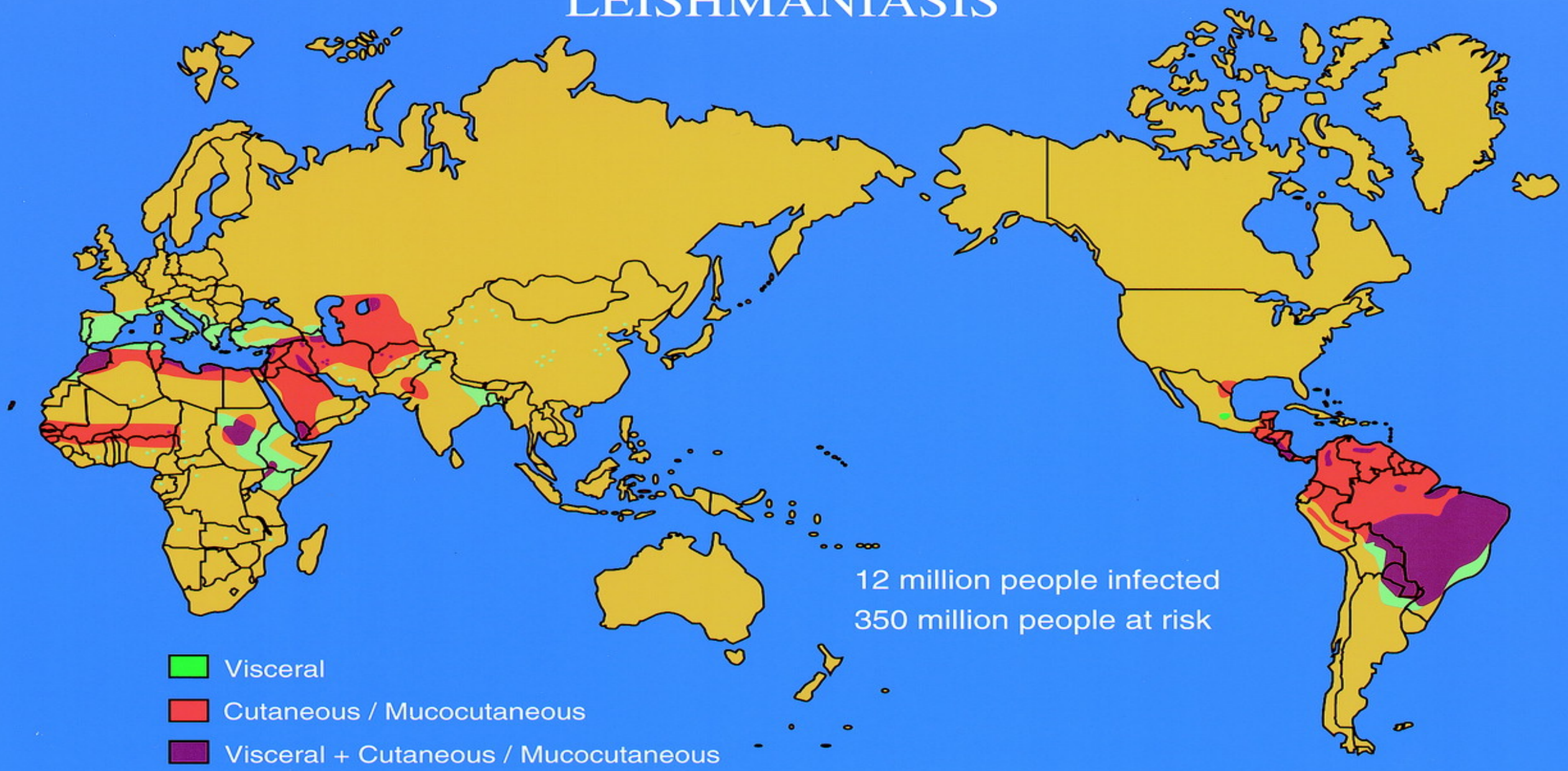
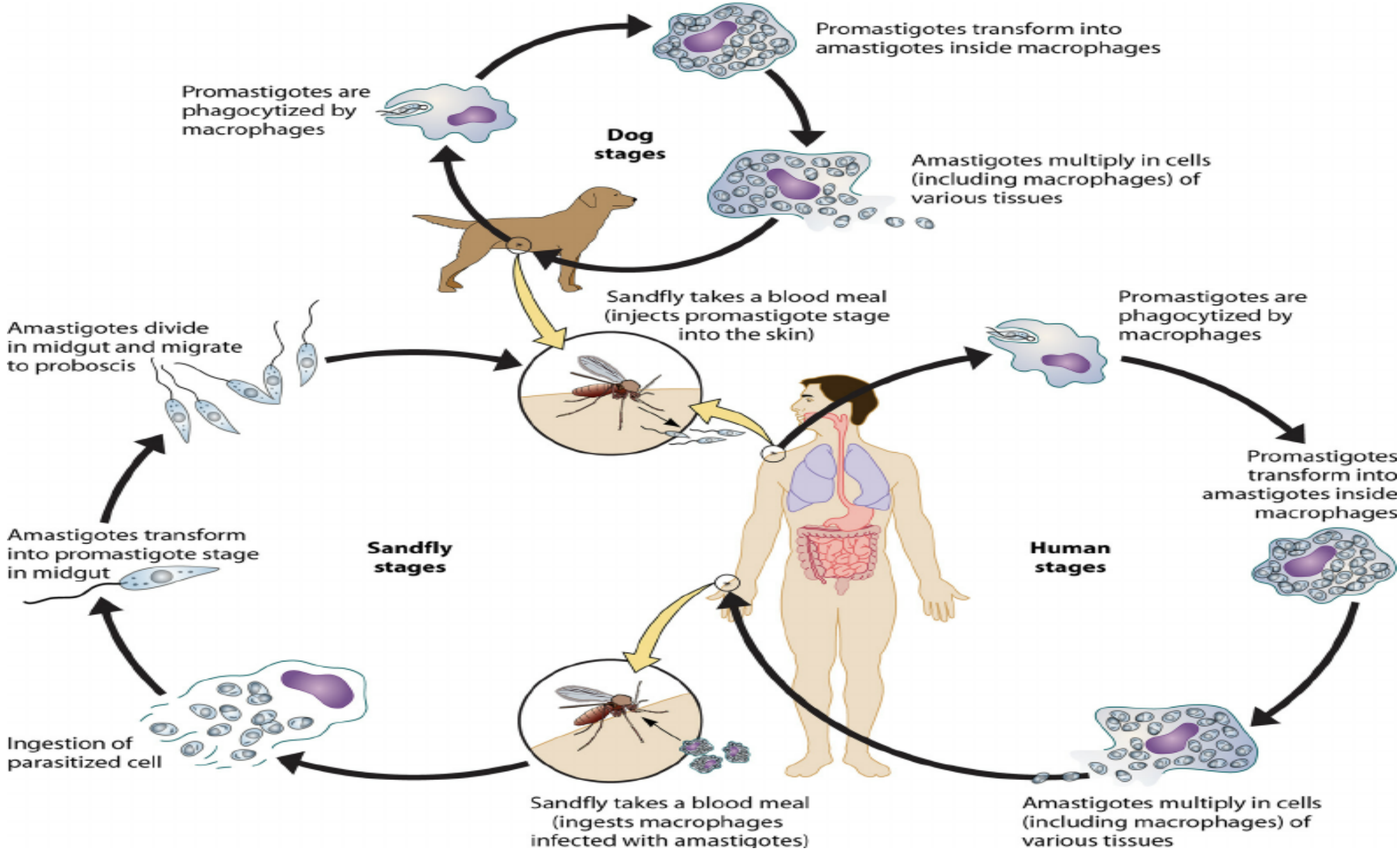
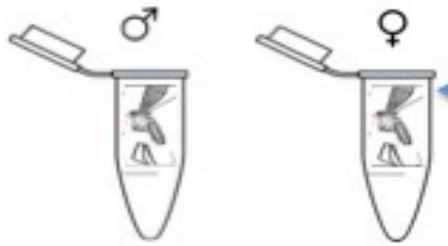
Identification of vector blood meal preferences

LEISHMANIASIS




Promastigotes transform into amastigotes inside macrophages
Promastigotes are phagocytized by macrophages
Dog stages
Amastigotes multiply in cells (including macrophages) of various tissues
Sandfly takes a blood meal (injects promastigote stage into the skin)
Promastigotes are phagocytized by macrophages
Amastigotes divide in midgut and migrate to proboscis
Promastigotes transform into amastigotes inside macrophages
Amastigotes transform into promastigote stage in midgut
Sandfly stages
Human stages
Ingestion of parasitized cell
Sandfly takes a blood meal (ingests macrophages infected with amastigotes)
Amastigotes multiply in cells (including macrophages) of various tissues



The remainder of the sand fly body preserved in 97% ethanol

♀ **DNA extraction**

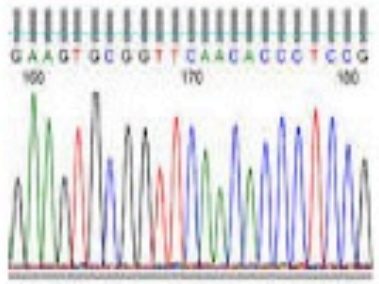
Collected sand flies

Stereoscopic dissection

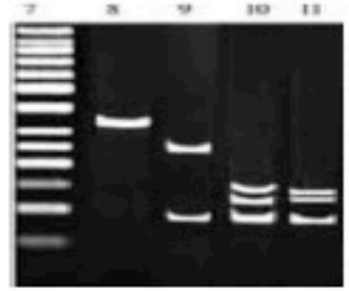
Taxonomical identification

Using head and the last 3 segments of the abdomen of ♀ and ♂

Blood meal identification
using mt DNA Cyt b-PCR



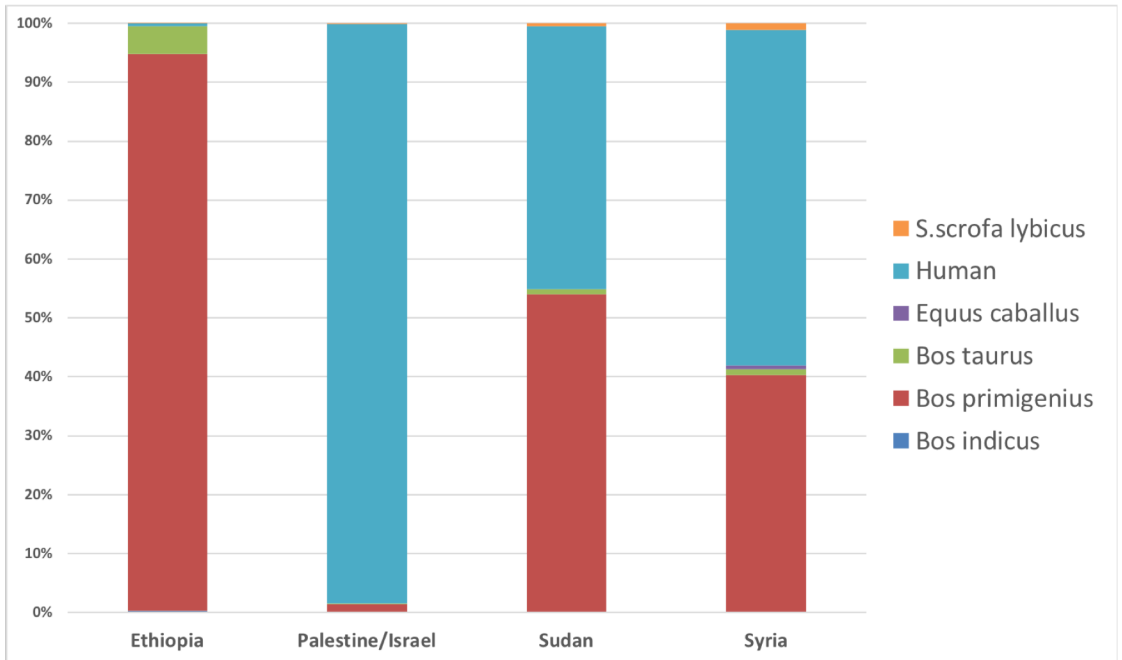
Lehshmania detection
using ITS1-PCR- RFLP

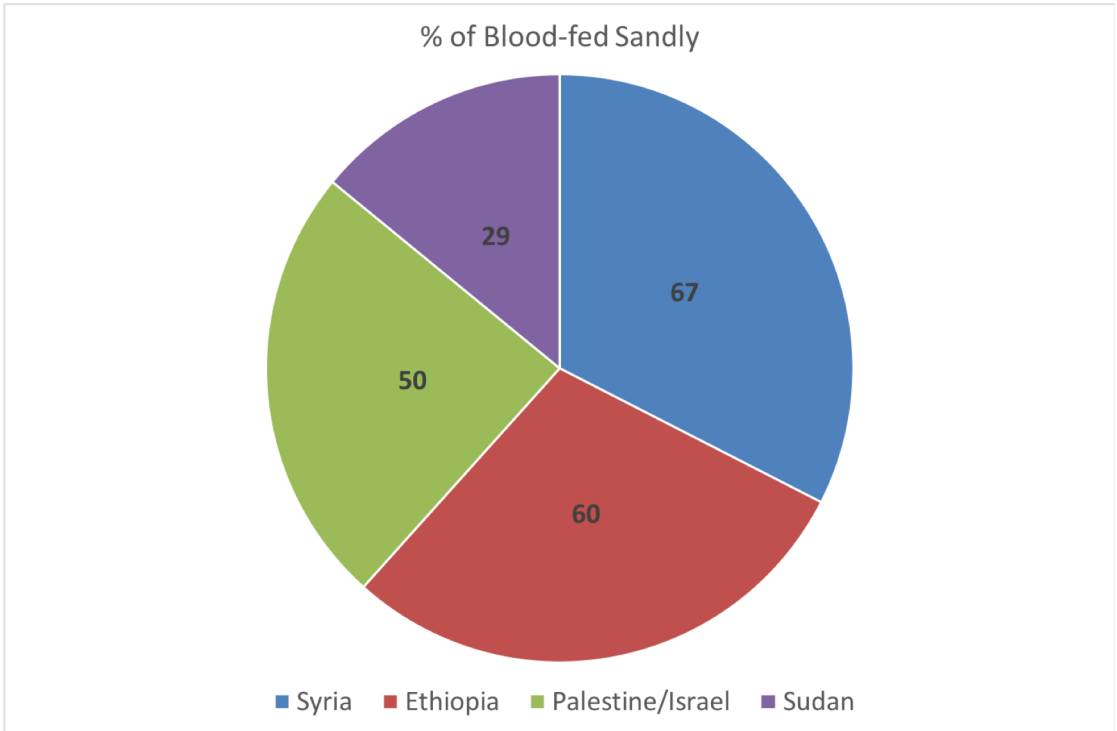


Host preferences

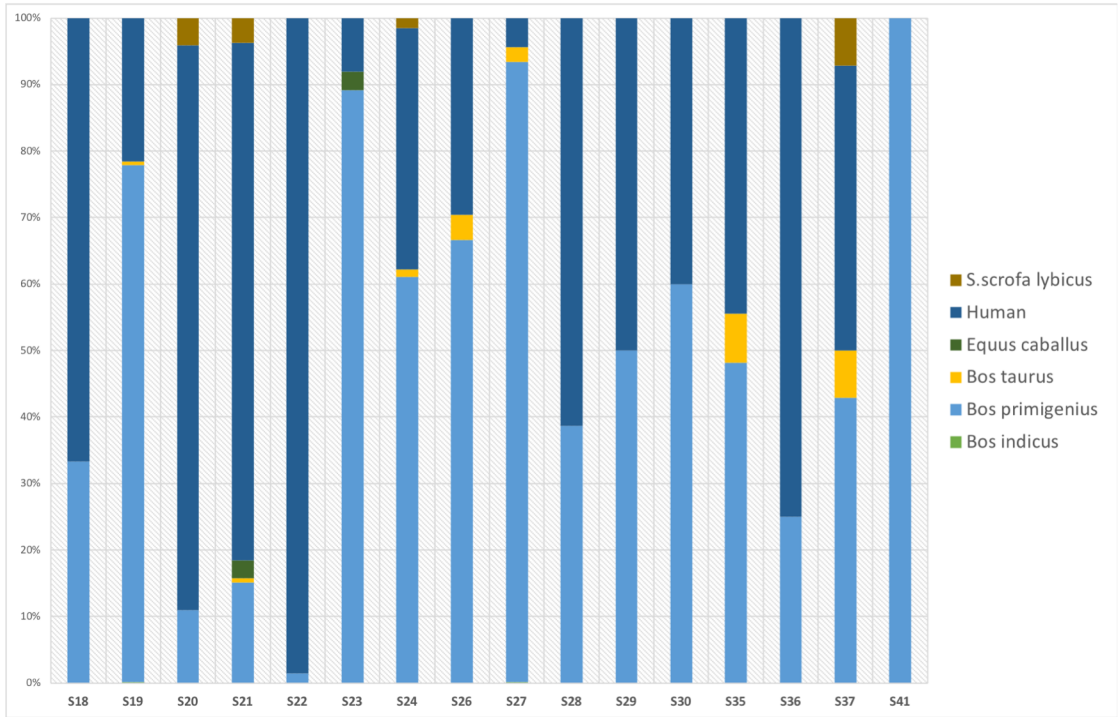
- Syria
- Ethiopia
- Sudan
- Palestine/Israel

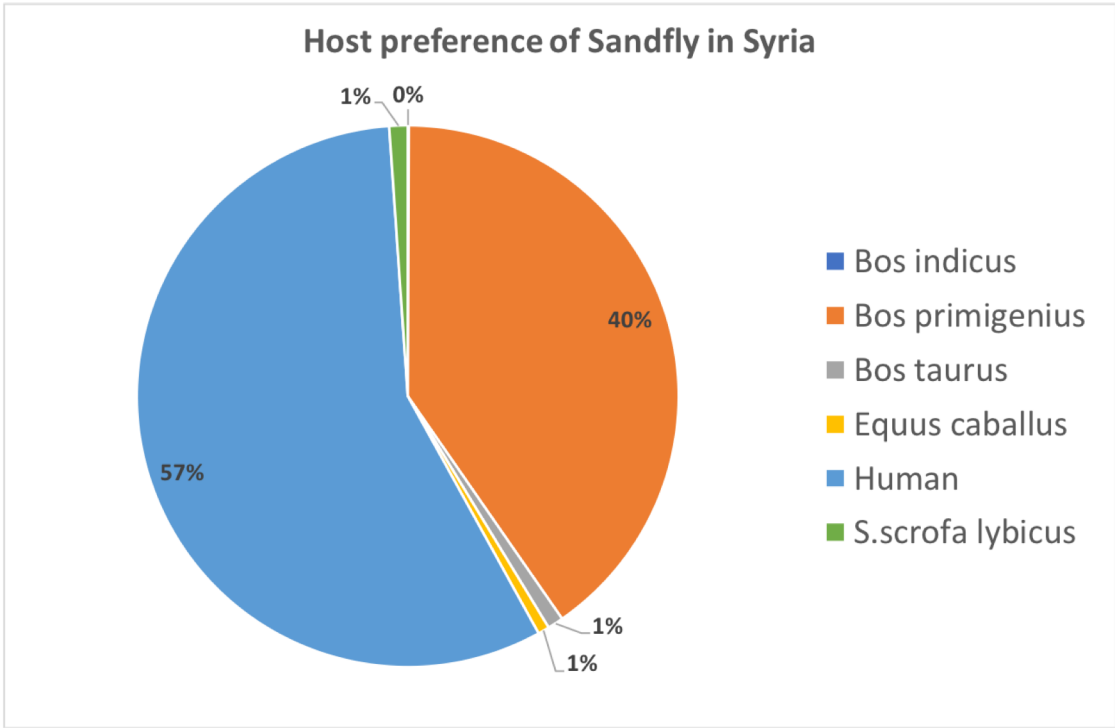
All Countries



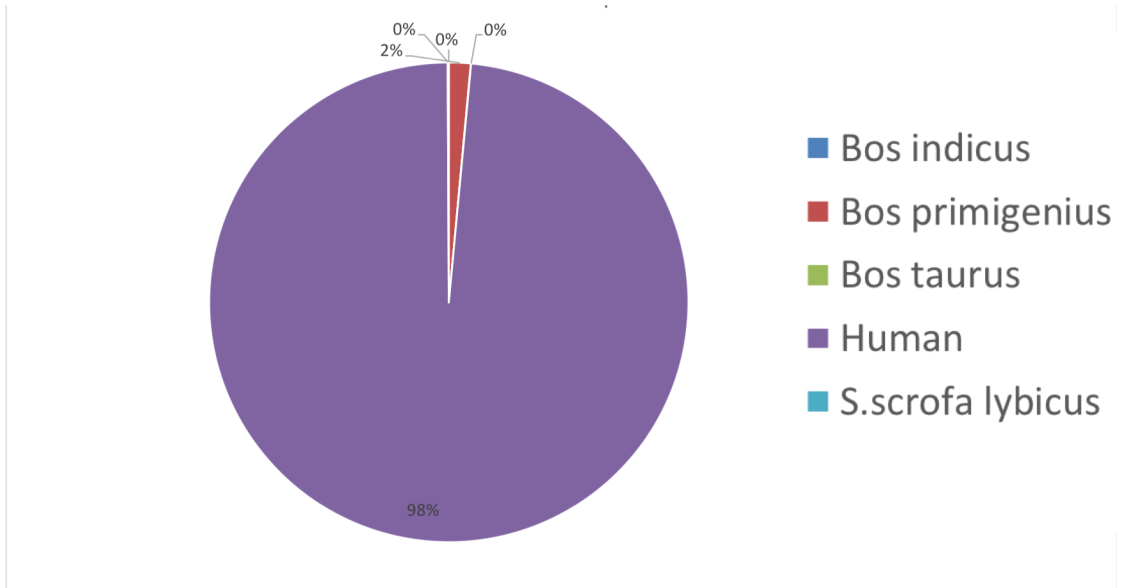


Syria

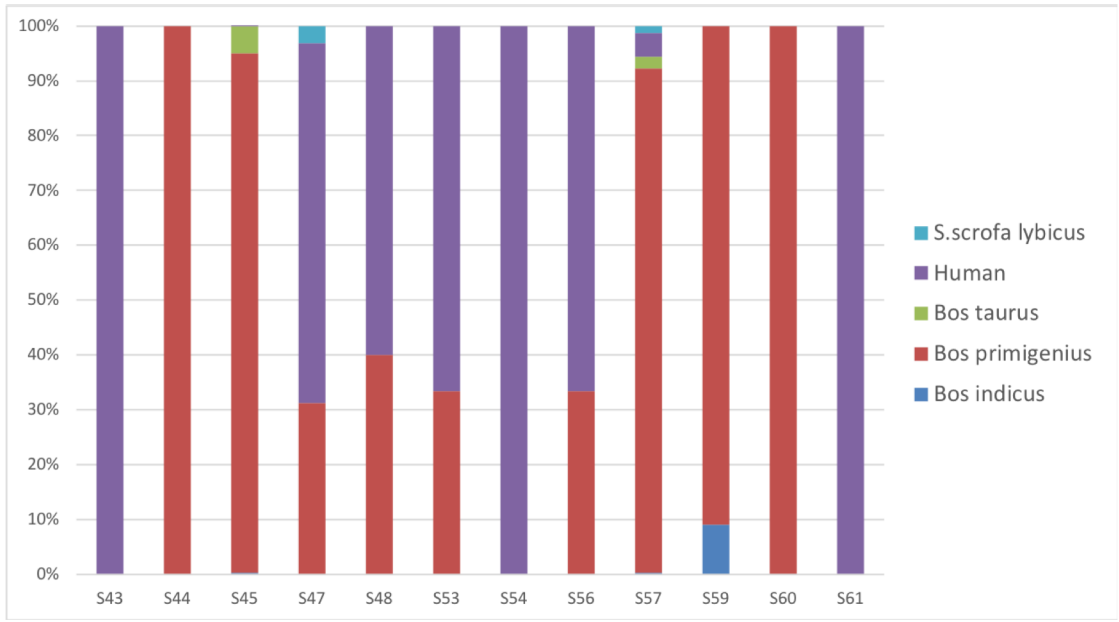

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
S18
S19
S20
S21
S22
S23
S24
S26
S27
S28
S29
S30
S35
S36
S37
S41
S.scrofa lybicus
Human
Equus caballus
Bos taurus
Bos primigenius
Bos indicus

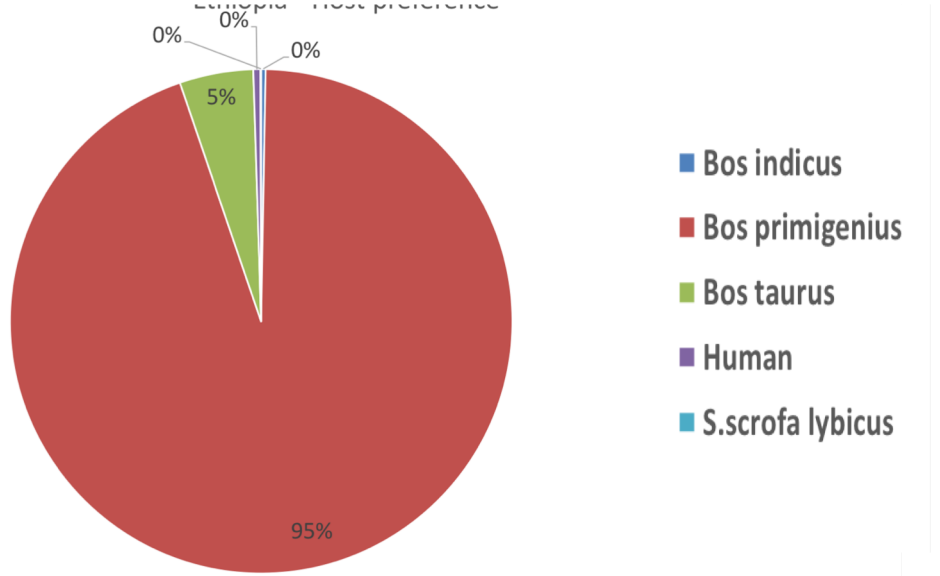

Host preference of Sandfly in Syria
1%
0%
40%
57%
1%
1%
Bos indicus
Bos primigenius
Bos taurus
Equus caballus
Human
S.scrofa lybicus

Palestine/Israel

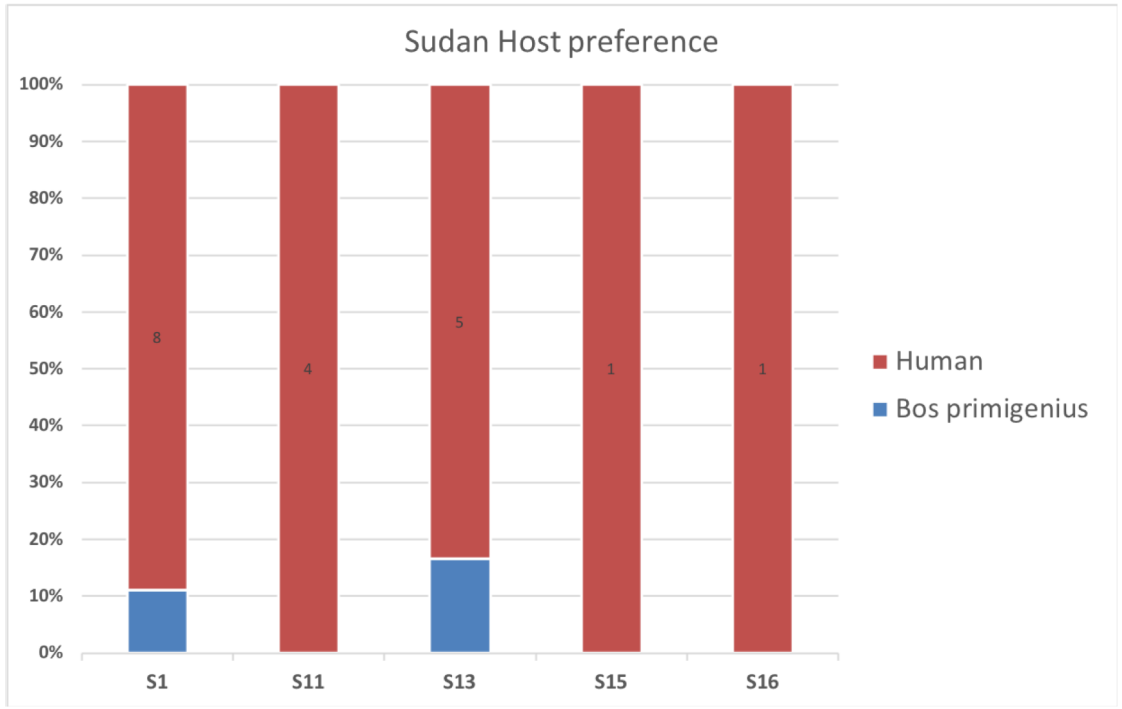


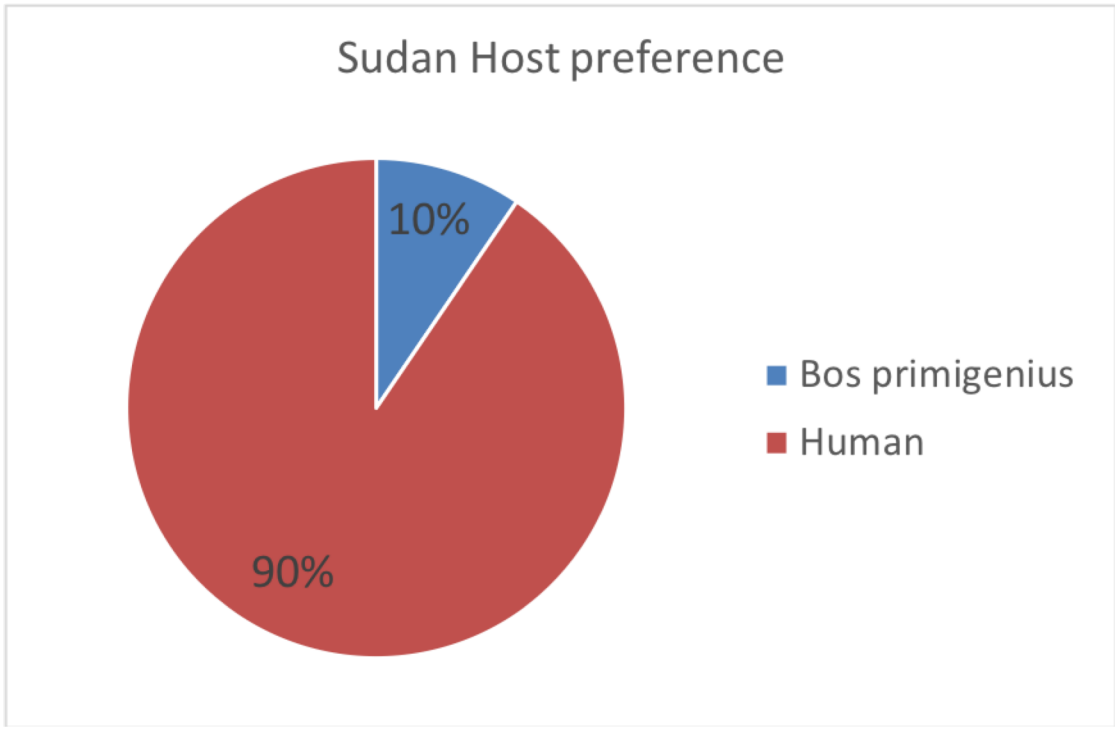
Ethiopia


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
S43
S44
S45
S47
S48
S53
S54
S56
S57
S59
S60
S61
S.scrofa lybicus
Human
Bos taurus
Bos primigenius
Bos indicus


0%
0%
0%
5%
95%
Bos indicus
Bos primigenius
Bos taurus
Human
S.scrofa lybicus

Sudan


Sudan Host preference
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
8
4
5
1
1
S1
S11
S13
S15
S16
Human
Bos primigenius


Sudan Host preference
10%
90%
Bos primigenius
Human

❖ **Epidemiology using NGS what does it tell you regarding the transmission of leishmania in different areas. Compare Syria/Israel, Ethiopia/Sudan. Which geographic areas do you expect to be more similar or different.**

- **Transmission cycle:**
  - **Syria = close to Zoonotic**
  - **Israel/Palestine = close to Anthroponotic**
  - **Sudan = close to Anthroponotic**
  - **Ethiopia = close to Zoonotic**

- **Cytochrome b – What could be one reason for the failure in the blood meal analysis and sequences = NNNNN :**
  - Failure in DNA extraction
  - Failure in DNA Purification

- **How else can you use NGS:**
  - Leishmania and Sandfly species
  - microbiome

- **Why do I see non-Leishmania kinetoplastida in NGS data? Why are there multiple mammals in cytochrome b.**
  - Similarity index to other non leishmania spp.
  - Sandfly can feed on multiple hosts and *cyt b* is very conservative gene.

❖ **Advantages of kDNA PCR (classical PCR) over ITS-1 and cytochrome b PCR systems.**

Importance and need of qPCR-kDNA system.

- kDNA is more sensitive than ITS1 and cyt b.
- kDNA present in higher copy number than ITS! And cyt b

❖ **Disadvantages of kDNA PCR (classical PCR) over ITS-1 and cytochrome b PCR systems.**

- Less specific

❖ **What factors can affect PCR sensitivity and specificity?**

- Quality and purity of DNA (sensitivity)
- Primer and gene of interest (specificity)

Thank You