



Nanopore Adaptive Sampling as a Rapid Mammalian Molecular Barcoding Tool

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INTRODUCTION

- Mammal identification is difficult for cryptic, elusive, and endangered species that are rare in nature and natural history collections.
- Traditional PCR is time-consuming, costly, not field friendly, and limited by quality and quantity of DNA and primer selection.^{1,2}
- We rapidly and accurately ID mammals with nanopore adaptive sampling (NAS), sequencing from tissue and feces in the field and lab.



Figure 1. Two bats species (*Carollia brevicauda* and *C. perspicillata*) common in the Neotropics have overlapping morphological features and cannot be distinguished in the field.

METHODS

- R9 or flongle flow cells
- NAS targeting mitochondrial genomes
 - High copy number
 - Barcoding genes (i.e. COI and cytb)
- Reference file for NAS: NCBI mitochondrial ref seq from a range of species.
- First ~300 bases of DNA molecules are mapped to the reference file in real-time
 - >70% matches sequenced



Figure 2: Fecal samples from USA. Tissue samples from Guyana.

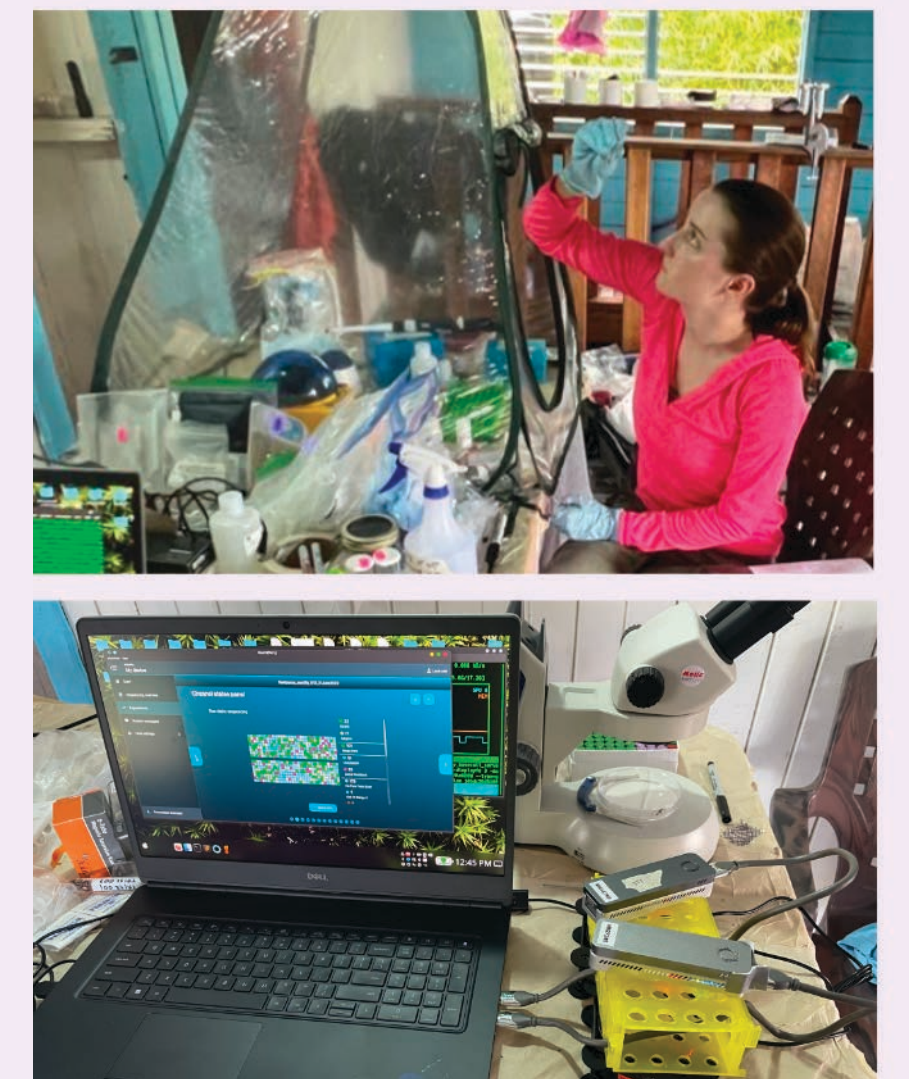
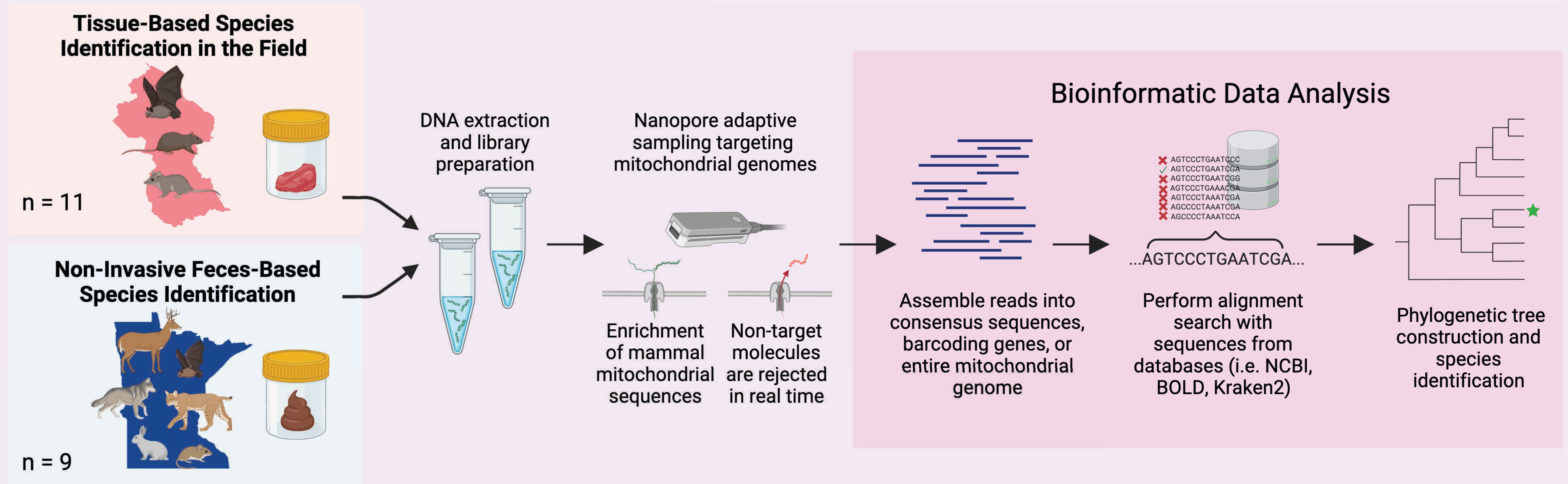


Figure 3: Mobile laboratory with portable MinION delivers molecular results in remote, resource-limited locations.

Accurate and rapid molecular barcoding of mammals is achieved with nanopore adaptive sampling targeting the enrichment of mitochondrial genomes.



RESULTS

- 9 Species IDs of Minnesota mammals from fecal samples
- 11 Species IDs of Guyana mammals from tissue in the field
- <36 Hours to achieve identifications

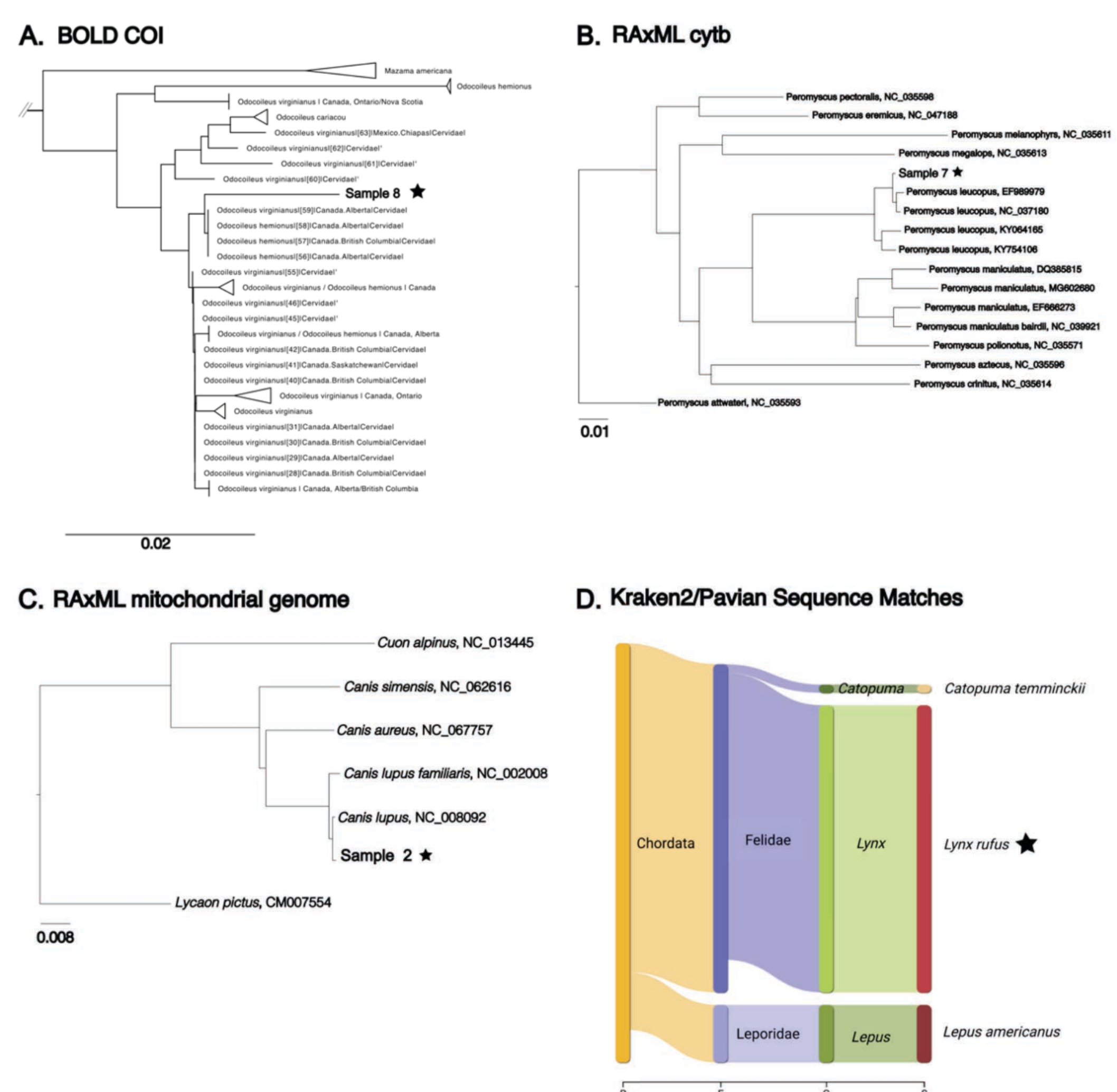


Figure 4: Bioinformatic methods for ID of fecal samples (black stars). A. Neighbor joining phylogenetic tree generated with BOLD. B. RAXML phylogenetic tree generated by cytb sequences. C. Mitochondrial genome RAXML phylogenetic tree. D. Pavian visualization of kraken2 results.

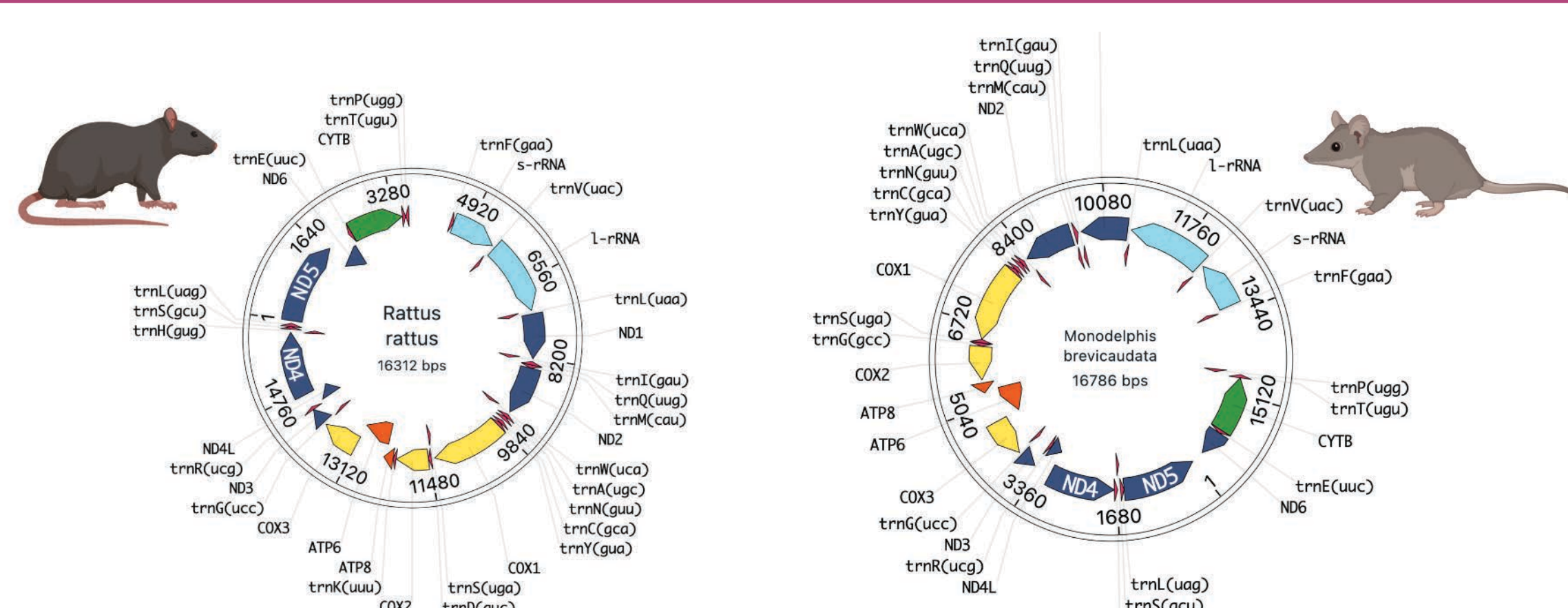


Figure 5: Whole mitochondrial genomes of Guyana species *de novo* assembled and annotated from data generated in field.

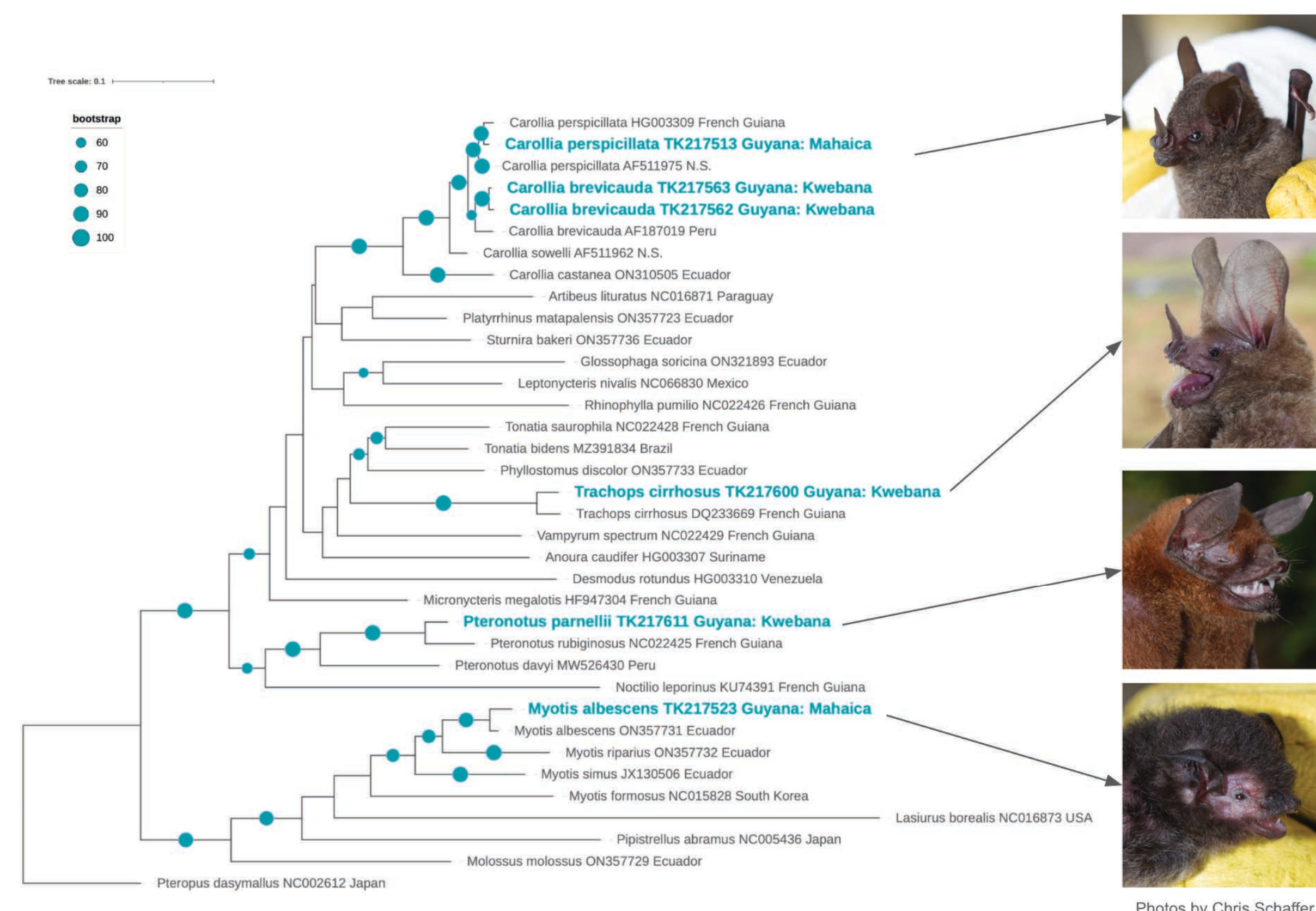


Figure 6: Field generated data identified species in real-time while conducting research in Guyana. Maximum likelihood tree of cytb sequences.

CONCLUSION

- Molecular ID possible with mobile lab under field conditions (i.e. heat, rain)
- Non-invasively collected feces can yield enough data to produce ID with NAS
- Challenges:
 - In the field: Inconsistency in cold chain, reliable power source
 - Fecal samples: Recommend minimum of >100 pores, >40 mitochondrial reads for confident identification
 - Mitochondrial barcoding: Species missing reference mitogenomes, incomplete lineage sorting, hybridization

We can use this method of mammal identification to...

Study mammals in the field



Monitor wildlife trade

Conserve threatened species



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