*, etc.
- viscerotropic and dermotropic variant

Leishmania d. donovani

- vector: *Phlebotomus orientalis*, *P. martini*
- reservoir: canids
- **“Kala azar“ „Dum dum fever“**
    - weight loss, weakness
    - cough, fever that lasts for weeks or months
    - enlarged spleen, enlarged liver
    - decreased production of red blood cells (RBCs)
    - bleeding, night sweats, thinning hair
    - scaly skin, dark, ashen skin
- estimated **200,000 to 400,000 infections each year** worldwide
- over 90% of new cases occur in 6 countries: Bangladesh, Brazil, Ethiopia, India, South Sudan and Sudan
- fatality rate in developing countries can be as high as 100 % within 2 years

Diagnosis of “Kala azar“ or „Dum dum fever“

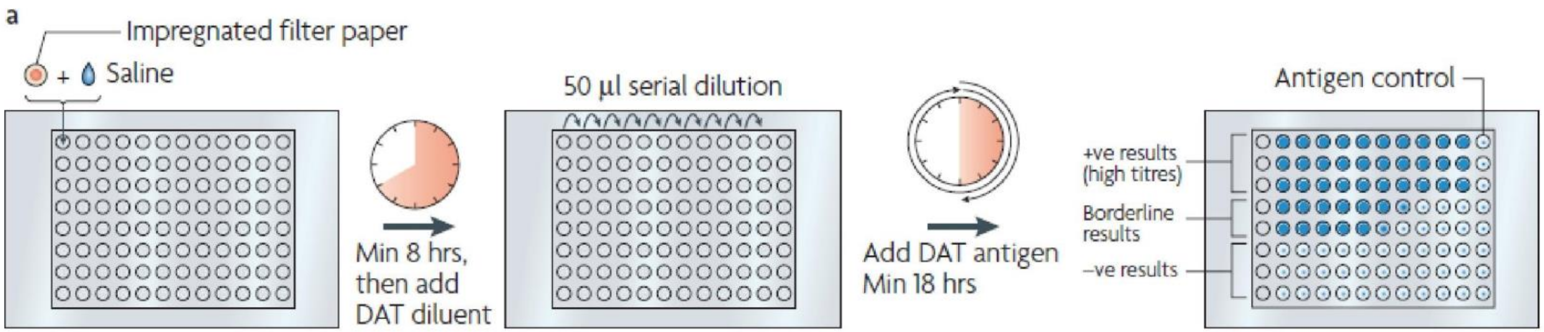
Leishmania d. donovani

- ✓ biopsy of the spleen and culture
- ✓ bone marrow biopsy and culture
- ✓ direct agglutination assay
- ✓ indirect immunofluorescent antibody test
- ✓ *Leishmania*-specific PCR test
- ✓ liver biopsy and culture
- ✓ lymph node biopsy and culture
- ✓ Montenegro skin test (not approved in the USA)
- ✓ skin biopsy and culture

Skin test (leishmanin test or Montenegro test) It is a delayed hypersensitivity skin test for survey of populations and follow-up after treatment - 0.2 ml(6-10 million/ml of killed promastigotes in 0.5% phenol saline) injected—erythema ≥ 5mm→ +ve after 6-8 weeks of cure.

Serological diagnosis of visceral leishmaniosis

direct agglutination test



rK39 immunochromatographic test

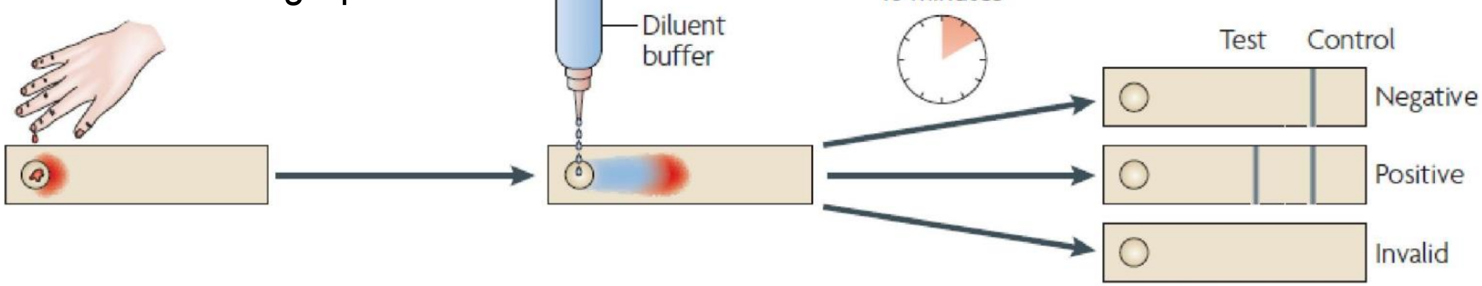
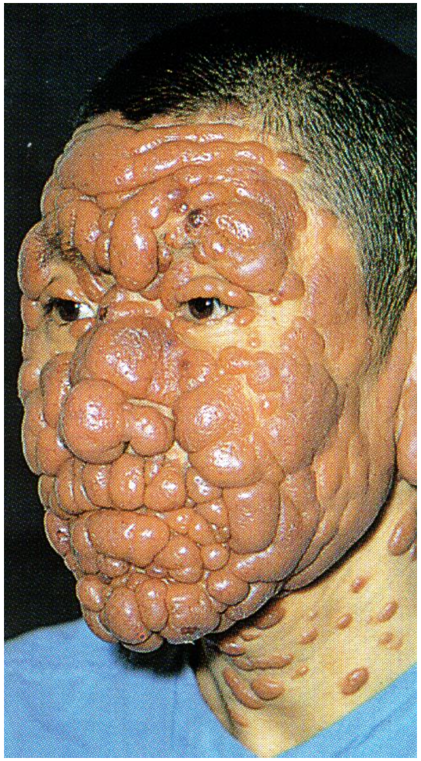


Figure 4 | **Serological tests for for visceral leishmaniasis. a** | The direct agglutination test. **b** | The rK39 immunochromatographic test strip.

The DAT and rK39 RDT are both based on the detection of antibodies in blood. Due to the persistence of antibodies over long periods, they cannot be used to differentiate between current and past infection. To overcome this limitation, tests are being developed that can detect VL antigen in urine and blood but their performance so far has been suboptimal.

Post-kala-azar dermal leishmaniasis (PKDL)

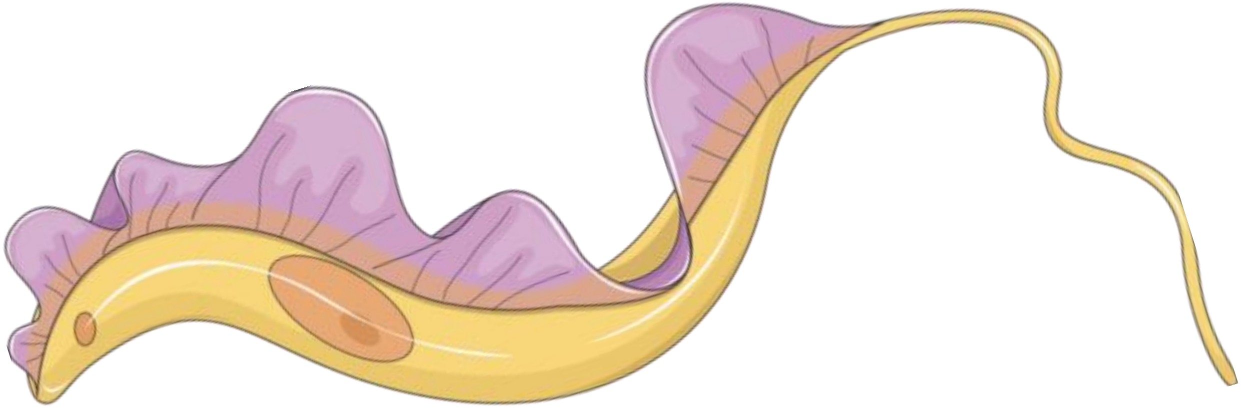
- developing usually 6 months to 1 or more years after kala-azar has apparently been cured, but can occur earlier
- occurring mainly in East Africa and on the Indian subcontinent, where 5-10% of patients with kala-azar are reported to develop the condition
- macular, papular or nodular rash on face, upper arms, trunks and other parts of the body
- human with PKDL considered a potential source of *Leishmania* infection




World Health Organization

WHO's work on leishmaniasis control involves:

- ✓ supporting national leishmaniasis control programs technically and financially to produce updated guidelines and make disease control plans, including sustainable, effective surveillance systems, and epidemic preparedness and response systems
- ✓ monitoring disease trends and assessing the impact of control activities which will allow raising awareness and advocacy on the global burden of leishmaniasis and promoting equitable access to health services
- ✓ developing evidence-based policy strategies and standards for leishmaniasis prevention and control and monitoring their implementation
- ✓ strengthening collaboration and coordination among partners and stakeholders
- ✓ promoting research and use of effective leishmaniasis control including safe, effective and affordable medicines, as well as diagnostic tools and vaccines
- ✓ supporting national control programs to ensure access to quality - assured medicines



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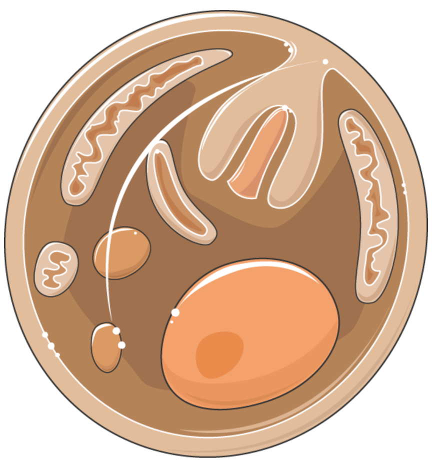

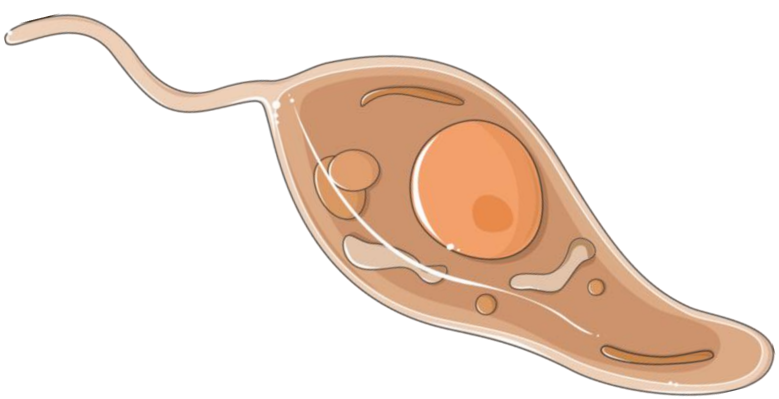

Lectures

- ✓ 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