

# Novel Vector-borne Parasites in Madagascar: implications for One Health

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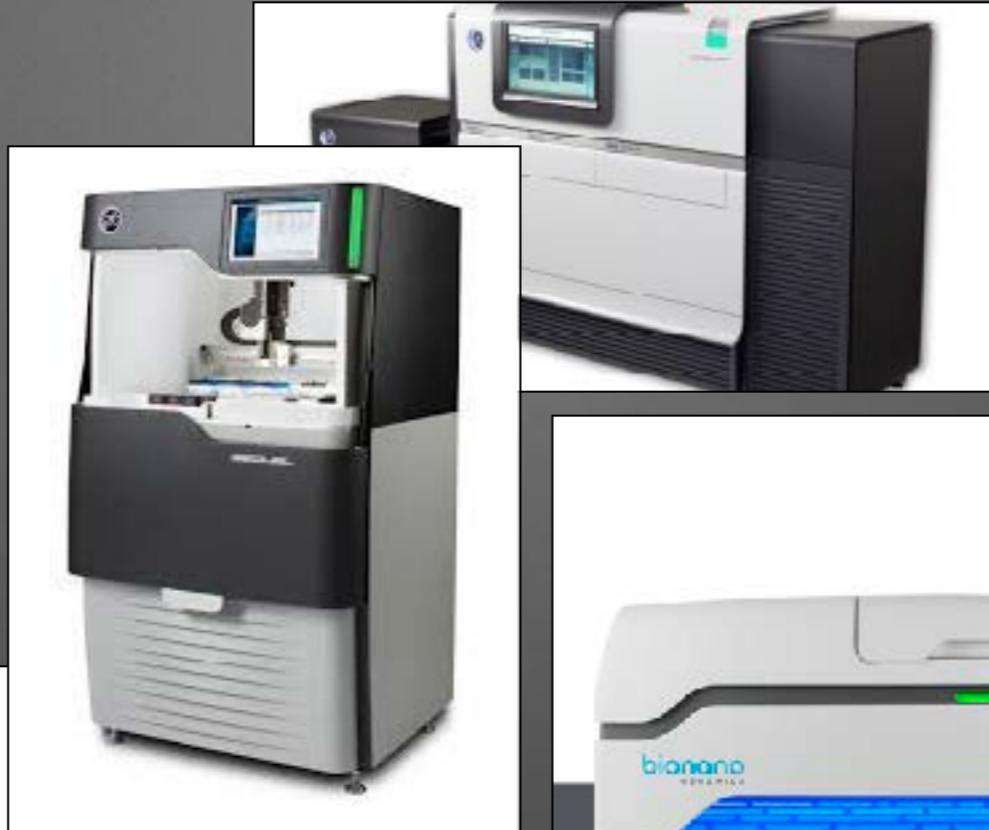
Duke University



# The Genomic Revolution



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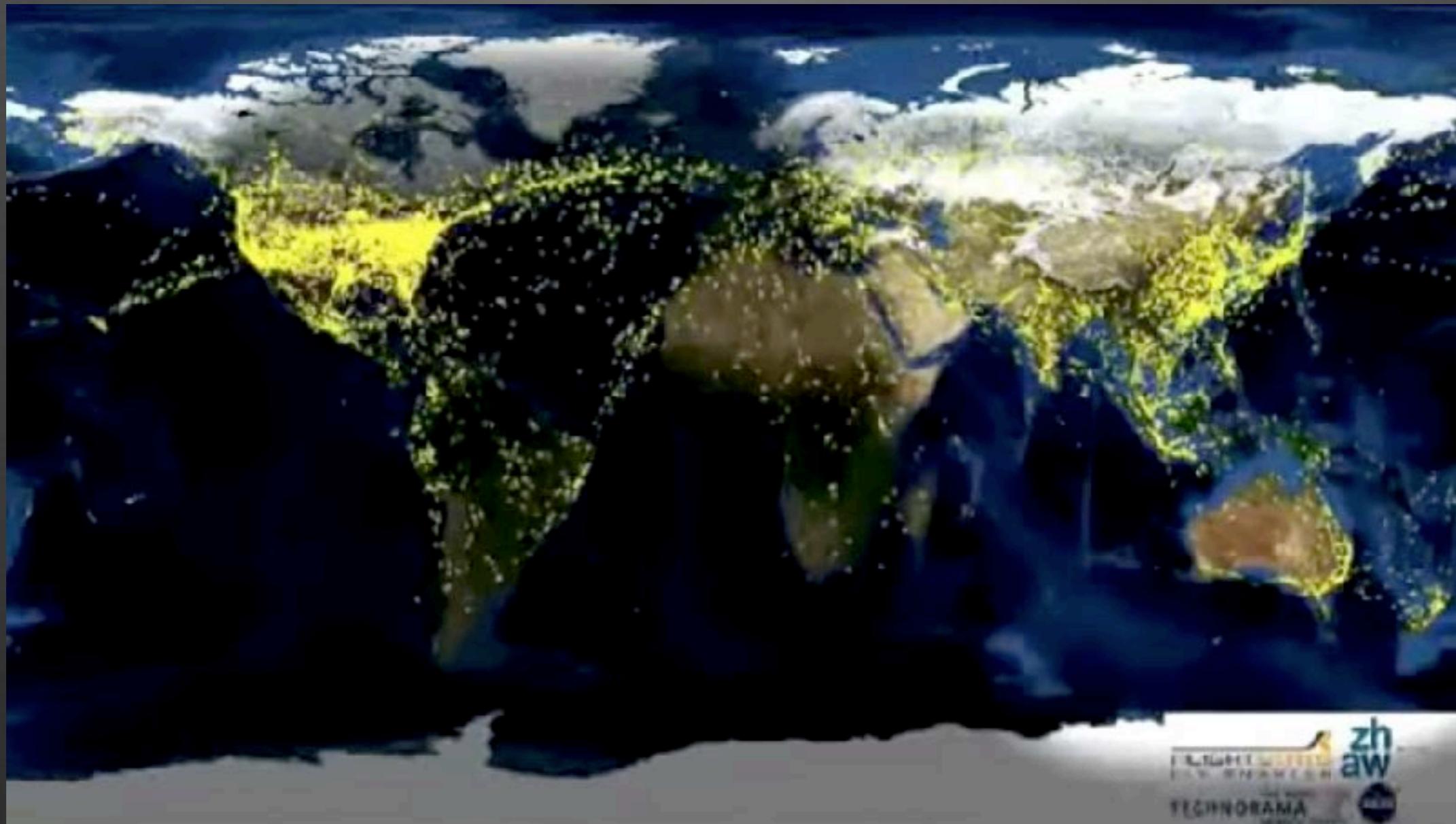
# Outline

- One World, One Health
  - Pathogen Discovery
  - Blood Transcriptomes
- Madagascar One Health Project
  - Why Madagascar?
  - Pathogen discovery
  - Ongoing and future work



# One World, One Health

- Zoonoses have potential for global impact
- Clear need for discovery and advanced surveillance



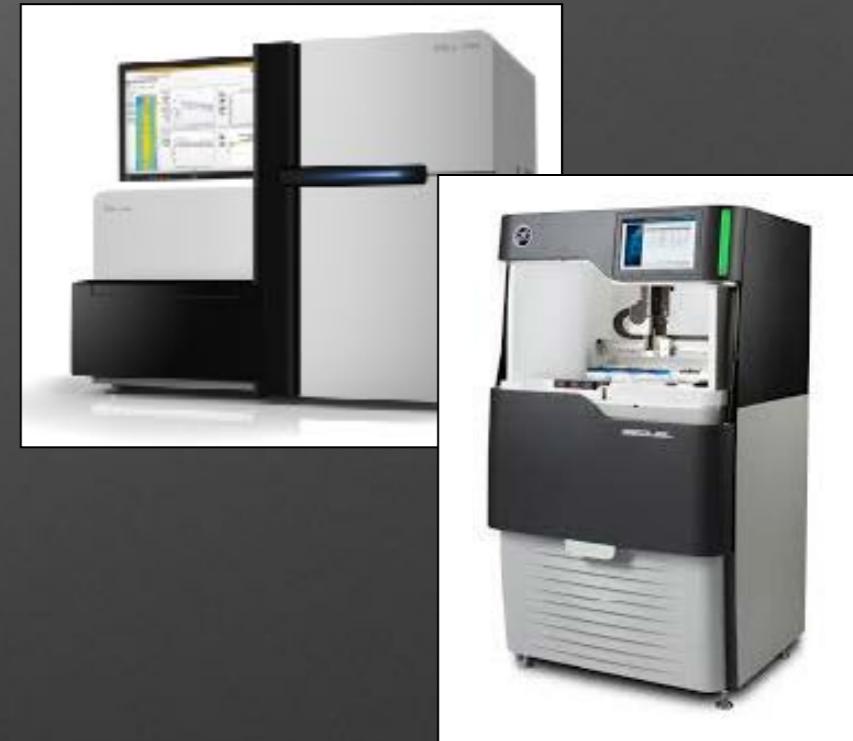
# One World, One Health

- Clear need for pathogen discovery in wildlife, domesticated animals, and humans
  - ~75% of emerging infectious diseases are zoonotic
  - Do we have a good understanding of pathogen biodiversity?
  - How can we use genomic tools to discover new pathogens?
    - In any species....



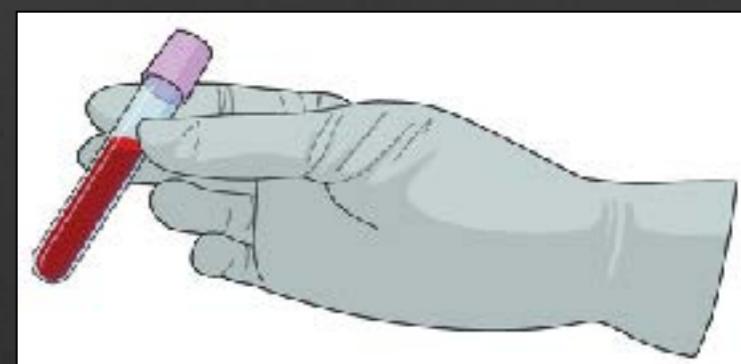
# Next-Gen Pathogen Discovery

- Metagenomic approaches
  - Deep sequence biological sample
  - Bioinformatically identify bacteria, viruses, parasites, etc.
  - No *a priori* information required
    - No PCR primers, probes, etc. needed
    - Can identify unculturable organisms
- One Medicine, One Science
  - Detection is first step
    - Determine pathogenicity
  - Use phylogenetics to classify and ID geographic origin
  - Use new information to design rapid diagnostics

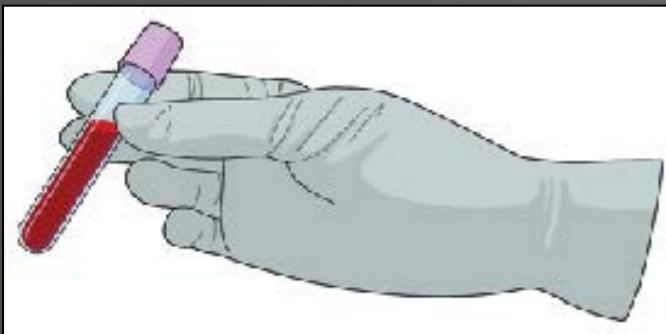


# Next-Gen Pathogen Discovery

- DNA and RNA metagenomics
  - DNA-seq: sequence entire pathogen genomes
    - requires deep level of coverage
    - pathogen enrichment by removing host DNA
  - RNA-seq: sequence the transcriptome (primarily expressed genes; lower depth of coverage required)
    - might miss pathogens (DNA viruses)
    - if biological sample is blood:
      - snapshot of host immune system



# Blood Transcriptome: experimental design



RNA stabilization  
(freezing or Tempus / PAX tubes)



Total RNA extraction

rRNA and hemoglobin purification

RNA-seq and/or Iso-seq  
(Illumina HiSeq, PacBio Sequel)

## Host Immune Profile

transcript mapping

gene expression profiles

antibody discovery

## Pathogen Discovery

remove host sequences

*de novo* transcriptome assembly

taxonomic assignment

develop qPCR assay for rapid screening



# Why Madagascar?

- Ideal natural laboratory for One Health projects:
  - Isolated for over 80 million years (no land bridges with Africa)
  - Fauna evolved in isolation (90% of species are endemic)
  - Humans, livestock, pets, etc. began colonizing ~5,000 years ago
  - Using genetics, we can identify endemic and invasive pathogens



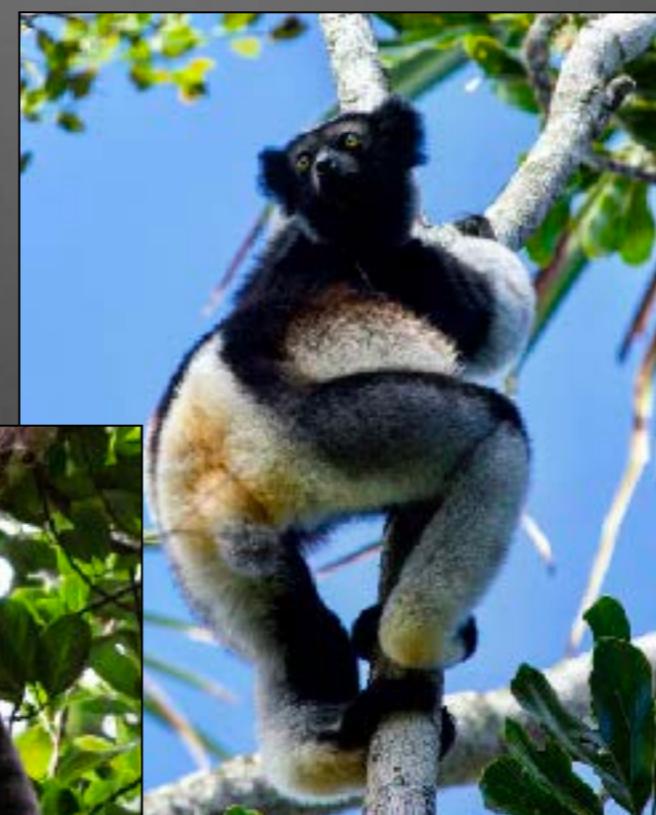
# Why Madagascar?

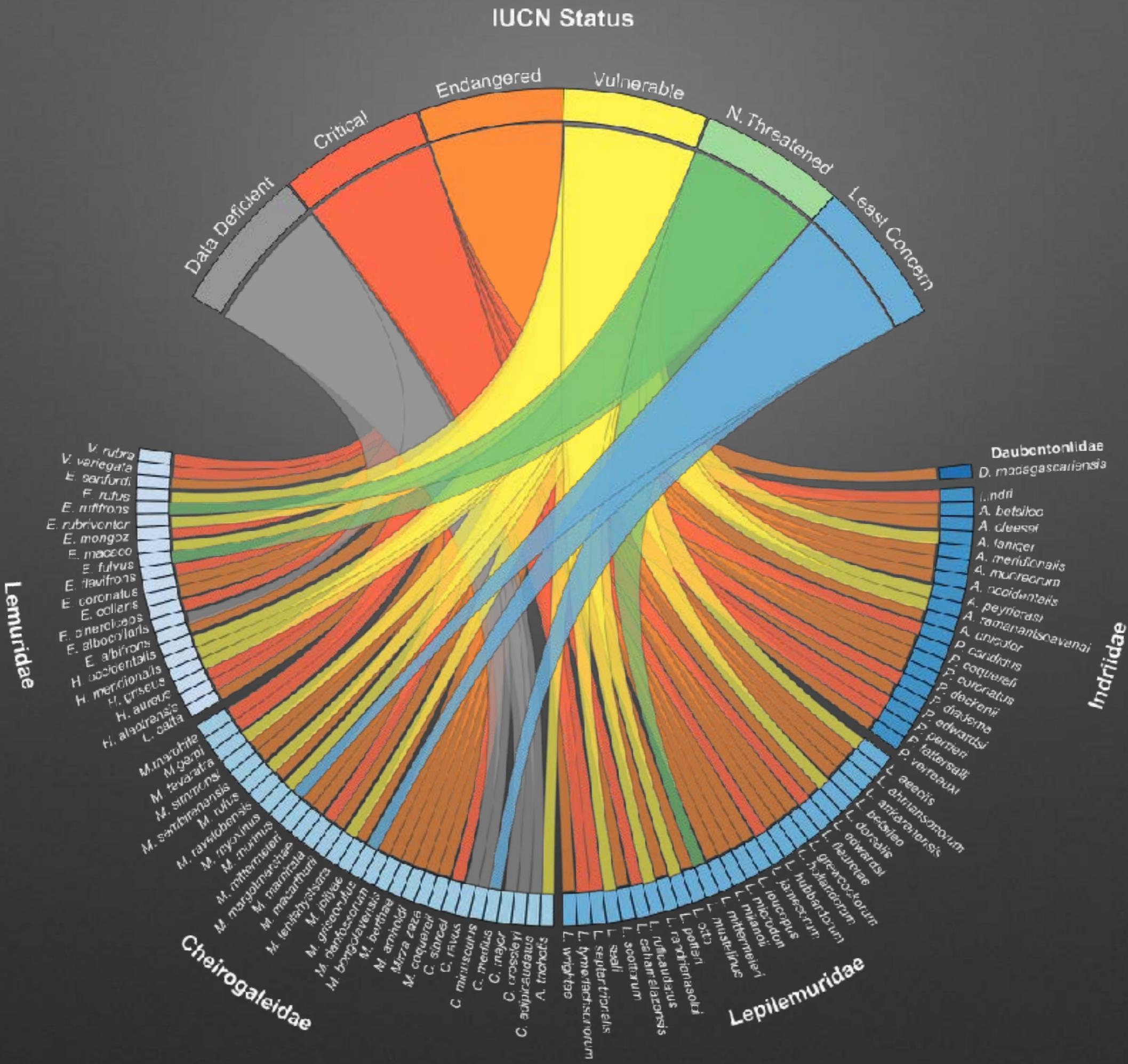
- Perfect storm for emerging zoonotic disease

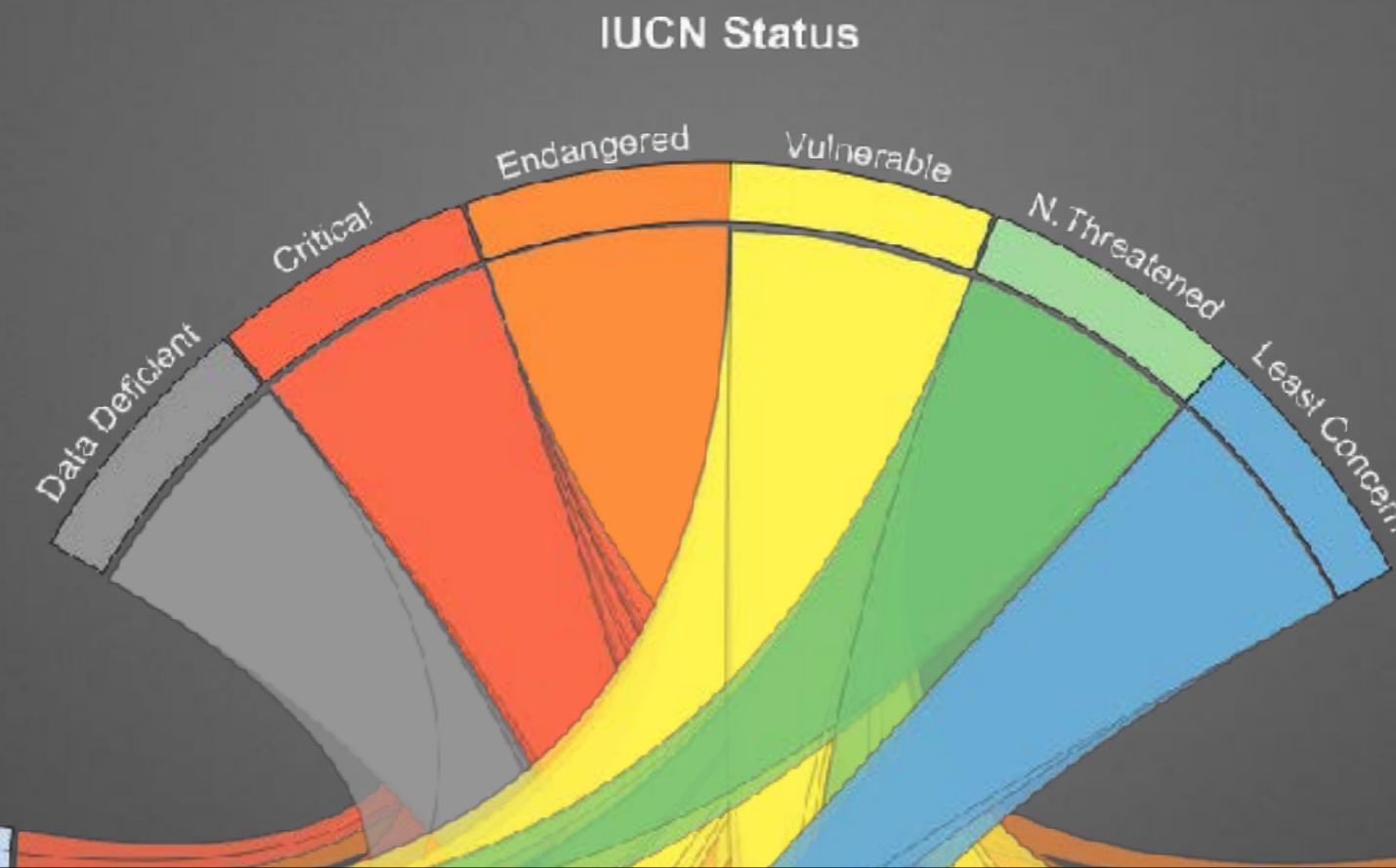


# Why Madagascar?

- Lemurs (103+ species endemic to the island)
  - World's most critically endangered group of primates

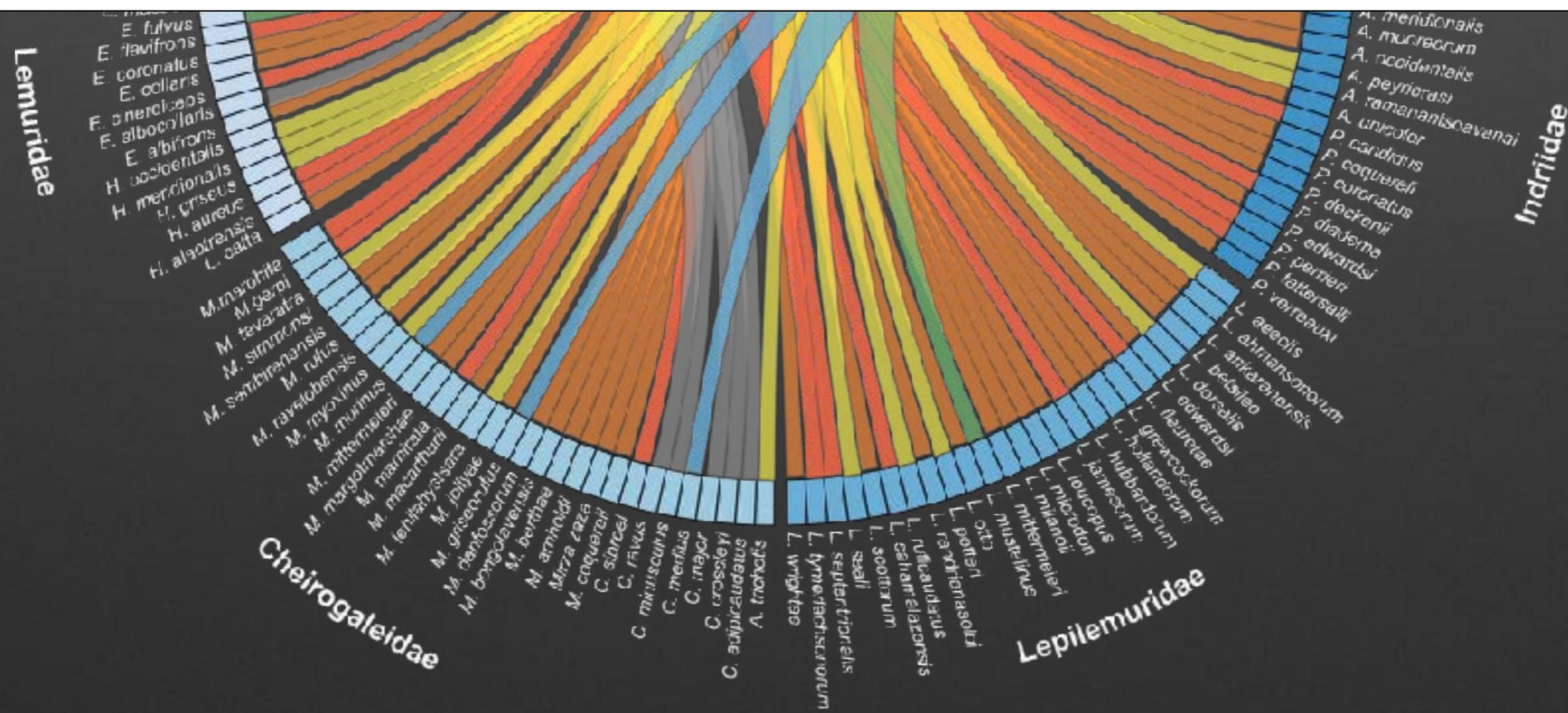






**Daubentonidae**  
*D. madagascariensis*

Advanced conservation approaches needed!!!



# Madagascar One Health Project

# Madagascar One Health

Phase 1: Pathogen Discovery  
(pilot study)

Phase 2: Design assays for rapid surveillance of many samples

Phase 3: form One Health Team (identify vectors, reservoirs, monitor health, etc.)

Throughout: inform conservationists, local vets and clinicians, and One Health community of discoveries



# Wild Lemur Pathogen Surveillance

- Traditional surveillance unreliable
  - Don't know what we're looking for
  - Increased potential for false negatives
  - Multiple invasions by humans and domesticated species
- Metagenomic discovery
  - No *a priori* information required to detect pathogens
  - Blood metatranscriptome
    - Minimally-invasive (critically endangered species)





Cathy Williams, DVM

- Team monitoring lemur health
- Routine blood sample collection
- Samples can be screened, using genomic tools, for pathogens
- Phase 1: NGS Discovery



*Indri indri*



*Propithecus diadema*

# Phase 1: Discovery

- Lemurs sampled from mid-altitude forests surrounding an active nickel mining site (Ambatovy)
- Highly fragmented landscape with many human pressures: hunting, logging, slash and burn agriculture



# Phase 1: Discovery

- RNA-seq: blood transcriptome approach
- Extracted total RNA from 6 blood samples
- Barcoded and pooled into single library and deep sequenced on 1 Illumina HiSeq 2000 lane
- ~37 million read pairs per individual
- Developed bioinformatic pipeline for *de novo* pathogen identification



**Pathogen biology**

Blood transcriptomes reveal novel parasitic zoonoses circulating in Madagascar's lemurs

Peter A. Larsen<sup>1</sup>, Corinne E. Hayes<sup>1</sup>, Cathy V. Williams<sup>2</sup>, Randall E. Junge<sup>3</sup>, Josia Razafindramanana<sup>4</sup>, Vanessa Mass<sup>5</sup>, Hajanirina Rakotondrainibe<sup>6</sup> and Anne D. Yoder<sup>1,2</sup>

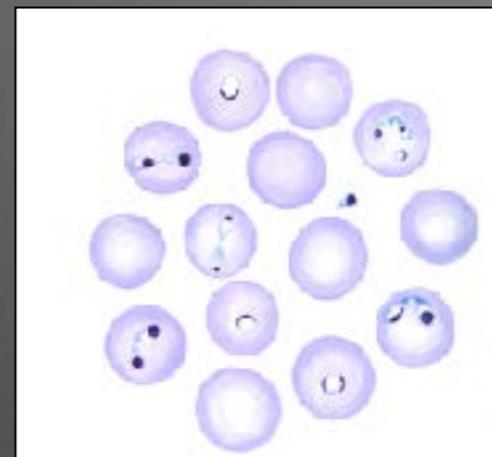
# What did we find?

Lots!

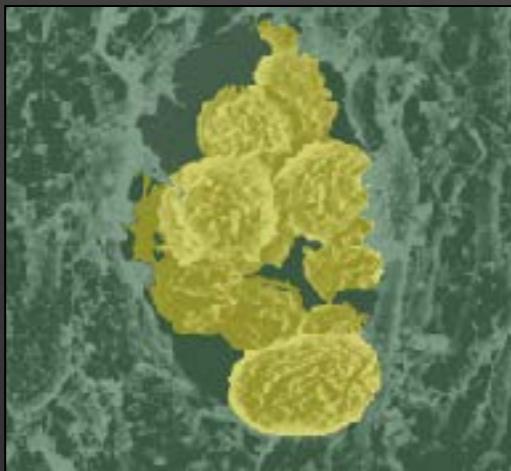
## Tick-borne



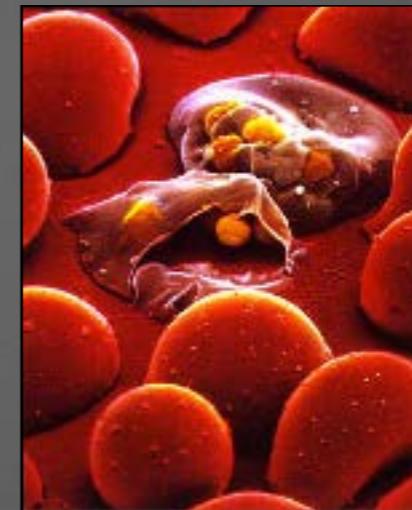
*Borrelia theileri*



*Babesia leo*  
and *B. canis*



New species of *C. Neoehrlichia*



3 new species of  
*Plasmodium*



New species of  
*Trypanosoma*

# Phase 1 Results

Tick-borne

	<i>Borrelia</i>	<i>Babesia</i>	<i>C. Neo..</i>	<i>Trypanosoma</i>	<i>Plasmodium</i>
	<i>I. indri</i> 1	✓		✓	
	<i>I. indri</i> 2		✓	✓	✓
	<i>I. indri</i> 3		✓	✓	✓
	<i>P. diadema</i> 1		✓	✓	✓
	<i>P. diadema</i> 2				✓
	<i>P. diadema</i> 3		✓	✓	✓



- Sequenced entire ribosomal and mitochondrial genes
- Results independently confirmed with DNA analyses



*Plasmodium*



*Trypanosoma*

# *Plasmodium spp.*

- 3 new species identified in *I. indri* and *P. diadema*



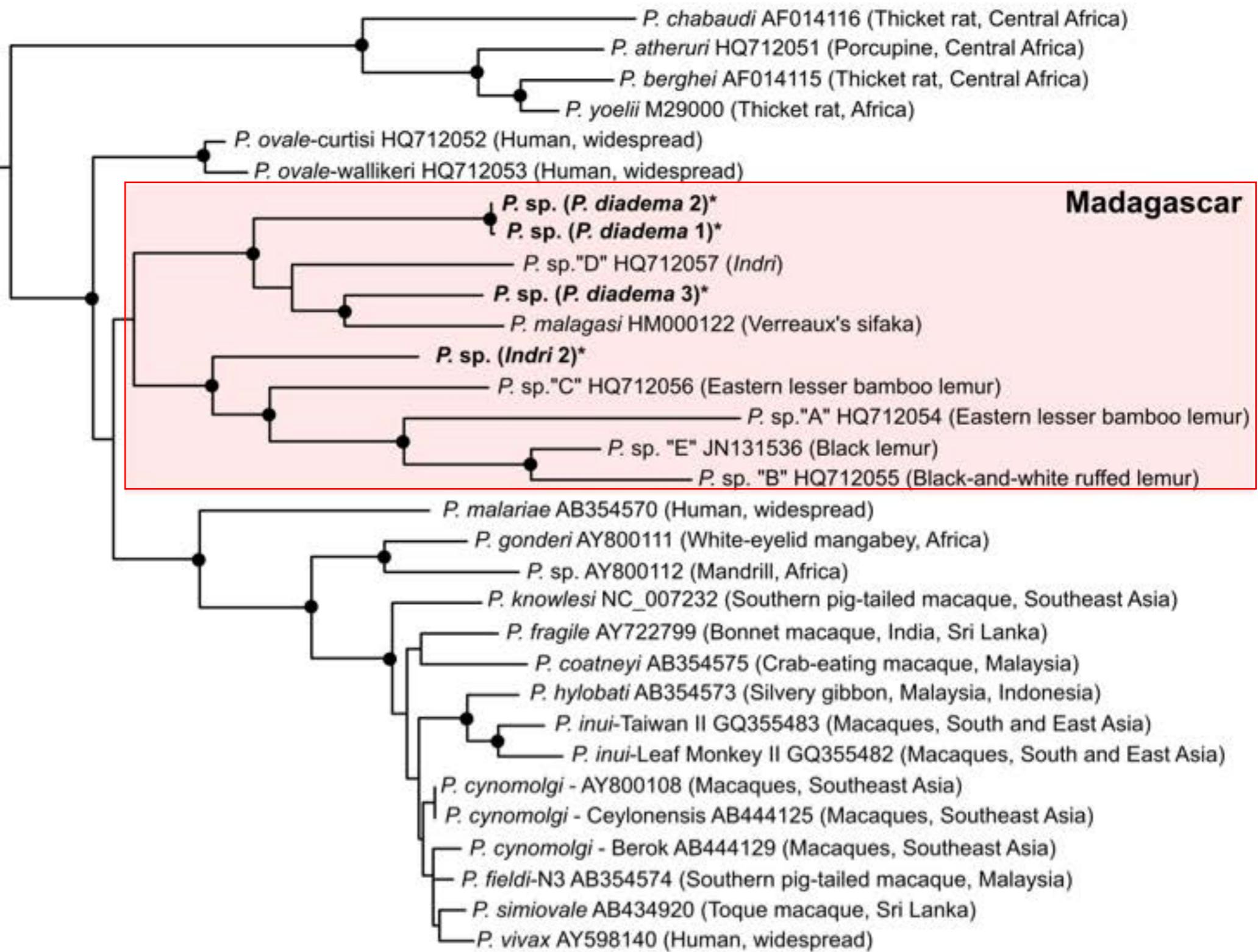
- Parasitic protozoa
- Mosquito vector and vertebrate host
  - 235 species of mosquitoes on Madagascar (59% endemic)!
  - ~3 species of *Plasmodium* that cause malaria in Madagascar (*falciparum*, *vivax*, *malariae*)



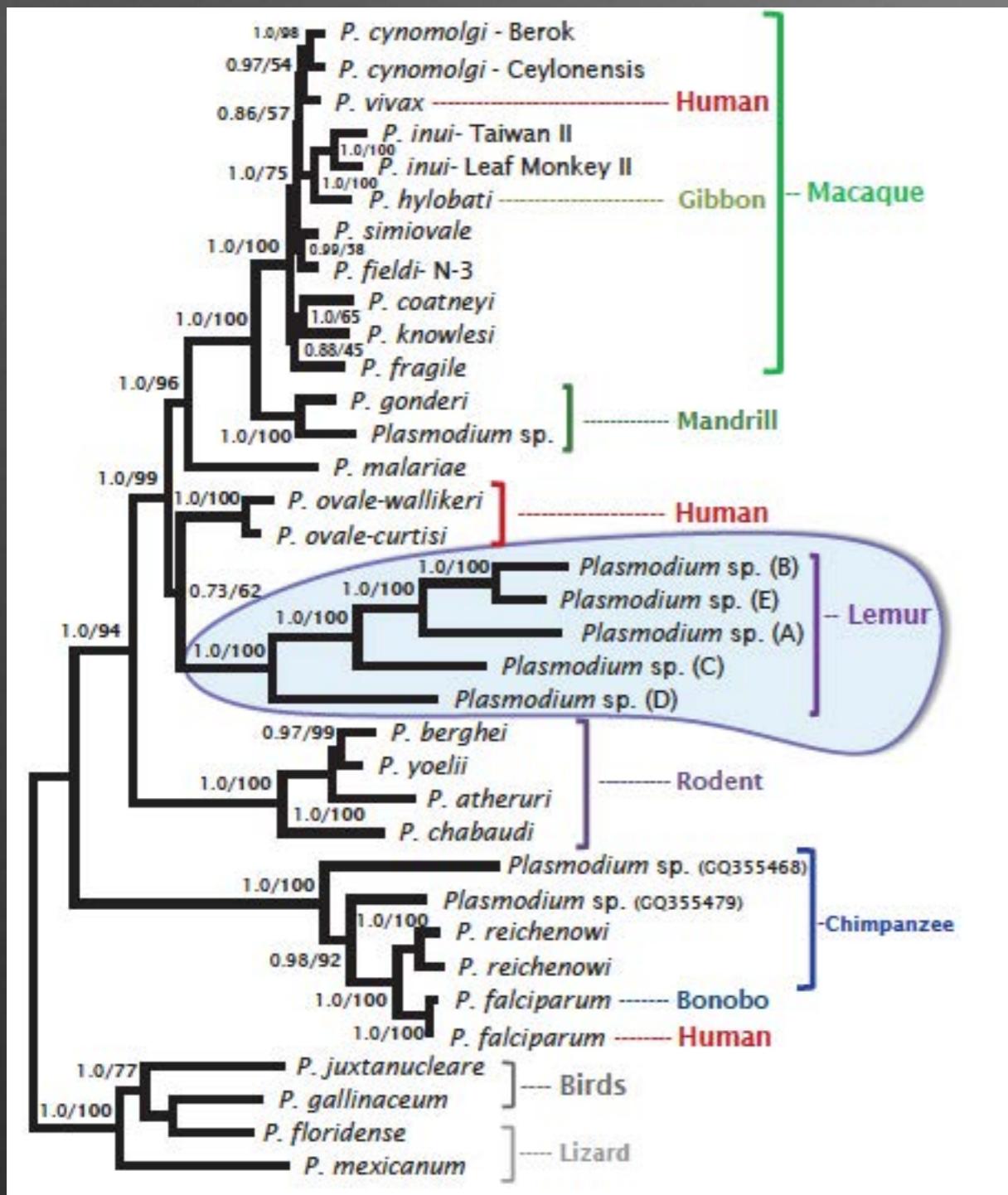
# Lemur Plasmodium Phylogenetics

A.

*Plasmodium*  
(COI)



# Lemur *Plasmodium*



- At least 8 species isolated from lemurs (3 from our study)
- Lemur *Plasmodium* evolved in isolation for ~20 million years (Pacheco et al. 2011)
- Likely many undescribed species in Madagascar!

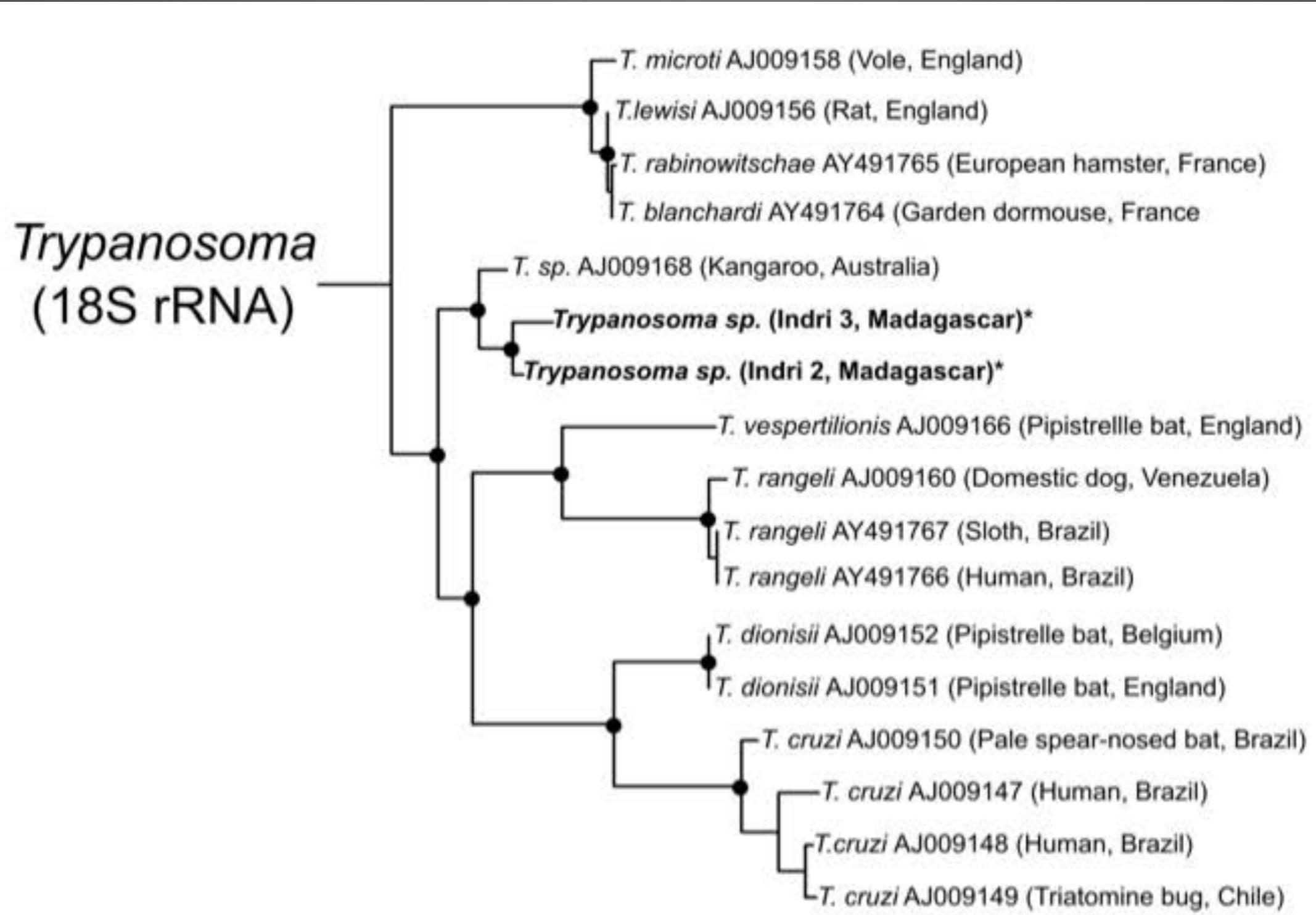
# *Trypanosoma* sp.

- 1 new species identified in *L. indri* and *P. diadema*



- Parasitic flagellate protozoa
- Transmitted by invertebrates. All are heteroxenous (require more than one host)
- *T. cruzi*: Chagas disease in South America; Triatoma (kissing bug)
- *T. brucei*: Sleeping sickness in Africa; tsetse fly

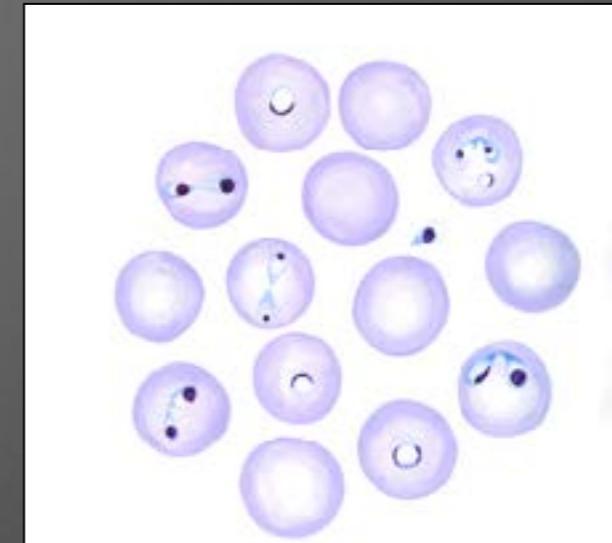
# Trypanosoma Phylogenetics



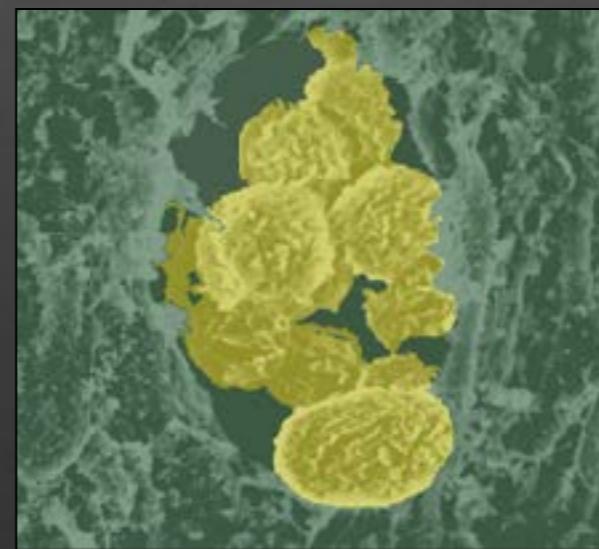
# Tick-borne Parasites



*Borrelia theileri*



*Babesia leo* and *B. canis*

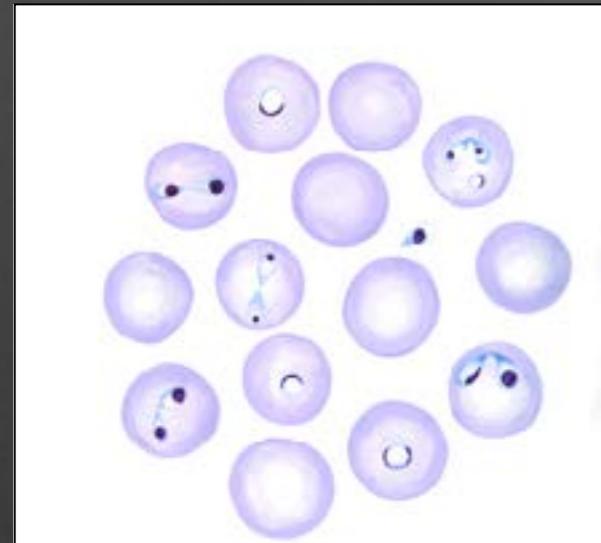


*C. Neoehrlichia* sp.

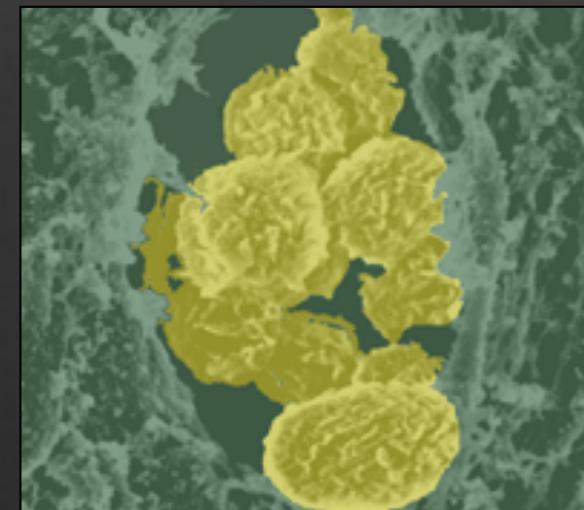
# Tick-borne Parasites



***Borrelia theileri*:** Bacterial spirochete.  
Causative agent of bovine borreliosis.  
Common in Africa. First sequence from a  
lemur.

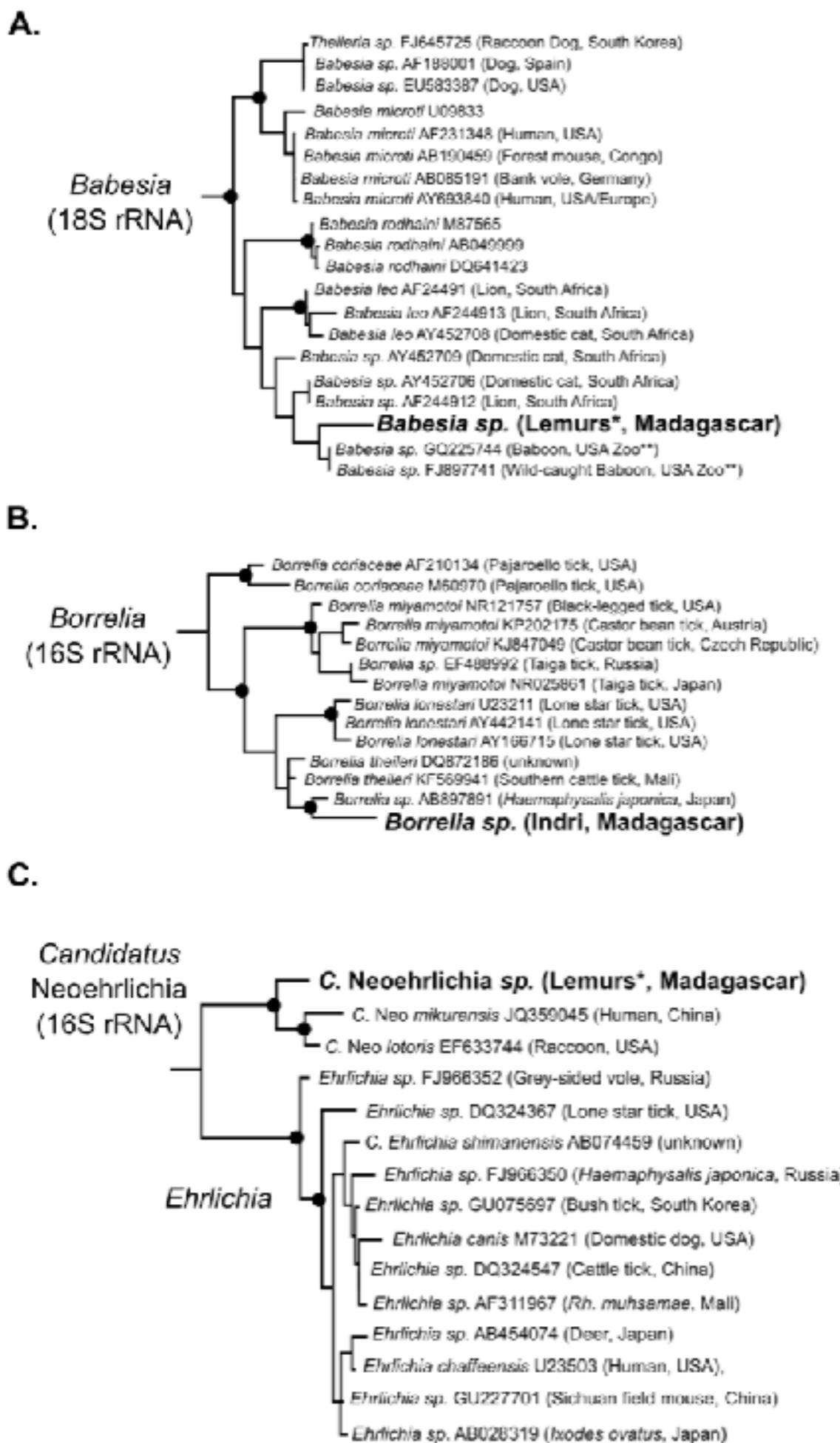


***Babesia* sp.:** Malaria-like prioplasma. Babesiosis:  
emerging in humans, wildlife, domesticated  
species. Multiple strains identified in lemurs  
(Springer et al. 2015 and Larsen et al. 2016). *B. leo*:  
South African cats. *B. canis*: global.



***C. Neoehrlichia*:** Bacteria that causes  
*neoehrlichiosis* in humans (anemia, deep vein  
thrombosis). First record from Madagascar!

# Tick-borne Parasites: phylogenetic analyses



- Phylogenetic analyses indicate the *Babesia* and *Borrelia* strains are not endemic to Madagascar
- Potential spillover from domesticated species (cats, dogs, and cattle)
  - *Babesia leo*, *B. canis*, *Borrelia theileri*
- Almost nothing known about *Neoehrlichia* (globally)

# The Opening of Pandora's Box



- 6 lemur blood samples; one Illumina HiSeq lane
- Discovered at least 5 new species of blood-borne parasites
- Several tick-borne parasites circulating in lemurs likely originated from domesticated animals (cattle, dogs, cats) imported to Madagascar from Africa.

# Phase 2: qPCR assays



- Designed qPCR probes for newly discovered tick-borne parasites
- *Avahi laniger, Indri indri, Lepilemur mustillinus, Propithecus diadema*
- Study site ~55 sq km
- Screened 102 banked samples (77 individuals, 25 recaptures)

# TBP Screening using qPCR

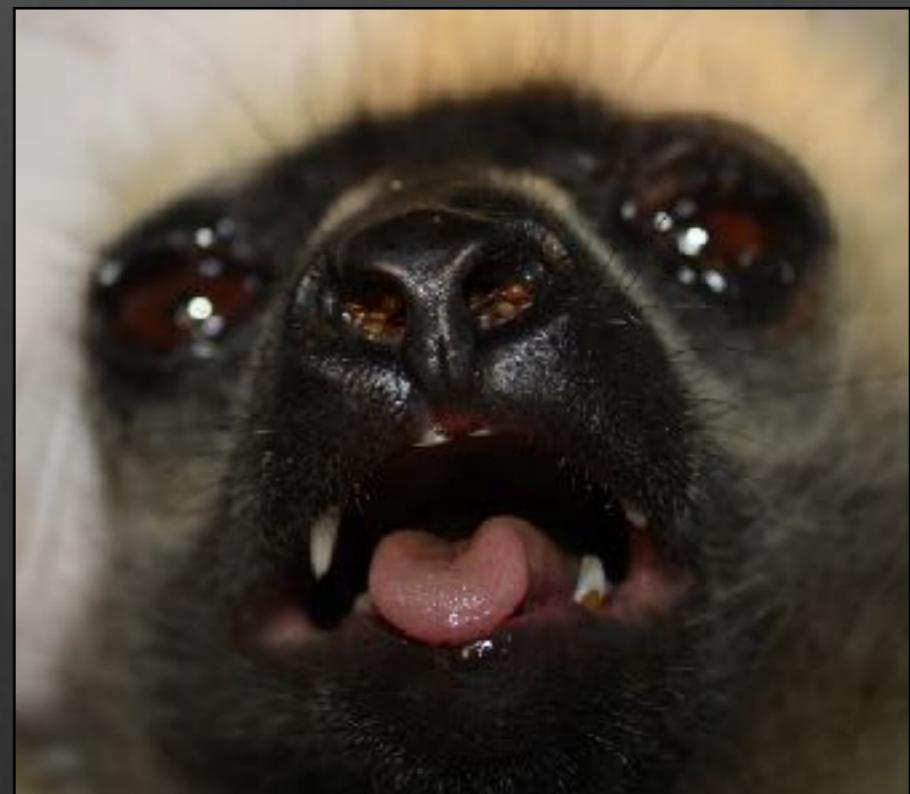
	<i>Babesia</i> +	<i>Borrelia</i> +	<i>Neoehrlichia</i> +
<i>Avahi laniger</i> (7)	7	0	1
<i>Indri indri</i> (15)	15	3	10
<i>Lepilemur mustillinus</i> (4)	0	2	0
<b><i>Propithecus diadema</i> (51)</b>	<b>51 (100%)</b>	<b>6</b>	<b>16 (31%)</b>

- *Babesia* infections are common
- *Neoehrlichia* previously unknown to exist on Madagascar.  
31 % infection rate in *P. diadema*!
- Coinfections: six individuals infected with all three parasites.

# *Babesia* +

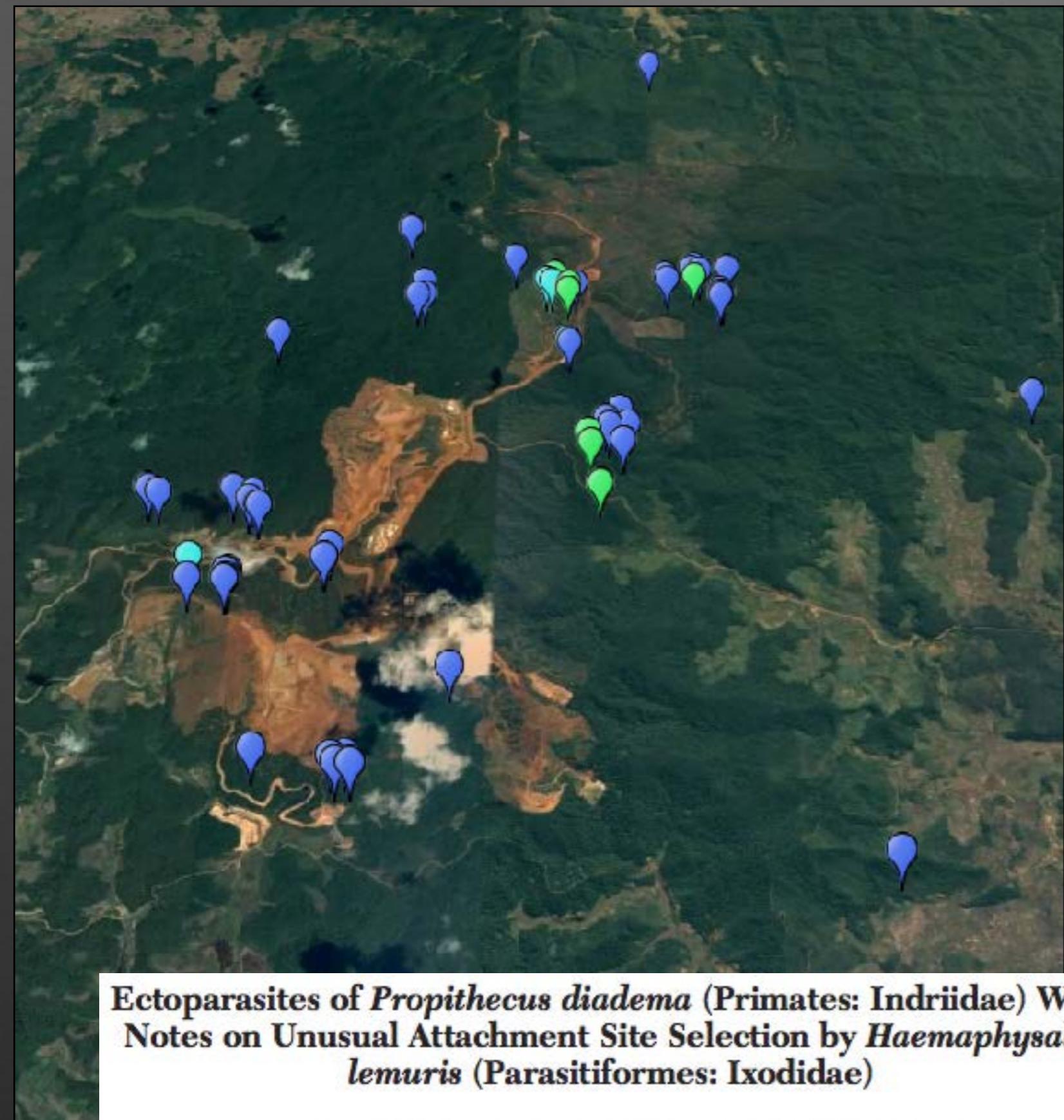
- *P. diadema* (51)
- *L. indri* (15)
- *A. laniger* (7)

*P. diadema*: heavy tick burden in nostrils. Not observed in other species.



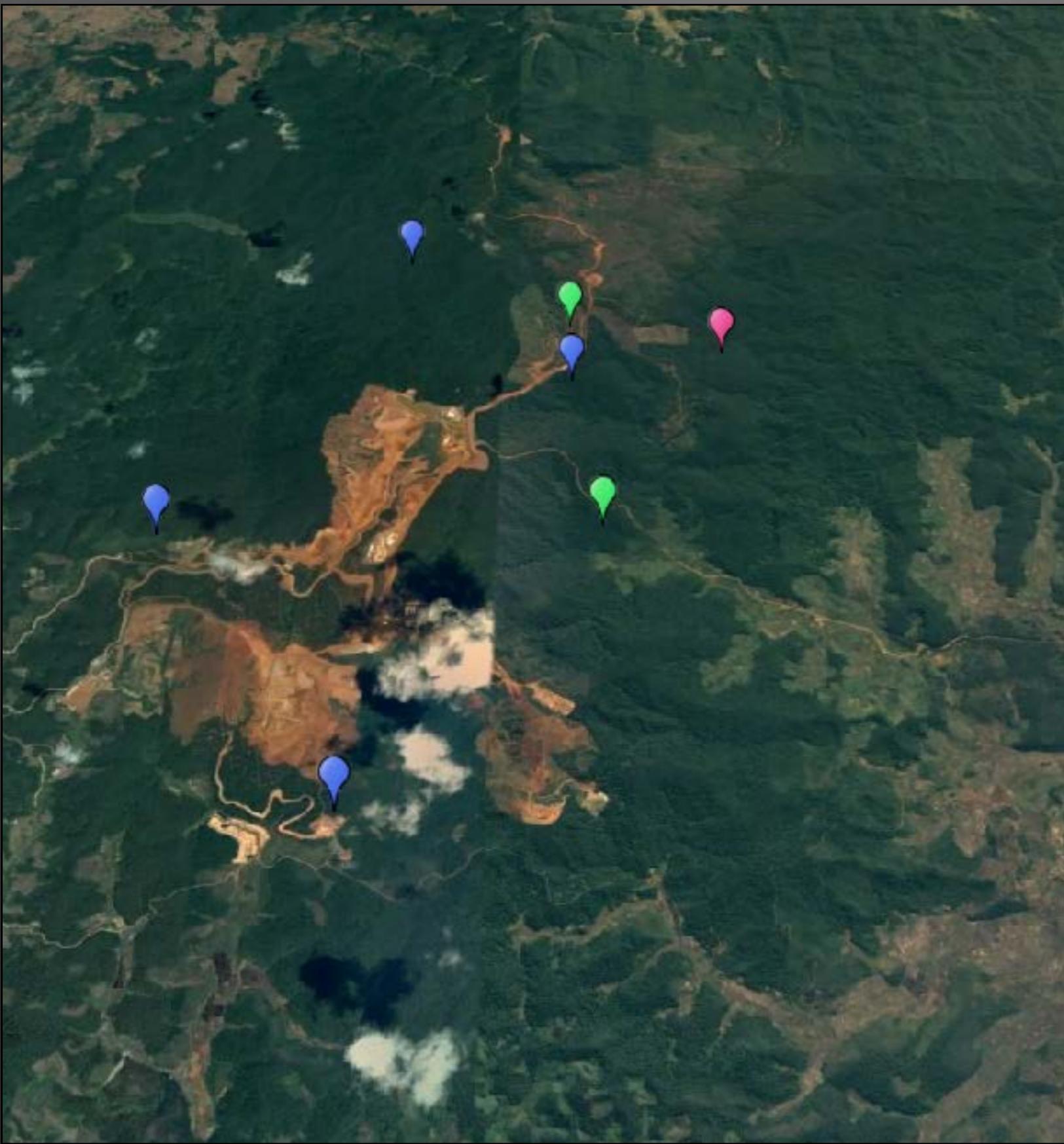
Ectoparasites of *Propithecus diadema* (Primates: Indriidae) With Notes on Unusual Attachment Site Selection by *Haemaphysalis lemuris* (Parasitiformes: Ixodidae)

HANS KLOMPEN,<sup>1,2</sup> RANDALL E. JUNGE,<sup>3</sup> AND CATHY V. WILLIAMS<sup>4</sup>



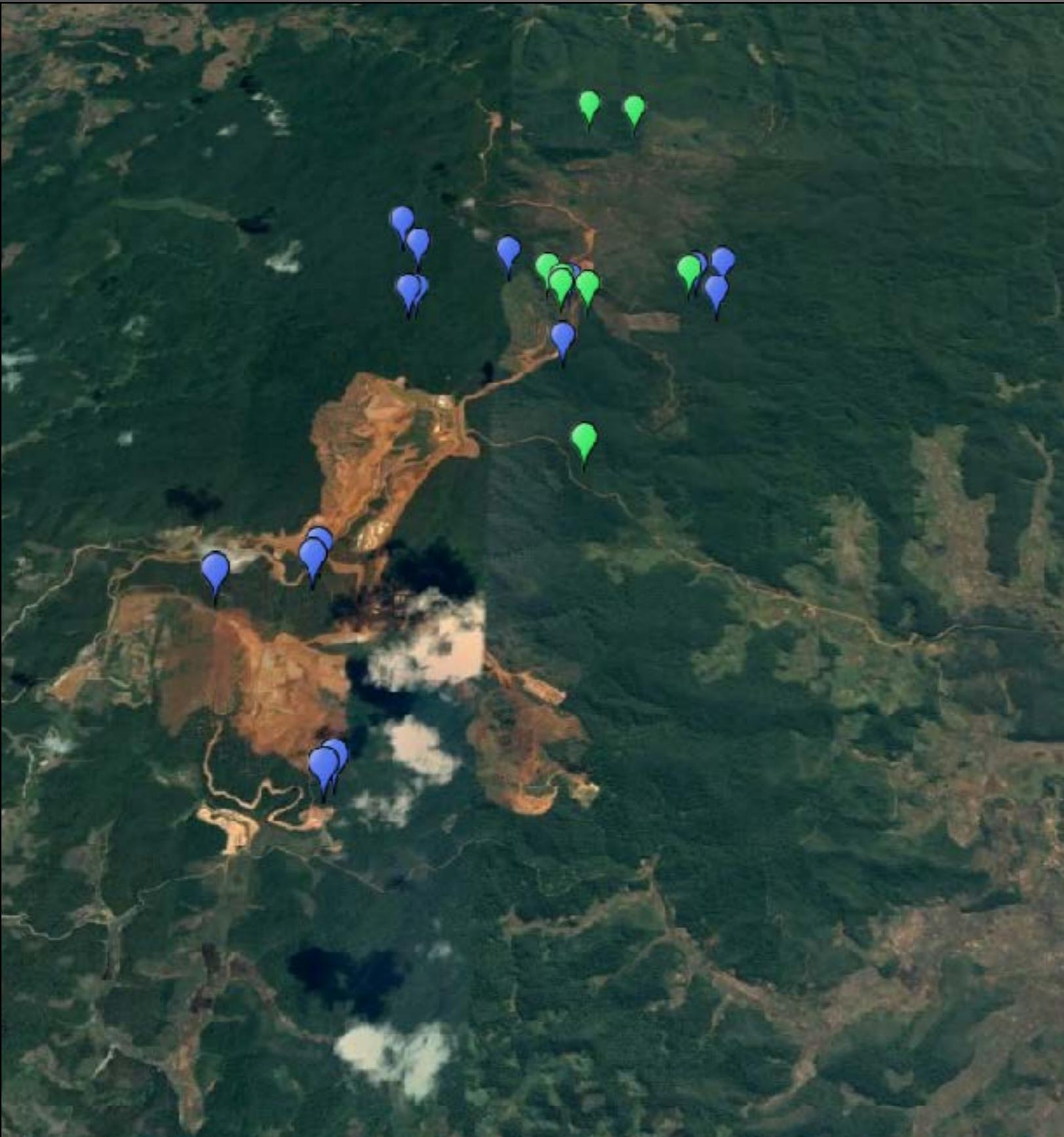
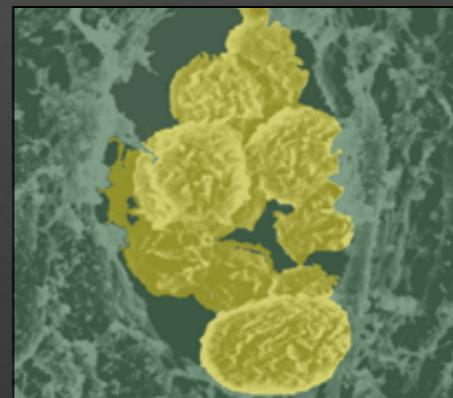
# *Borrelia* +

- *P. diadema* (6)
- *I. indri* (2)
- *L. mustelinus* (3)



# *Neoehrlchia* +

- *P. diadema* (16)
- *I. indri* (10)
- *A. laniger* (1)



# Emerging Tick-borne Pathogens in Madagascar?

- Closely related to strains known to cause disease...are these strains causing disease?
- Are these same strains circulating in humans, livestock, pets?
- Geographic distribution in Madagascar?
- Which tick species are serving as vectors?

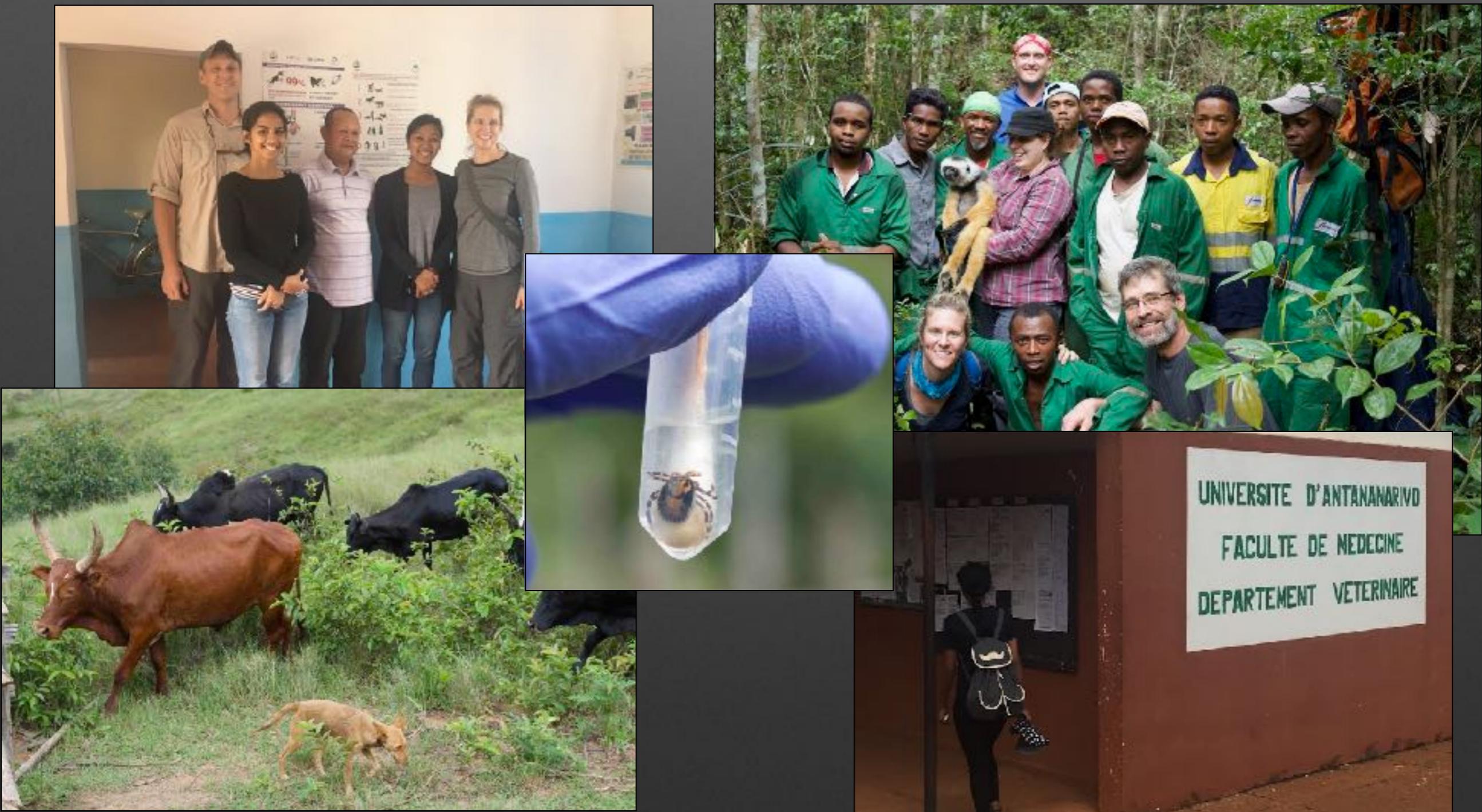


# Phase 3: Madagascar One Health (March 2017 Expedition)

- Objectives:
- Identify and collaborate with local veterinarians
- Identify tick species that are serving as vectors for the new TBP
- Sample blood and ticks from lemurs, cattle and dogs throughout the study area



# Phase 3: Madagascar One Health (March 2017 Expedition)



# Phase 3: Madagascar One Health

- Collected blood and ticks:
  - 40 lemurs (two species)
    - Ticks: ~50 *H. lemuris*
    - Hippoboscid flies: (~12)
  - 32 Zebu cattle (6 separate herds)
    - Ticks: ~30 *Amblyomma variegatum*, ~20 *Rhipicephalus microplus*
  - 10 dogs (no ticks)



# Phase 3: Madagascar One Health

- Screening all samples using qPCR assays
  - Identified invasive *Babesia leo* in endemic tick, *H. lemuris*
  - African *Borrelia theileri* is circulating in domestic dogs on Madagascar



# Phase 3: Madagascar One Health

- Cattle ticks: tropical bont tick and asian blue tick
  - Major disease vectors throughout the tropics
  - Huge impact on livestock (heartwater disease, bovine anaplasmosis, borreliosis [*Borrelia theileri*], etc.)
  - Vectors for Crimean-Congo hemorrhagic fever, Dugbe virus, yellow-fever, etc.



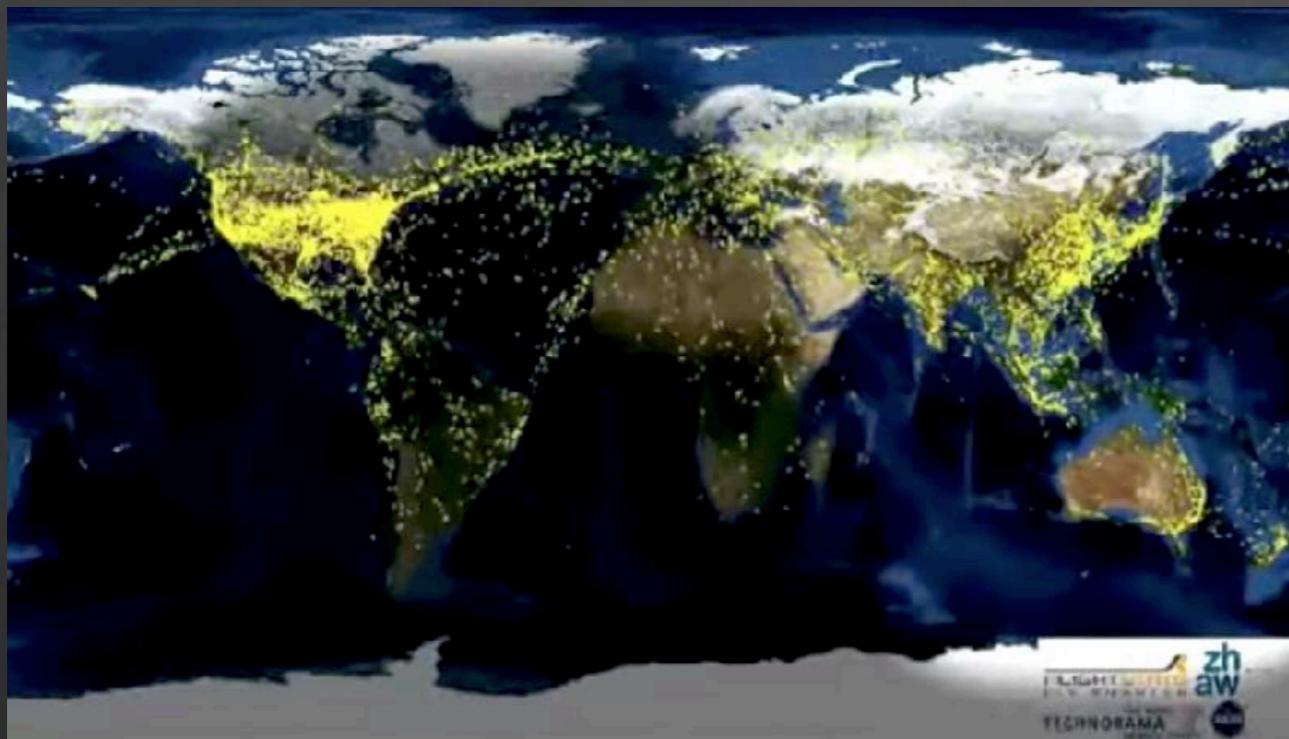
*Amblyomma variegatum*



*Rhipicephalus microplus*

# Implications for One Health

- Madagascar as a model system
  - Zoonotic potential of invasive pathogens
    - Tick-borne parasites known to cause disease in African species are now circulating in endemic wildlife
  - Do we have a good understanding of biological diversity / pathogen diversity?



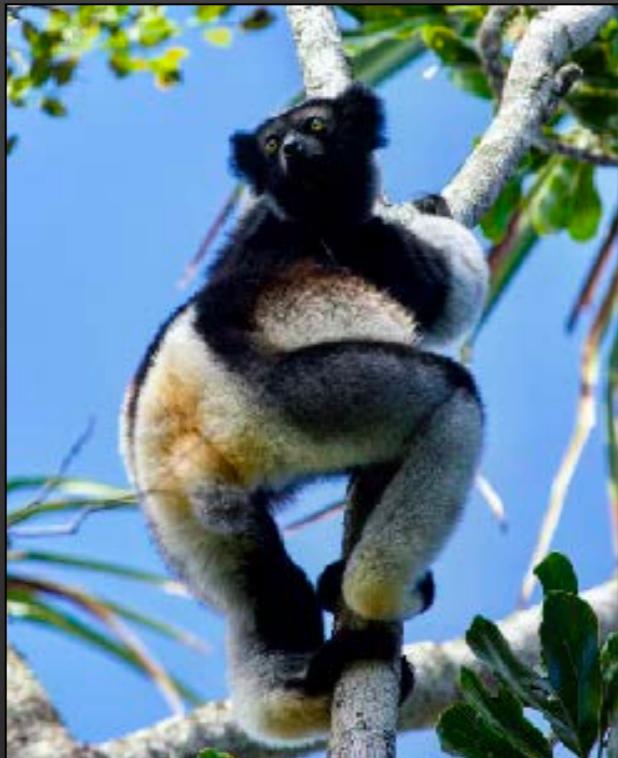
# Ongoing and Future Work

- Many important questions!
- Are these TBP negatively impacting health of humans, domesticated animals, and wildlife on the island?
- Collaborate with clinicians and veterinarians



# Conclusions

- We used blood metagenomics to discover multiple vector-borne parasites that were previously unknown to be circulating in lemurs (or unknown to exist on Madagascar).
- Developed new qPCR assays for rapid cost-effective surveillance.
- These approaches can be streamlined and deployed in almost any study system.



# Thank you!

## Collaborators

Barb Quroollo, DVM

Cathy Williams, DVM

Randy Junge, DVM

Ed Breitschwert, DVM

Jose Razafindramanana, PhD

Vanessa Mass, PhD

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Tsiky Rajaonarivelo, DVM

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