

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

*In the name of God, the Beneficent, the Merciful*



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# Vectors of Leishmaniasis

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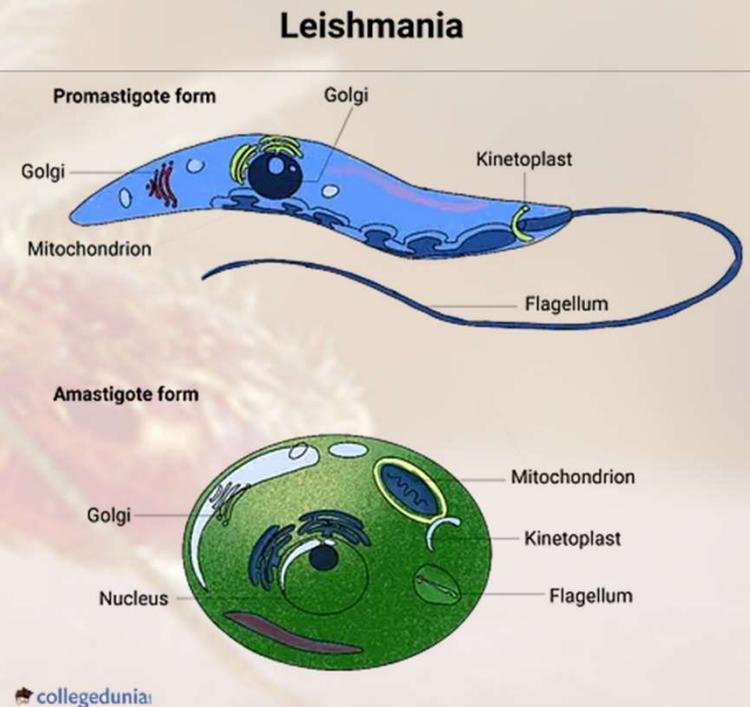
# Introduction

- 200 million insects alive per each human at any given point
- 14,000 species feed on blood, some with potentially severe implications for human health
- Vector-borne diseases account for more than 17% of all infectious diseases, Cause at least 700,000 deaths annually
- Neglected Tropical Diseases (NTDs)



# Leishmaniasis

- Among the NTDs, leishmaniasis is associated with significant incidence, morbidity, and mortality (the deadliest NTD, according to recent global estimates)
- Leishmaniasis is a spectrum of diseases caused by around 20 *Leishmania* species, transmitted by different phlebotomine sand fly species



# Sand flies

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- The first report was by **Bonanni** in Italy (1691)
- Its medical importance in the transmission of *Leishmania* by **Bueno**, a Spanish physician (1764)
- They are found in all geographical areas except New Zealand and the Pacific Islands
- Around 1000 sand fly species/subspecies were validated/described thus far around the world
- Only about 81 species are vector
- Important vectors of diseases such as leishmaniasis, papatasi fever, carrion
- Very small adults(1.5-4 mm), with a hairy body, big eyes, long legs , a pair of wings and look like the letter v during rest
- Only females are bloodsuckers

# Taxonomy

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- Domain: Eukaryota
  - Kingdom: Animalia
    - Phylum: Arthropoda
      - Class: Insecta
        - Order: Diptera (two-winged flies)
          - Sub-order: Nematocera
            - Family: Psychodidae
              - Sub-family: Phlebotominae
                - *Phlebotomus* (13 subgenera), *Sergentomyia* (10 subgenera), and *Chinius* (4 species) from the **Old World**
                - *Lutzomyia* (26 subgenera and groups), *Brumptomyia* (24 species), and *Warileya* (6 species) from the **New World**

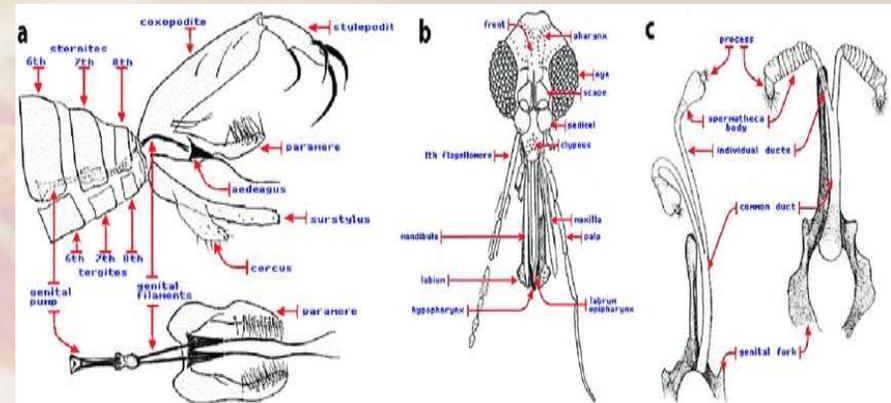
# Taxonomical classification of sand flies

➤ Initially, the taxonomical classification of sand flies was based on morphological analyses

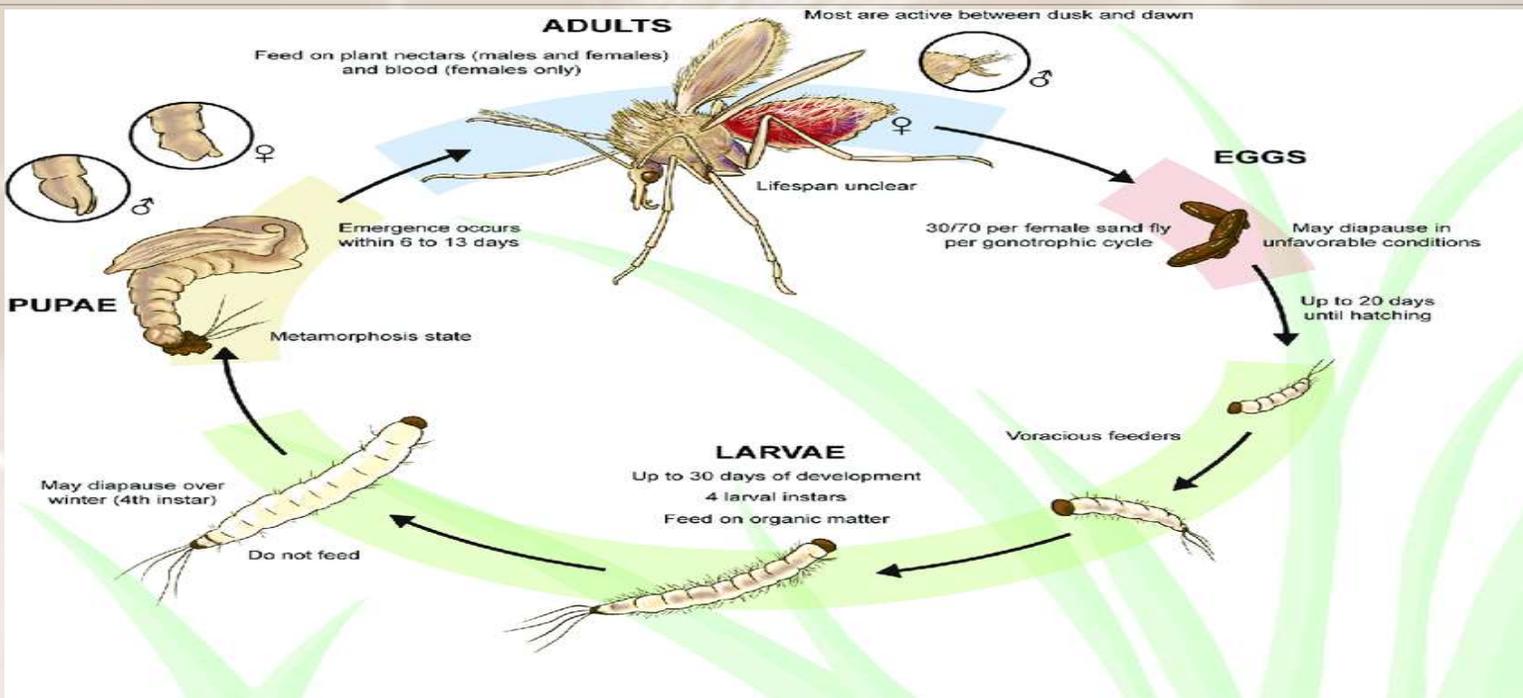
- Male genitalia
- Determination of the wing venation indices
- Spermathecae
- Cibarium
- Pharynx

➤ More recently, modern methods including:

- Chromosome analysis
- Isoenzyme analysis
- Molecular and phylogenetic analyses (DNA barcoding and Next-Generation Sequencing)
- Mass spectrometry



# Sand flies' life cycle



Schematic representation of the sand flies' life cycle. The sand fly life cycle comprises four major stages: eggs (orange background), larvae (four instars: green background), pupae (yellow background), and adults (blue background). In the latter two stages, different morphological features (highlighted within the circles) can be used to distinguish the gender.

# Blood-feeding

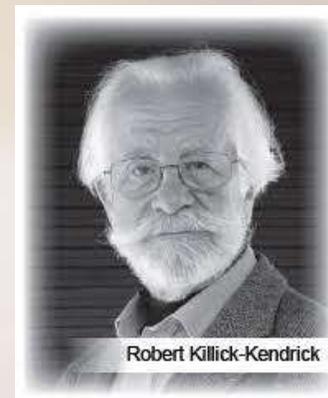
- The need for blood to give rise to a new sand fly generation
- Most sand fly species are exophagic (feed outside of dwellings)
- Although some are known to be endophagic and endophilic (feeding and resting in human and animal dwellings)
- Sand flies will take blood from the closest permissive available source
- Humans are generally accidental *Leishmania* hosts
- Sand flies are blood-pool feeders
- Through salivation, sand flies introduce several pharmacologically active molecules into the skin, to facilitate feeding



# Proven vectors

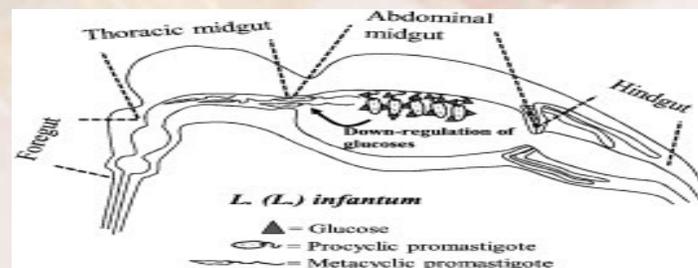
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- Among the 1000 sand fly species/subspecies validated/described, only one-tenth (10%) are proven or suspected vectors of *Leishmania* parasites
- Proven vectors (Killick-Kendrick and the WHO Expert Committee on the control of Leishmaniases) :
  - ✓ (i) They feed on humans (are anthropophilic),
  - ✓ (ii) They also feed on the relevant reservoir hosts in the case of zoonotic agents,
  - ✓ (iii) They are found in nature infected with the same parasites (*Leishmania* species) circulating in humans (from the same geographical area);
  - ✓ (iv) They support the complete development of the *Leishmania* parasites circulating in humans, including after the defecation of the bloodmeal remnants;
  - ✓ (v) They are able to transmit those parasites to susceptible hosts when they take a bloodmeal



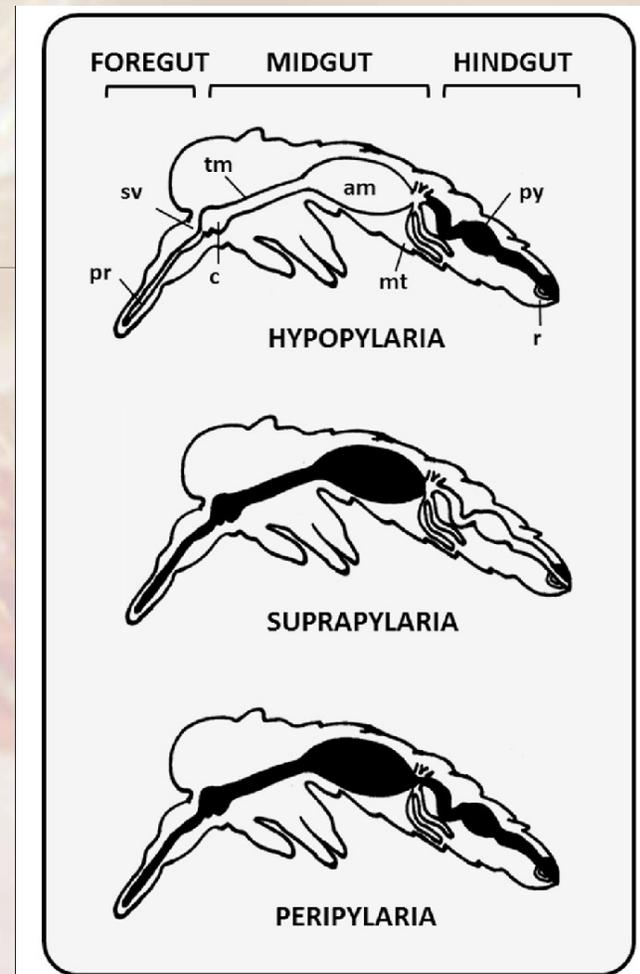
# Ligand–receptor interactions

- The attachment of parasites to the midgut :
  - ❖ The binding of *Leishmania* lipophosphoglycan (LPG) molecules to a specific sand fly midgut receptor, a galectin ( $\beta$ -galactoside binding family of lectins)
- Although LPG molecules are very abundant surface proteins, found in all *Leishmania* species, they are also polymorphic (particularly the 10–30 phosphoglycan repeating units) and different
- The attachment-detachment processes of parasites to the midgut dependent on the specificity of ligand–receptor interactions

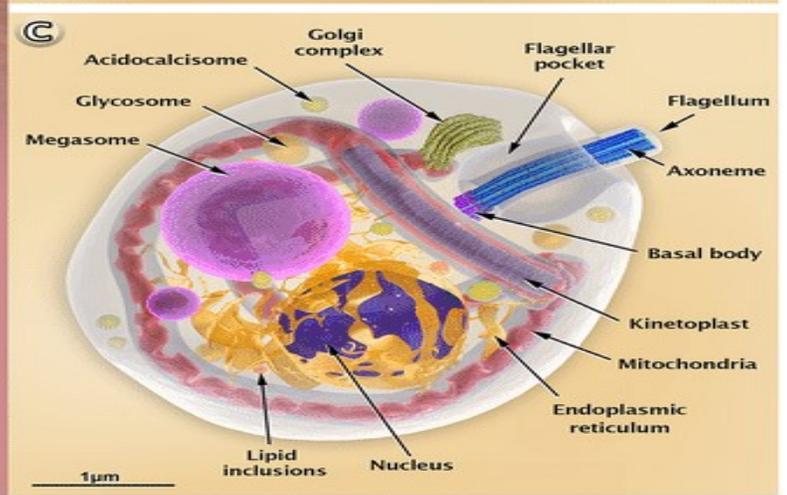
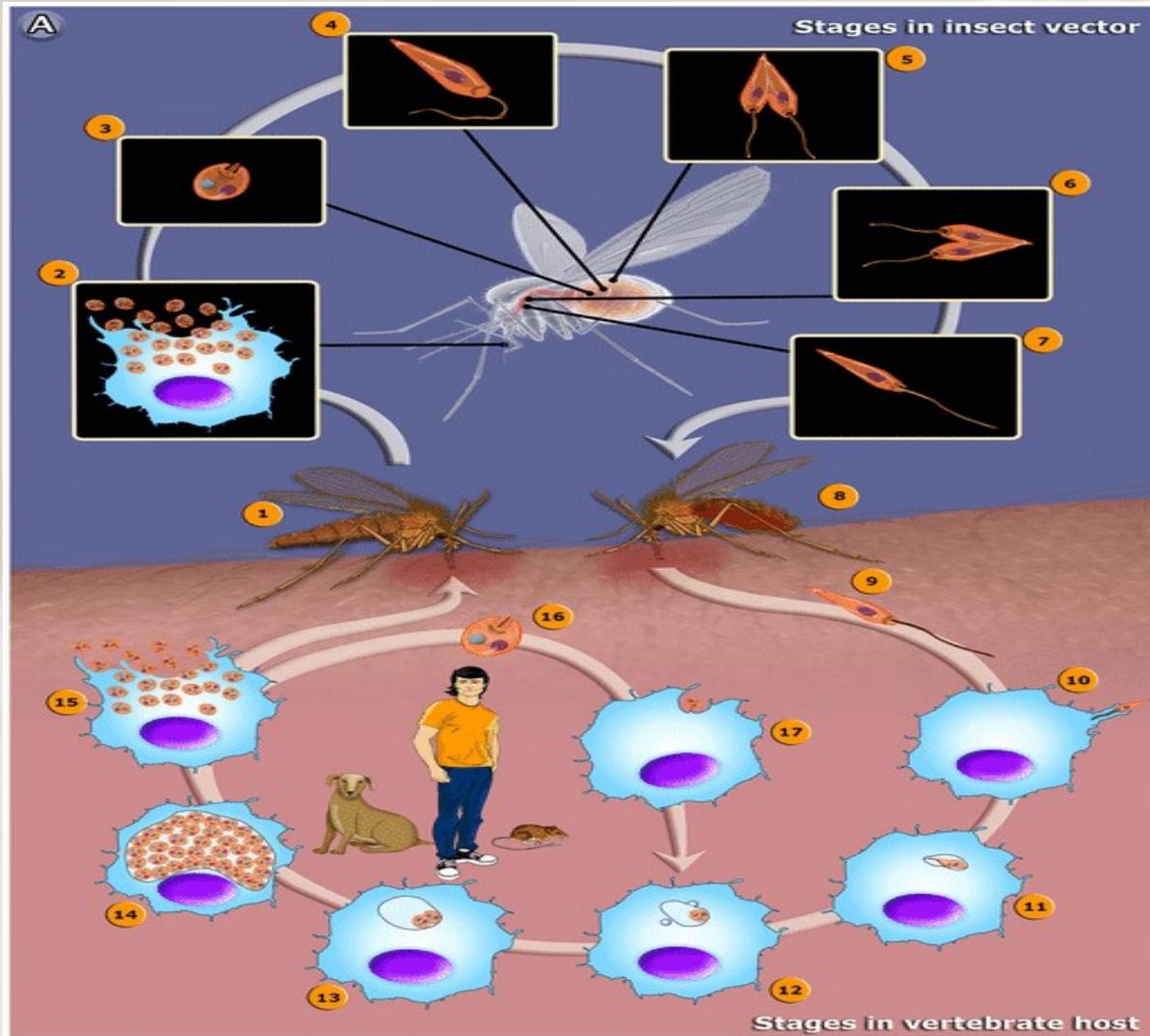


# Classification based on the growth of the parasite in the digestive system of the sand fly

- **Hypopylaria:** Lizard leishmaniasis, Transmission through ingestion
- **Suprasyplaria:** *L.mexicana*  
*L.donovani*  
*L.infantum*  
*L.tropica*  
*L.major*
- **Peripyplaria:** *L.brazilliensis*, New World  
*L.tarentolae*, Lizard leishmaniasis  
*L. adleri* , Lizard leishmaniasis



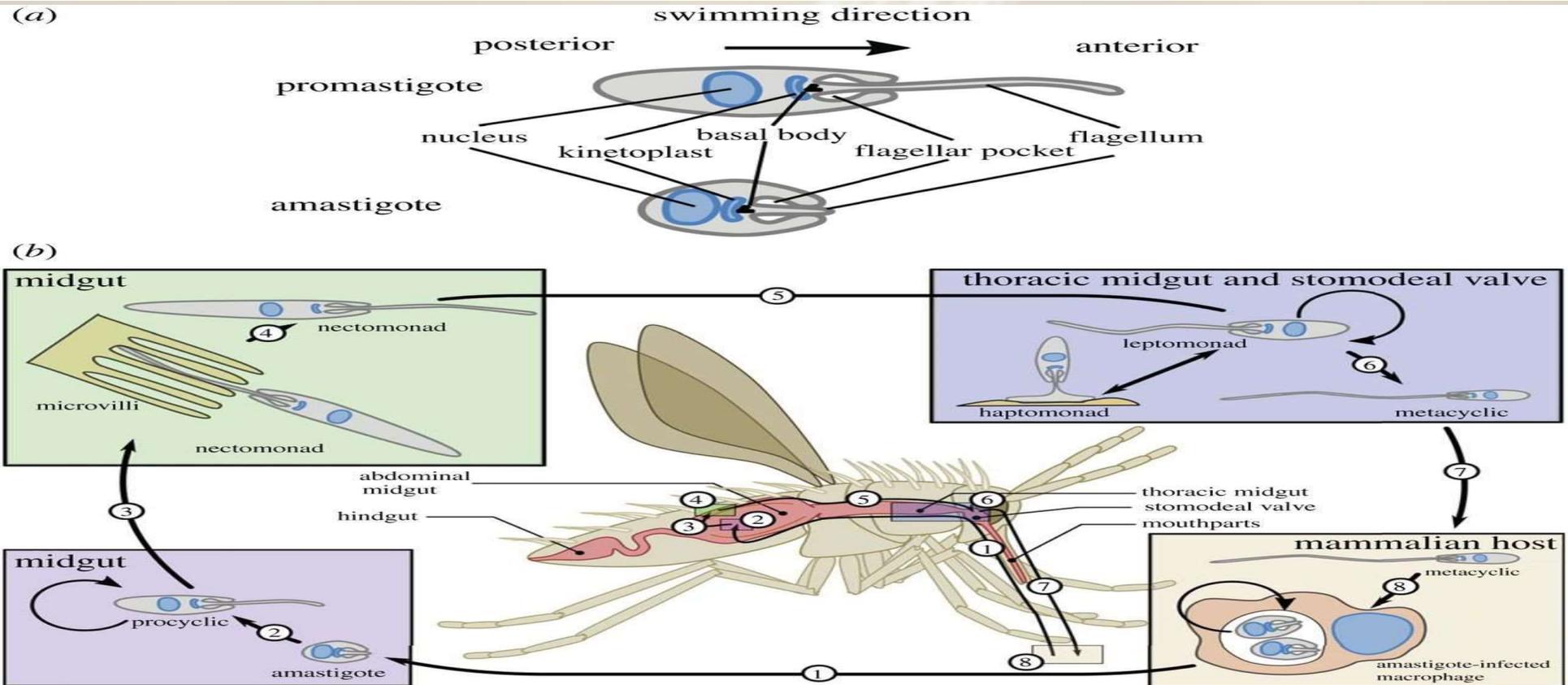
Diagrammatic representation of the three Leishmania sections proposed by Lainson & Shaw (1979).



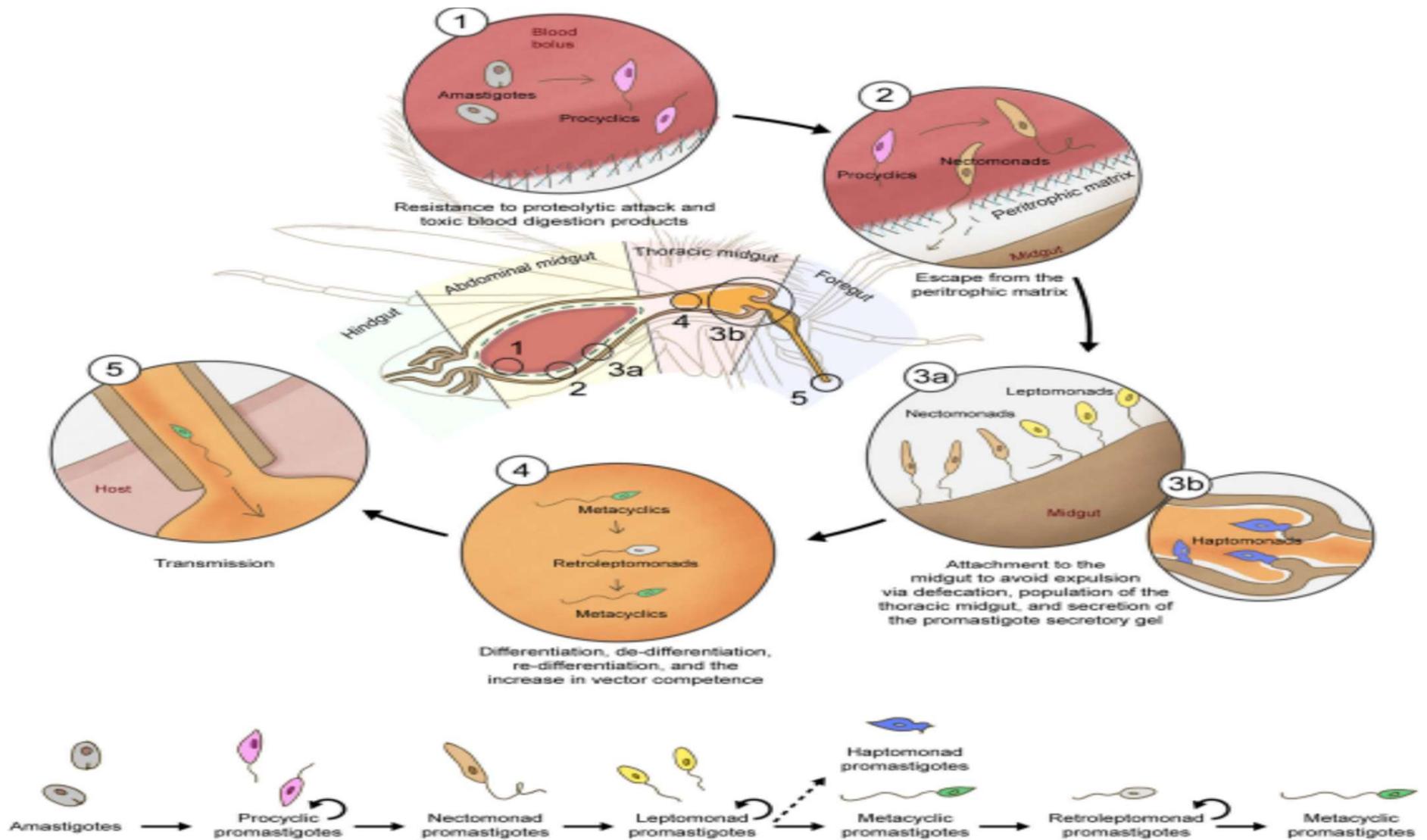


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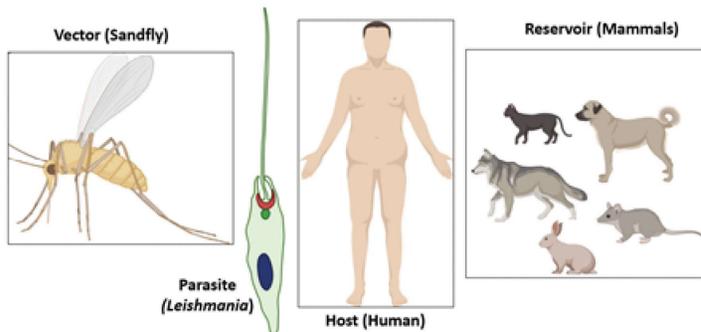
# Sand fly-Leishmania interactions toward the development of mature infections



Schematic of promastigote and amastigote morphologies and the *Leishmania* life cycle with the different cell types highlighted. (a) Promastigote and amastigote morphologies aligned along the posterior anterior axis with key structures in the cells indicated. (b) Cartoon of the current understanding of the *Leishmania* life cycle with critical events and different cell types highlighted. A sand fly takes a blood meal from an infected mammalian host and ingests a macrophage containing *Leishmania* amastigotes. Once in the sand fly midgut, the amastigotes differentiate into procyclic promastigotes. Next, the procyclic promastigotes become nectomonad promastigotes, which escape the peritrophic matrix and then attach to the microvilli in the midgut before moving to the thoracic midgut and stomodaeal valve where they differentiate into leptomonad promastigotes. Here, the leptomonad promastigotes differentiate into either haptomonad promastigotes which attach to the stomodaeal valve or metacyclic promastigotes that are the mammalian infective form, which are transmitted when the sand fly next takes a blood meal. Proliferative stages are indicated by a circular arrow.

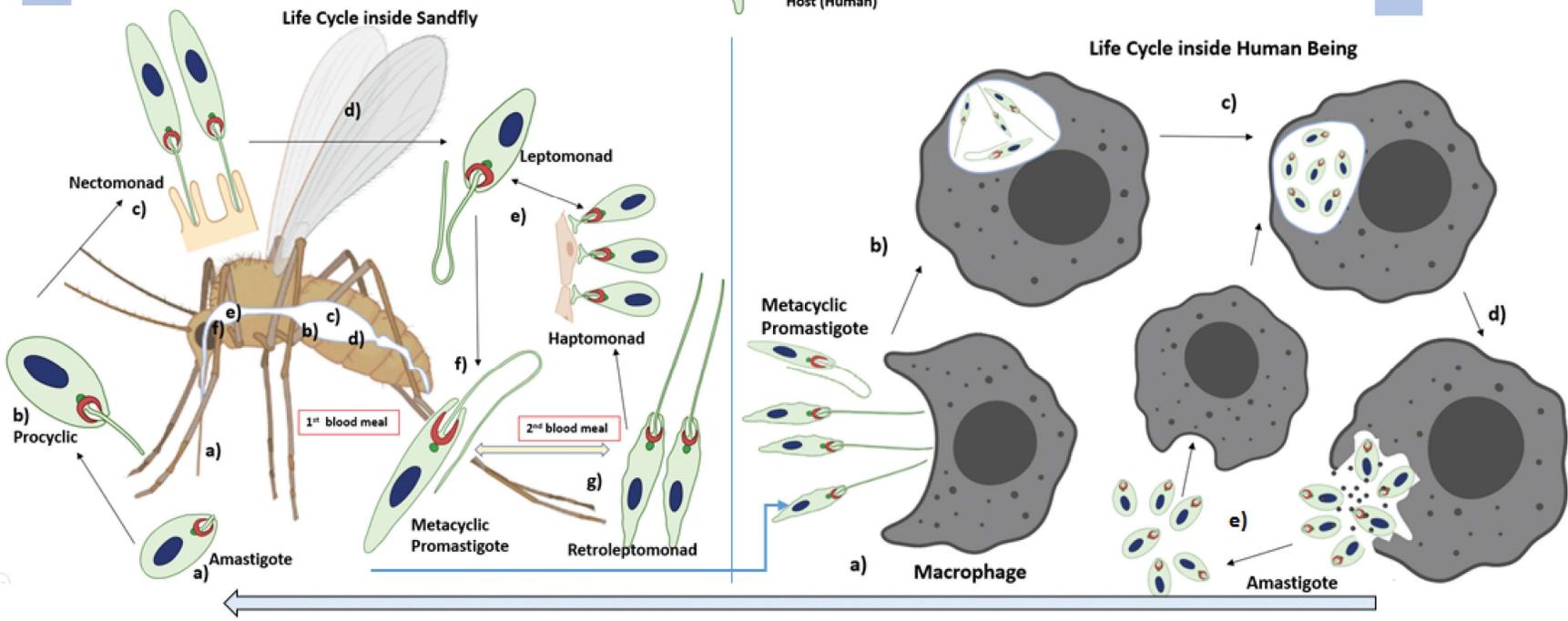


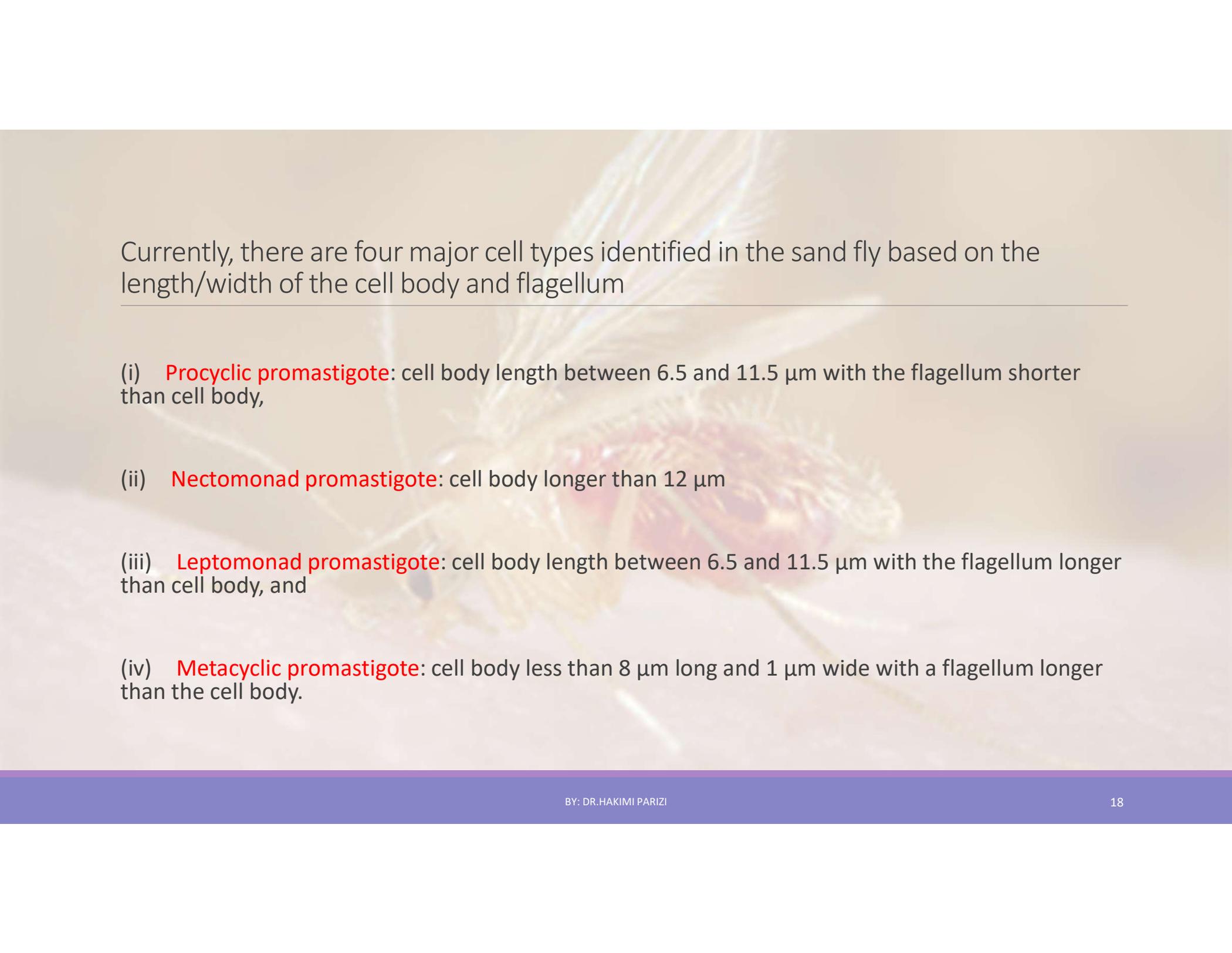
# LEISHMANIASIS



**A**

**B**





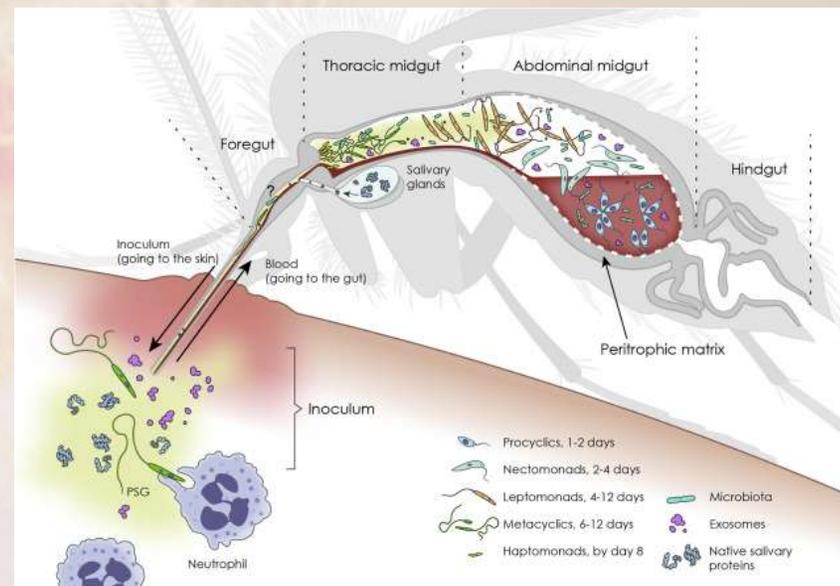
Currently, there are four major cell types identified in the sand fly based on the length/width of the cell body and flagellum

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- (i) **Procyclic promastigote**: cell body length between 6.5 and 11.5  $\mu\text{m}$  with the flagellum shorter than cell body,
- (ii) **Nectomonad promastigote**: cell body longer than 12  $\mu\text{m}$
- (iii) **Leptomonad promastigote**: cell body length between 6.5 and 11.5  $\mu\text{m}$  with the flagellum longer than cell body, and
- (iv) **Metacyclic promastigote**: cell body less than 8  $\mu\text{m}$  long and 1  $\mu\text{m}$  wide with a flagellum longer than the cell body.

# *Leishmania* transmission: the infectious inoculum

- The deposition of metacyclic parasites into the host's skin is dependent on their **regurgitation** by the sand fly vector
- Leptomonad promastigotes
  - PSG
  - Damage of the stomodeal valve
- Proteophosphoclycan
- Sand fly saliva
- Sand fly gut microbiota



# Gonotrophic cycle



# Vectors of leishmaniasis in Iran

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## • ACL

- *Phlebotomus sergenti*
- *Ph.papatasi*
- *Ph.caucasicus*

## • ZCL

- *Ph.papatasi*
- *Ph.caucasicus*
- *Ph.alexandri*
- *Ph.ansari*
- *Ph.salehi*

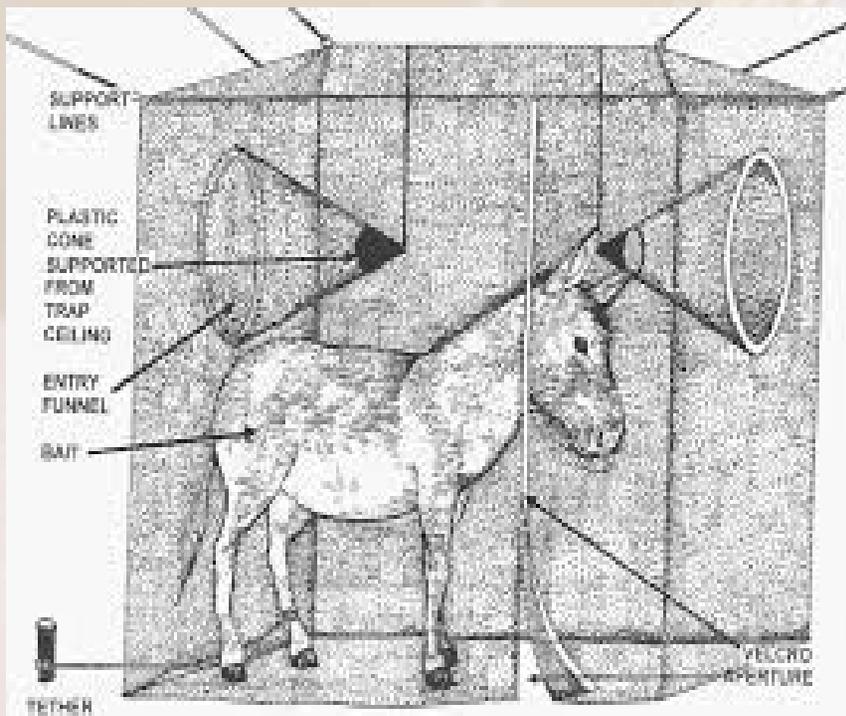
## • VL

- In Fars Province
  - *Ph.major*
  - *Ph.keshishiani*
  - *Ph.alexandri*
- In Ardabil Province
  - *Ph.perfiliewi*
  - *Ph.kandelakii*

# Methods of catching and studying sand flies



# Methods of catching and studying sand flies



# Microscope slides of Phlebotomine sandflies and identification



FIGURE 4: Specimens of *Lutzomyia longipalpis* 14 months after preparation using Kieffer glycerin gelatin. (A1) Female head showing cibarium (200X)

# Vector control for leishmaniasis

## ❖ Chemical control

- WHO-recommended insecticide-treated
- Indoor residual spraying for endophilic sandflies
- ULV
- Insecticide-impregnated bed nets (long-lasting)
- Repellents
- Insecticide-impregnated dog collars

## ❖ Environmental management

- local sanitation and improved housing

**Leishmaniasis**  
Information for internally displaced persons and refugees

**Source of infection**  
Vector-borne disease transmitted through the bites of infected female phlebotomine sandflies, which feed on blood to produce eggs.

**Type of exposure & prevention**  
It is caused by bite of an infected female sandfly (phlebotomine), a tiny 2-3 mm long insect vector. Internally displaced persons and refugees are at high risk of leishmaniasis because of increased chances of contact with sandflies. Control it by:

- Remaining vigilant of sandflies, especially when outdoors
- Keeping your home clean from waste and sewerage
- Covering windows to avoid sandflies from entering
- Avoiding sleeping outside or on ground
- Destroying sandfly breeding sites through use of insecticides
- Covering full body with clothing and using insecticide-treated nets to avoid sandfly bites

**Symptoms**  
Leishmaniasis has three forms: visceral (Kala-Azar, most serious form); cutaneous (most common); and mucocutaneous. Depending upon its type it can be fatal. Symptoms include:

**Visceral leishmaniasis**

- Irregular fever
- Anaemia
- Weight loss
- Spleen and liver enlargement
- Rash usually on face, upper arms, trunk and other parts of the body

**Cutaneous leishmaniasis**

- Ulcers on exposed parts of the body (face, arms and legs)
- Disfigured skin lesions after recovery

**Mucocutaneous leishmaniasis**

- Lesions in the mucous membranes (nose, throat or mouth)

**Actions to take in case of symptoms:**  
Treatment is complex and should be administered by highly experienced health personnel. See a doctor in case of symptoms.

World Health Organization  
www.who.int/emergencies

# Important components of planning and control operations

Evidence-based decision-making, which requires prior knowledge of :

- ✓ Local epidemiology
  - Ecology of area
  - Seasonality of transmission
  - Anthroponotic or zoonotic modes of transmission
- ✓ Vector bionomics
  - Namely species distribution
  - Ecology
  - Behavior such as endophilic, peridomestic or sylvatic
- ✓ Intra- and intersectoral collaboration involving collaboration among health and non-health sectors
- ✓ Advocacy for greater allocation of resources
- ✓ Social mobilization for community engagement



# Outstanding questions



- Most of what we know about *Leishmania* transmission by sand flies is based on laboratory evidence. Therefore, the translation of these notions to the natural context, or, in other words, their validation is still needed.
- Can we modulate immunity in sand flies to make them refractory to *Leishmania* parasites and use this as a vector-based strategy for the control of leishmaniasis?
- What extent do vector-derived factors impact host immunity and how can we overcome such responses in a significant fashion?
- Most laboratories interested in leishmaniasis do not have access to sand flies. Therefore, most of the information generated may not be translatable to the natural context.

# References

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- Cecílio P, Cordeiro-da-Silva A, Oliveira F. Sand flies: Basic information on the vectors of leishmaniasis and their interactions with *Leishmania* parasites. Communications biology (2022) 5:305, <https://doi.org/10.1038/s42003-022-03240-z> [www.nature.com/commsbio](http://www.nature.com/commsbio)
- Rassi Y& Hanafi AA. Phlebotomine Sand-flies, vectors of Leishmaniasis: morphology, biology , ecology.2006. Tehran: noavaran elm.



Thank you for your attention