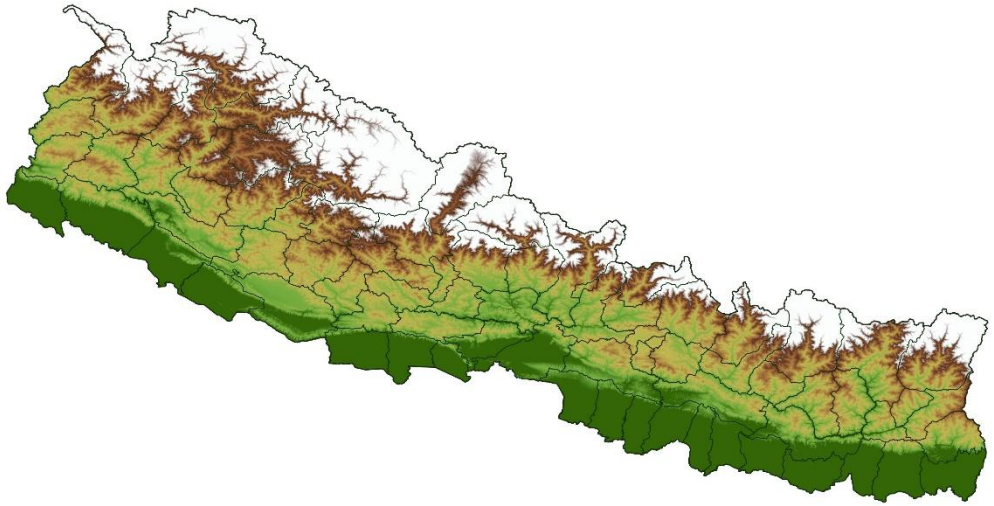
Mapping the distribution of phlebotomine sand fly species with emphasis on *Leishmania* vectors in Nepal and exploring the potential of DNA barcoding for their identification



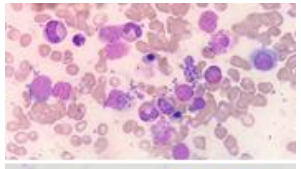
Lalita Roy, MSc, PhD

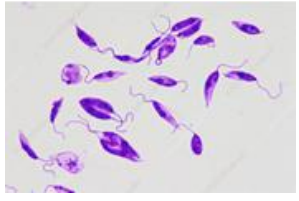
Medical Entomologist

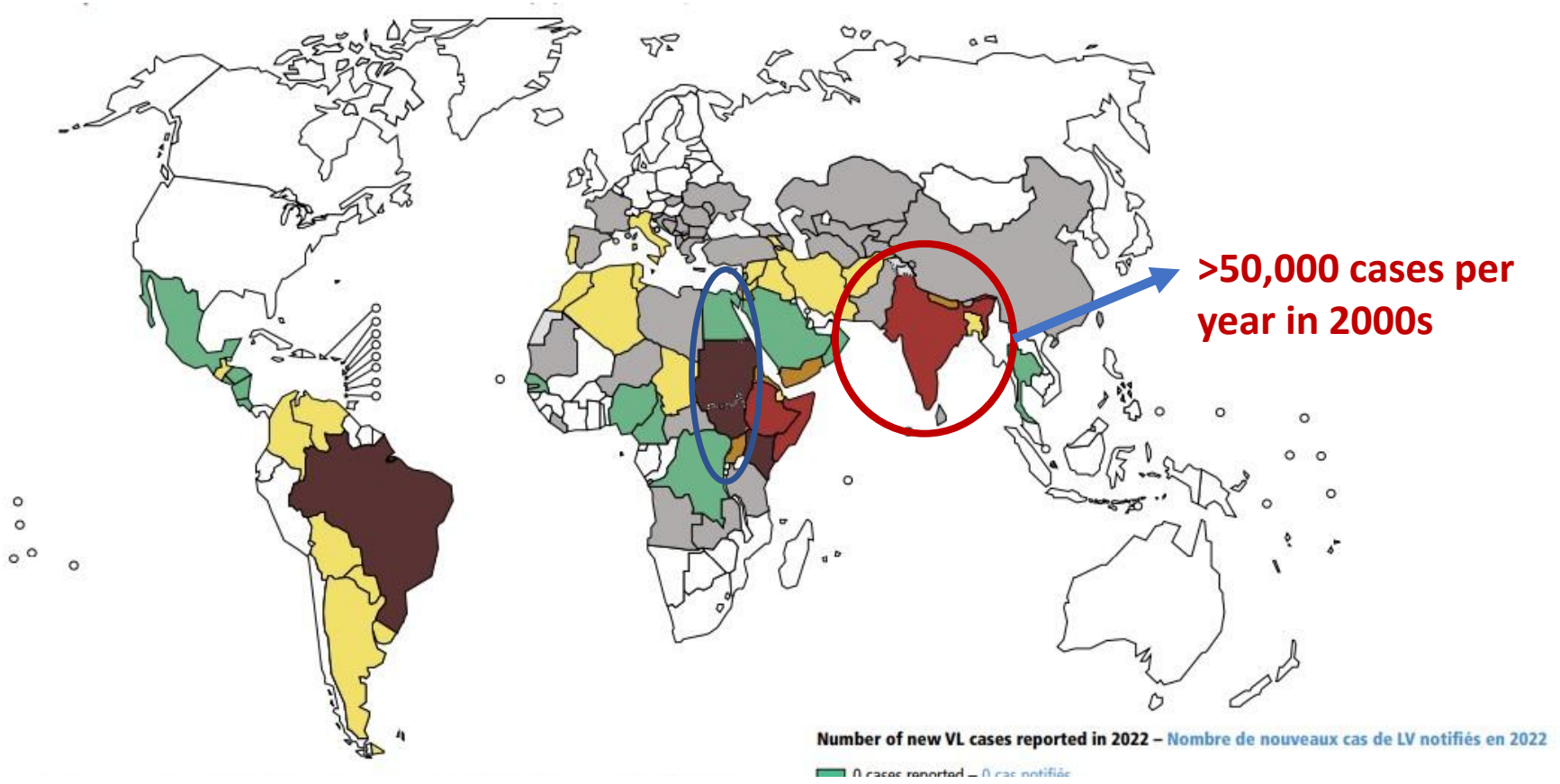
B.P. Koirala Institute of Health Sciences, Dharan Nepal

Background: Visceral Leishmaniasis

- Visceral leishmaniasis → Kala-azar in Southeast Asia
- Protozoan parasite → *Leishmania donovani*
- Vector → *Phlebotomus argentipes*







Background: Visceral Leishmaniasis

- **Elimination campaign (VL elimination programme)** started in 2005 as a joint venture of India, Bangladesh and Nepal, and WHO
  - Included Bhutan and Thailand in 2014
- **Target:** reduce annual incidence of VL to less than 1 per 10,000 population at district level in Nepal and sub-district level in India and Bangladesh.

Health Ministers signing memorandum of understanding

Background: Visceral Leishmaniasis

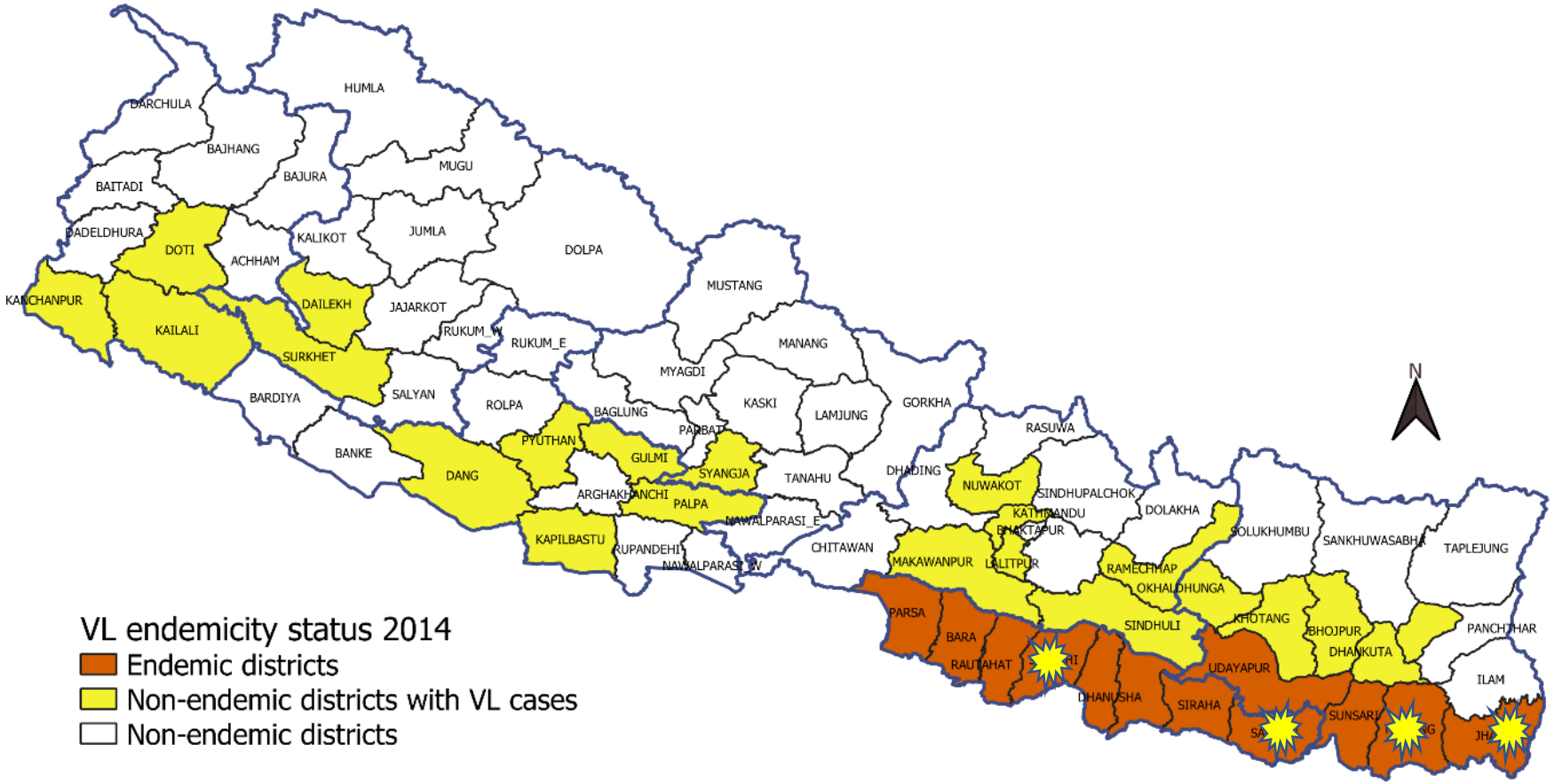
- **Strategies of VL elimination programme**

  - Early diagnosis and complete treatment

  - Vector control through indoor residual spraying

Background: Visceral Leishmaniasis

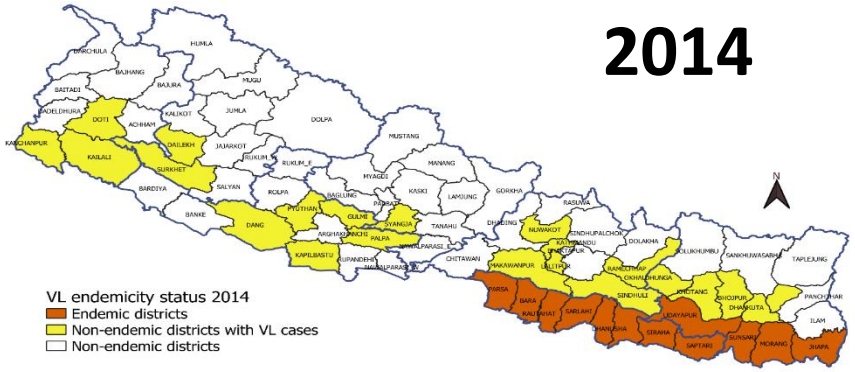
- **Trends in endemicity status of VL since 2014 to 2024**
  - **Resurgence** or **outbreaks**



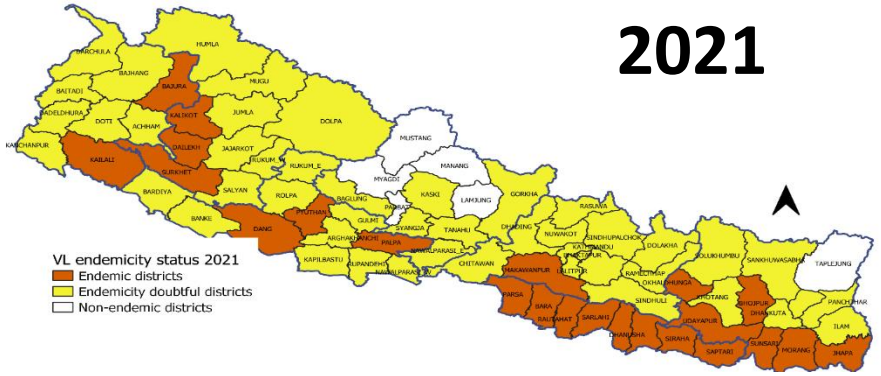
Introduction: VL in Nepal

- **Trends in endemicity status of VL since 2014 to 2024**
  - **Wide geographical distribution of the cases**

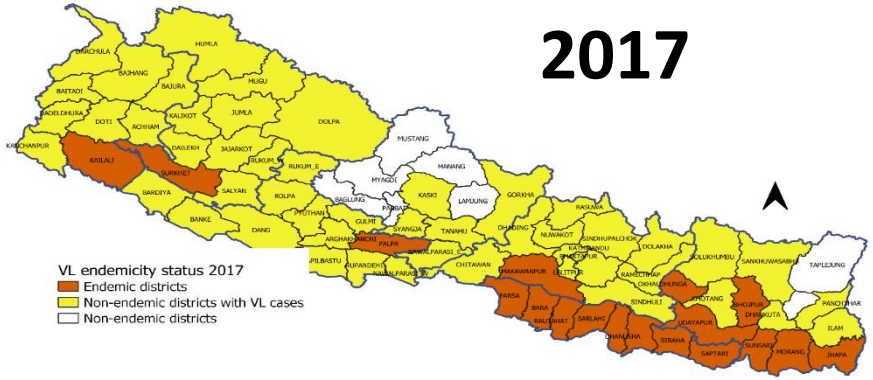
2014



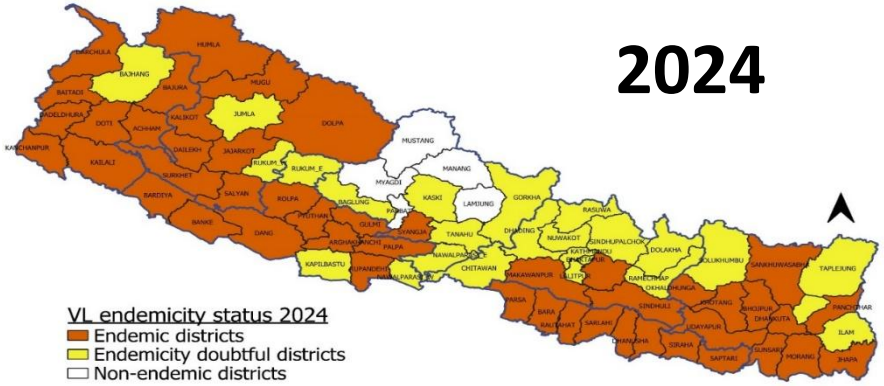
2021



2017



2024



Rationale

- **Knowledge gaps**

  - **Resurgence** or **outbreaks** in some of the pocket areas in classical endemic districts

  - **Changing epidemiology** - increasingly wide geographical distribution of VL cases in naïve areas including hilly & mountainous region – **entomological evidence of potential local transmission**

- **Monitoring the circulating vectors** in broader areas – essential for planning and implementation of tailored vector control interventions

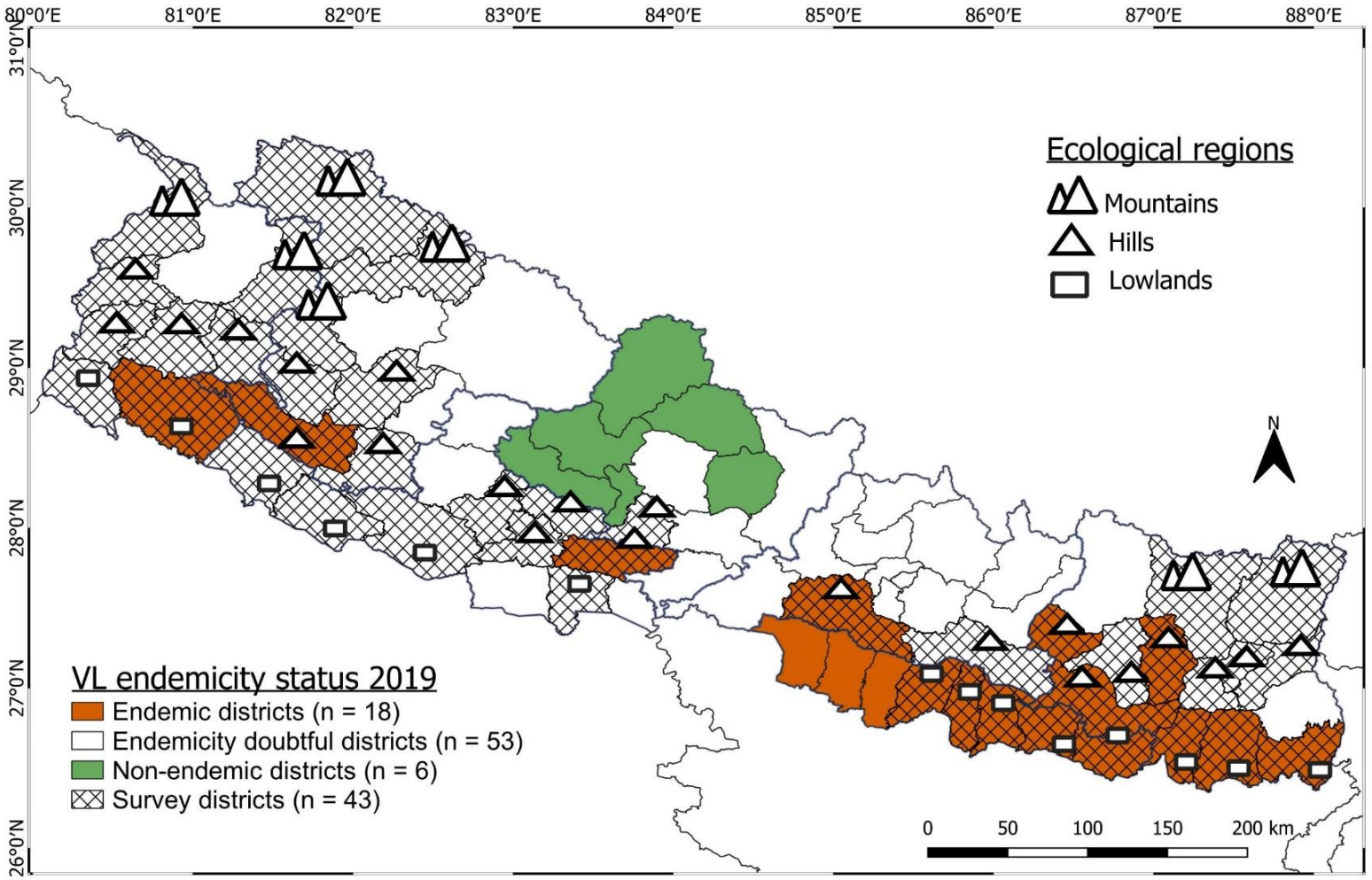
Objectives

- **Main Objective**
  - Assess the status of vector and other Phlebotomine sand flies in areas with VL cases
  - Assess and validate DNA barcoding method as complimentary method for sand fly species identification

Methodology (I)

Methodology (I)

- **Selection of districts:**
  - number of VL cases reported during each of the previous two years
  - Accessibility
- **Number of survey districts:**
  - 43
- **Duration**
  - 2019 - 2022



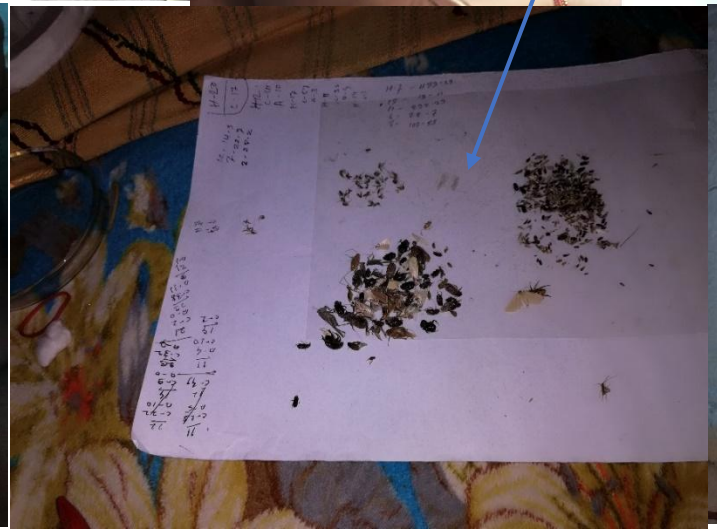
Methodology (I)

Sandfly collection

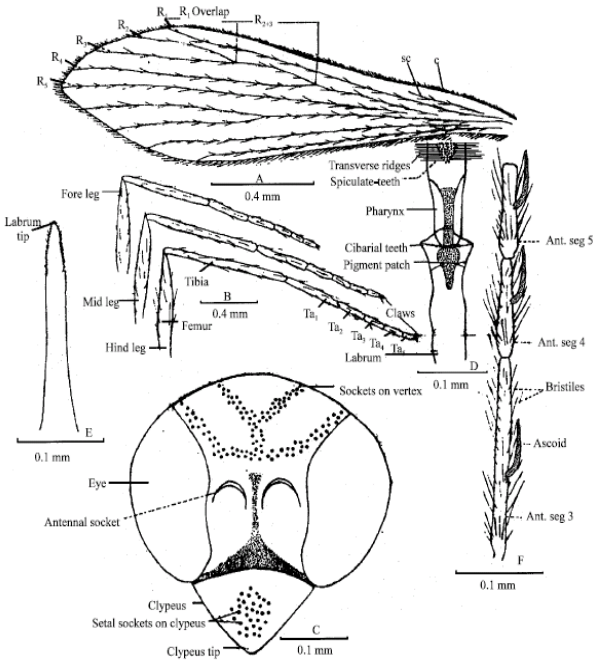
- Sites
  - 6 hhs/clusters
  - households and/or cattle sheds or
  - mixed dwellings

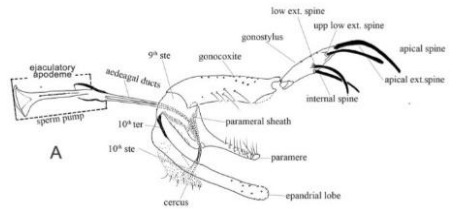
- CDC light traps and mouth aspiration
  - Two consecutive nights

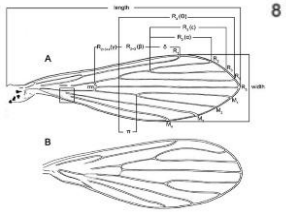
Methodology (I)

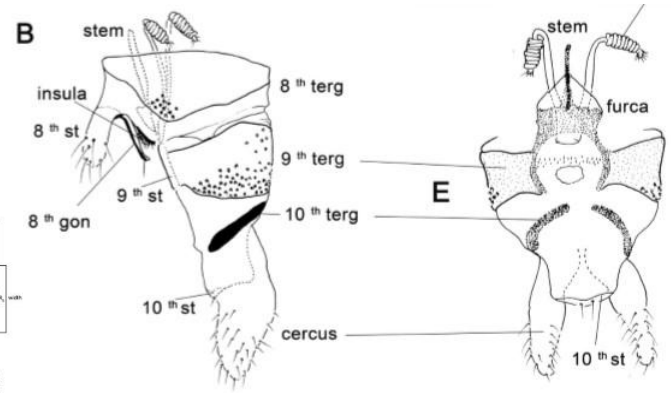


Methodology (I)



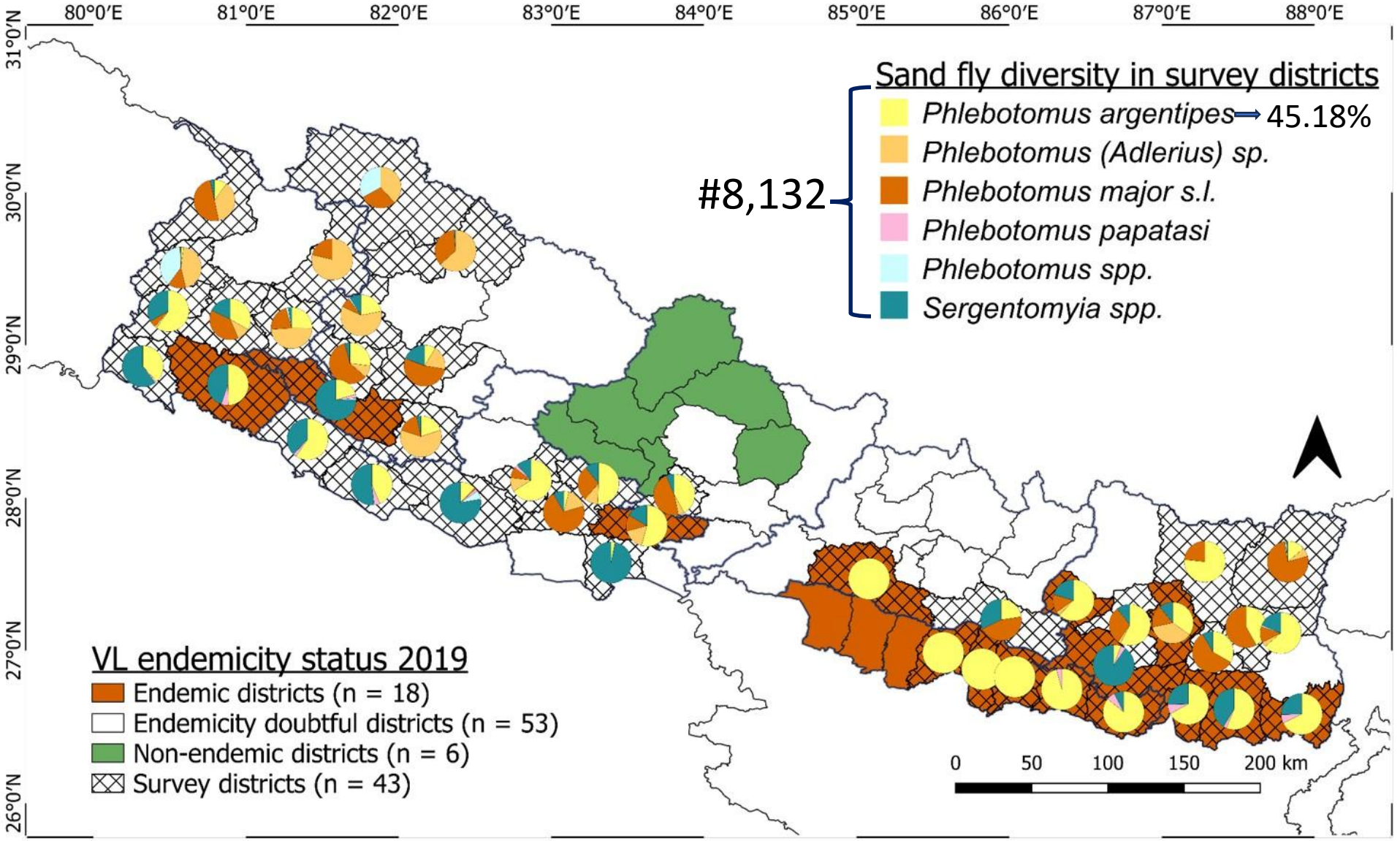






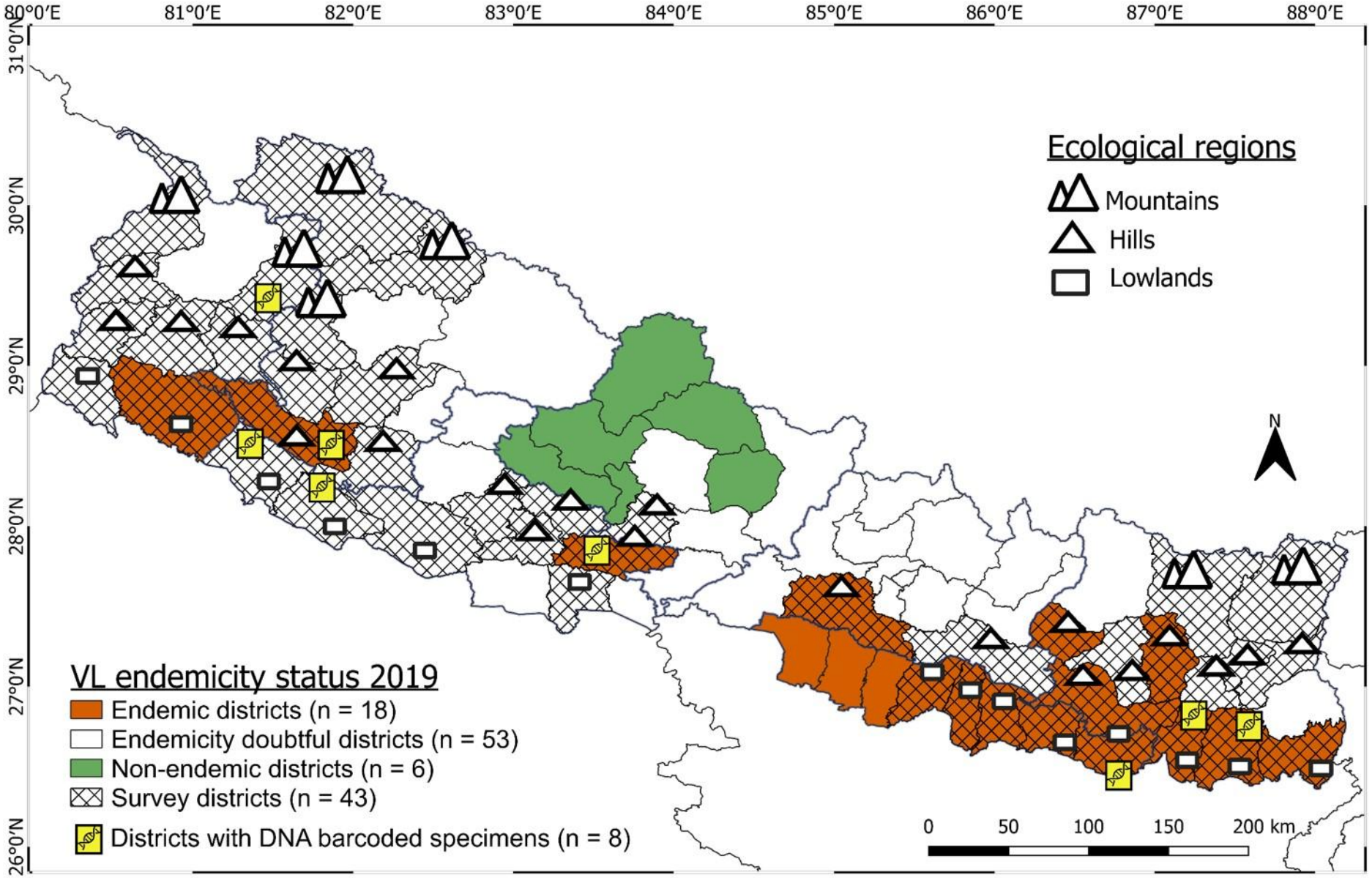
Morphological Identification:- Regional Key (Kalra and Bang, 1988; Lewis DJ, 1978)

Results (I)


80°0′E
81°0′E
82°0′E
83°0′E
84°0′E
85°0′E
86°0′E
87°0′E
88°0′E
31°0′N
30°0′N
29°0′N
28°0′N
27°0′N
26°0′N
Sand fly diversity in survey districts
#8,132
Phlebotomus argentipes
45.18%
Phlebotomus (Adlerius) sp.
Phlebotomus major s.l.
Phlebotomus papatasi
Phlebotomus spp.
Sergentomyia spp.
VL endemicity status 2019
Endemic districts (n = 18)
Endemicity doubtful districts (n = 53)
Non-endemic districts (n = 6)
Survey districts (n = 43)
0
50
100
150
200 km

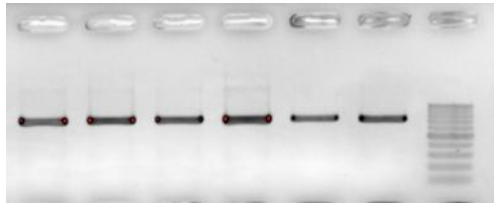
Methodology (II)

Methodology (II)



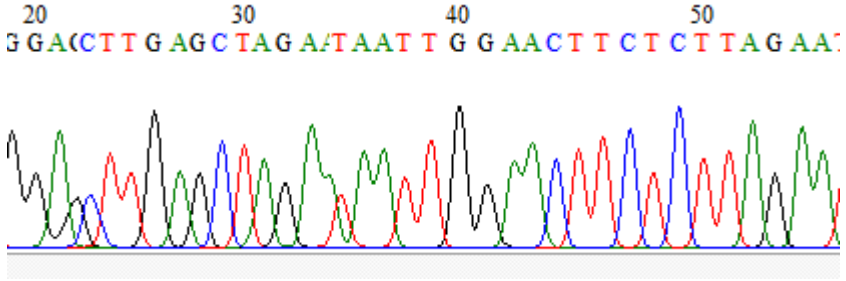
Methodology (II)

Phlebotomine sand flies = **315**



PCR assay targeting mitochondrial

***COI* gene (~650 bp)**



Sequencing

Results (II)

- The species identification success rate of generated *COI* barcode sequences - 97% (305/315).

- 101 haplotypes (639bp) representing 12 taxa
- Haplotype diversity – 0.933
- Nucleotide diversity – 0.078

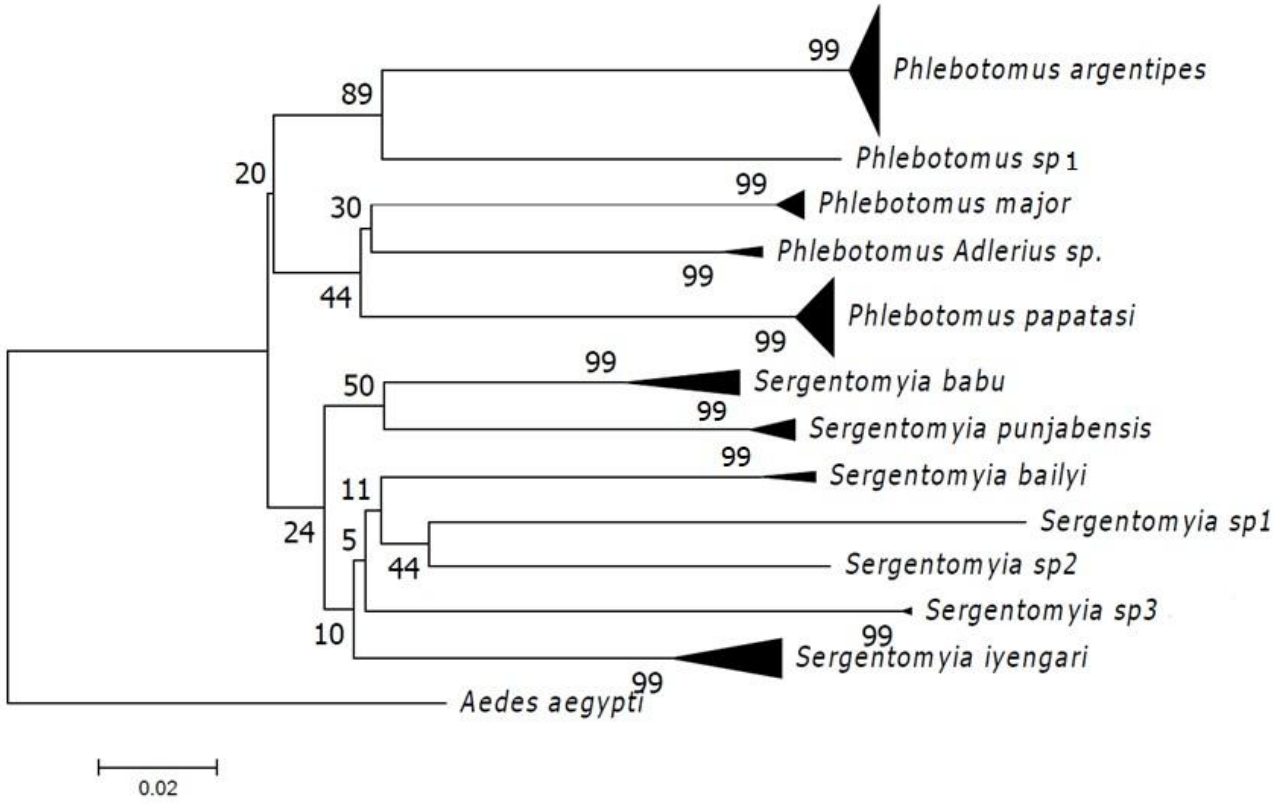
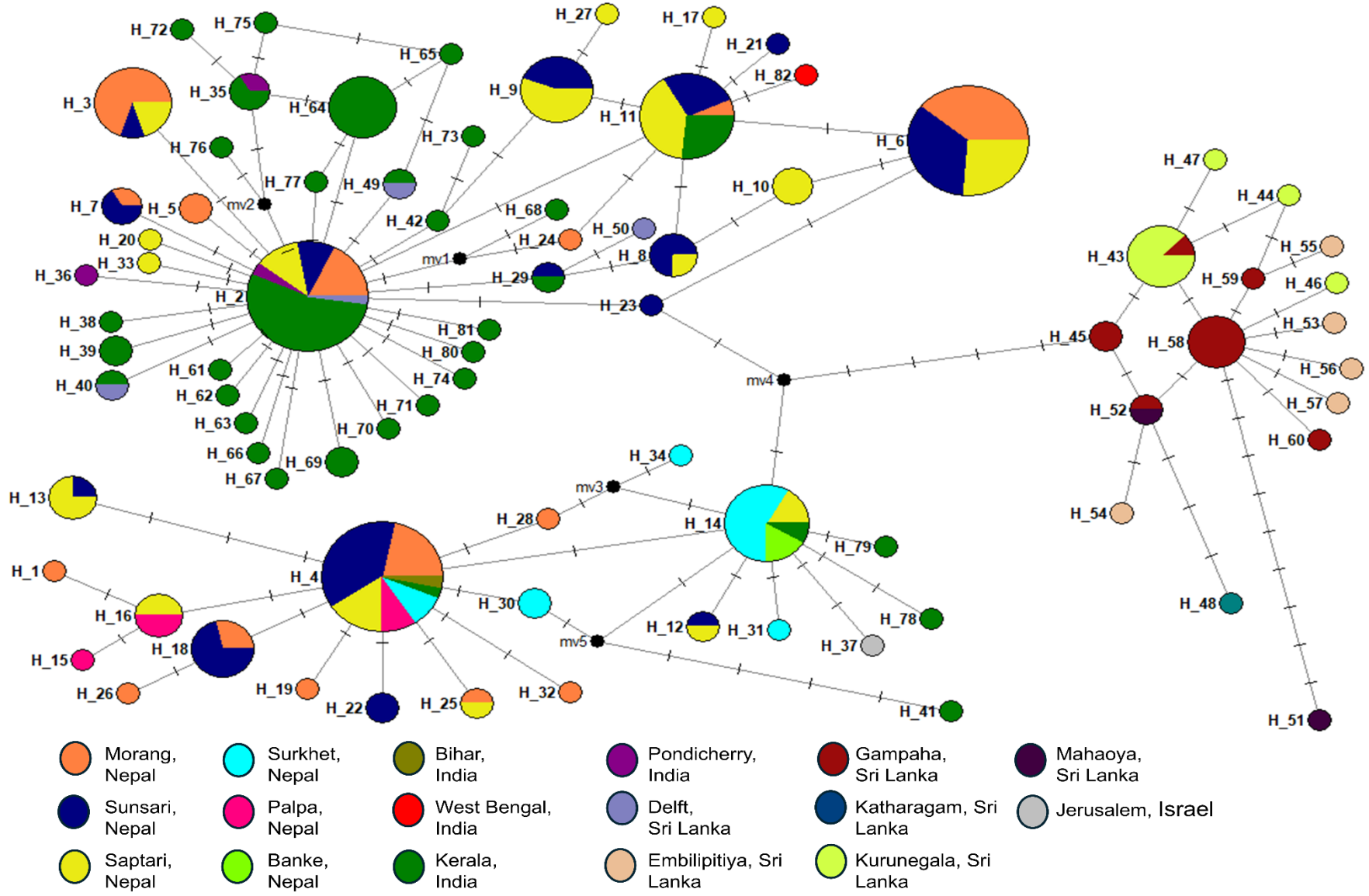


Figure: Bootstrapped Neighbor-Joining (NJ) tree

Results (II)



- Median-joining network of *Ph. argentipes* (n = 422) from Nepal, India, Sri Lanka and showing genetic relationships among COI haplotypes.

Conclusion

- Presence of known vector of *Leishmania donovani* - clusters with VL cases
- Presence of other potential vectors of *Leishmania* species at higher altitude regions
- DNA barcoding useful to complement the morphological ID of sand flies

Acknowledgment

B.P. KOIRALA INSTITUTE OF HEALTH SCIENCES 1993

INSTITUTE OF TROPICAL MEDICINE ANTWERP

University of Antwerp

Thank you for your kind attention

Dr. Lalita Roy is a prominent Nepalese medical entomologist at the B.P. Koirala Institute of Health Sciences (BPKIHS) in Dharan, specializing in the surveillance and control of vector sand flies transmitting *Leishmania* parasites causing **visceral leishmaniasis (kala-azar) and cutaneous leishmaniasis** in Nepal. She did her PhD at the Institute of Tropical Medicine and the University of Antwerp, Antwerp, Belgium. Her research focused on providing critical entomological evidence for Nepal's national disease elimination program. Her work notably highlights the challenges of insecticide resistance and utilizes molecular tools like DNA barcoding for sand fly species identification and tracking sand fly diversity.