

# DNA Barcoding Bats

## Global campaigns and regional focuses



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### INTRODUCTION

- The order Chiroptera (bats) has the second highest species richness among mammals but has followed a unique life history leading to flight.
- There are currently 1116 recognized bat species (~20% of the total mammalian species diversity).
- The incidence of overlooked taxa in bats is likely to be high due to their cryptic behaviour and morphology
- Due to the relatively small number of species, a campaign to survey the world's bat diversity using COI has proceeded rapidly and now includes specimens from 54 countries (Figure 1). There are currently more than 11,000 bat barcodes representing ~40% of known species.



Figure 1: Sampling locations included in current global bat campaigns.

- Several regional campaigns have been amassed into this global survey. Here I will focus on our work in the Neotropical regions encompassing 11 countries in Central and South America (Figure 2).



Figure 2: Countries surveyed in Neotropical bat projects.

- Three objectives of this research campaign are highlighted in this study:
  - Comparative analyses of phylogeographic patterns between DNA Barcode sequences and traditional cytochrome b analyses.
  - Adjustments to species distributions and support for recently recognized species.
  - Taxon identification within a focal region, Guyana.

### METHODS

- Specimen skins and skeletons are deposited as permanent vouchers in the Department of Natural History, Royal Ontario Museum (ROM), Toronto, Canada.
- Total genomic DNA was extracted by glass fiber protocols (Ivanova et al. 2006) from small pieces (1 mm<sup>2</sup>) of tissue from cryogenically stored tissue associated with the ROM permanent voucher collection.
- DNA barcode sequences were amplified and sequenced using standard protocols (Clare et al. 2007), (Ivanova et al. 2007).
- Cytochrome b sequences were amplified and sequenced from total genomic DNA or downloaded from Genbank.
- Analyses were performed using BOLD and MEGA 3.

### RESULTS

#### Phylogeographic Patterns

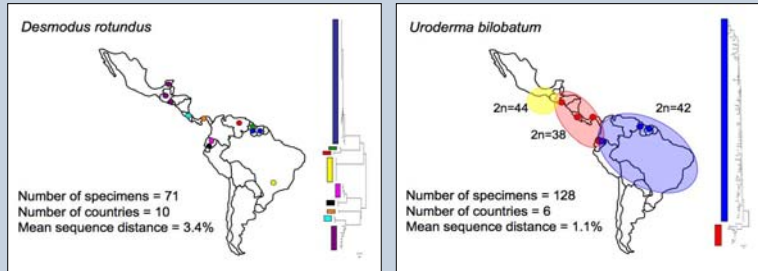


Figure 3: The vampire bat, *Desmodus rotundus* has ~9 distinct lineages established by DNA barcode. Similar results exist for cytochrome b. These lineages correspond to the last major uplifting of the Andes mountain range and to the "Great American Interchange" event that occurred with the formation of the Central American land bridge. These patterns suggest that both vicariance and dispersal events may have established the pattern of mitochondrial divergence.

Figure 4: Two haplotype groups within *Uroderma bilobatum* correspond to geographic lineages previously identified by cytochrome b and karyotype analysis (numbers indicated) (Hoffman et al. 2003). A third lineage is hypothesized to exist with a distinct *cyt b* sequence array and karyotype (yellow range) (Hoffman et al. 2003 but specimens from this local have not yet been surveyed by DNA barcoding. Lineages revealed by DNA barcoding are consistent with the central American origin of this species.

#### Updating Species Distribution

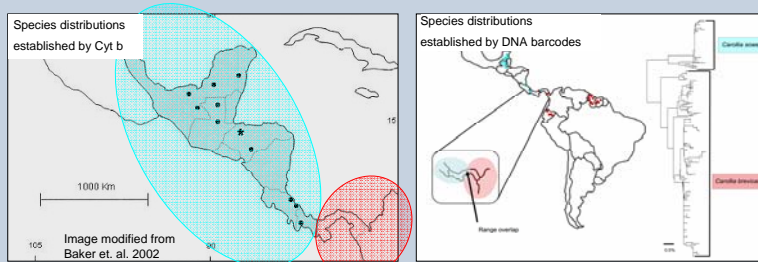


Figure 5: *Carollia sowelli* was described in Baker et al. (2002) based on cytochrome b deviation from its morphologically identical sister taxa, *C. brevicauda*. Subsequent investigations were unable to establish a sympatric range for these two species though it was hypothesized that this overlap did exist. Such an overlap would help support the genetic species description by confirming reproductive isolation without a hybrid zone. Continual screening of the ROM databases with DNA barcodes has found two distinct genetic groups corresponding to *C. sowelli* and *C. brevicauda* and DNA barcodes do place these two groups living sympatrically in central Panama.

#### Taxonomy within bats of Guyana (Clare et al. 2007)

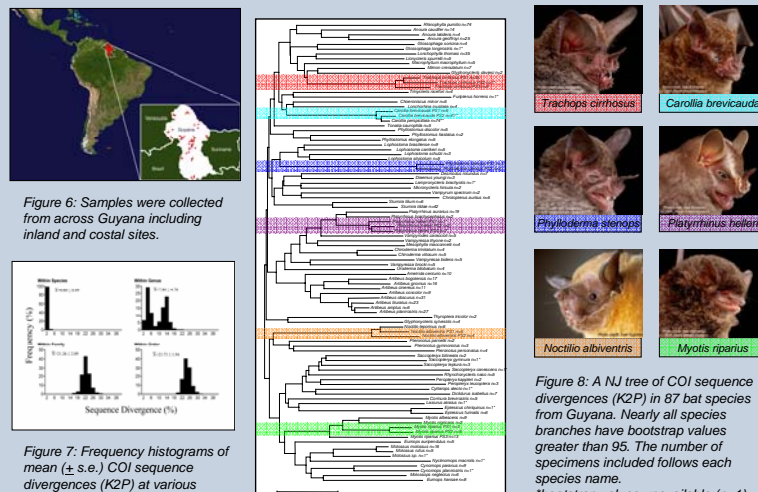


Figure 6: Samples were collected from across Guyana including inland and coastal sites.

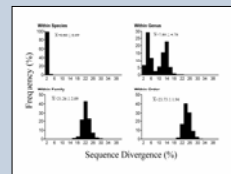


Figure 7: Frequency histograms of mean (± s.e.) COI sequence divergences (K2P) at various levels of the taxonomic hierarchy for bat species from Guyana.

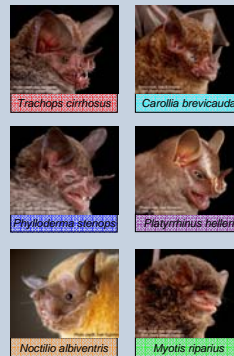


Figure 8: A NJ tree of COI sequence divergences (K2P) in 87 bat species from Guyana. Nearly all species branches have bootstrap values greater than 95. The number of specimens included follows each species name. \*bootstrap values unavailable (n=1) •6 species (above - colour coded to tree) show evidence of cryptic speciation

### CONCLUSIONS

- DNA barcoding of bats has proven to be extremely successful for species identification (100% successful in Neotropical bats).
- Cytochrome b sequences are commonly found in mammal literature as part of phylogenetic and phylogeographic literature. The correspondence between *cyt b* and COI sequences is extremely high.
- Structural rearrangements of the nuclear genome (karyotype alterations) correlate well to lineages established through DNA barcoding.
- DNA barcode sequences reaffirm phylogeographic patterns and thus present a useful record of historical events such as the "Great American Interchange" and the uplifting of the northern segments of the Andes.
- Intensive DNA barcoding surveys of Neotropical bats are helping to clarify species range patterns particularly among newly recognized species where information is limited (e.g. *Carollia sowelli*).

### FUTURE

- Continue the Neotropical bat survey
- Augment the distribution of sampling targeting phylogeographically important areas such as the western side of the Andes mountain range.
- Correlate karyological changes with DNA barcode lineages



- Explore nuclear DNA variation for supplemental confirmation of DNA barcode lineages to establish species status.
- Explore vocal variations within echolocation bats to establish whether DNA barcode lineages can be identified by vocal changes which may act as a reproductive barrier.

### REFERENCES

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For more information see Clare, E.L., Lim, B.K., Engstrom, M.D., Eger, J.L. & Hebert, P.D.N. (2007) *Mol. Ecol. Notes* 7 (2), 184-190, or contact ELC at: eclare@uoguelph.ca