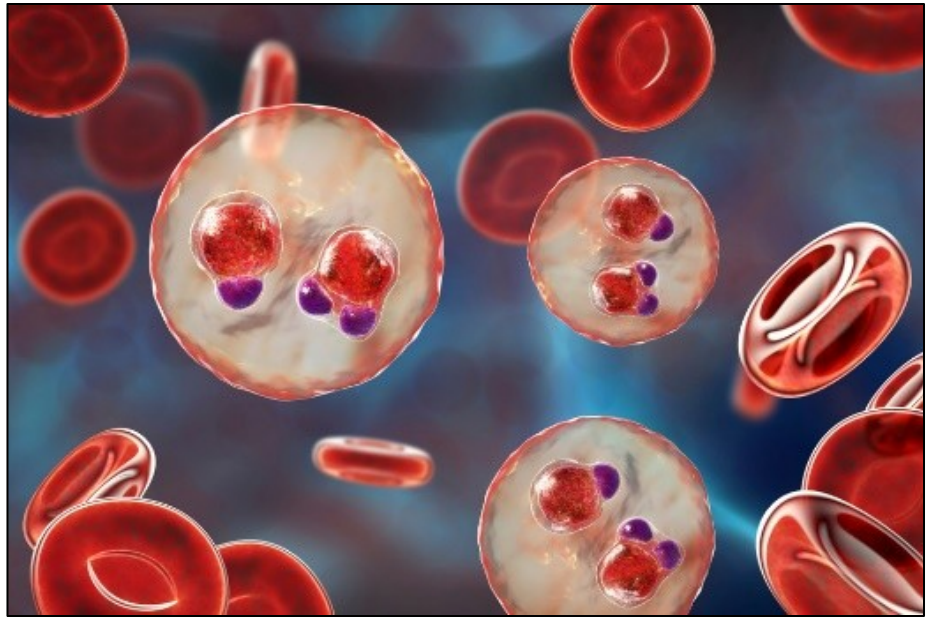
Buněčné biologie prvoků

Parazitičtí prvoci



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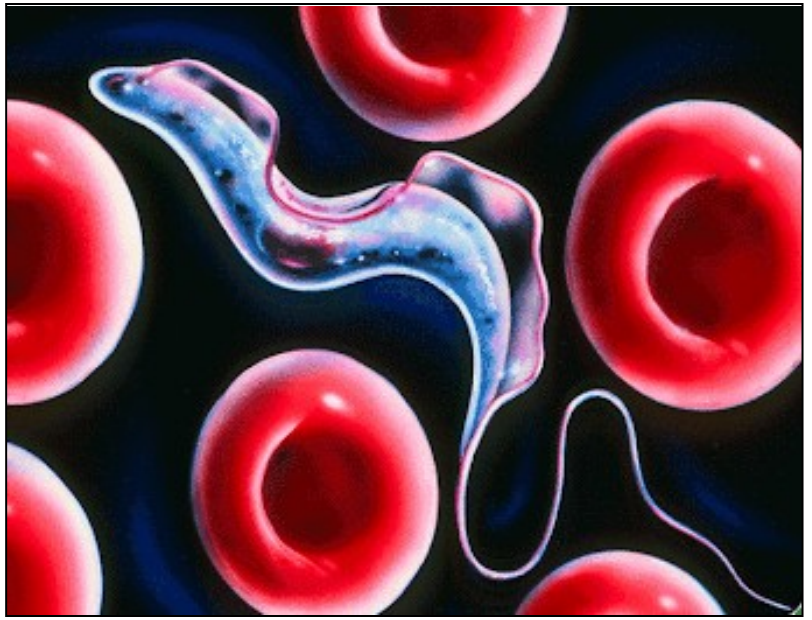
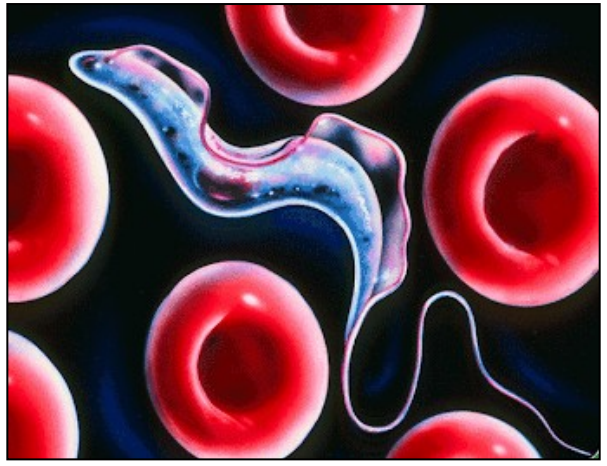
Biology Centre
Ceske Budějovice



Parasites

Parasitos = parasite of the classical Greek antiquity was a tolerated, but not invited co-eater during a guest meal.

Parasites are organisms that live in or on another organism (host) and derive nutrients at the expense of the host.



unicellular organisms (protists)

helminths (worms)

arthropods

Parasites

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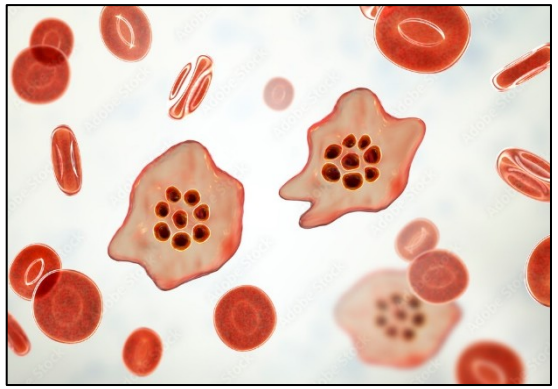
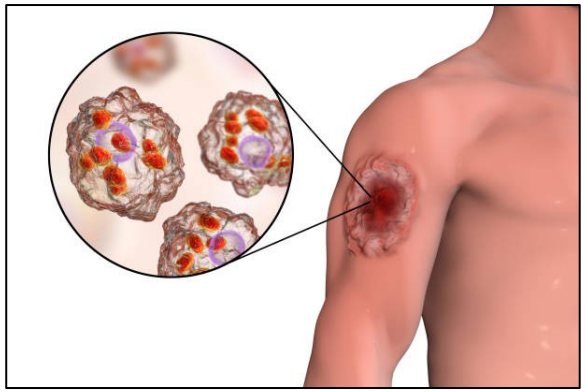
Toxoplasma



Plasmodium



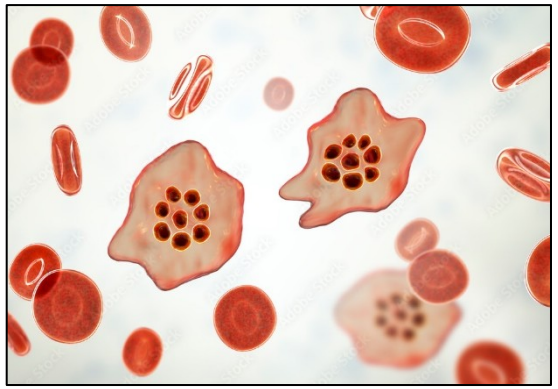
Leishmania

Trypanosoma

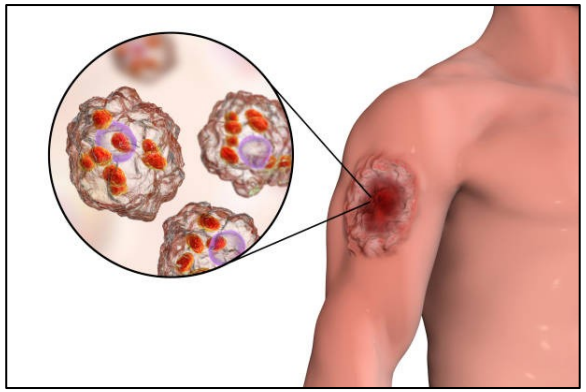
Parasites

- **Global health impact:** Parasitic diseases affect millions worldwide.
- **Economic implications:** Agricultural and livestock parasitism.
- **Scientific discovery:** Unveiling unique features and pathways.

Toxoplasma



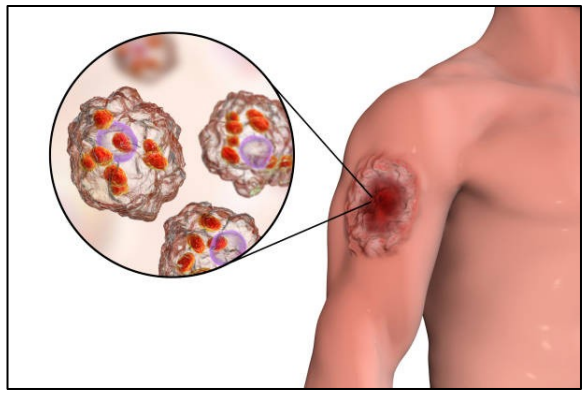
Plasmodium

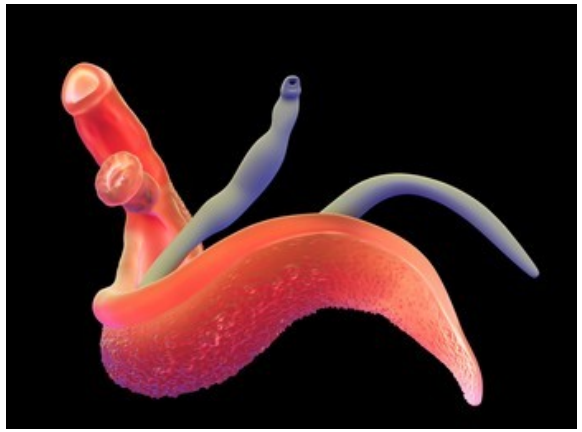


Leishmania

Trypanosoma

Overview of Parasitology
The Relevance of Parasitology





Disease name	Frequency* (total number of cases)	Parasitic organism	Transmission
PROTOZOAL DISEASES			
Malaria	216,000,000	*Plasmodium*	Mosquito bites
Leishmaniasis	1,200,000	*Leishmania*	Sandfly bites
African sleeping sickness	10,000**	*Trypanosoma brucei*	Tsetse fly bites
Chagas' disease	8,000,000	*Trypanosoma cruzi*	Kissing bug feces
PARASITIC WORM DISEASES			
Schistosomiasis	200,000,000	*Schistosoma*	Water
Filariasis	120,000,000	*Wuchereria bancrofti*	Mosquito bites
River blindness	25,000,000	*Onchocerca volvulus*	Blackflies bites
Hookworm infection	740,000,000	*Necator americanus*	Soil
Ascariasis	1,200,000,000	*Ascaris lumbricoides*	Soil

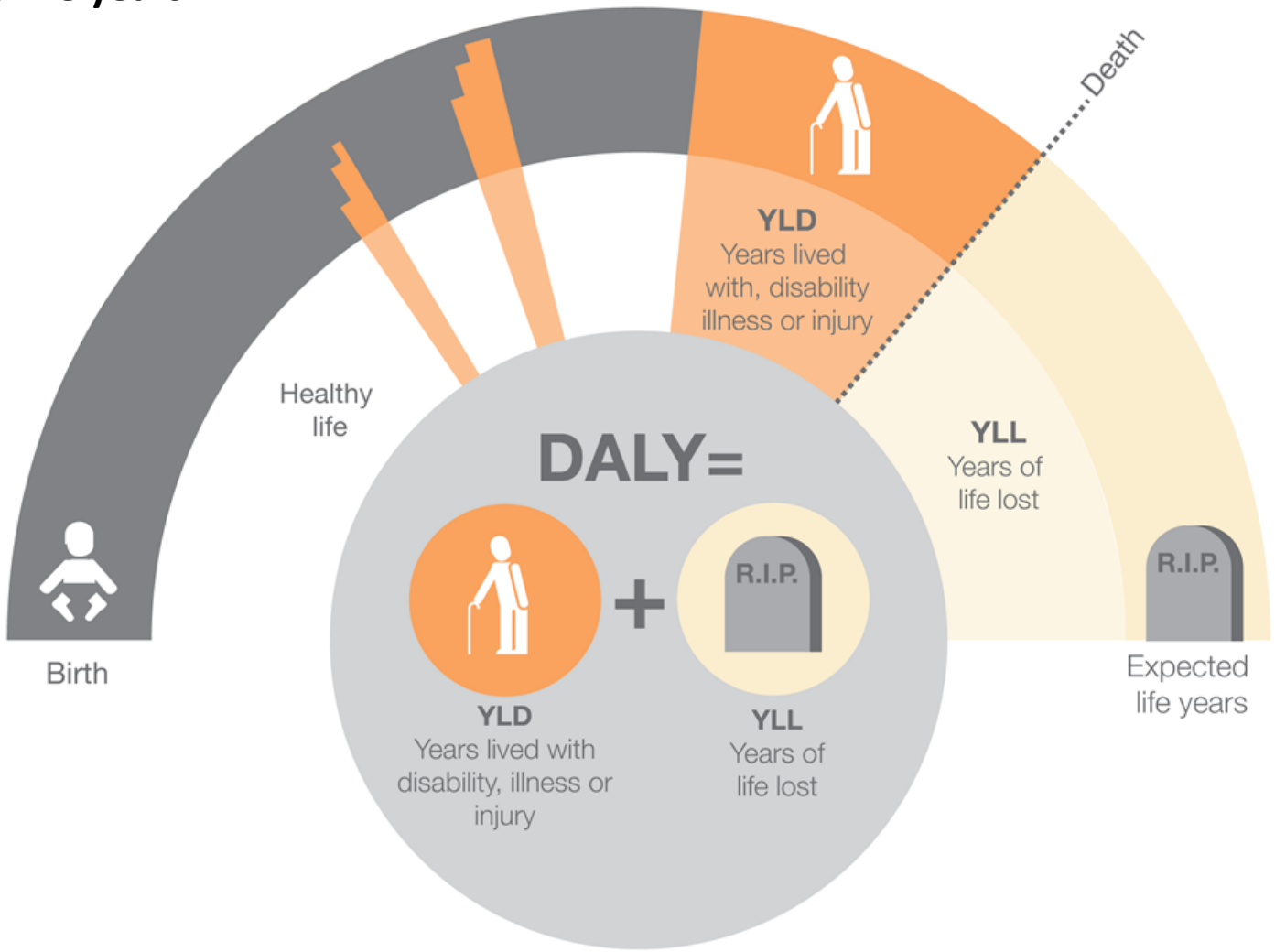
Disease burden

	Disease	Health burden (millions DALYs)[a]	Deaths (per annum)
Viral	**HIV/AIDS***	**89**	**1.1 millions**
	Rabies	1.46	26,400
	Dengue	0.83	14,700
Bacterial	**Tuberculosis**	**36**	**1.6 millions**
	Trachoma	**0.33**	**0**
Protozoal	**Malaria***	**42**	**670, 000**
	Sleeping sickness	1.6	13,000
	Chagas disease	0.6	13,000
	Leishmaniasis	3.32	59,000
Helminthic	Schistosomiasis	3.31	15,000
	Onchorcerciasis	1.0	0
	Filariasis	2.78	0
	Soiled-transmitted	5.19	2,700

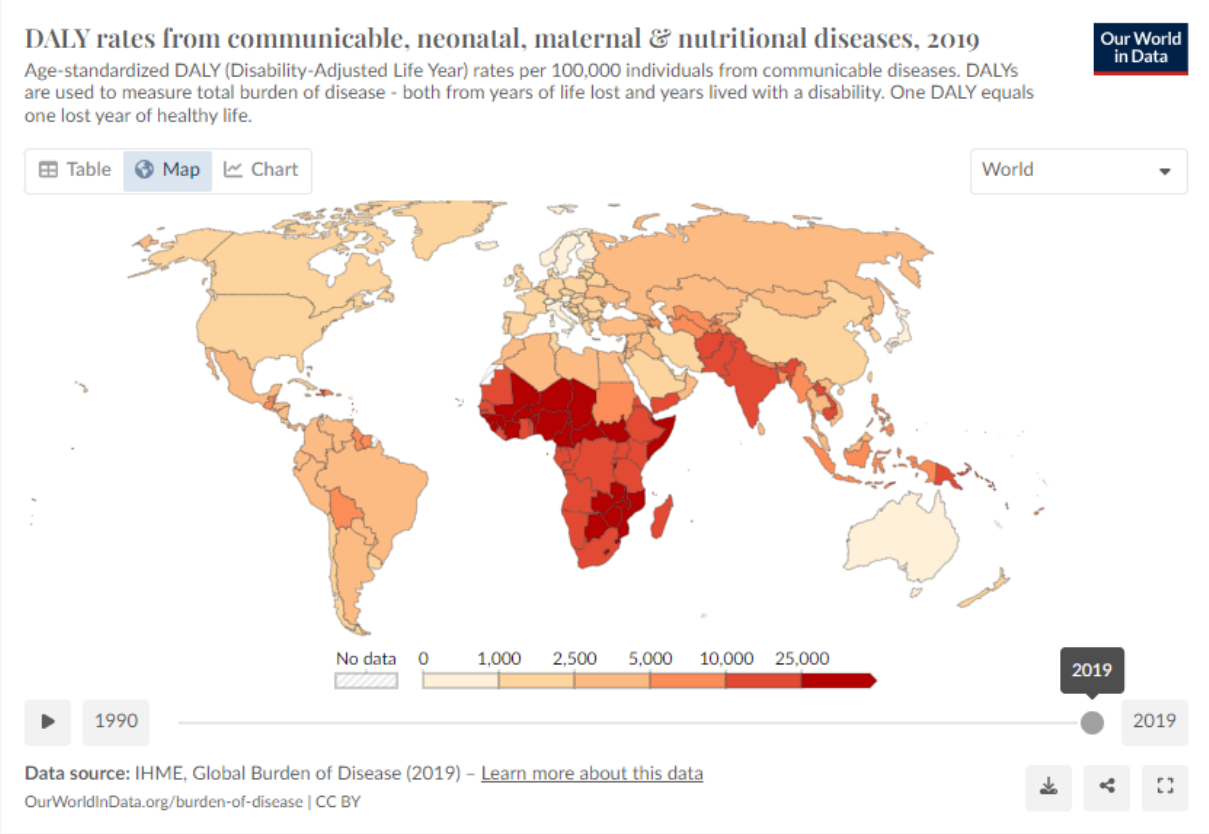
* *World Health Report estimates, 2012*

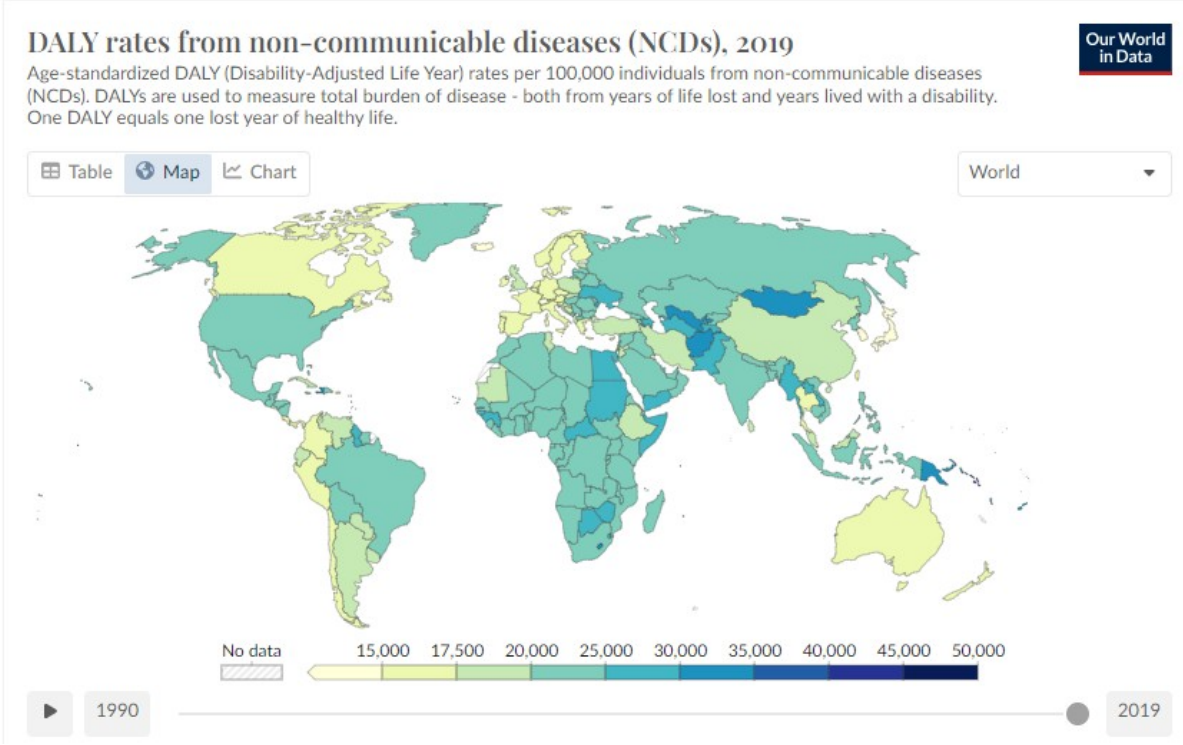
Disease burden in DALYs

DALYs – disability adjusted life years



Communicable vs non-communicavle diseases





- **NEGLECTED TROPICAL DISEASES**

WHAT DOES IT MEAN TO BE "NEGLECTED"?

THOMSON REUTERS

RESEARCH PAPERS (1992-2011)

= 5,000 research papers

= 10 million people

Neglected Tropical Diseases impact daily life for billions of people globally. However, funding for research and treatment of them pales in comparison to "first-world impacted or supported" diseases such as HIV/AIDS.

Social and moral questions arise when trying to understand why some diseases are favored over others, adding a new perspective on what it means to be truly neglected.

Thomson Reuters Global Research Report: *Neglected Tropical Diseases*
http://researchanalytics.thomsonreuters.com/grr/

DIABETES

346 Million Afflicted

194,481
RESEARCH PAPERS

ELEPHANTIASIS LYMPHATIC FILARIASIS

120 Million Afflicted

1,858
RESEARCH PAPERS

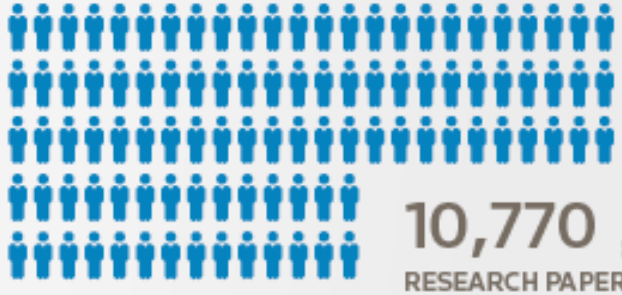
HIV/AIDS

34 Million Afflicted

154,562
RESEARCH PAPERS

INTESTINAL WORMS SOIL-TRANSMITTED HELMINTHIASIS

~1 Billion Afflicted



10,770
RESEARCH PAPERS

Sources: Thomson Reuters Web of Knowledge, World Health Organization

Neglected Tropical Diseases

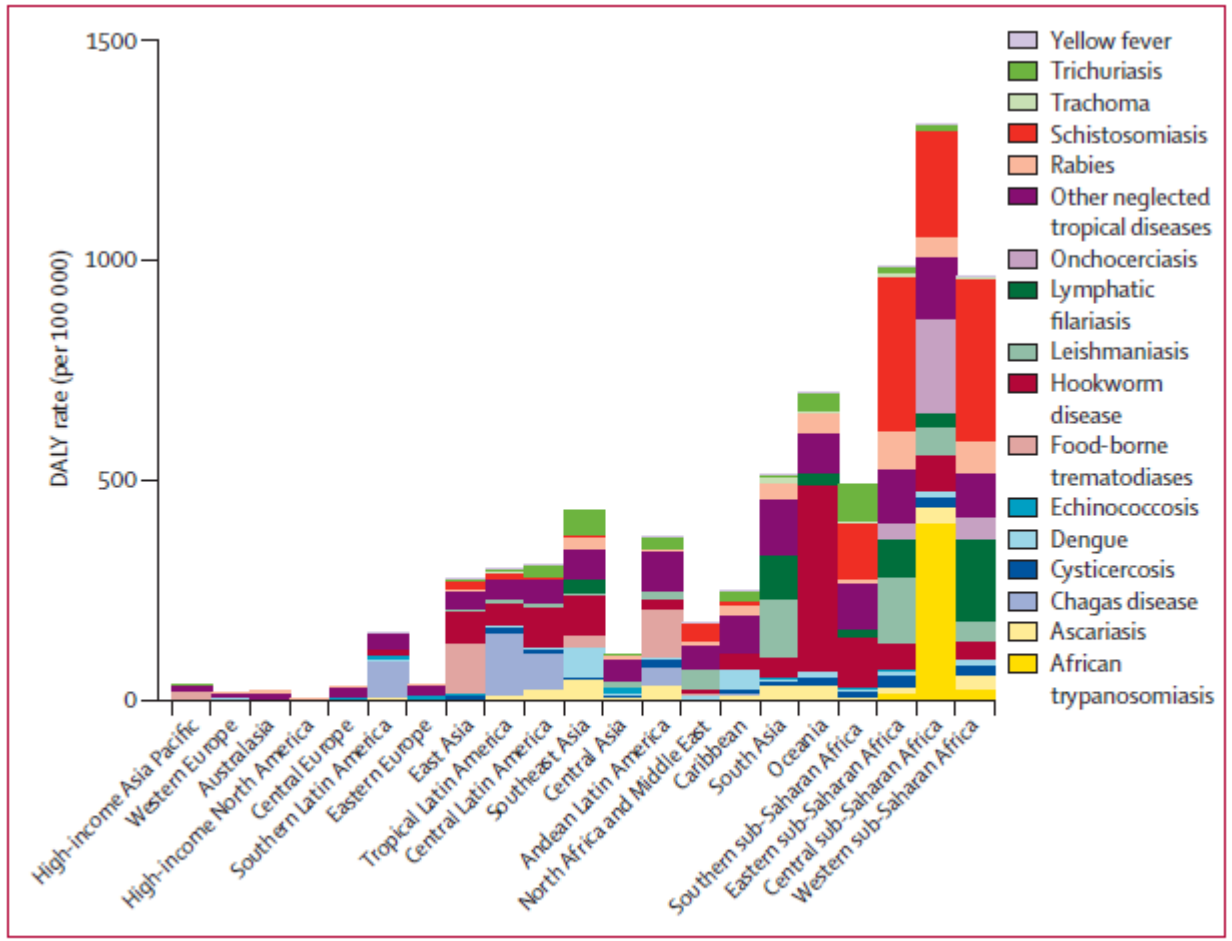
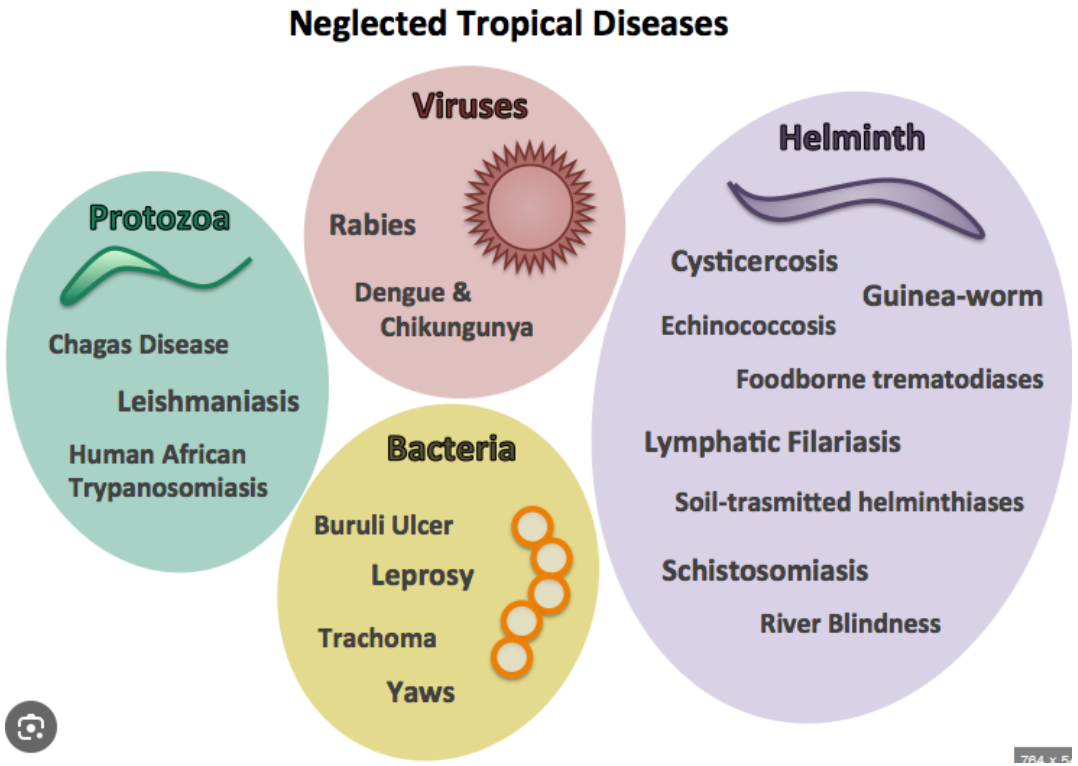
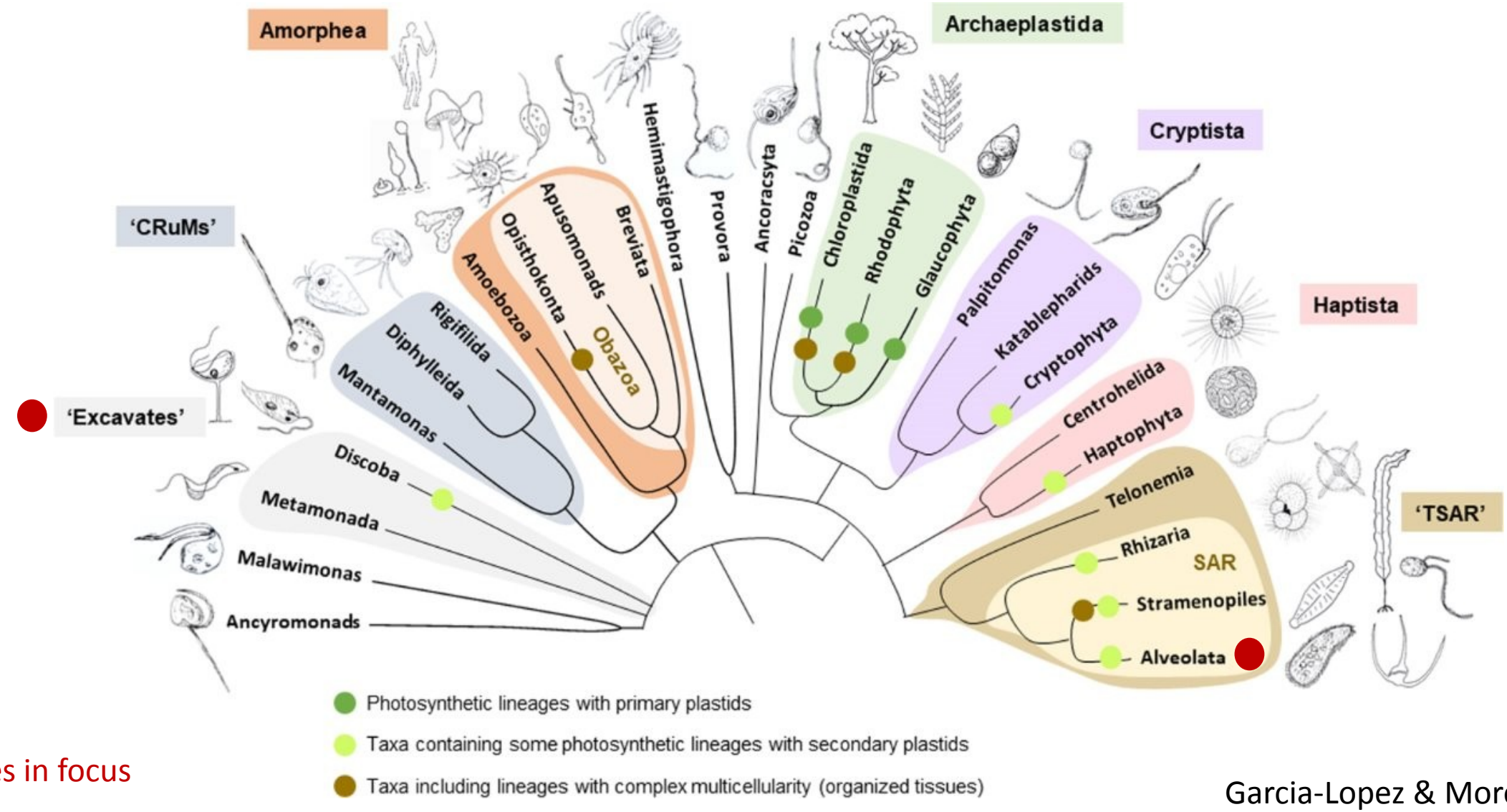


Figure 9: **Neglected tropical disease disability-adjusted life year rates by cause and region in 2010**
This figure excludes malaria.



Parasites in focus



● Parasites in focus

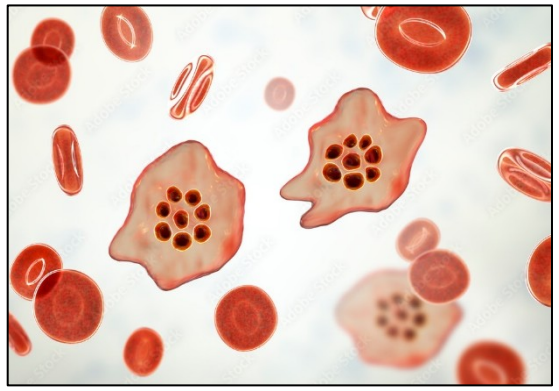
Garcia-Lopez & Moreira 2023

Model Organisms in Parasitology

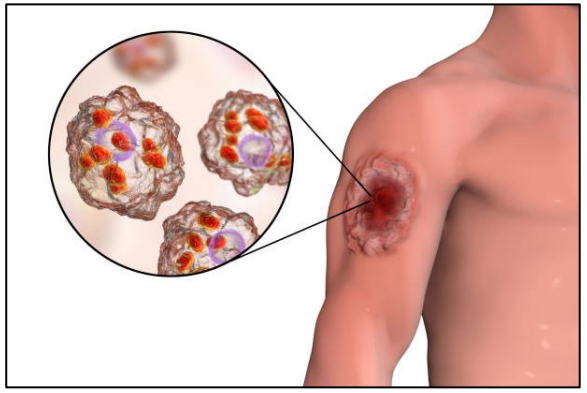
Model organisms serve as valuable tools for understanding the biology, genetics, and pathogenic mechanisms of parasites.

- **Genetic Manipulation:** Ease of genetic modification facilitates the study of specific genes and their functions.
- **Short Reproductive Cycles:** Allows for quick generation of experimental data and observations.
- **In Vitro Cultivation:** Facilitates controlled experimentation and observation.
- **Conservation of Biological Processes:** Many biological processes are conserved across species, allowing extrapolation of findings.

Toxoplasma



Plasmodium



Leishmania

Trypanosoma

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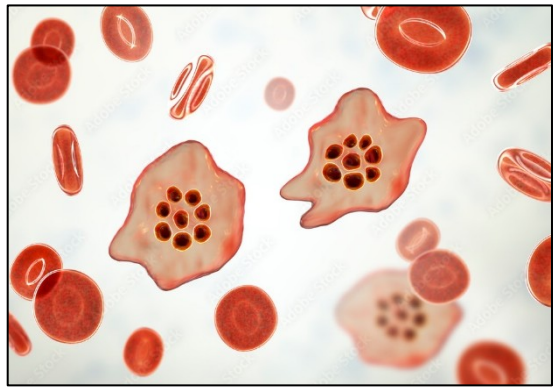
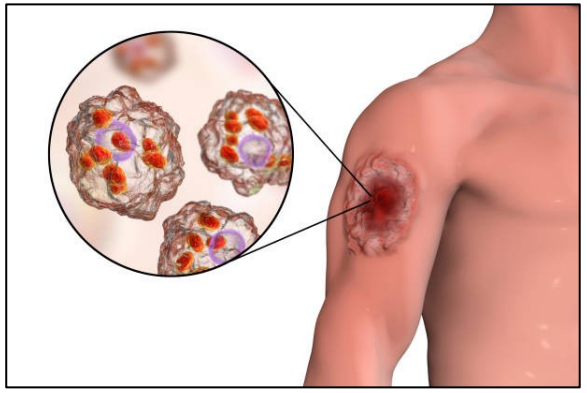


Plasmodium

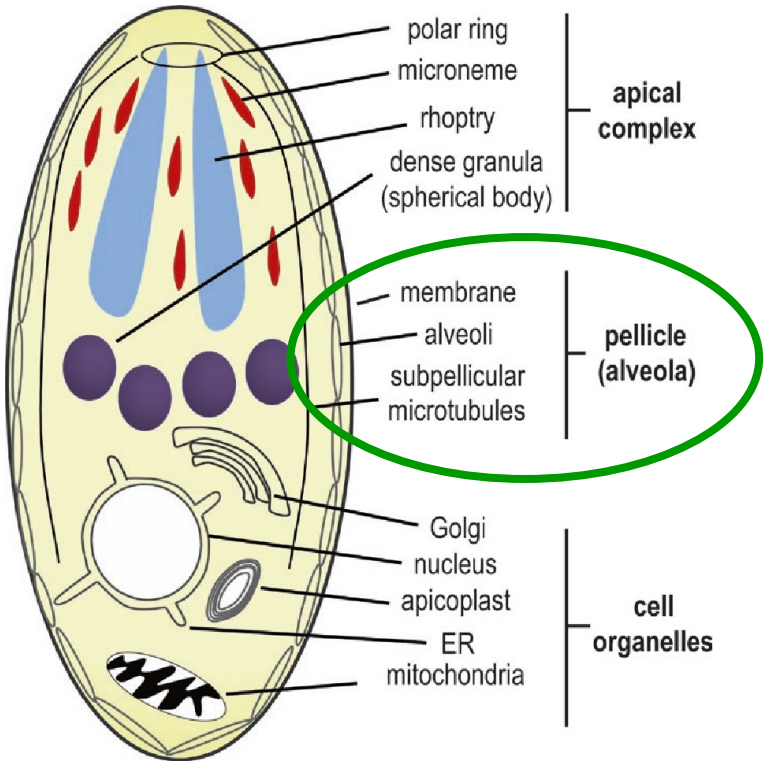


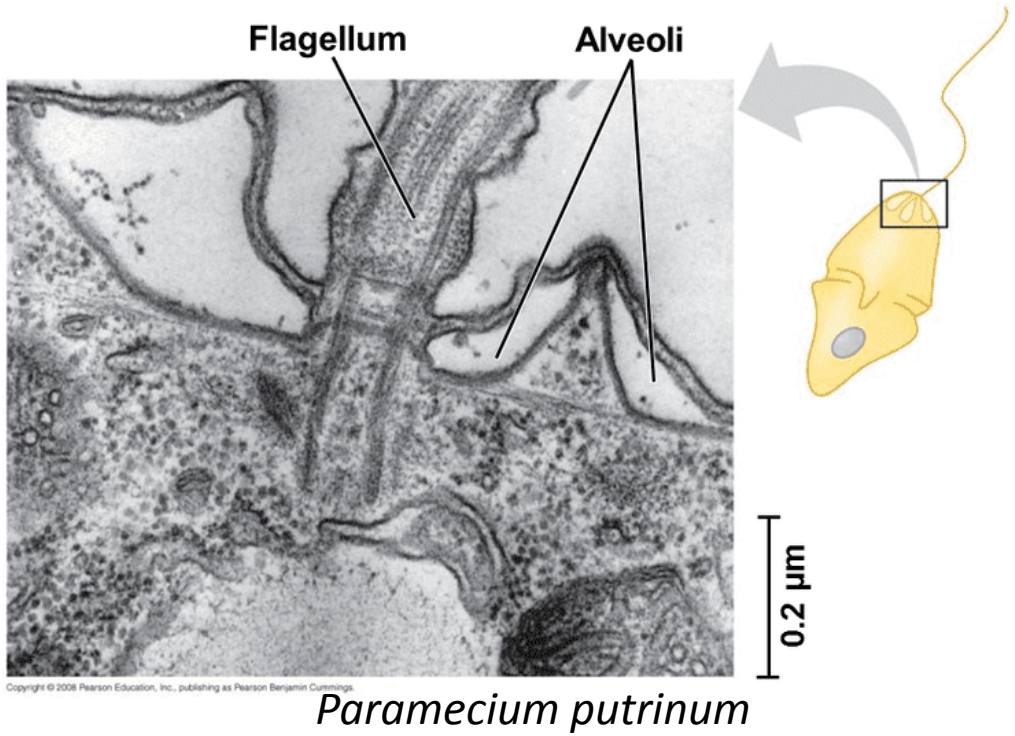
Leishmania

Trypanosoma

SAR CLADE: ALVEOLATA (meaning "with cavities„)

presence of cortical (outer-region) alveoli (sacs)

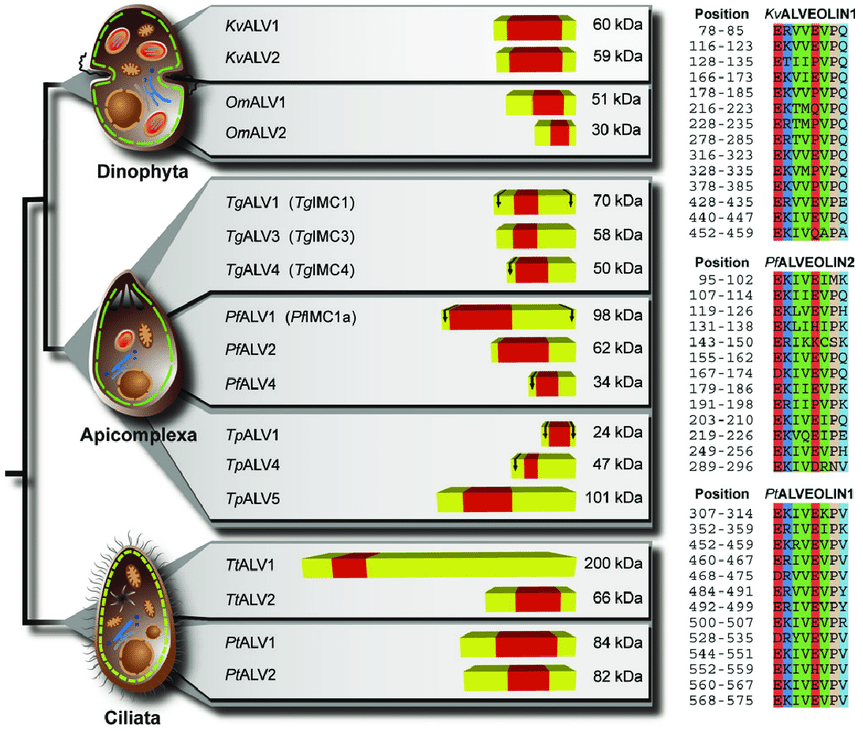




Paramecium putrinum

flattened vesicles (sacs) packed into a continuous layer just under the membrane and supporting it, typically forming a flexible pellicle

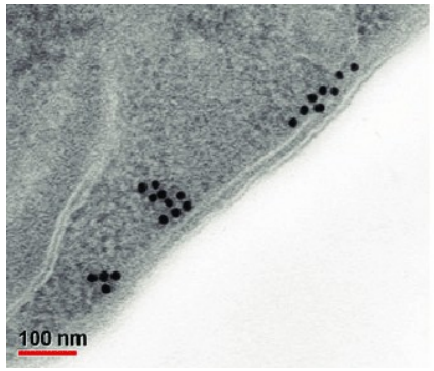
SAR CLADE: ALVEOLATA (meaning "with cavities„)

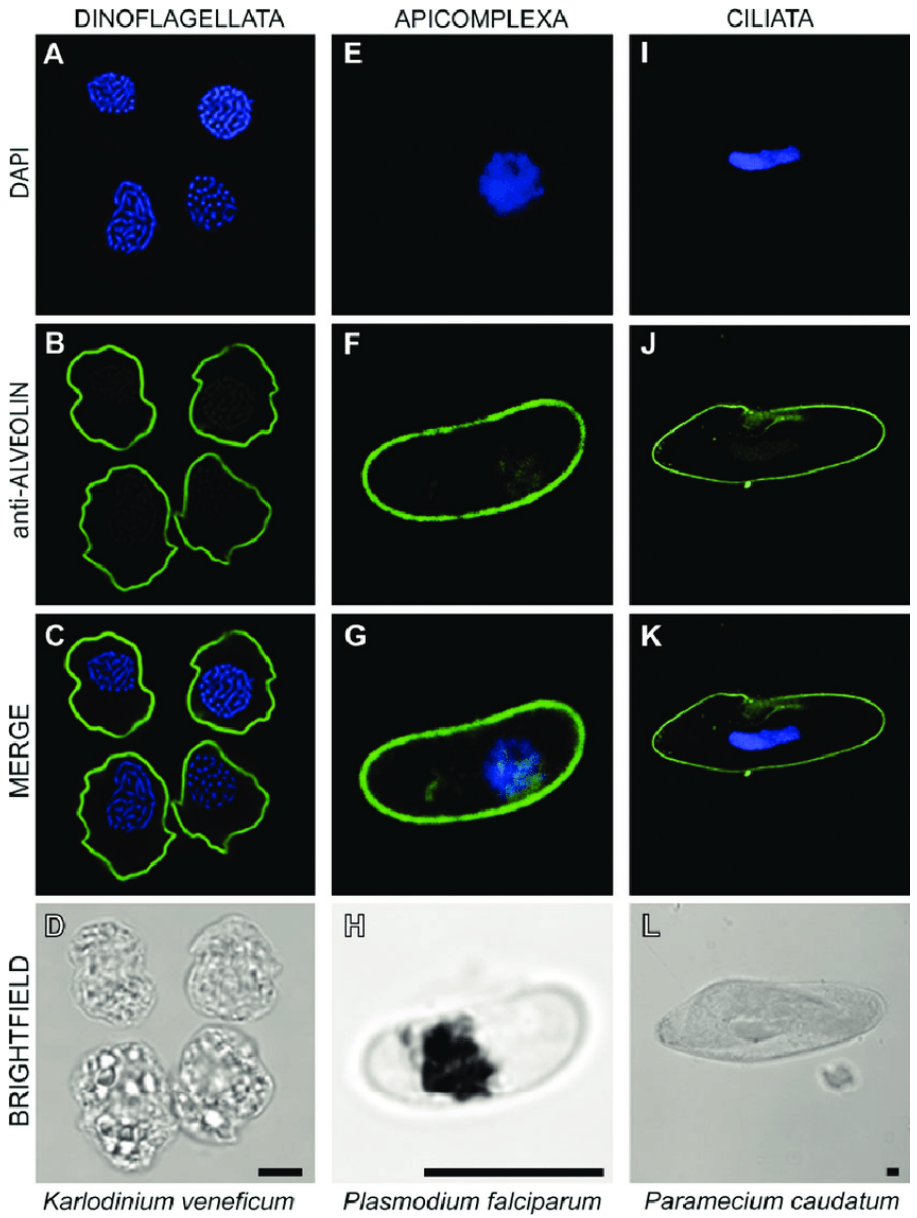


Position	*Kv*ALVEOLIN1
78-85	ERVVEVPQ
116-123	EKVVEVPQ
128-135	ETIIPVPQ
166-173	EKVIEVPQ
178-185	EKVVPVPQ
216-223	EKTMQVPQ
228-235	ERTMPVPQ
278-285	ERTVPVPQ
316-323	EKVVEVPQ
328-335	EKVMPVPQ
378-385	EKVVPVPQ
428-435	ERVVEVPE
440-447	EKIVEVPQ
452-459	EKIVQAPA

Position	*Pf*ALVEOLIN2
95-102	EKIVEIMK
107-114	EKIIEVPQ
119-126	EKLVEVPH
131-138	EKLIHIPK
143-150	ERIKKCSK
155-162	EKIVEVPQ
167-174	DKIVEVPQ
179-186	EKIIEVPK
191-198	ERIIPVPK
203-210	EKIVEIPQ
219-226	EKVQEIPE
249-256	EKIVEVPH
289-296	EKIVDRNV

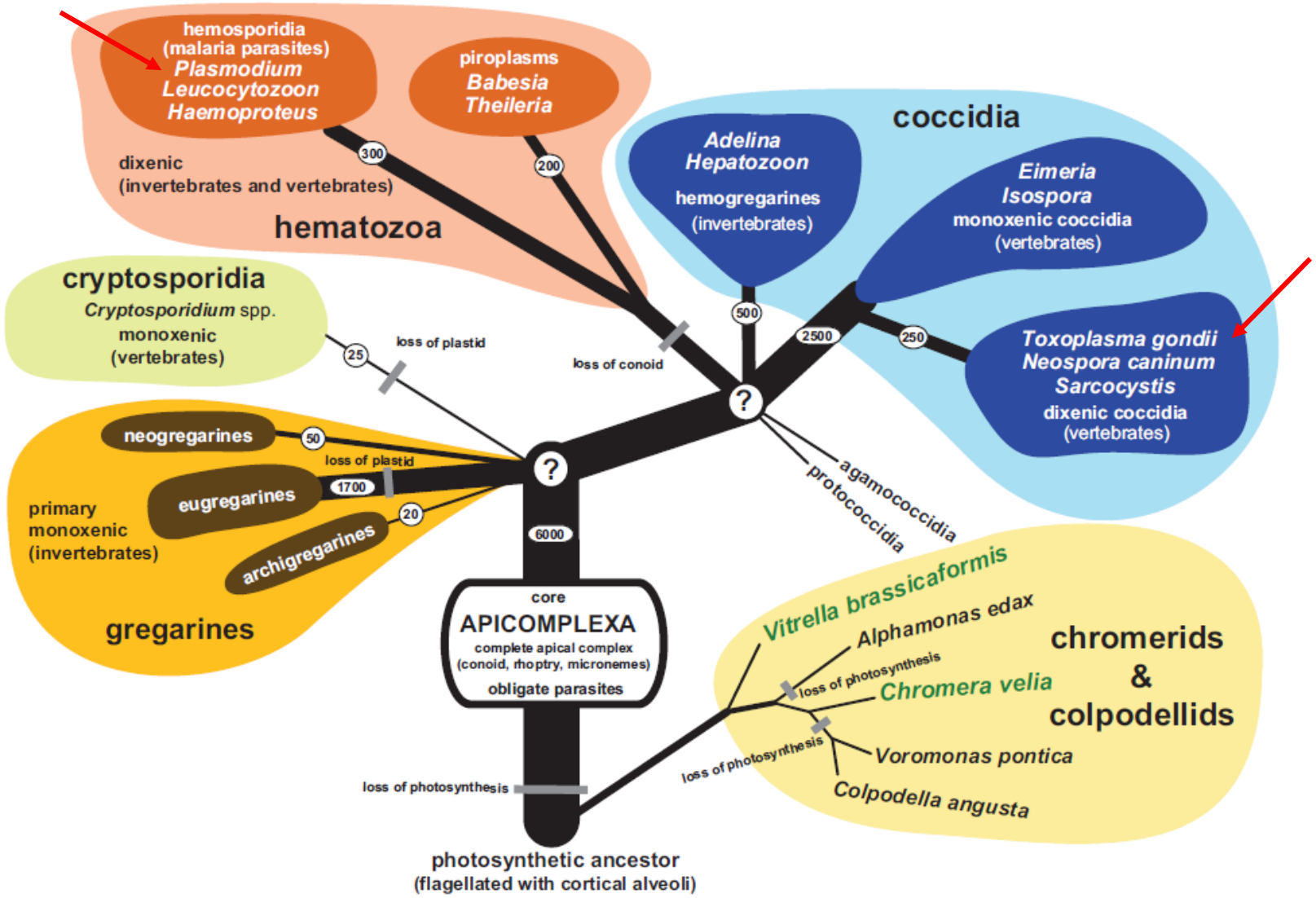
Position	*Pf*ALVEOLIN1
307-314	EKIVEKPV
352-359	ERIVEIPK
452-459	EKRVEVPV
460-467	ERIVEVPV
468-475	DRVVEVPV
484-491	ERVVEVPY
492-499	ERIVEVPY
500-507	EKIVEVPR
528-535	DRYVEVPV
544-551	EKIVEVPV
552-559	EKIVHVPV
560-567	EKIVEVPV
568-575	EKIVEVPV





Gould et al., 2008

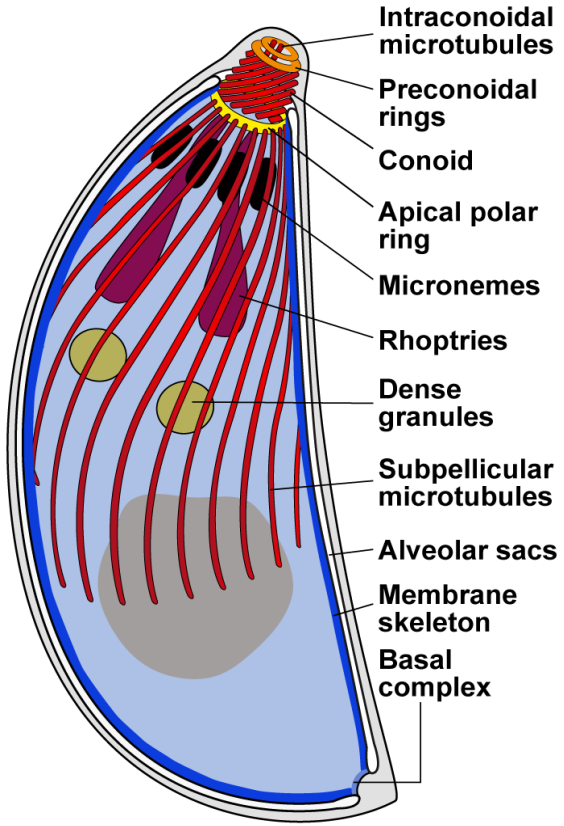
APICOMPLEXA



Šlapeta and Morin-Adeline (2011)

APICOMPLEXA - VÝTRUSOVCI

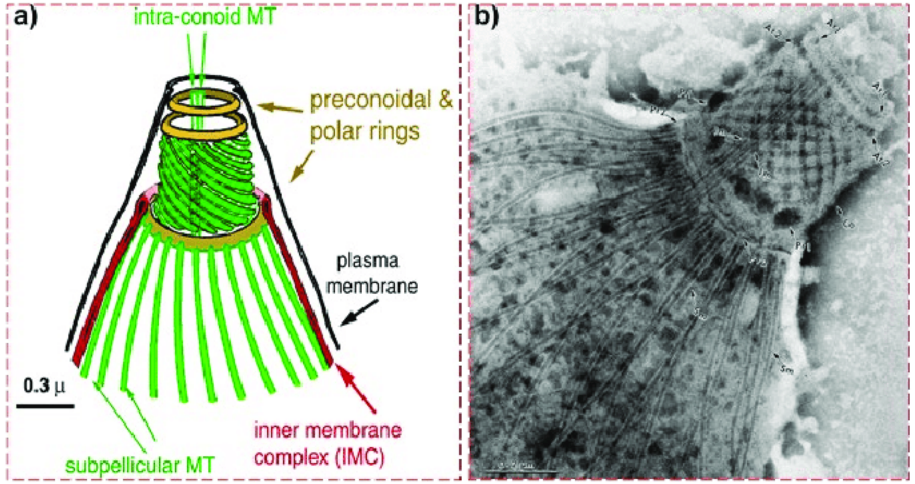
Apical complex

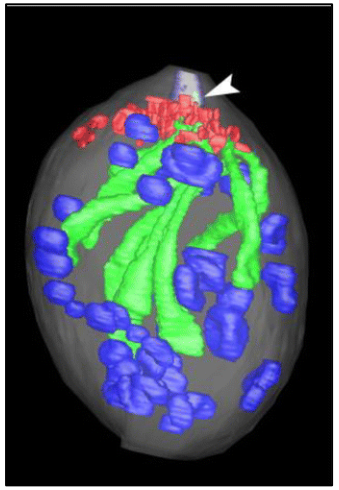


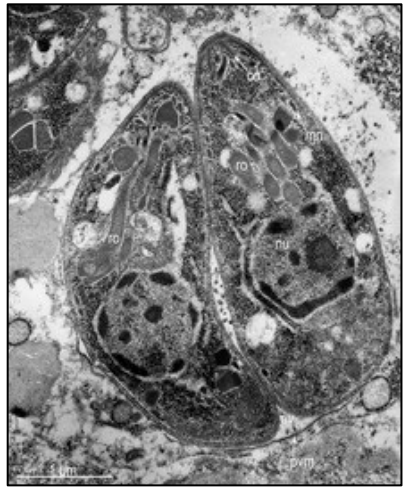
a group of structures and organelles

consists of **structural** components and **secretory** organelles

required for **invasion of host cells** during the parasitic stages of the Apicomplexan life cycle

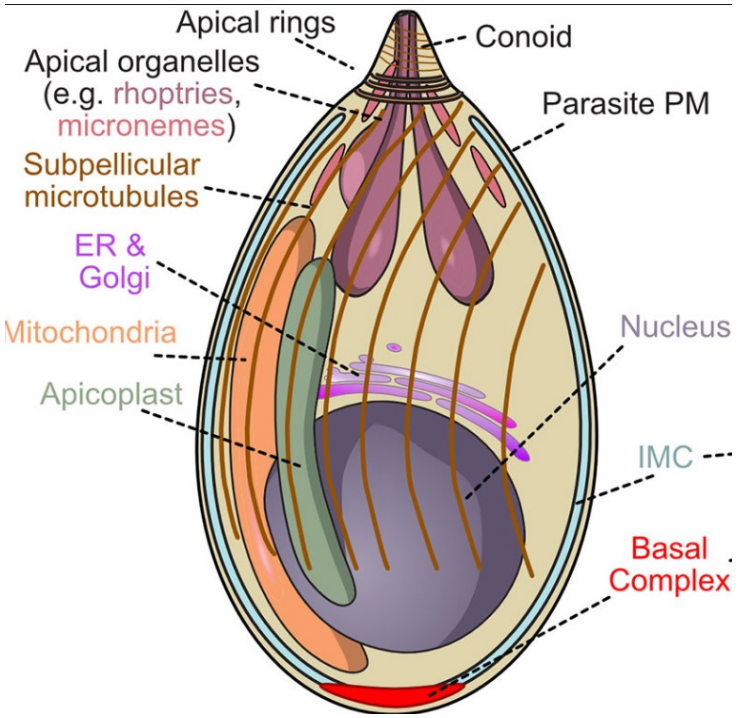




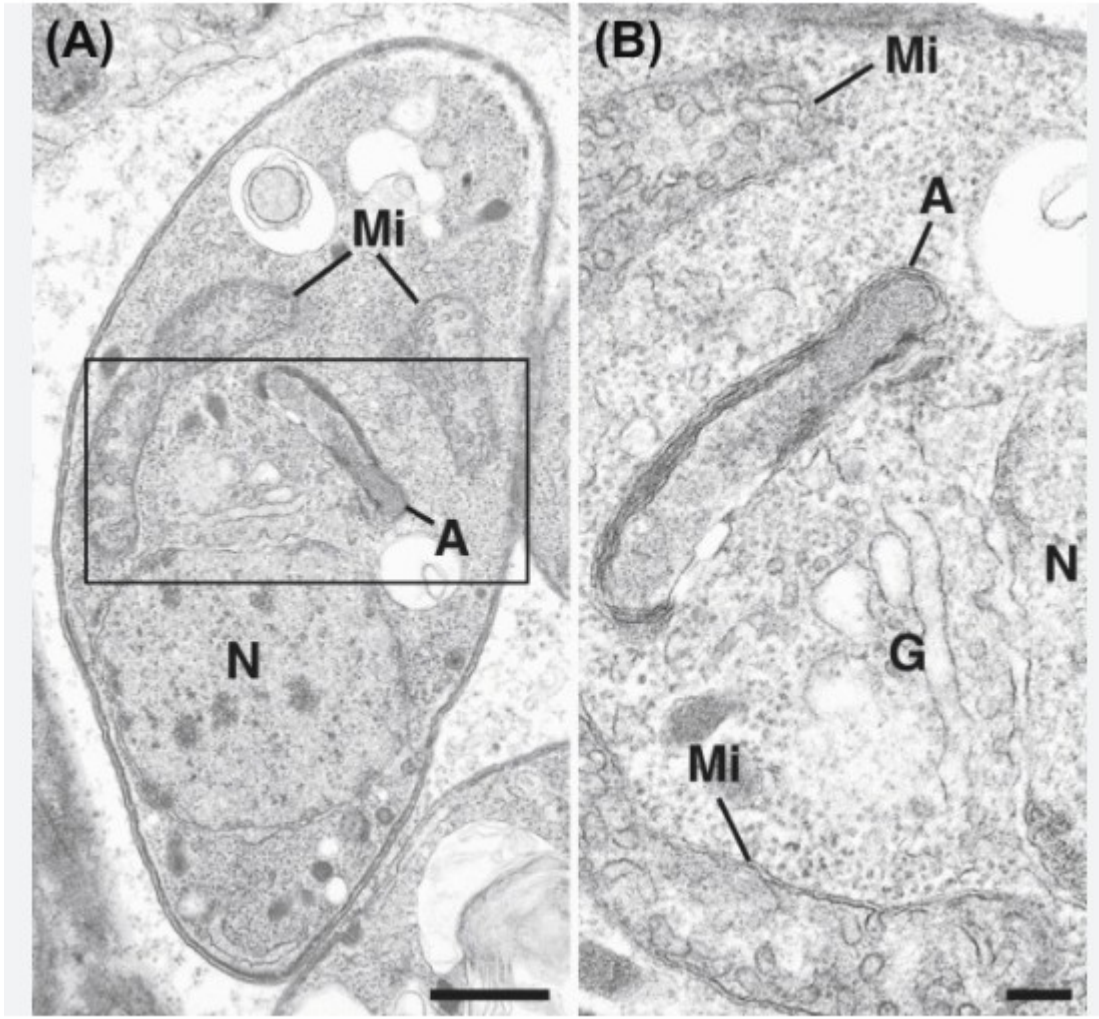


APICOMPLEXA

Apicoplast



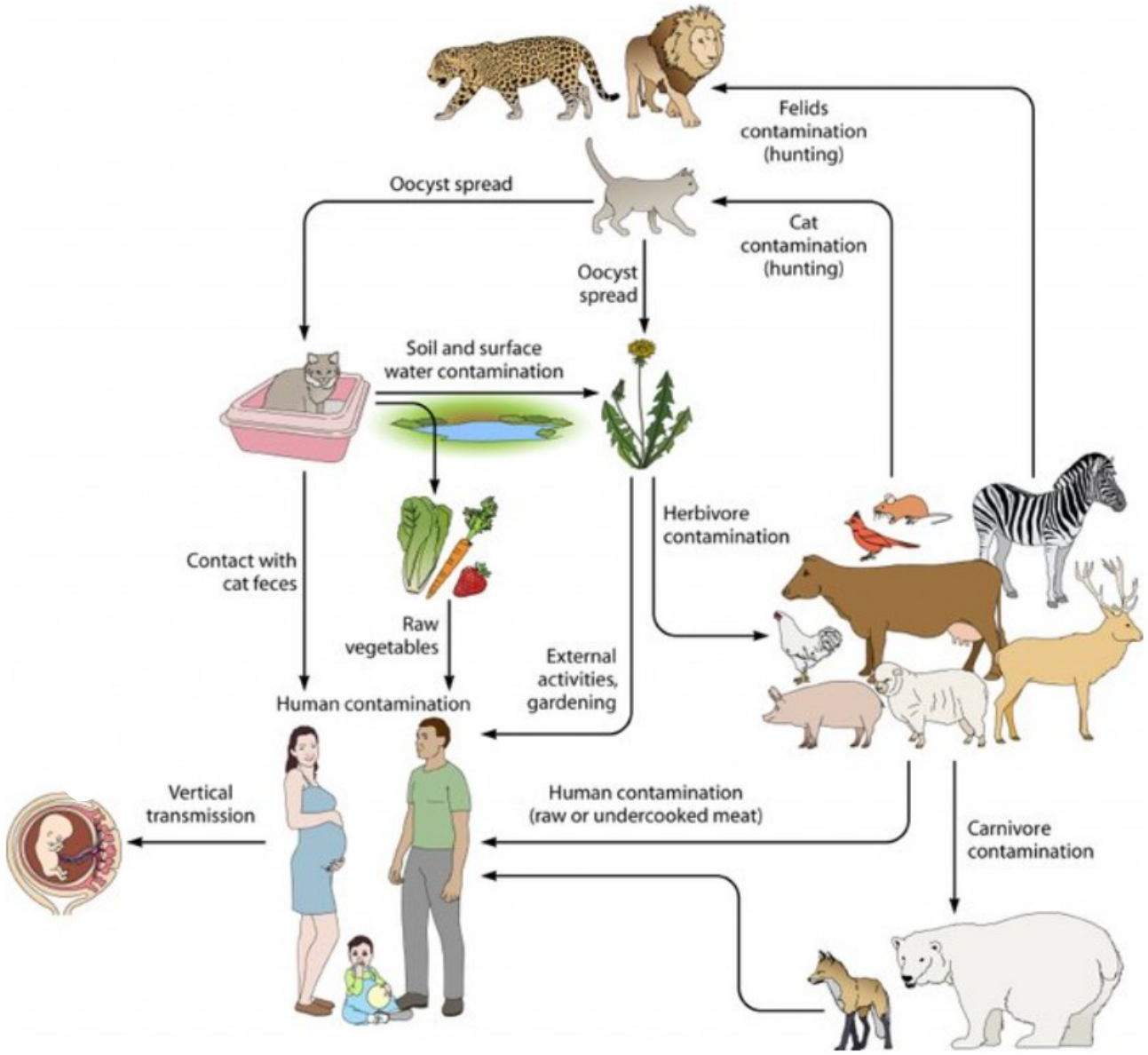
a derived non-photosynthetic plastid
originated by **secondary endosymbiosis** (4 membranes)
has its **own genome**
essential **metabolic** pathways
drug target



Toxoplasma gondii
Seeber et al., 2014

Toxoplasma gondii

- **coccidia**
- found **worldwide**
- capable of infecting virtually all warm-blooded animals (incl. birds)
- but **felids** such as domestic cats are the only known **definitive hosts** in which the parasite may undergo sexual reproduction
- one of the most common parasites in developed countries



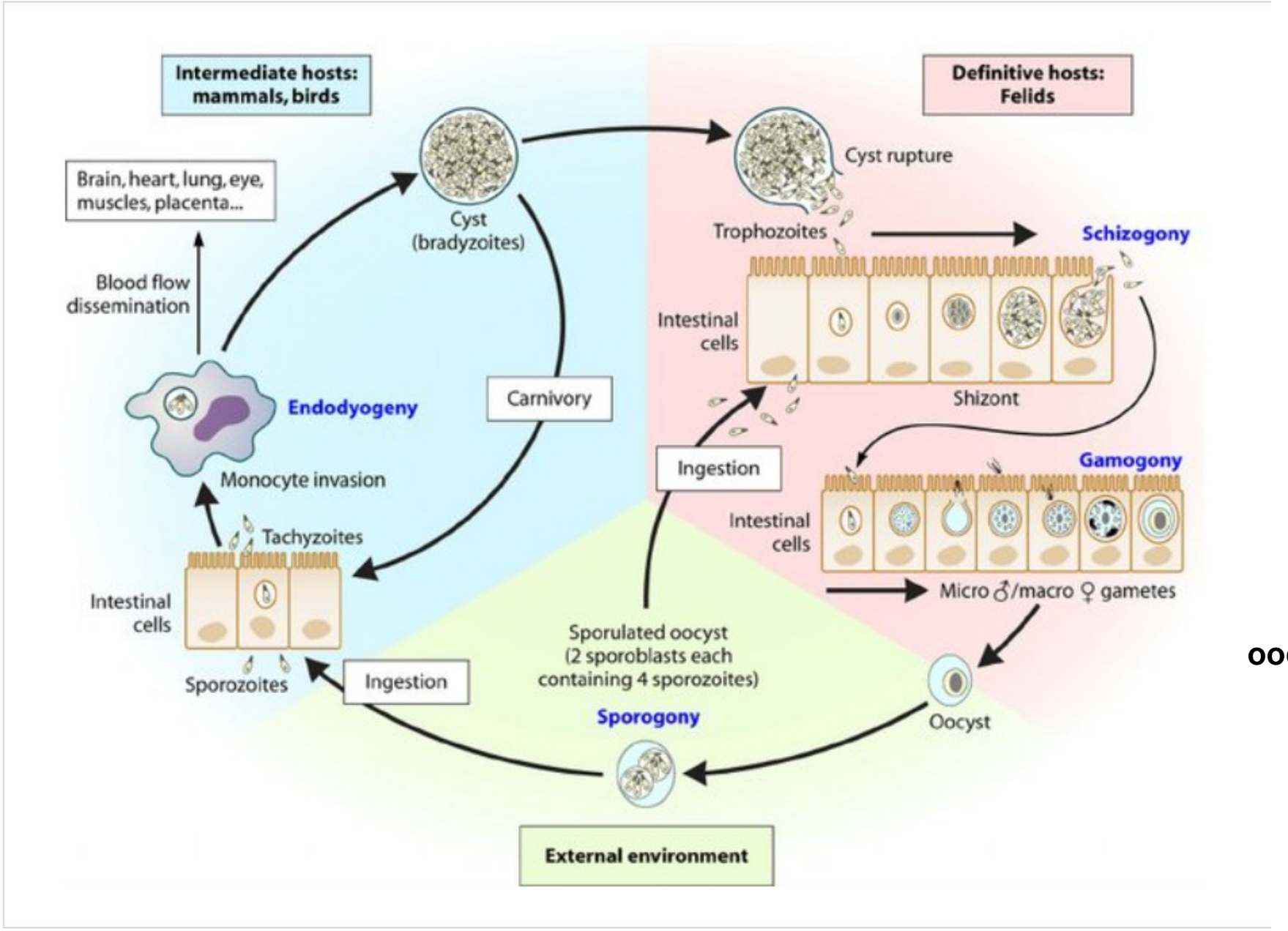
Toxoplasmosis

- **significant health consequences**, particularly in immunocompromised individuals and pregnant women (risk of congenital toxoplasmosis).
- serological studies estimate that 30–50% of the global population has been exposed
- France 84% prevalence, **CZ 30% prevalence**

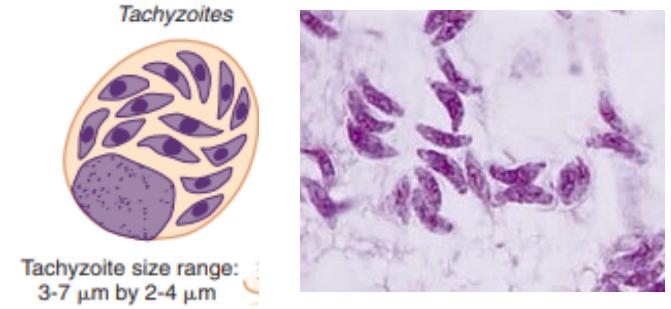
Manipulation theory

- Rats
- Mice
- Human (Ig Nobel prize to Jaroslav Flegr)

Toxoplasma gondii

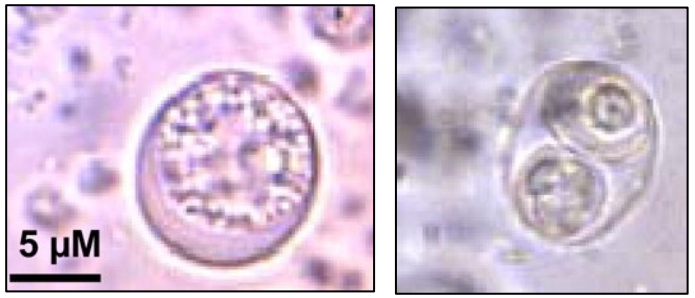


tachyzoites → rapid growth and repli



bradyzoite → sessile, **slow-growing f**

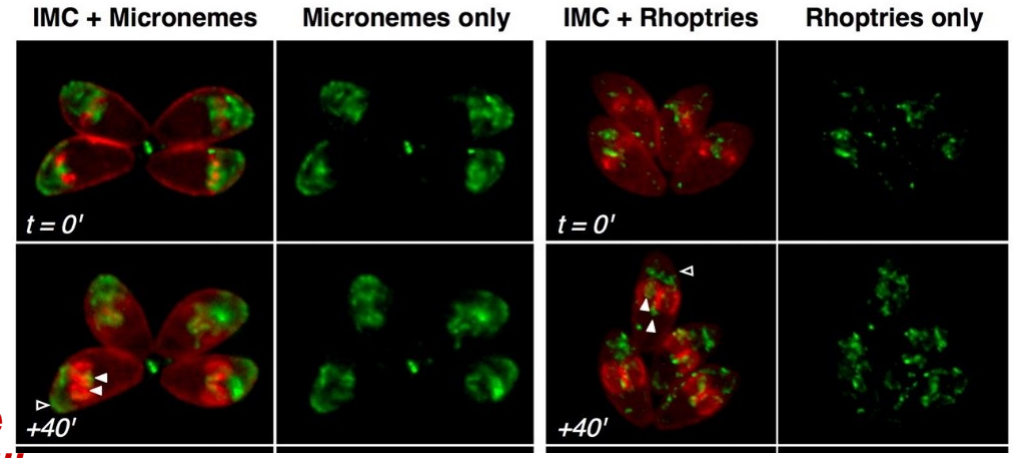
oocyst → mature oocyst containing sporozo



unsporulated and sporulated oocysts

Toxoplasma gondii as model organism

- the best model system to study the biology of the Apicomplexa
- life cycle can be completed *in vitro* ← controlled experiments to investigate various biology aspects ease of ***in vitro* culture**
- the **mouse animal model** is well-established
- readily amenable to **genetic manipulation** in the laboratory
  - the high efficiency of **transient and stable transfection**
  - **gene knockout, gene tagging, and transgenic expression**
  - the availability of many **cell markers**
  - advanced **microscopic techniques**
  - **functions of specific genes and pathways involved in parasite**
  - **heterologous expression of apicomplexan proteins in *T. gondii***

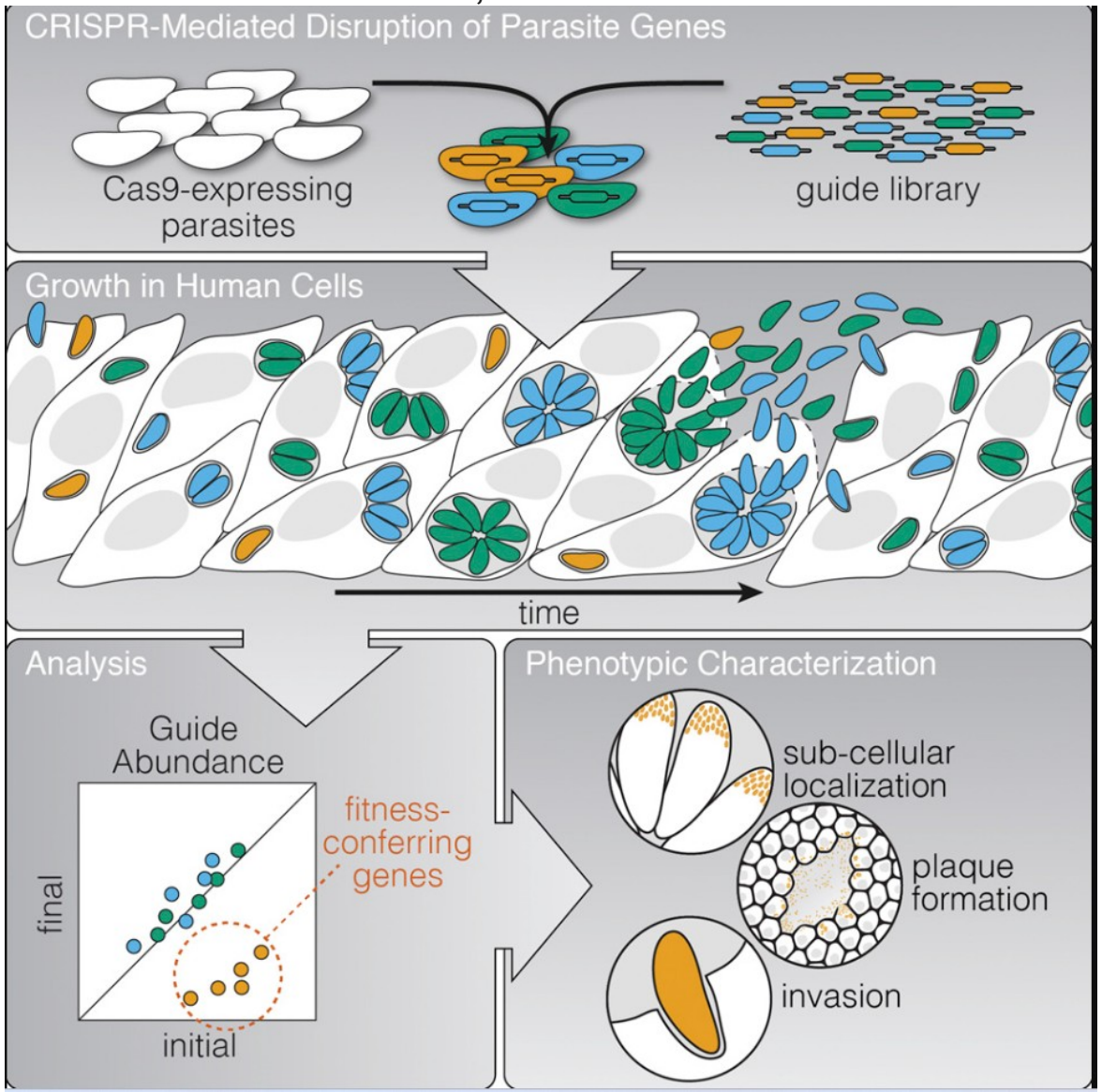


IMC = the inner membrane complex

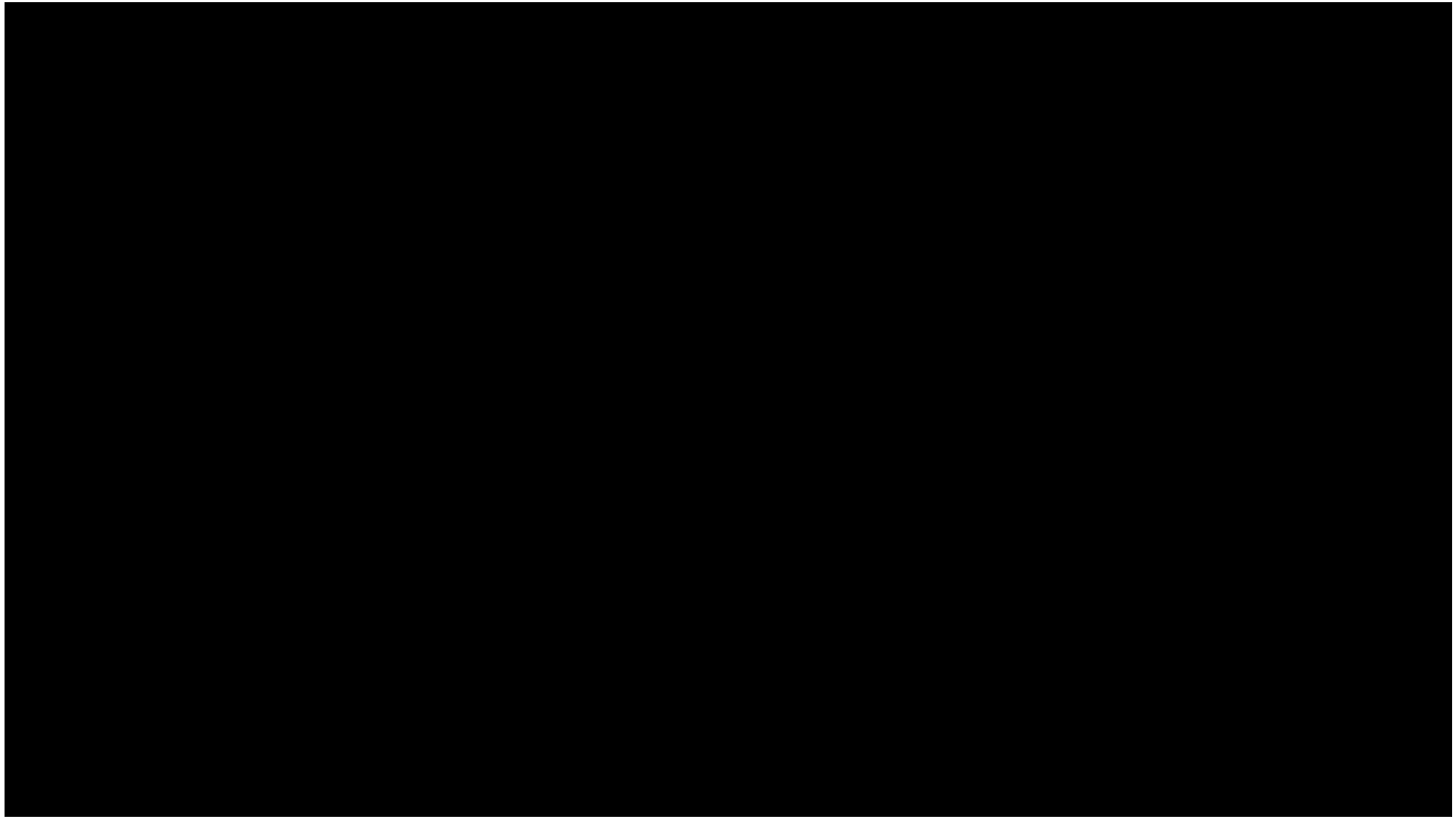
- **molecular mechanisms of host-parasite interactions, host cell invasion, and immune evasion strategies**
- (← intracellular parasitsm)
- **mechanisms of drug resistance, the biology of the apicoplast**
- **interactions with different hosts and the resulting pathogenesis** (← wide host range)

A Genome-wide CRISPR Screen in *Toxoplasma* Identifies Essential Apicomplexan Genes

Sidik et al., 2016. Cell



Lourido talk on basics of Toxoplasma



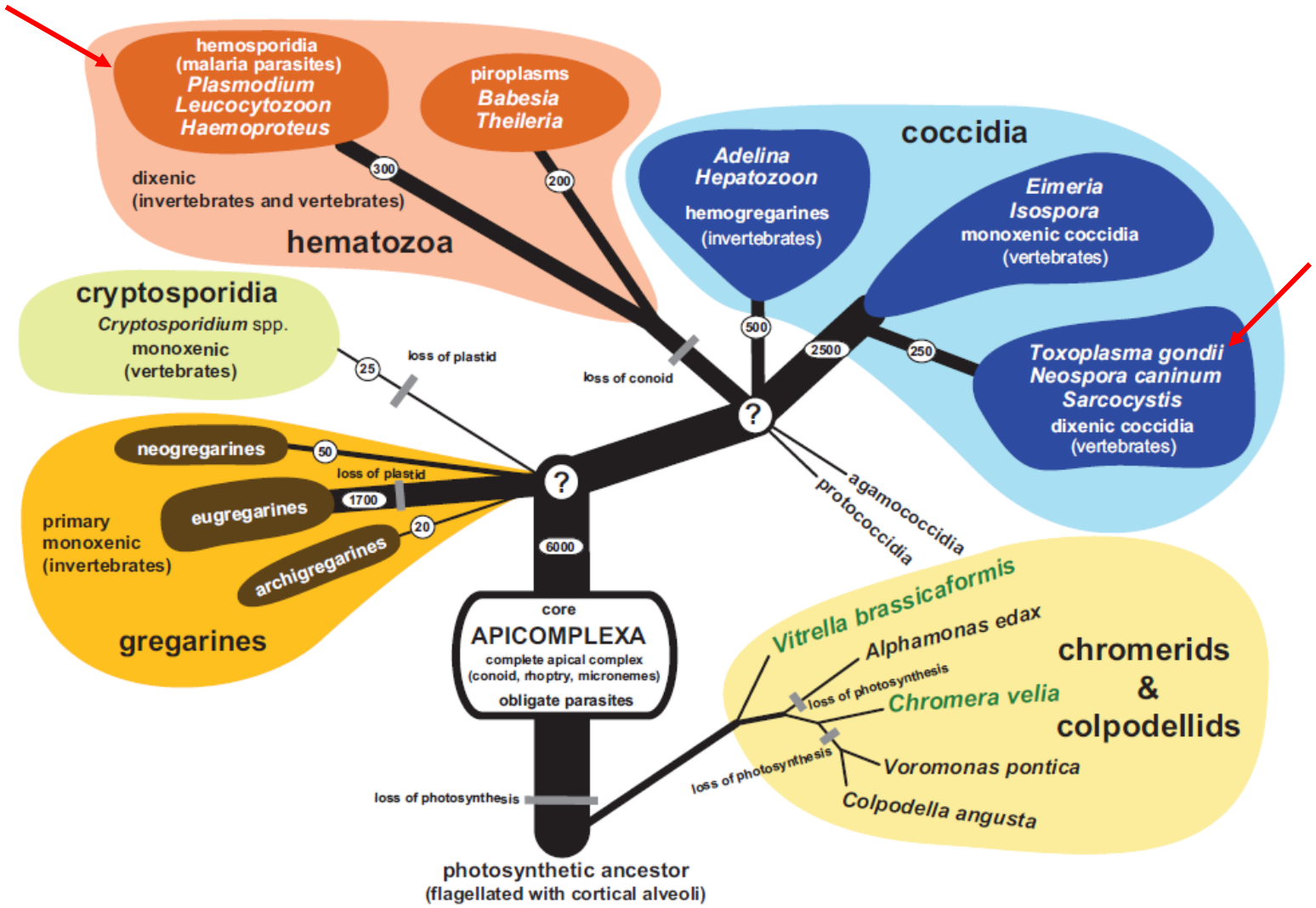
https://www.youtube.com/watch?v=wML68MA--Kw

The life cycle of Toxoplasma gondii - Part 01

https://www.youtube.com/watch?v=YGTe6Kk9w8E

The life cycle of Toxoplasma gondii - Part 02

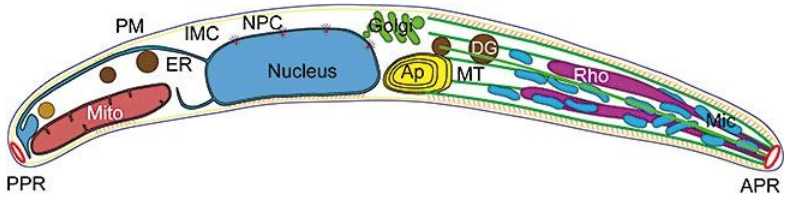
APICOMPLEXA



Plasmodium

- **Hematozoa**
- many species were discovered in various hosts and classified
- mammals ~ 50 species; birds ~ 40 species; reptiles ~ 60 species
- **five species that regularly infect human**
  - *P. vivax, P. falciparum, P. malariae, P. ovale,* and *P. knowlesi*

ACONOIDEA
= „zoit“ lacks conoid

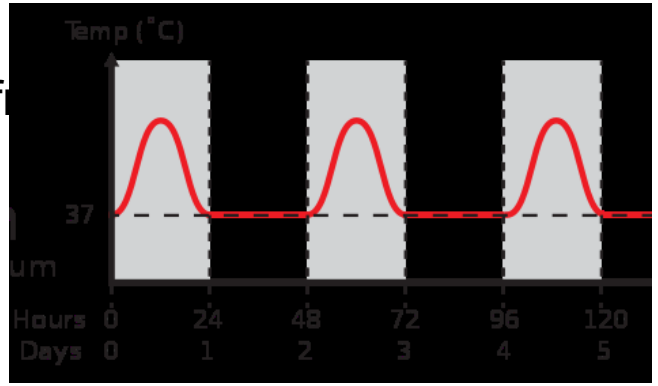


MALARIA („mal aria“ = špatný vzduch)

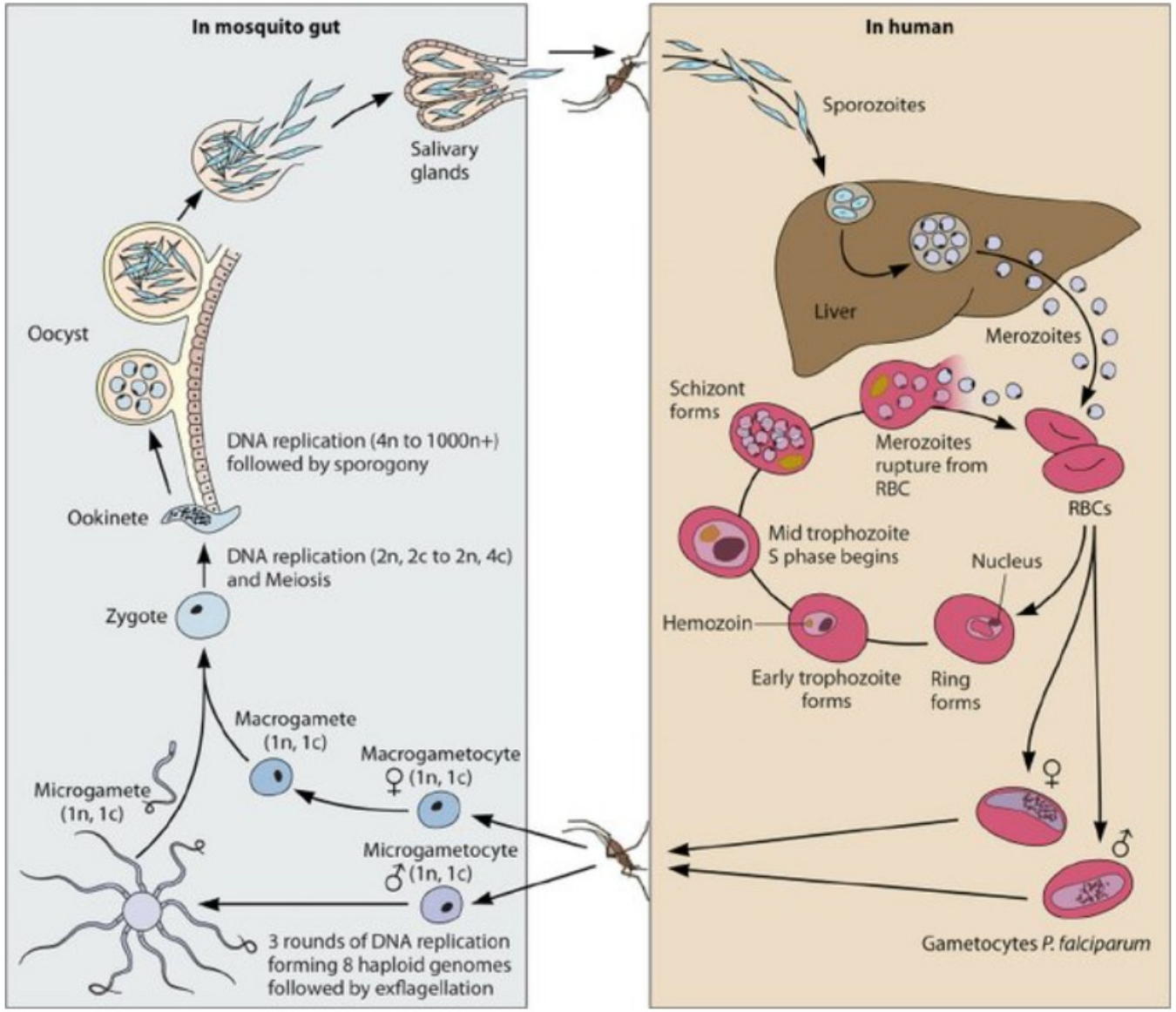
300 mil infections/year
2.5 billions in endemic area

P. falciparum

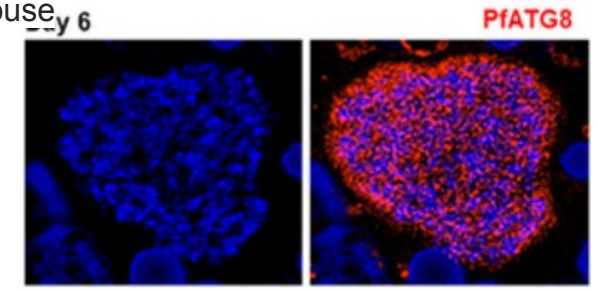
- „falx“ = „srp“
- also called malignant or falciparum malaria
- **the most dangerous form of malaria**
- the highest rates of complications and mortality
- as of 2006 - an estimated 247 million human malarial infections (98% in Af
- almost every malarial death is caused by *P. falciparum*
- **disease: malignant tertian malaria (36-48h), „tropicana“**



Plasmodium spp. life cycle

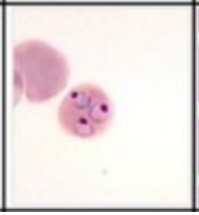
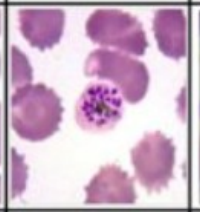
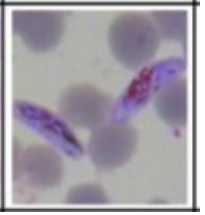


Expression of ATG8 by liver forms of *Plasmodium falciparum* in a humanized mouse

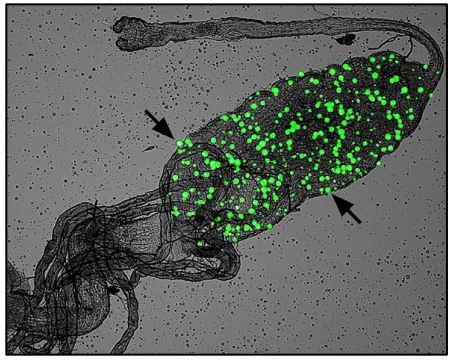


Blood cell life cycle stages

Human Malaria

Species \ Stages	Ring	Trophozoite	Schizont	Gametocyte
P. falciparum				

A. funestus midgut with greater than 300 *P. berghei* oocysts at 7 days post-infection.



Plasmodium as model organism

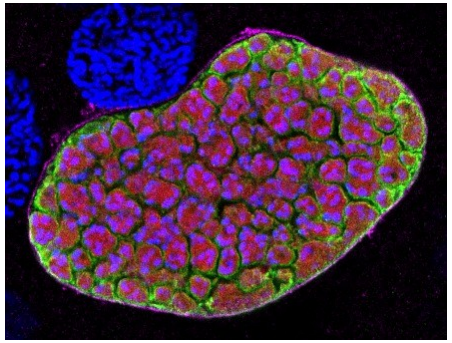
P. falciparum

- *in vitro* cultivation
- challenging mosquito infection („***in vitro* feeding**“)
- **genetic manipulation** has historically been challenging
  - the availability of many **cell markers**
  - advanced **microscopic techniques**

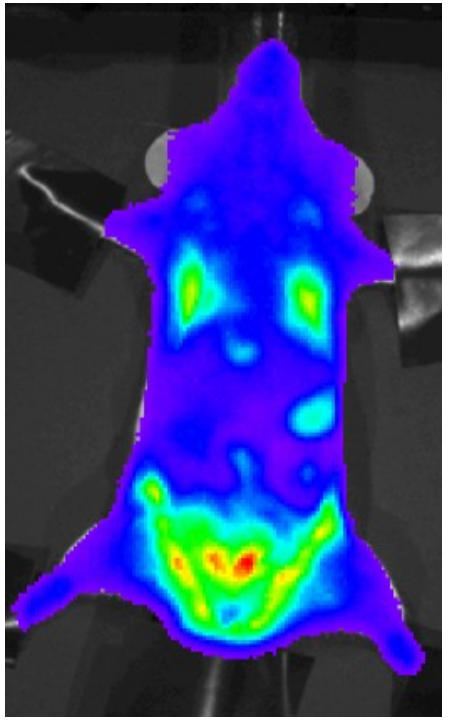
P. berghei

- a popular model organism for the study of human malaria
- can be **genetically manipulated** more easily than the species which infect humans
- the **mouse animal model** is well-established
  - experimental **cerebral malaria**
- easy **infection of mosquitoes** incl. transmission
- **development and screening of anti-malarial drugs**
- **development of an effective vaccine against malaria**

A liver cell with *P. berghei* expressing mCherry (red).

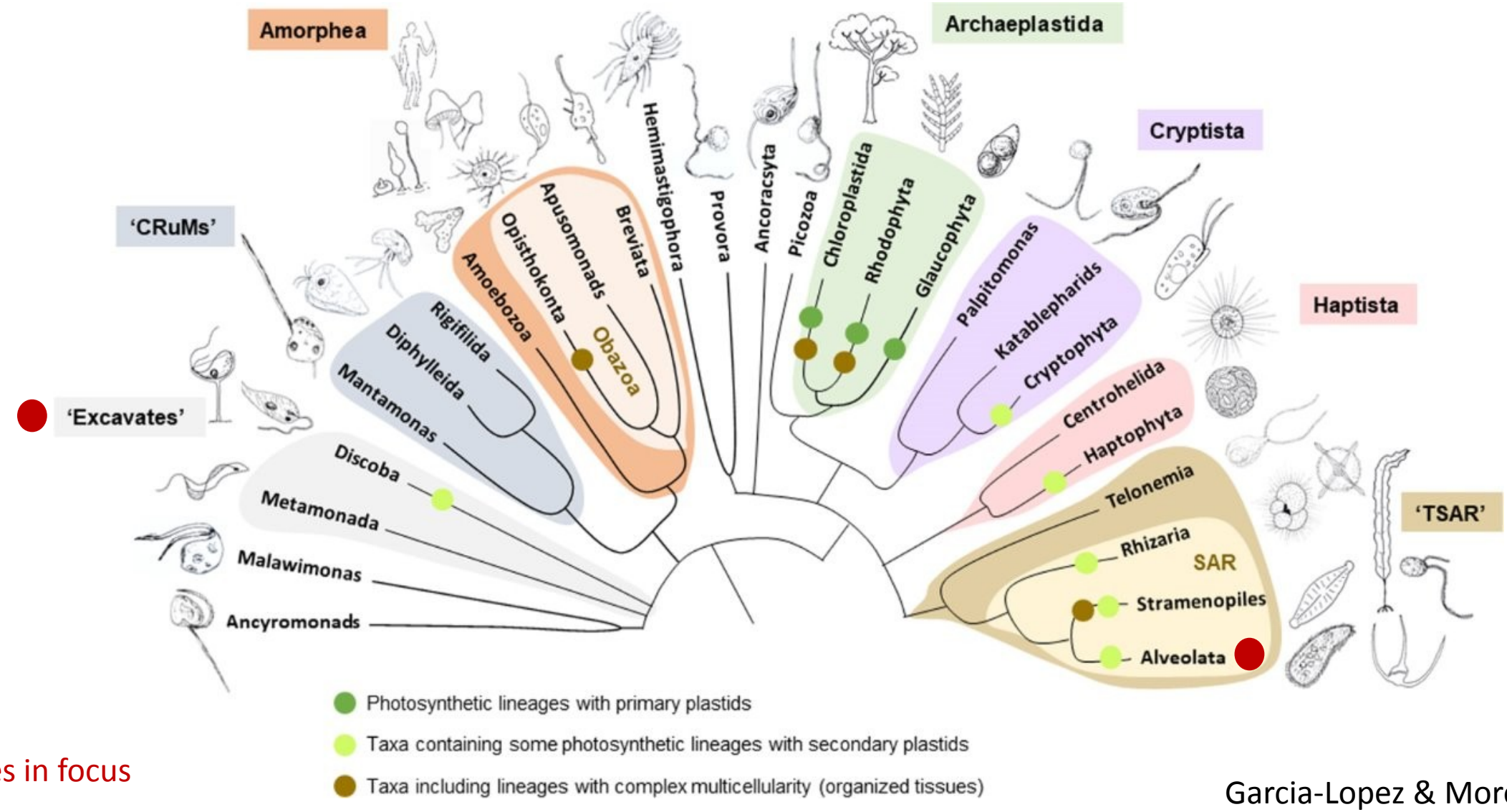


P. berghei expression of bioluminescent reporter protein Luciferase



Overview of Parasitology

Classification of Parasites



Parasites in focus

Garcia-Lopez & Moreira 2023

„EXCAVATA“

DISCOBA

- **Euglenozoa** — parasites, one group with plastids (chloroplast)
- **Heterlobosea** — alternate between flagellate and ameboid forms
- **Jakobea** — free living

METAMONADA

- **Preaxostyla** — free-living or living in the hindguts of insects
- **Fornicata** — mostly symbiotes and parasites of animals
- **Parabasala** — generally intestinal commensals of insects, some human pathogens

Trypanosoma gambiense

Leishmania sp.

Giardia intestinalis

Trichomonas vaginalis

„EXCAVATA“

DISCOBA

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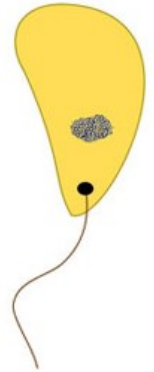
Trypanosoma gambiense



Leishmania sp.

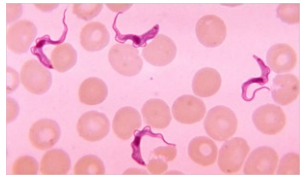
Giardia intestinalis

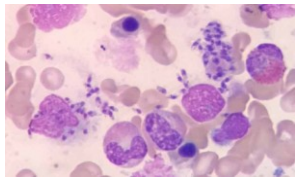
Trichomonas vaginalis

Euglenozoa

Kinetoplastea

Free-living, **parasitic**





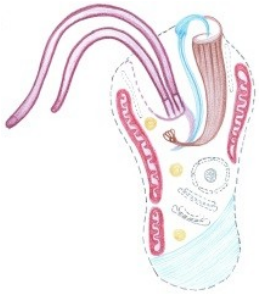
Trypanosoma brucei, T. congolense, T. vivax, causative agent of African Trypanosomiases

T. cruzi, Chagas disease

Leishmania spp., causative agent of leishmaniases

Diplonemea

Free-living (in most cases)



Diplonema papillatum
D. japonicum

Euglenids

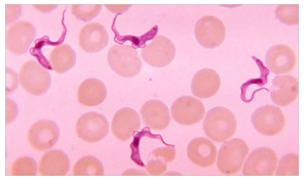
Free-living (auto- and heterotrophs)

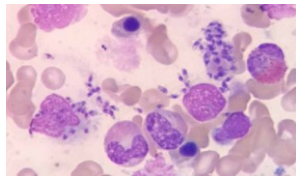
Euglena gracilis

Kinetoplastida, Trypanosomatida

Kinetoplastea

Free-living, **parasitic**

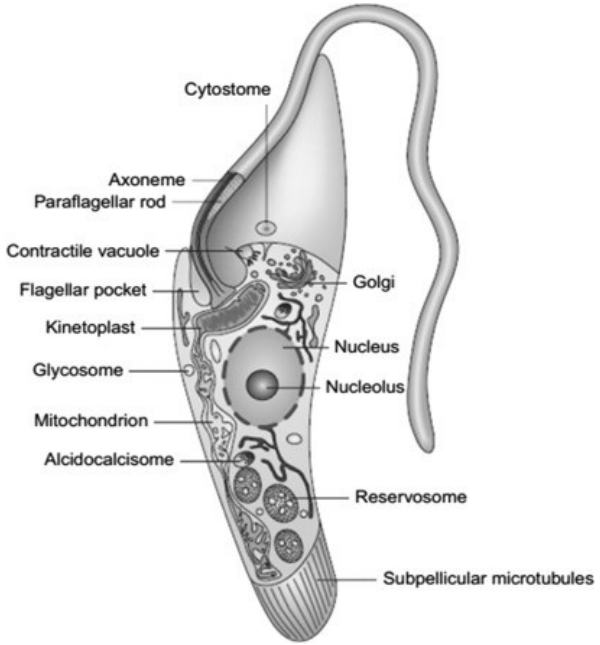


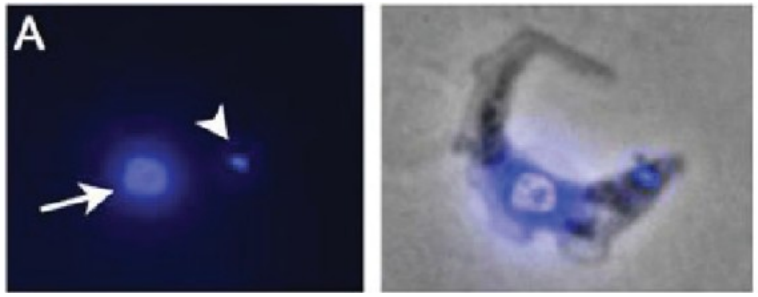


Trypanosoma brucei, T. congolense, T. vivax, causative agent of African Trypanosomiases

T. cruzi, Chagas disease

Leishmania spp., causative agent of leishmaniases





Arrow indicates the nucleus and arrowhead indicates the kinetoplast of *T. brucei.*

› the presence of an organelle with a large massed DNA called **kinetoplast**

› **glycosomes**

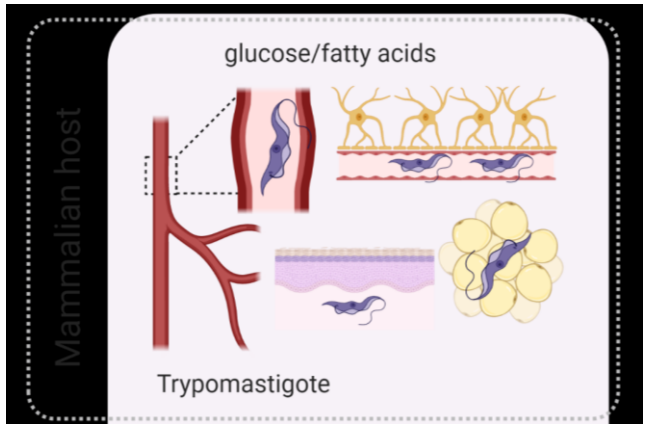
› **acidocalcisoms**

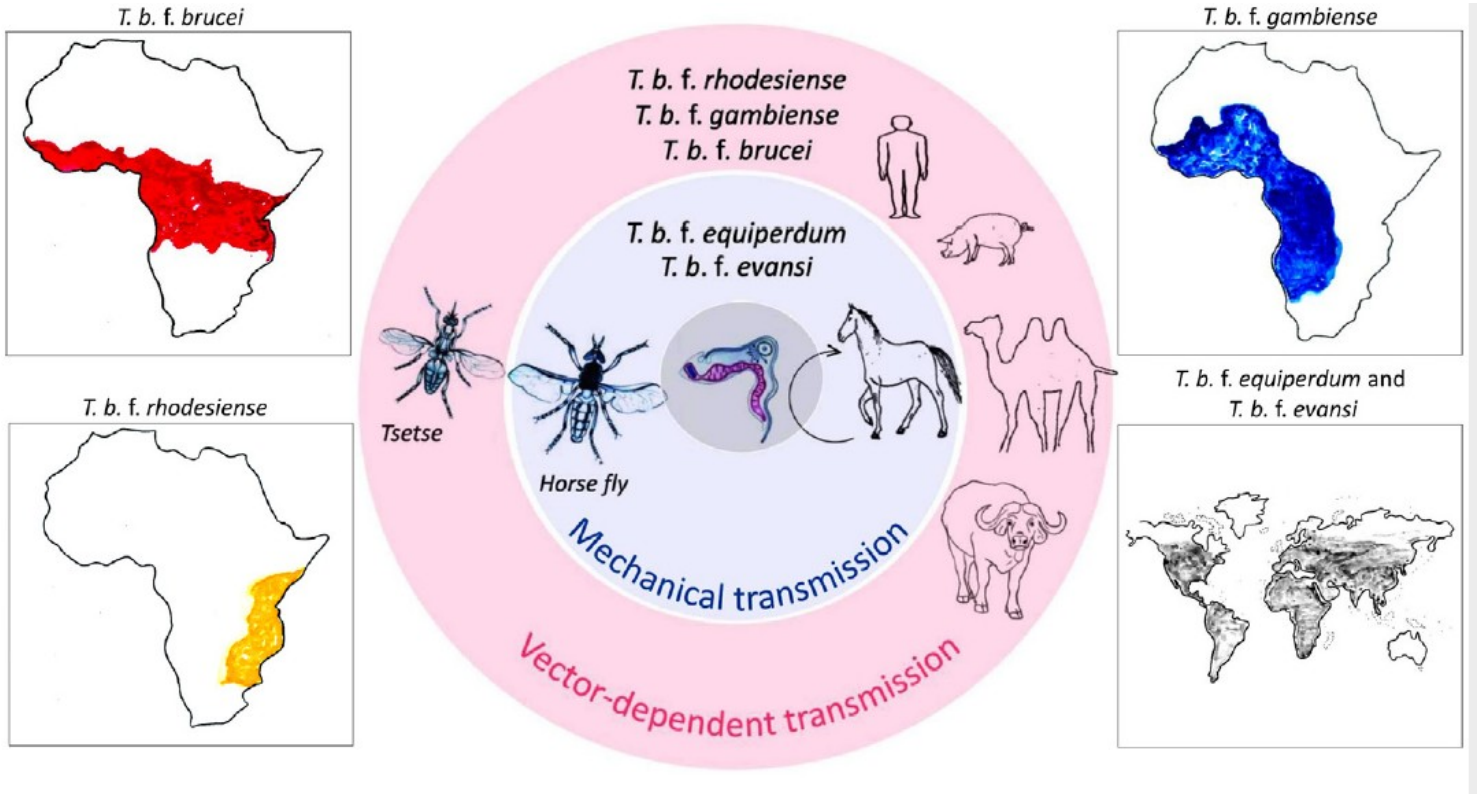
African Trypanosomes

Trypanosoma brucei *(T. b. brucei, T. b. gambiense, T. b. rhodesiense, T. b. evansi, T. b. equiperdum)*
T. congolense, T. vivax

- Human African Trypanosomiasis (HAT)
  - 36 African states
  - 50 millions in affected areas
  - Always lethal if untreated

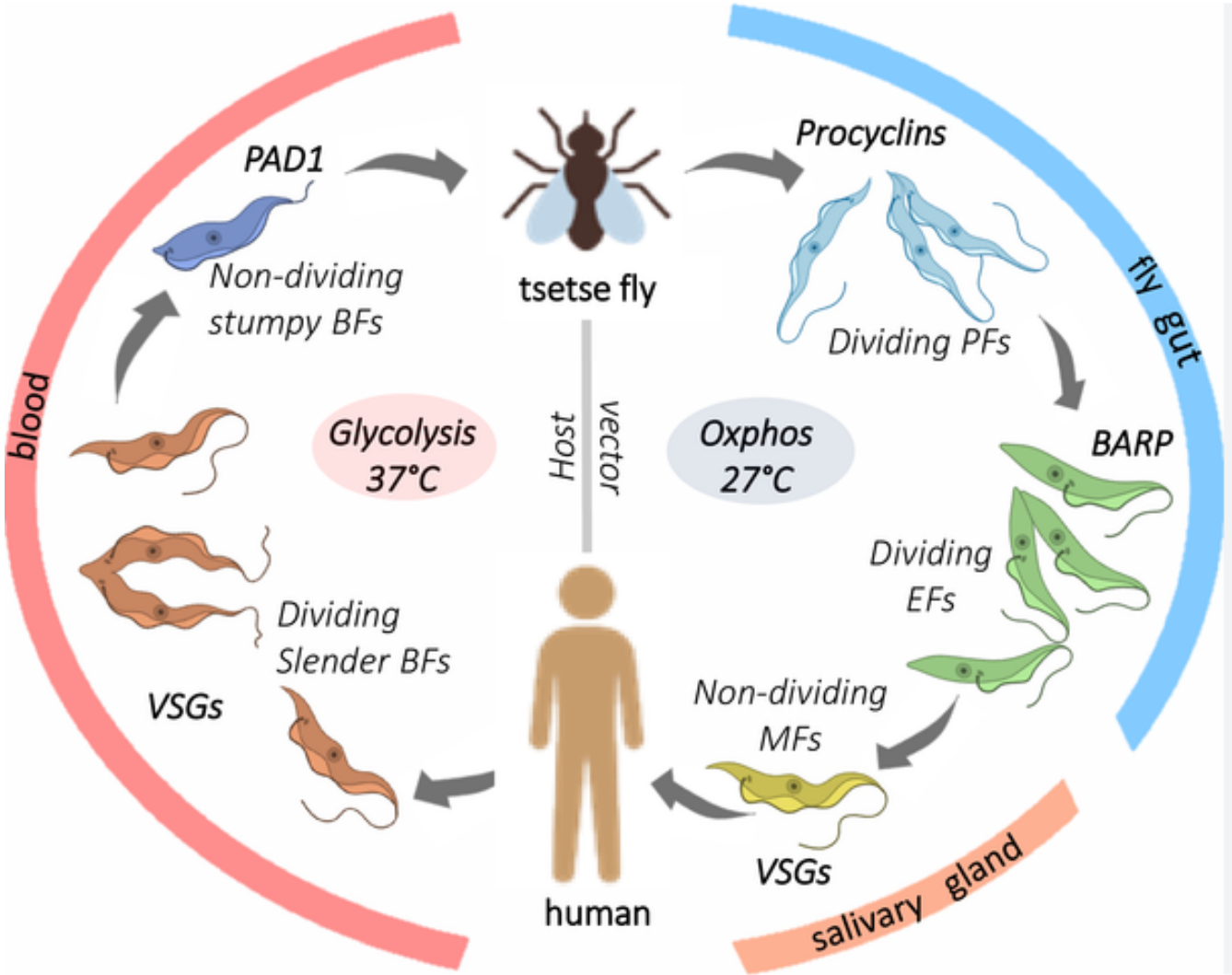
- Animal African Trypanosomiasis (AAT)
  - Direct loos of livestock products
  - Loss of crop productivity due to loss of the animals draught power

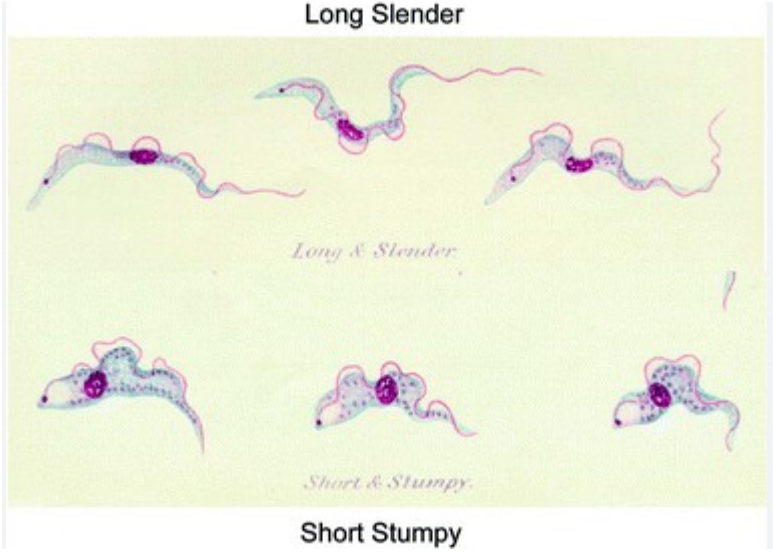


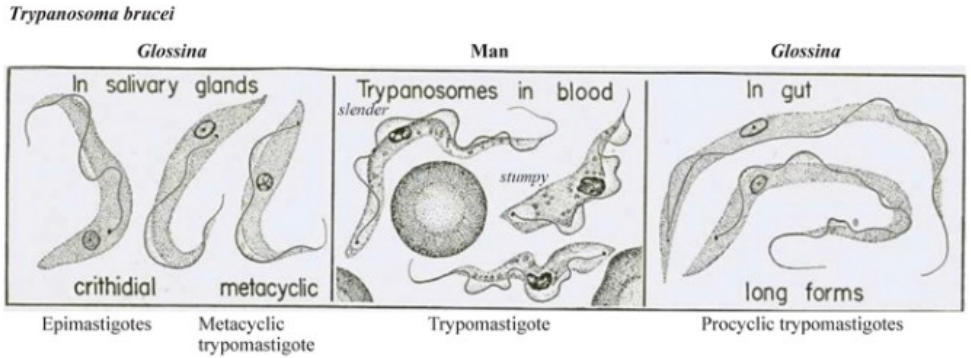


Adapted from Lukeš et al., 2022. Trends in Parasitol.

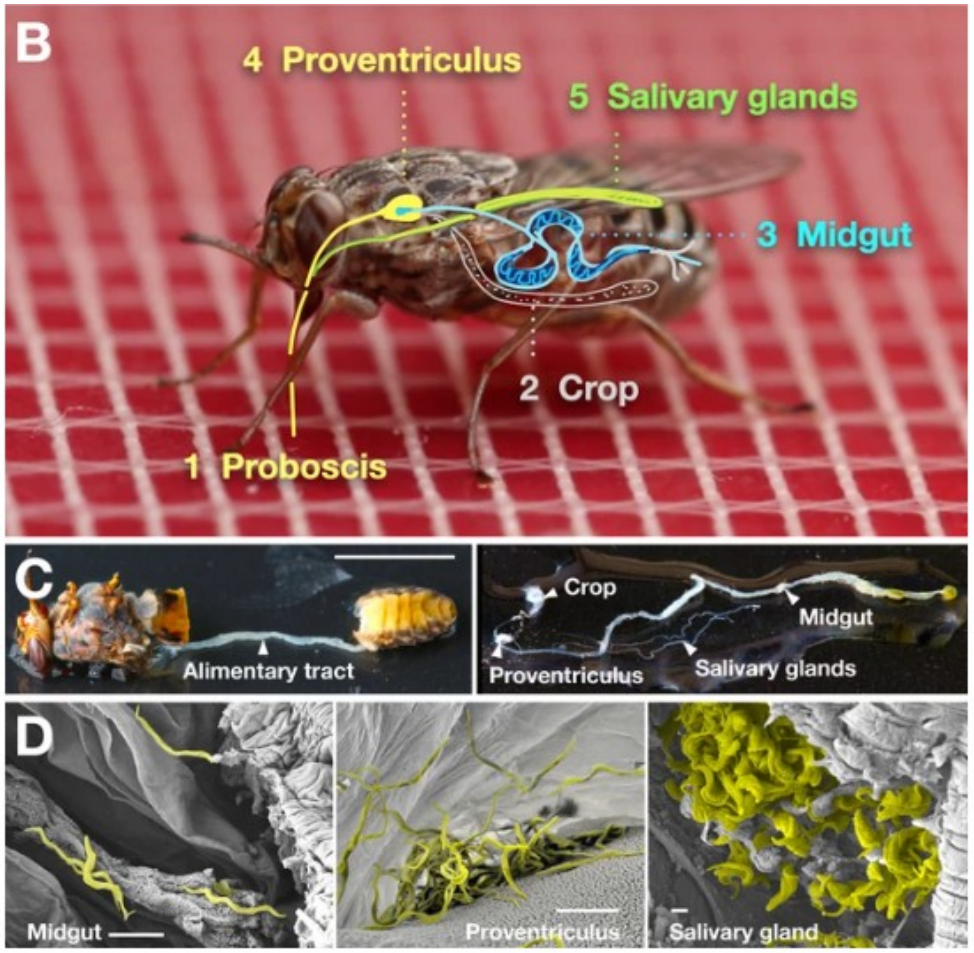
T. brucei life cycle

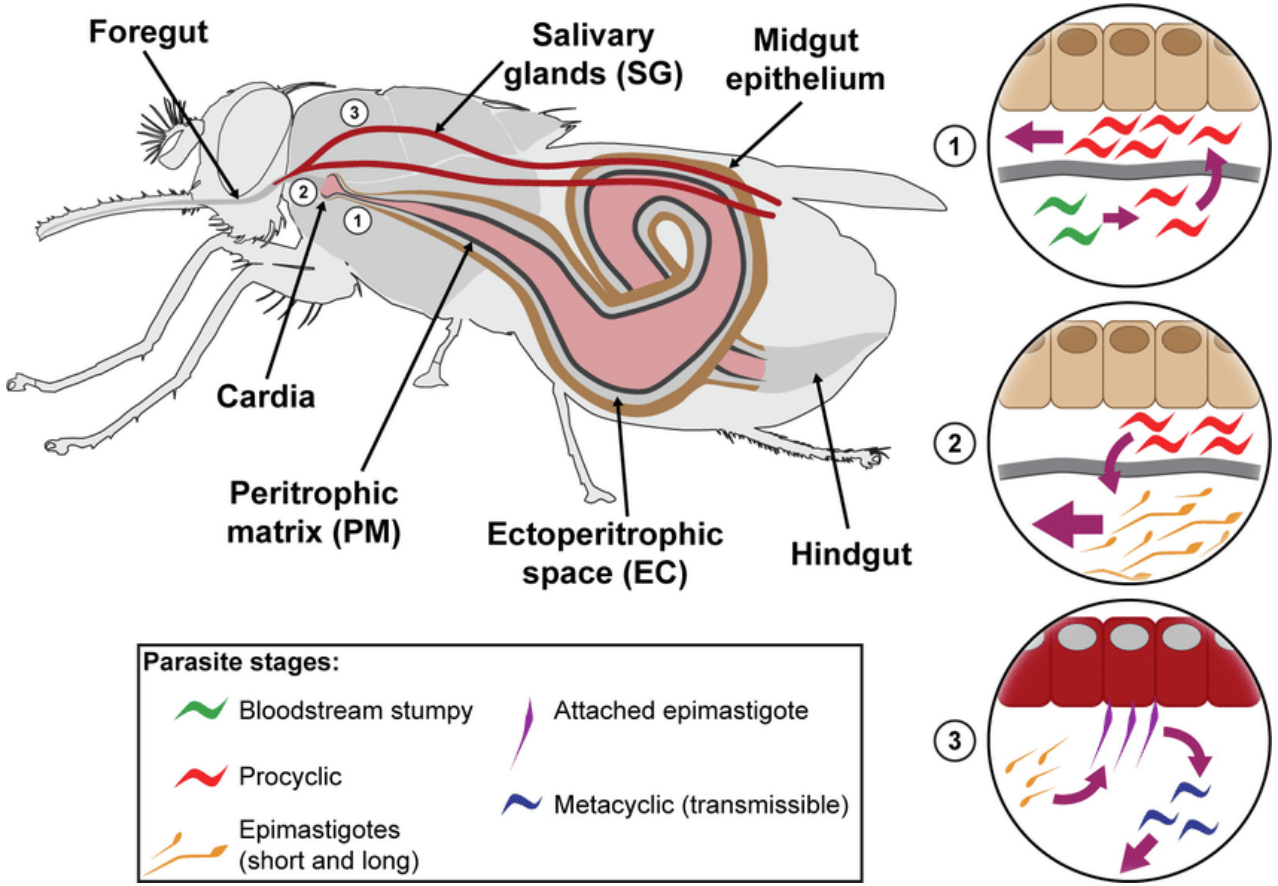






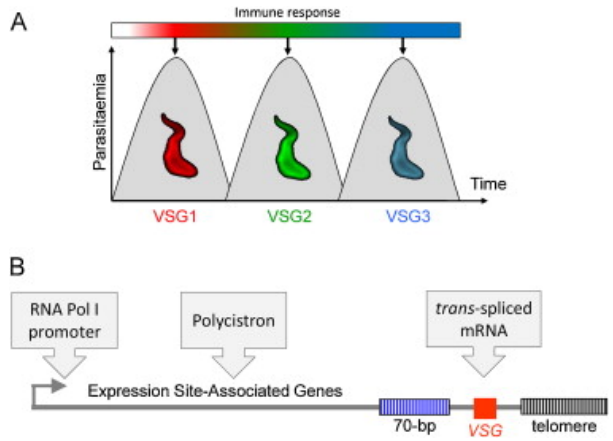
T. brucei life cycle – insect forms development





Trypanosoma brucei as model organism

- a model organism for the kinetoplastids
- ***in vitro* culture of both bloodstream and procyclic stages**
- the mouse animal model is well-established
  - infection in rodents provide valuable platforms for studying disease pathogenesis, immune responses, and drug efficacy.
- well-established tools for **genetic manipulation**

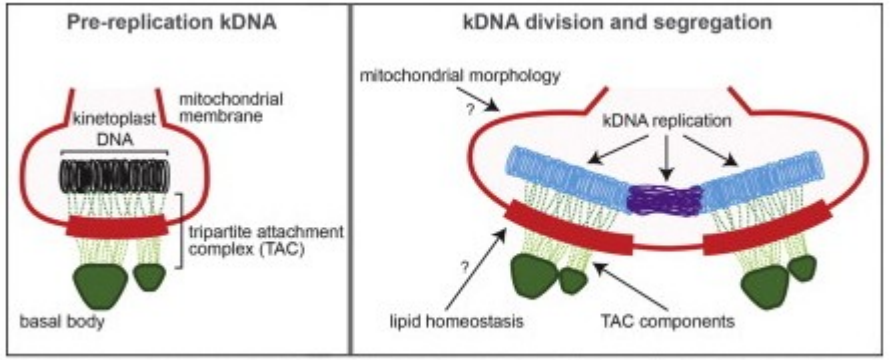


- **unusual nuclear architecture** compared to those of other eukaryotic model organisms
  - genome organization and nuclear gene expression regulation

- **kinetoplast** structure: minicircles and maxicircles
  - **RNA editing**

- **antigenic variation**

- **glycosomes** → dealing changes in nutrient availability



The mitochondrial DNA of kinetoplastids (kDNA), must be replicated once per cell cycle. Diverse activities affect kDNA division and segregation, leading to new models for these processes.

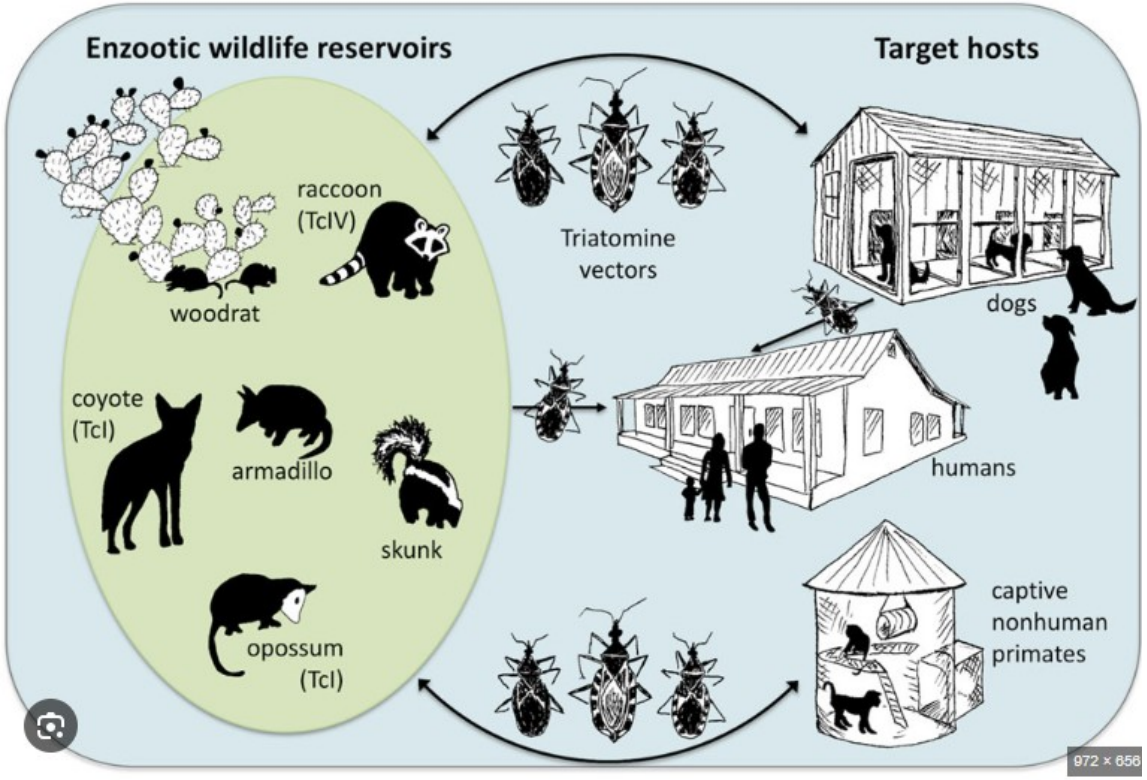
Trypanosoma cruzi

T. cruzi

6 definovaných skupin, různé kmeny

Zoonóza

Přenášena plošticemi rodu Triatominae (kissing bugs)



Chagasova choroba

16-18 mil infikovaných

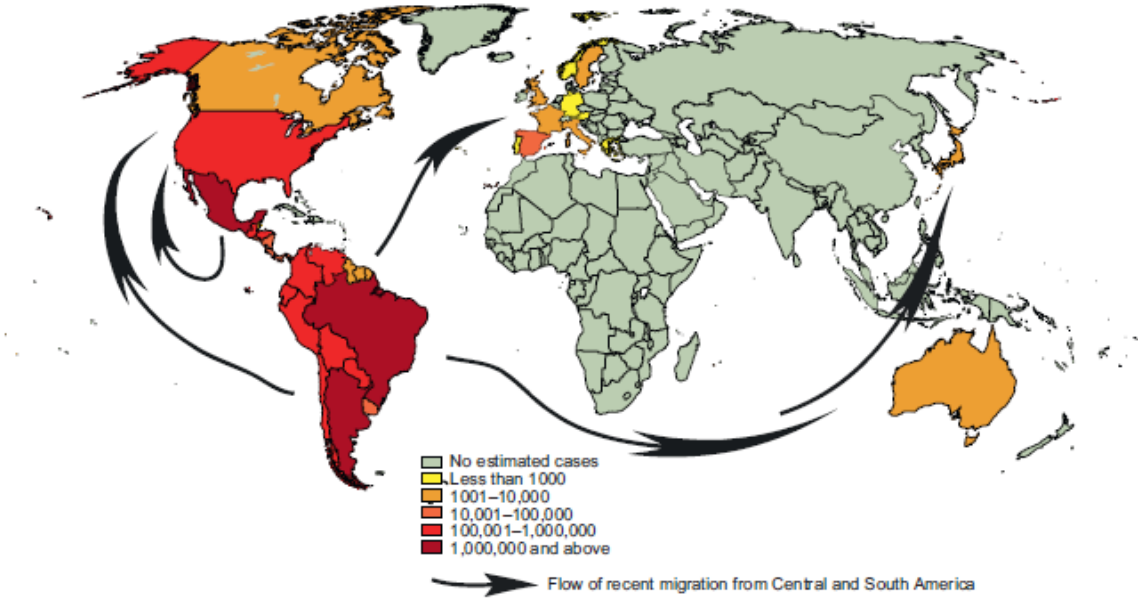
90 mil žijících v rizikových oblastech

Akutní a chronická faze (kardio-, gastrointestinální kompli

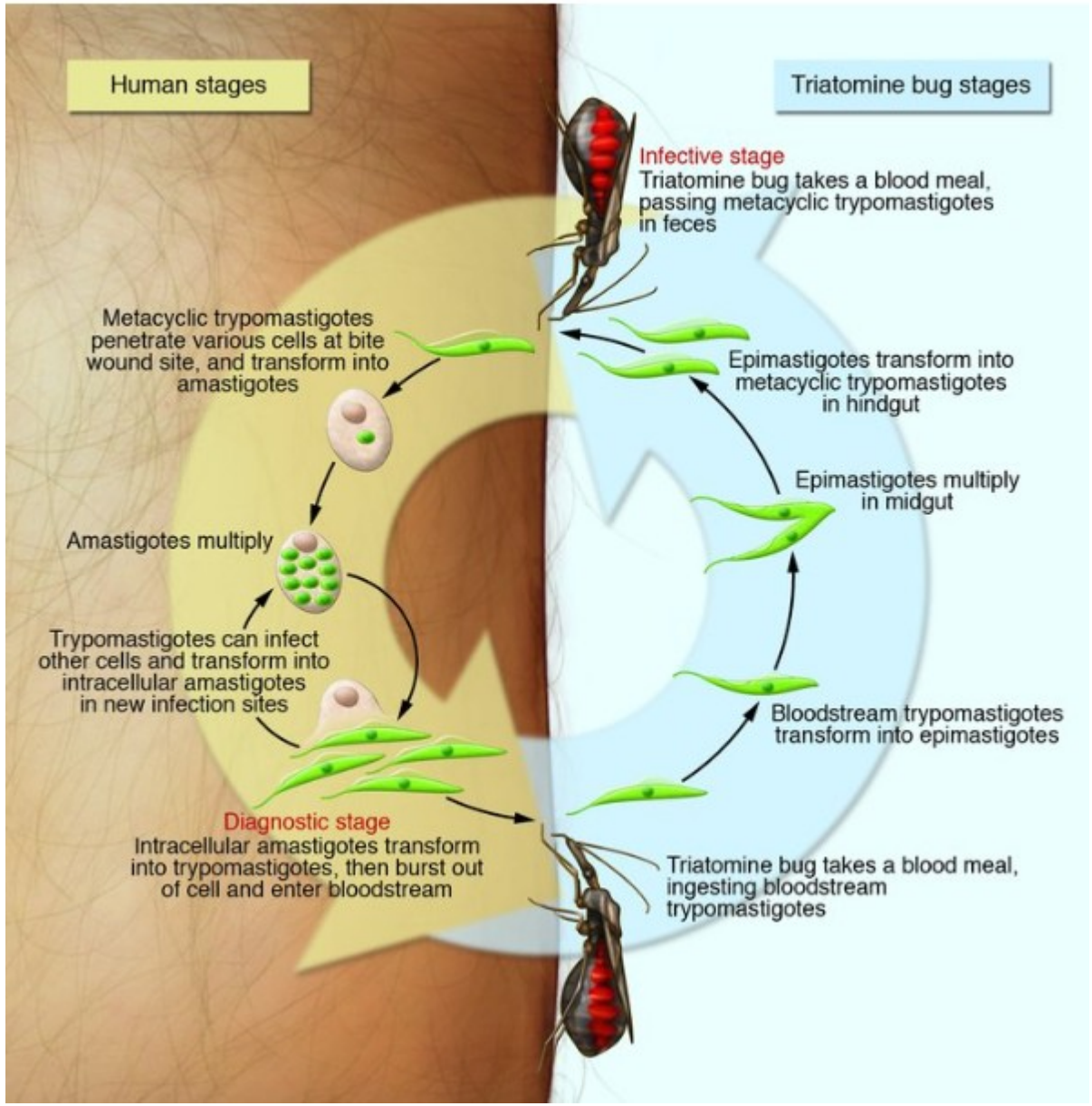
úmrtí až v 10%

Treatment – benznidazole, nifurtimox

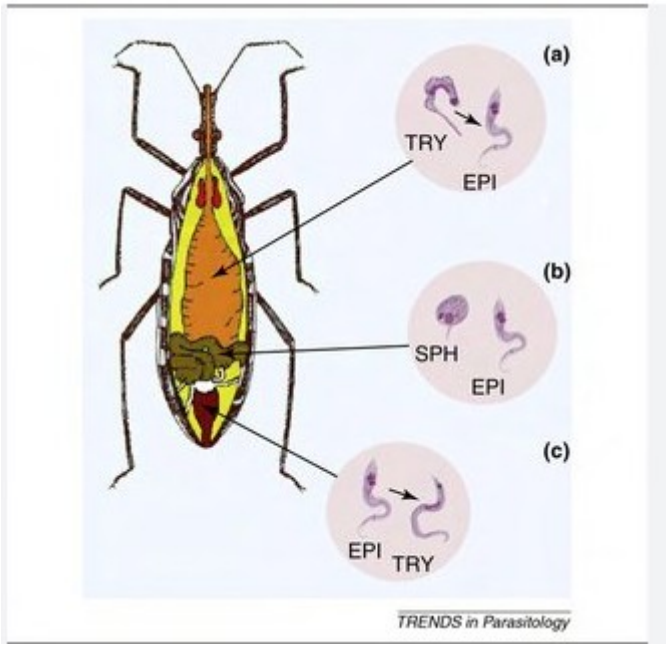
Geographical distribution



Trypanosoma cruzi life cycle

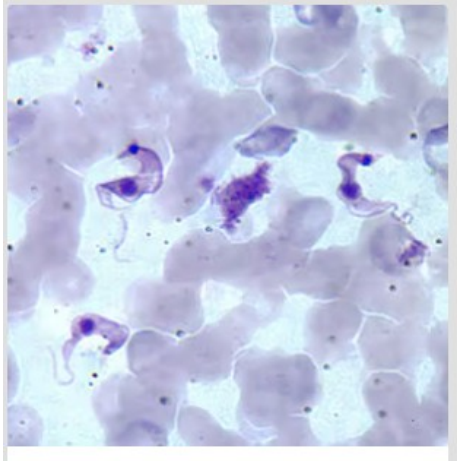


INSECT VECTOR

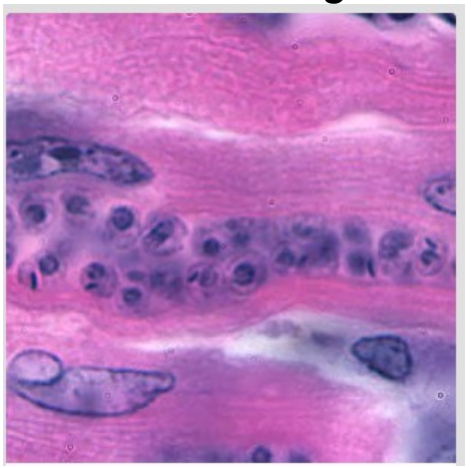


MAMMMALIAN HOST

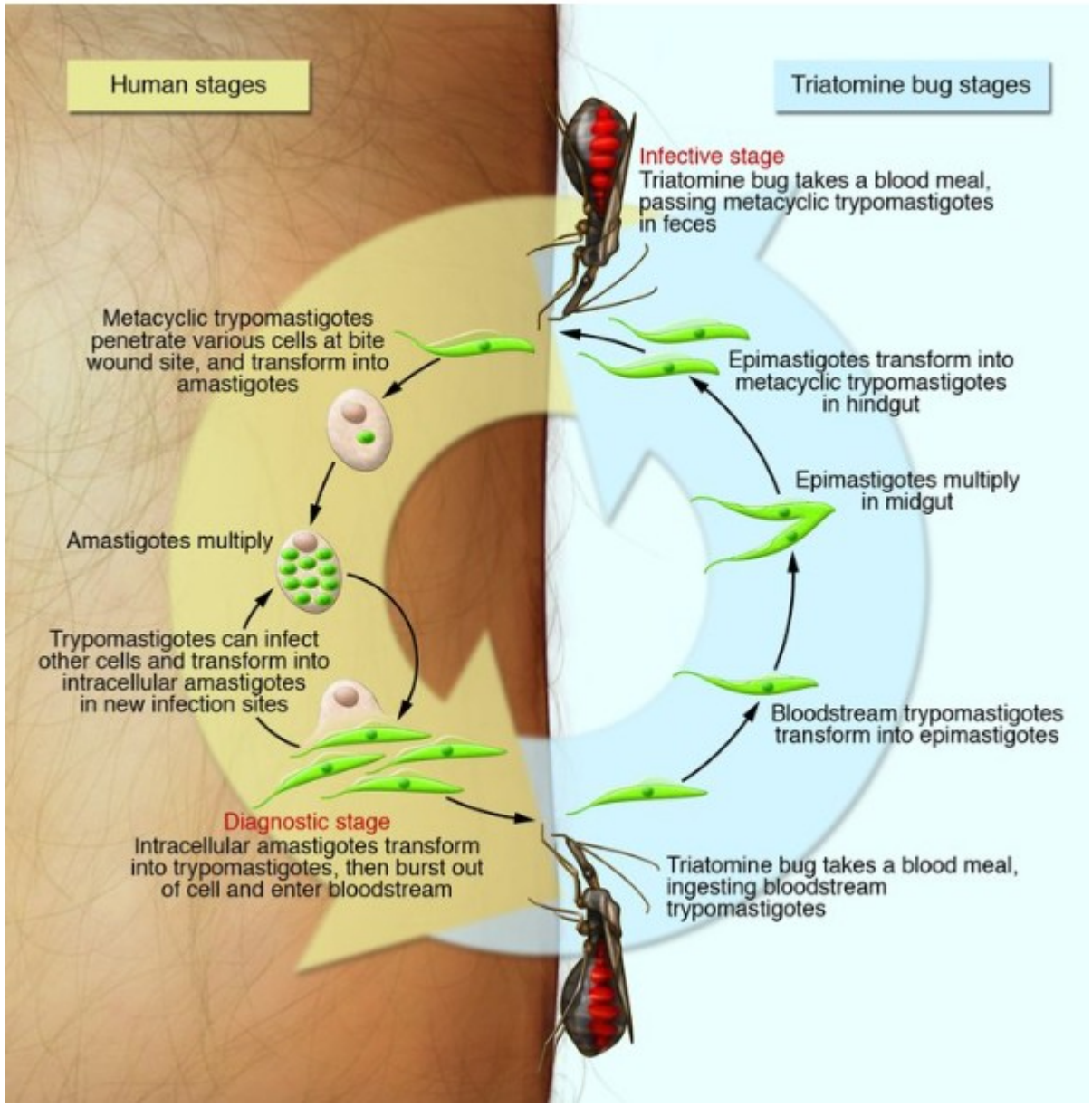
T. cruzi trypomastigote

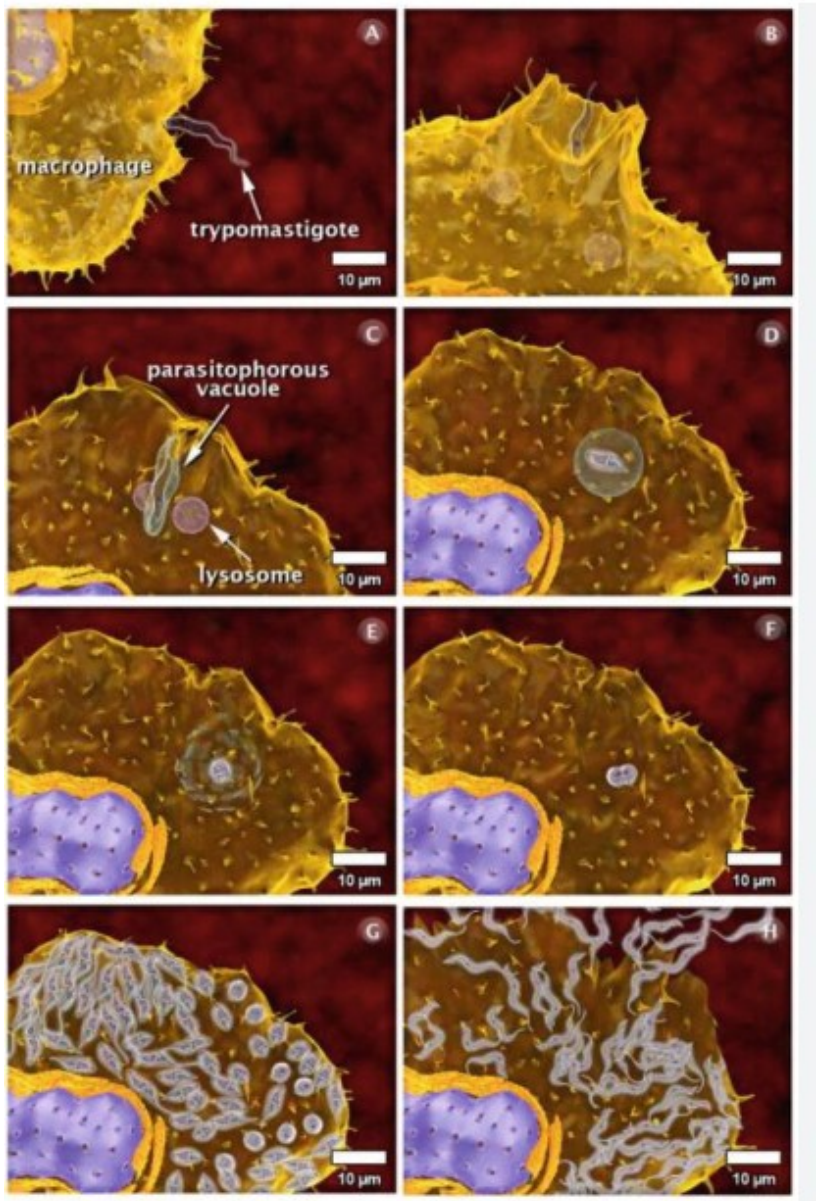


T. cruzi amastigote



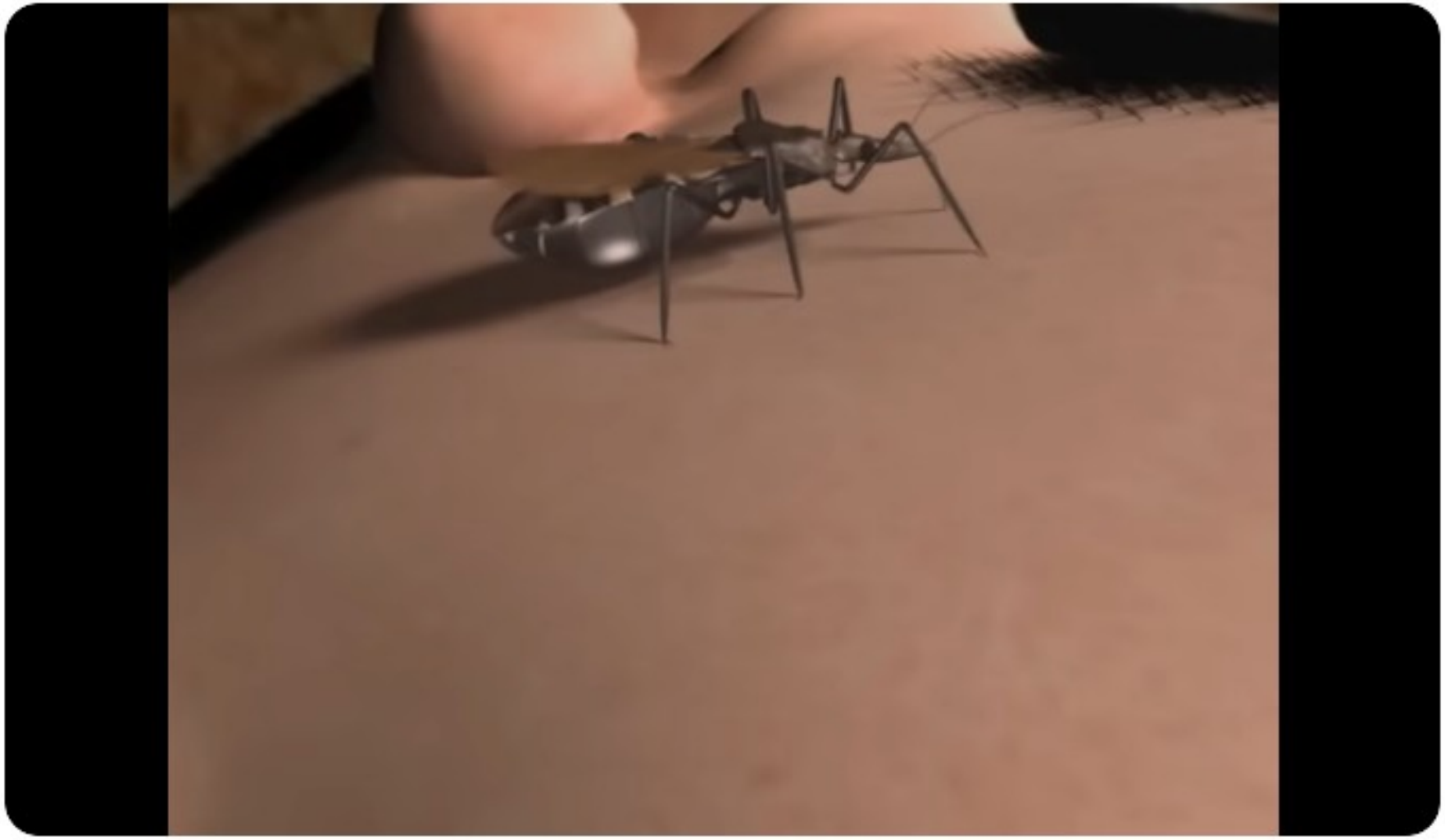
Trypanosoma cruzi life cycle





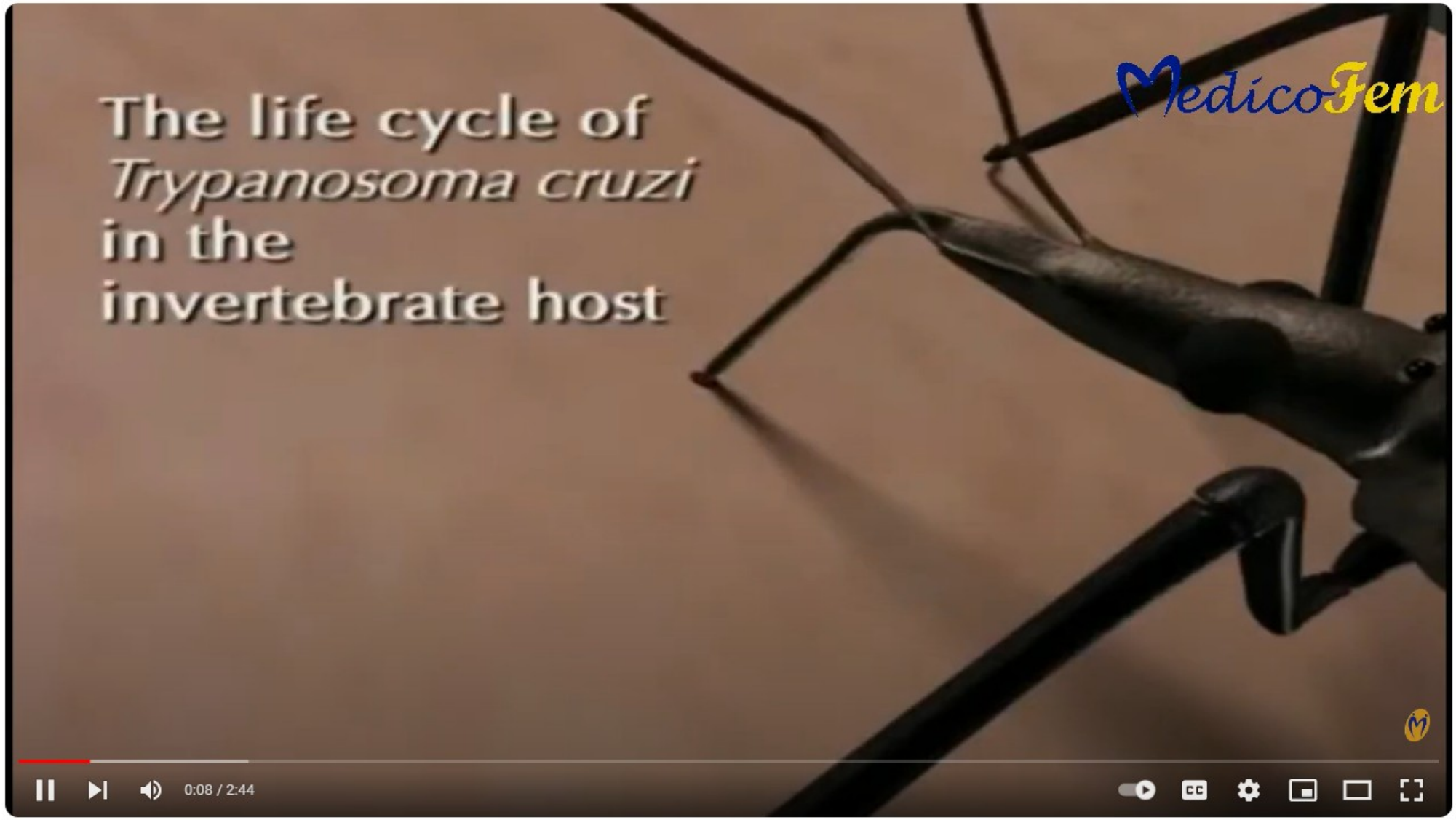
Trypanosoma cruzi life cycle

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



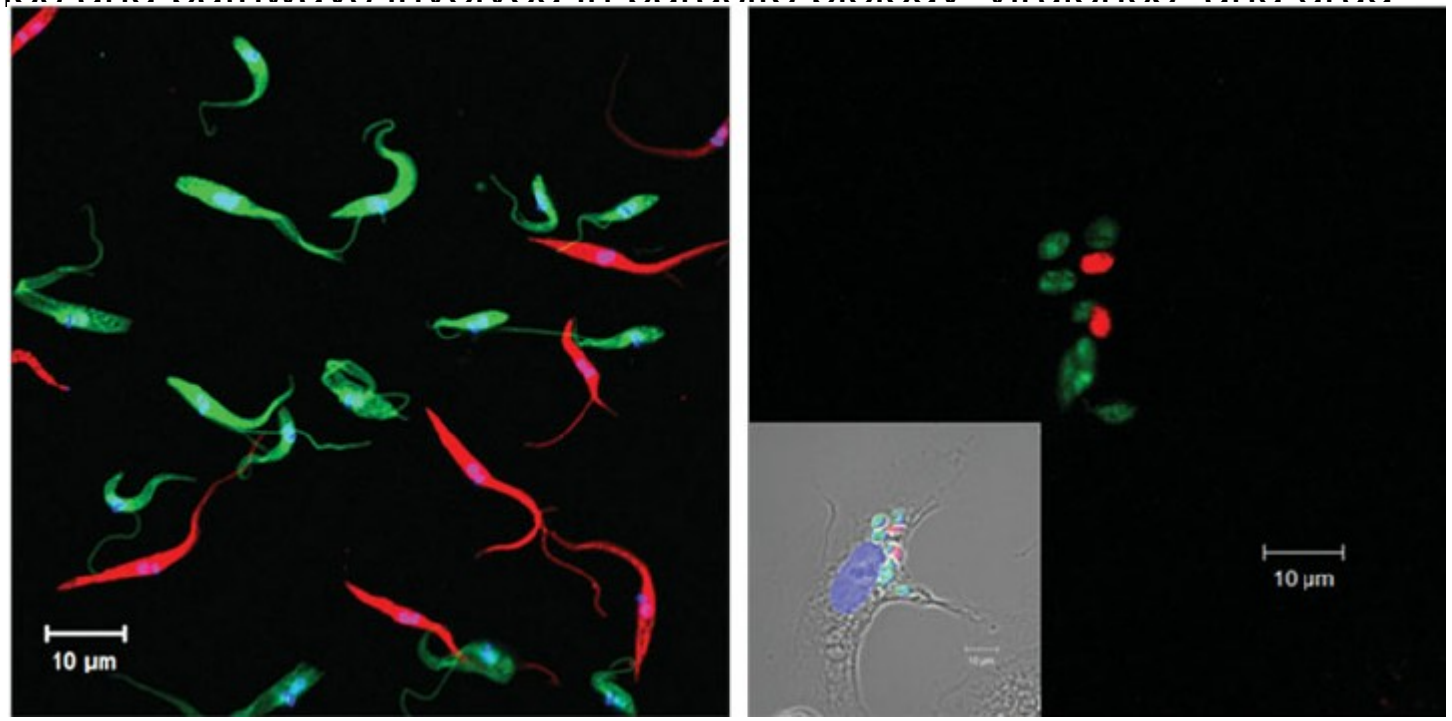
Trypanosoma cruzi life cycle

https://www.youtube.com/watch?v=_mZIzMU10OY



Trypanosoma cruzi as model organism

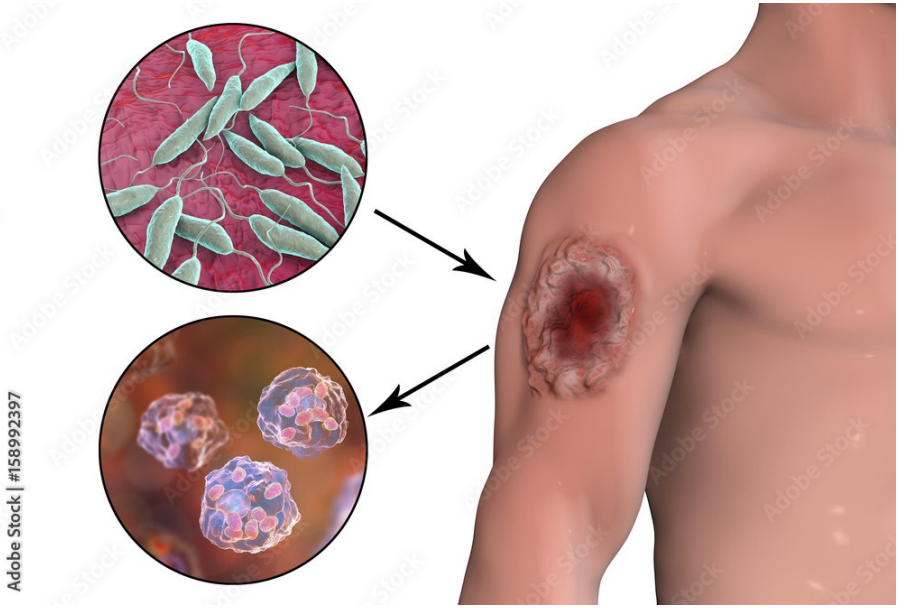
- ***in vitro* culture → parasite biology, drug susceptibility, and host interactions**
- the mouse animal model is established
  - disease pathogenesis, immunopathology, and vaccine development
  - host-parasite interactions, and drug resistance in Chagas disease
- well-established tools for **genetic manipulation**
  - specific genes and pathways involved in parasite biology, virulence, and drug resistance

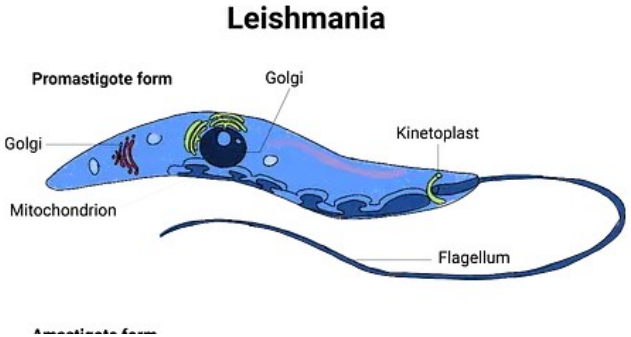


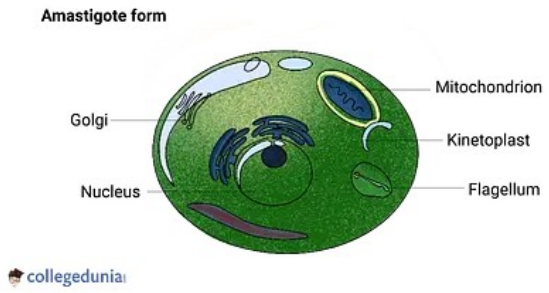
Transgenic *Trypanosoma cruzi* expressing GFP or DsRed

Leishmania spp.

- causative agents of Leishmaniasis, a group of diseases that affect both humans and animals.
- over 20 different species of Leishmania that are known to cause disease in humans and animals.
- Leishmaniasis is found in tropical and subtropical regions around the world, including parts of South America, Africa, Asia, and the Mediterranean.
  - Population at risk: 350 mil
  - Infected: 12 mil
  - Deaths: 51 000 (VL)







Leishmania

Leishmaniasis

- **Cutaneous Leishmaniasis (L. tropica, L.aethiopica,**

It is the **most common form of Leishmaniasis** that causes ulcers on the skin and skin damage that leads to long-term scars.

•treatment may not be necessary., It can speed healing and prevent further complications.

- **Mucocutaneous Leishmaniasis (L. braziliensis, L. panamensis)**

It is a rare form of disease. It is caused by the cutaneous form of the parasite and can arise several months after the skin ulcers heal.

•The disease does not heal on its own and mostly requires treatment**.**

- **Visceral Leishmaniasis (L. donovani. L. infantum)**

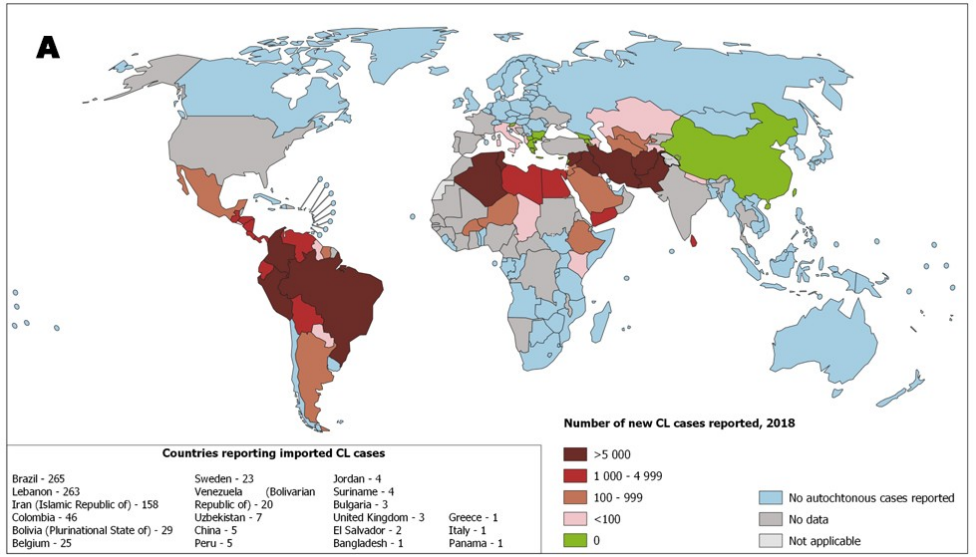
It is also known as Systemic Leishmaniasis. It is likely to occur two to eight months after being bitten by an infected sandfly.

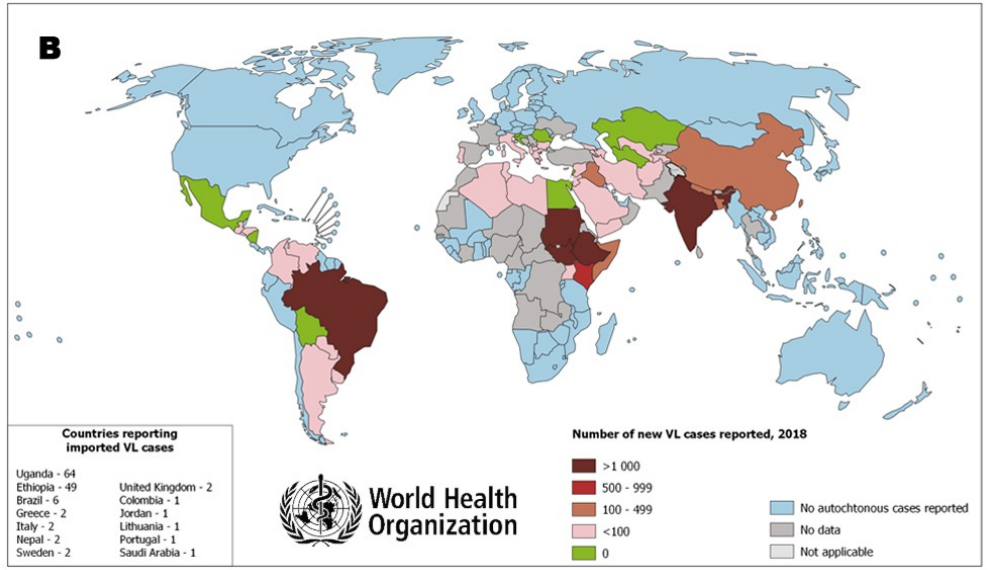
•It causes anemia, weight loss, and irregular periods of fever.

•It **damages internal organs** such as the spleen and the liver.

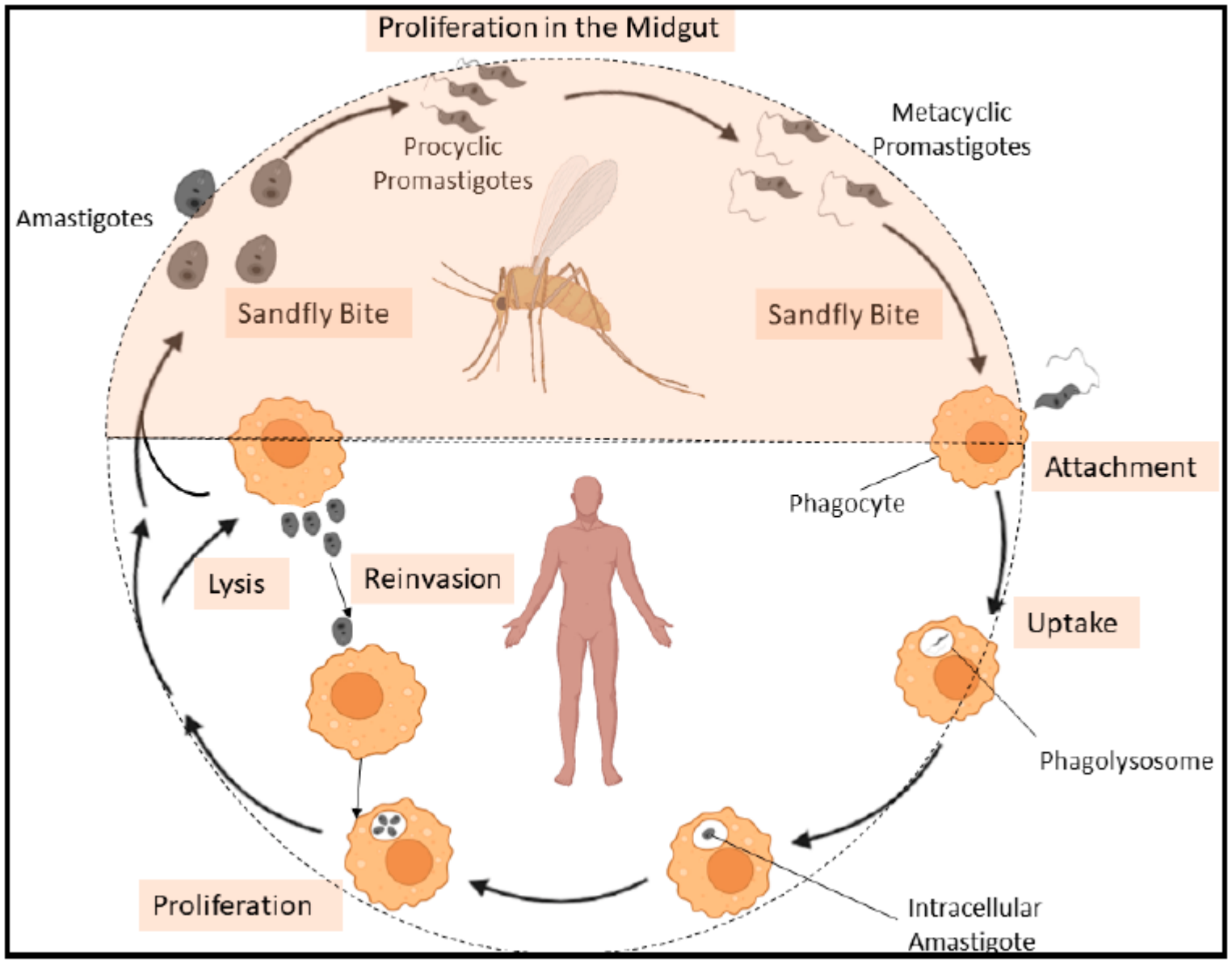
•It affects the **bone marrow** as well. It **weakens the immune system** by the damage of these organs.

•If not treated, this form of condition is almost always fatal.





Leishmania spp. life cycle



Leishmania as model organism

Experimental accessibility

- › ***in vitro* culture → parasite biology, drug susceptibility, and host interactions**
- › the mouse animal model is established → gene expression, pathogenesis

Genetic tractability

- › relatively small genome that is genetically tractable
- › **genetic manipulation** → gene function and impact on parasitic biology.

Complex life cycle → mechanisms by which parasites adapt to **different host environments**

Drug resistance → studying **mechanisms of drug resistance** and identifying new drug targets

Immune evasion → various strategies to evade the host immune system → chronic infections

Comparative studies → comparative genomics between *Leshmania* and related parasites → evolution of parasitic traits and host-parasite interactions