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Comparing Two Species: Historical Phylogeography and Species Delimitation of Myiarchus Flycatchers in the West Indies.

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Comparing two species:

historical phylogeography and species delimitation of *Myiarchus* flycatchers in the West Indies

Emily Staden

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Objectives

Objectives

- **To determine the evolutionary relationship between**
  - La Sagra's flycatcher (*Myiarchus sagrae*)
  - Stolid flycatcher (*Myiarchus stolidus*)
- **To be accomplished by integrating**
  - Demographic history
  - Phenotypic characteristics

Potential Hypotheses

- **H_0: *M. stolidus* and *M. sagrae* represent two distinct phylogenetic species**
  - Prediction: samples from each species epithet will most closely relate to conspecific samples
- **H_1: *M. stolidus* and *M. sagrae* represent a single phylogenetic species**
  - Prediction: samples from each species epithet should be unrelated

Potential Hypotheses

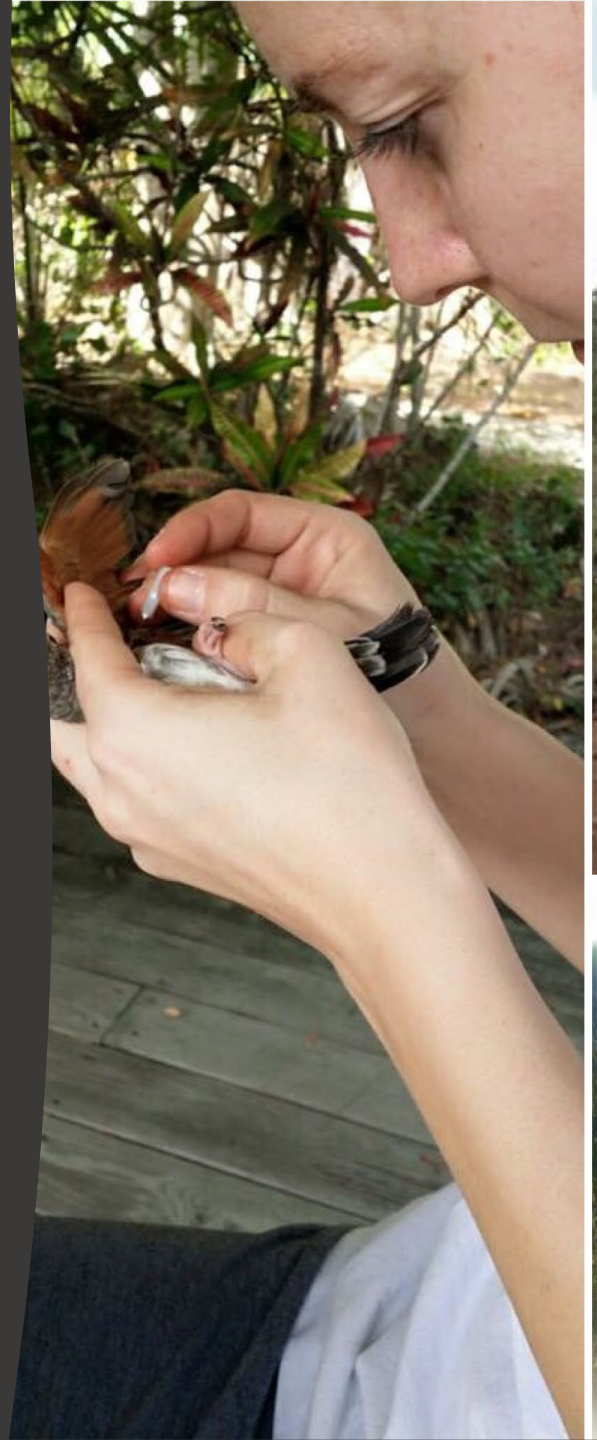
- **H_2: *M. stolidus* is replacing *M. sagrae* in the Bahamas**
  - Prediction: Replacement only detectable if occurring within past 130 yrs. in 1 of 7 locations sampled at both time points
- **H_3: *M. stolidus* and *M. sagrae* are hybridizing**
  - Prediction: Hybridization only be detectable if occurring within past 130 yrs. in 1 of 7 locations sampled at both time points

Inspiration for Research

Inspiration for Research

- **A suspicious capture…**
  - Documented distribution suggested that the individual was *M. sagrae*
  - However, the plumage and coloration argued that the individual was *M. stolidus*

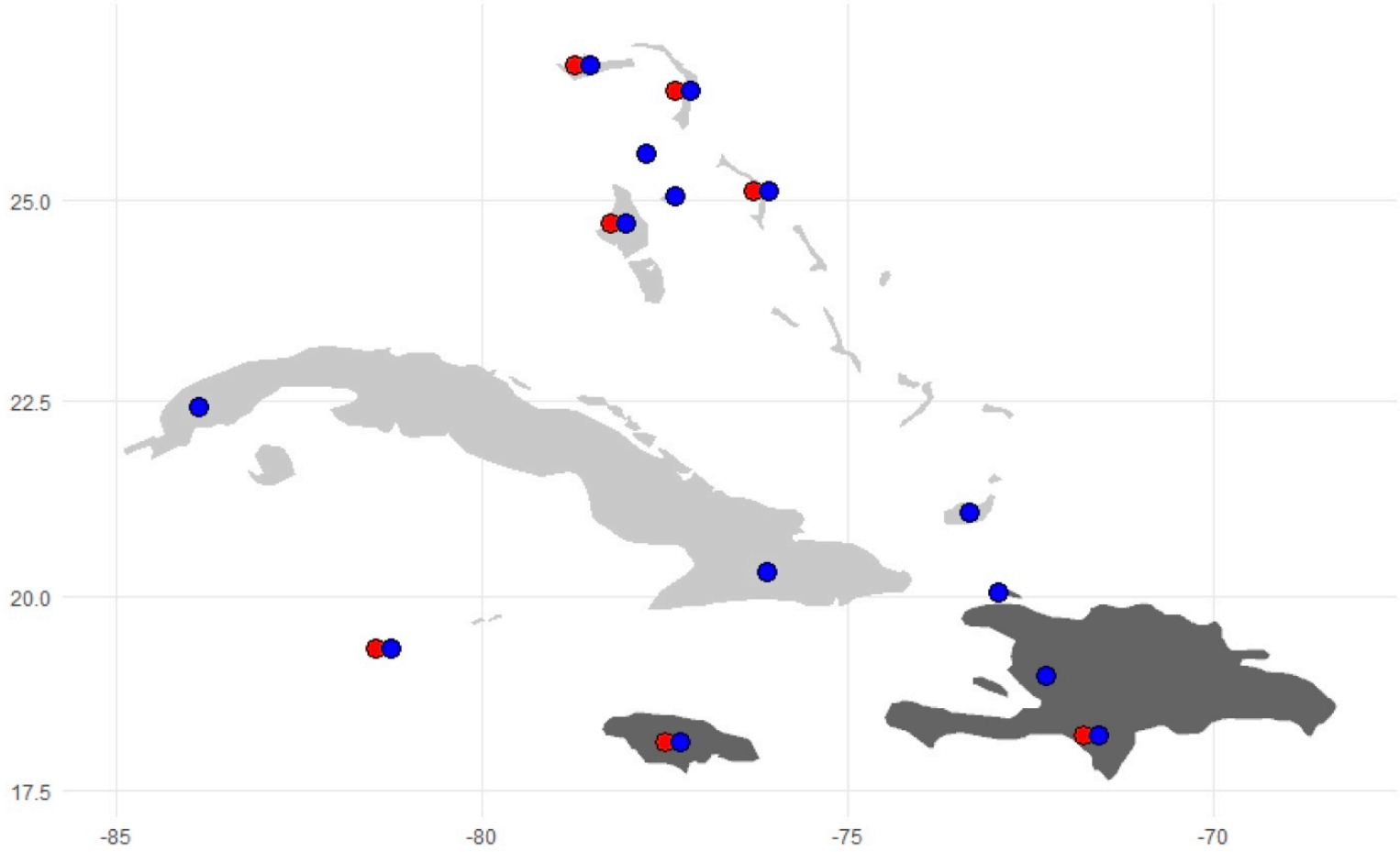
- **It was an event that would inspire further research…**



- ***Myiarchus sagrae***
  - Upper parts are olive brown with a short crest
  - Greyish white throat transitioning to a dull white belly

- ***Myiarchus stolidus***
  - Upper parts are olive brown with a short crest
  - Bright white throat
  - Characterized by pale yellow abdominal plumage

Ricklefs Lab 2016 Field Expedition to Bahamas



- **Light gray islands**
  - *M. sagrae* distribution
- **Dark gray islands**
  - *M. stolidus* distribution
- **Red dots**
  - Sampling locations for the Ricklefs collection
- **Blue dots**
  - Sampling locations for museum specimens

*Overlapping locations displaced for clarity

Species Range Map & Sampling Localities

Methods & Applications

Methods & Applications

- **Preliminary Analysis**
- **Data Set Expansion**
- **Morphological Variation Analysis**

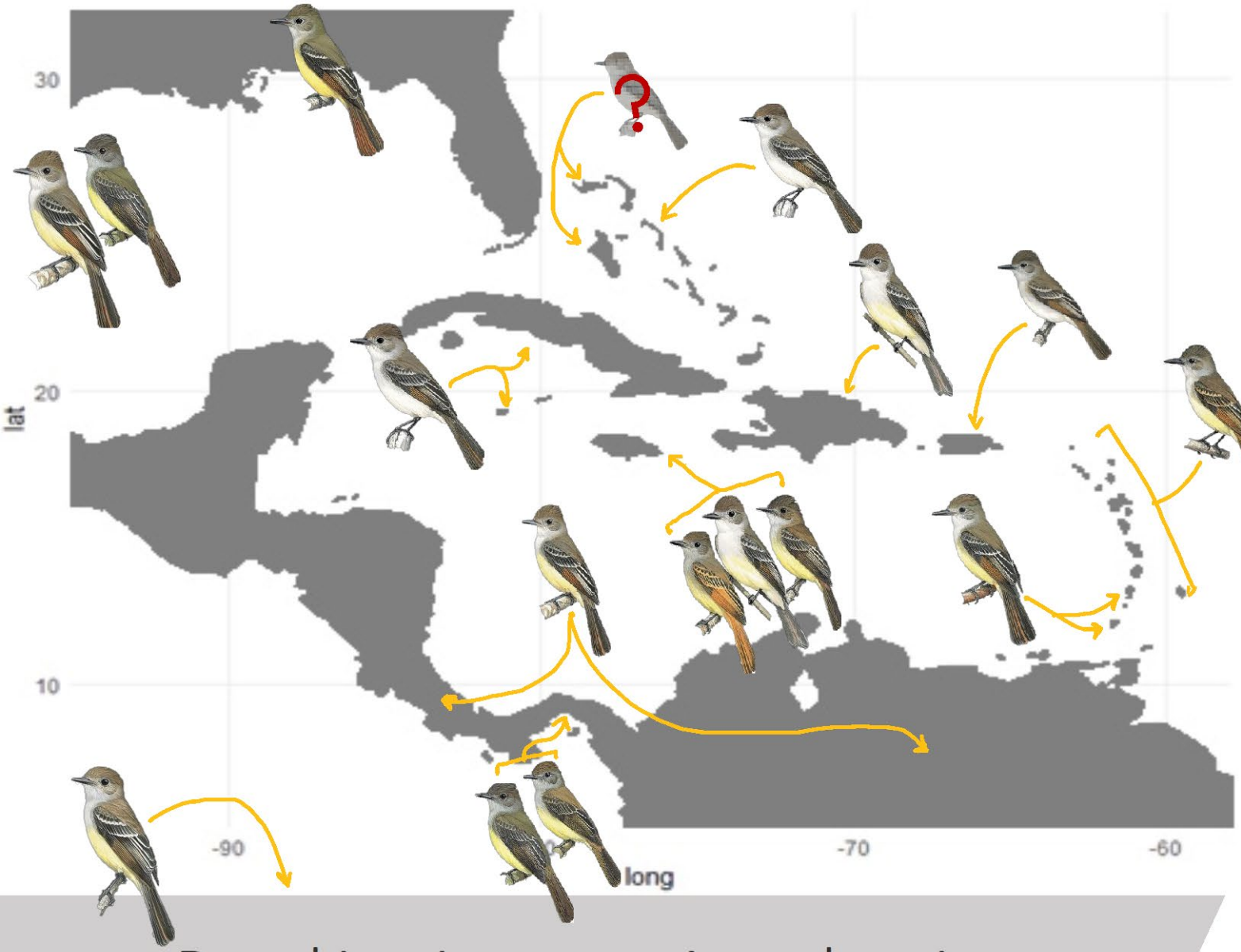
Preliminary Analysis

Methods & Applications
[Preliminary Analysis]

- **Nuclear DNA Analysis**
  - Vimentin gene *(VIM)*
    - Type III intermediate filament (IF) protein
      - Expressed in the mesenchymal cells of all animals
    - Plays a significant role in supporting and anchoring position of organelles in the cytosol

Methods & Applications
[Preliminary Analysis]

- **Mitochondrial DNA (mtDNA) Analysis**
  - Mitochondrial cytochrome *b* gene *(CYTB)*
    - A protein found in the mitochondria of eukaryotic cells
      - Part of the electron transport chain
    - Used for determining phylogenetic relationships between organisms due to its sequence variability



Preliminary Analysis of *CYTB* Variation

- **Conflicts with species delimitation**
  - *M. sagrae*
  - *M. stolidus*

- **Analysis of Genetic Relationships**
  - 104 individuals of 14 *Myiarchus* species
    - Distributed across 20 islands of the West Indies
    - Inland locations around the Caribbean Basin

Data Set Expansion

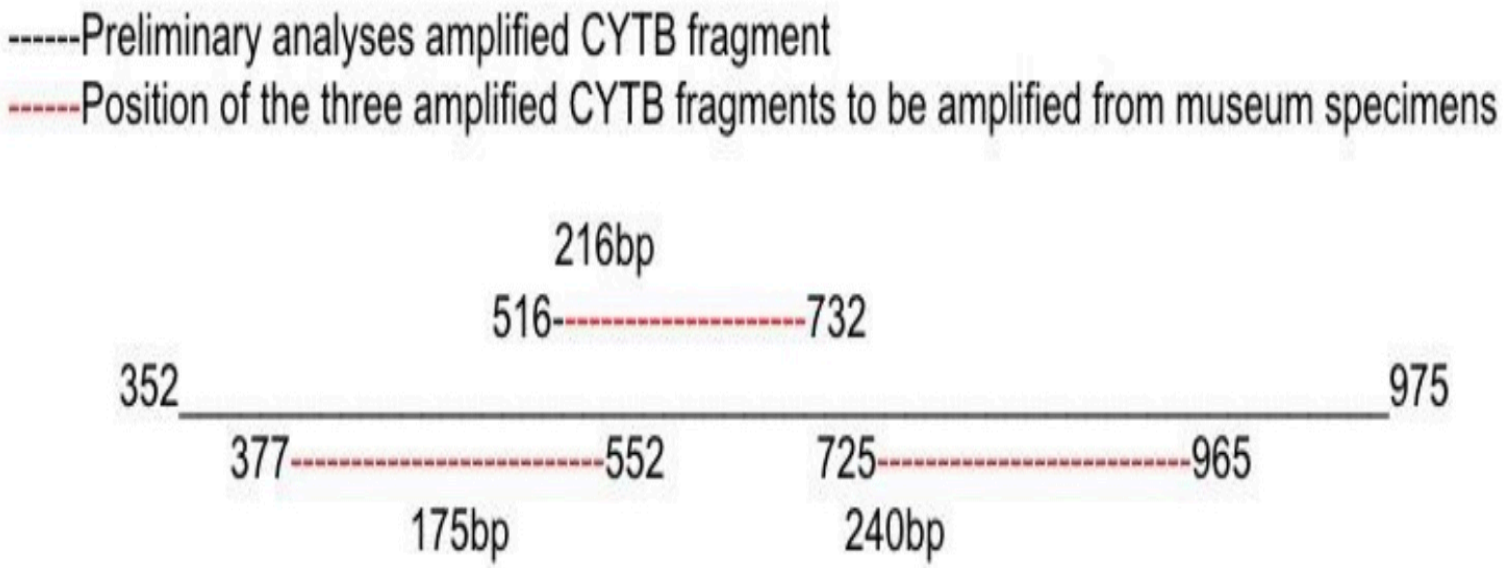
Methods & Applications [Data set Expansion]

- **Ancient DNA Extraction**
  - DNA Extraction from toe pads of museum specimens
    - Granted from the Field Museum of Natural History in Chicago

Methods & Applications
[Data set Expansion]

- **Mitochondrial DNA (mtDNA) Analysis**
  - Cytochrome b ($CYTB_{frag}$)
    - Museum specimen age was ~130 years
      - Not ideal for typical PCR and gene sequencing
        - Specific primers and protocols were designed

Application of CYTB Amplification in Each Collection



- ***CYTB* Function in Contemporary Collection**
  - Amplified a ~600bp continuous fragment

- ***CYTB* Function in Ancient Collection**
  - Concatenated fragments amplified from newly developed primers
    - Reduce the length of target fragments
    - Maximizing data recovery

Morphological Variation Analysis


M. tagoas hesperiens

Methods & Applications [Morphological Variation Analysis]

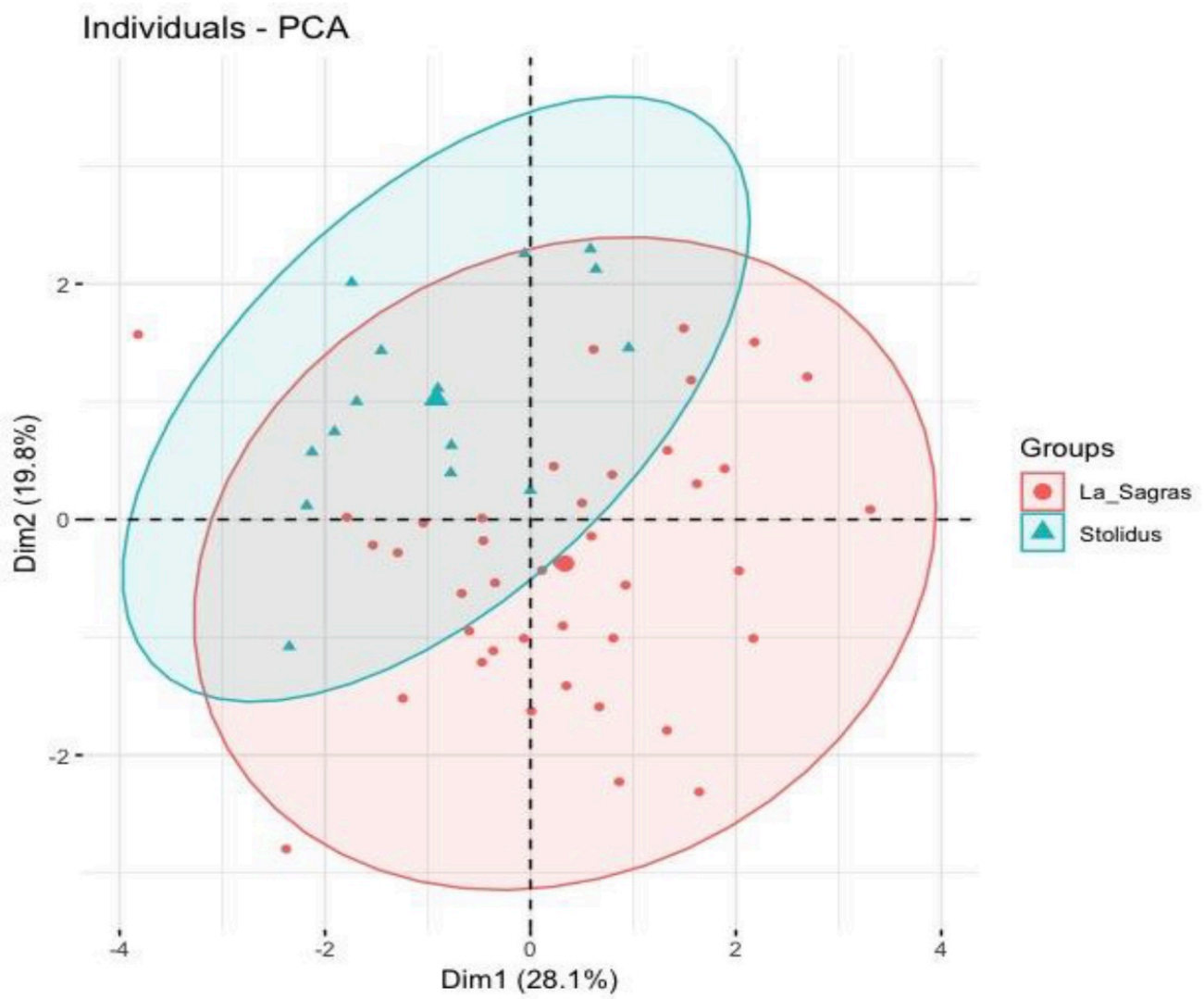
- **Analysis 1st Phenotypical Character**
  - Tests species limits between *M. stolidus* and *M. sagrae*
    - Attention to dimensions of bill size and body size
      - Principal components analysis
      - Discriminant function analysis


A3


A3
Cascades


405
406
383
384


A3
Cascades
ENVIRO100



Principal Components Analysis of Morphological Variation

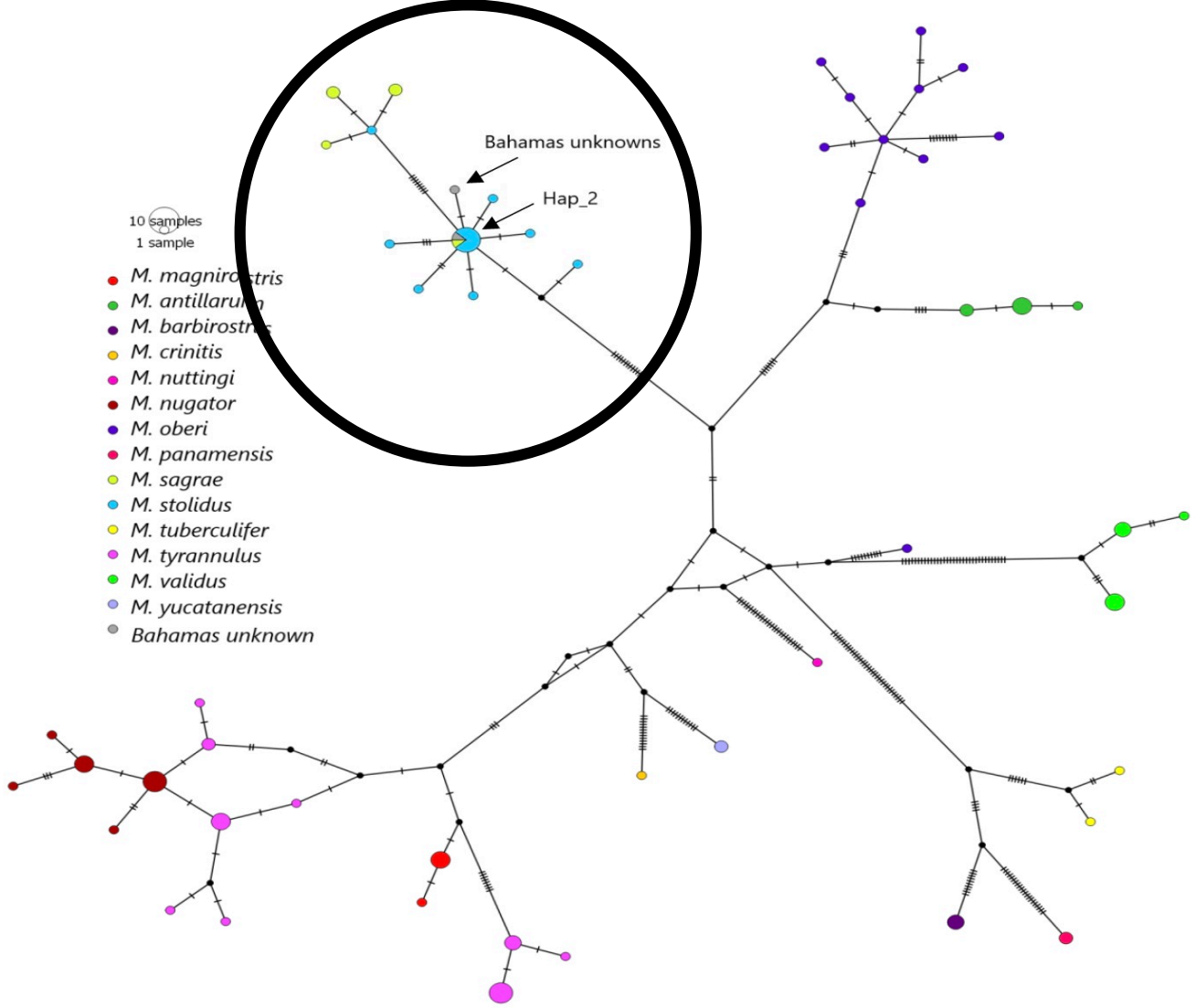
- **Morphometric**
  - Measuring:
    - Bill length
    - Bill depth
    - Bill width
    - Tarsus length
    - Wing Chord
    - Tail Length
    - Body Length

- **Preliminary PCA Results**
  - Indicate substantial overlap in morphological space

Results

Results

- **Lab work is currently in progress…**
  - Upon reviewing the literature
    - Joseph et al[1] reports:
      - *M. sagrae* as paraphyletic within a comprehensive phylogeny
      - *M. stolidus* forms a polyphyletic grouping sharing a most recent common ancestor with *M. sagrae*



Median-joining Haplotype Network of *CTYB*

- **Ignoring ROI…**
  - Congruent with previous phylogenetic hypotheses

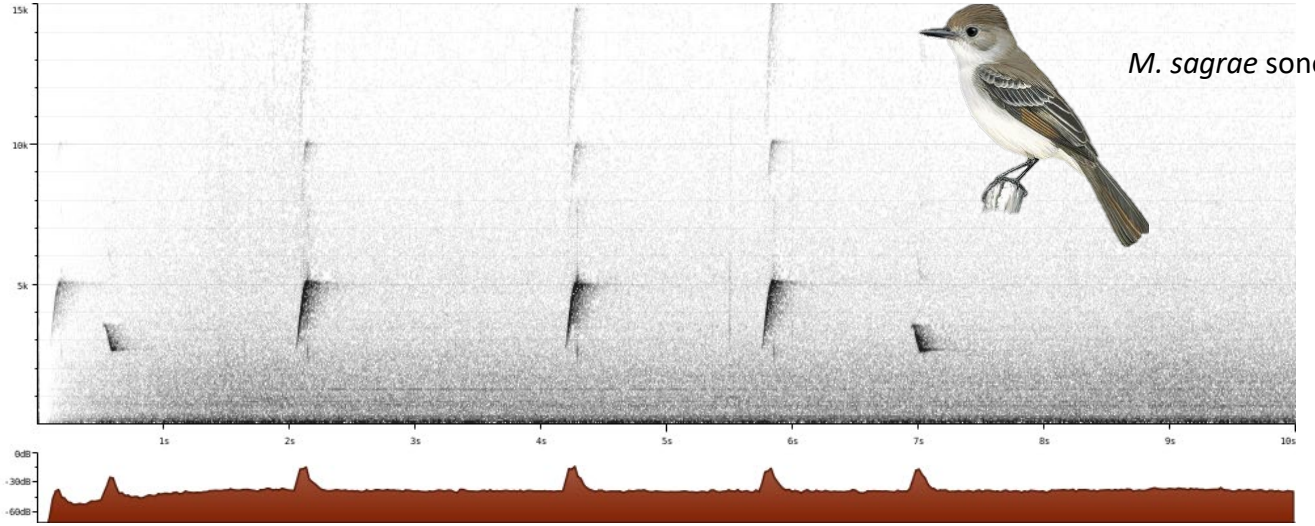
- **Region of Interest**
  - Demonstrates several genetically inconsistent species assignments
    - One haplotype (Hap_2) is shared by individuals classified as either of these species
    - Unknown individual from Bahamas
  - 8 point mutations separate Hap_2 from *M. stolidus* haplotype, which itself gives origin to three additional *M. sagrae* haplotypes

Upcoming Research

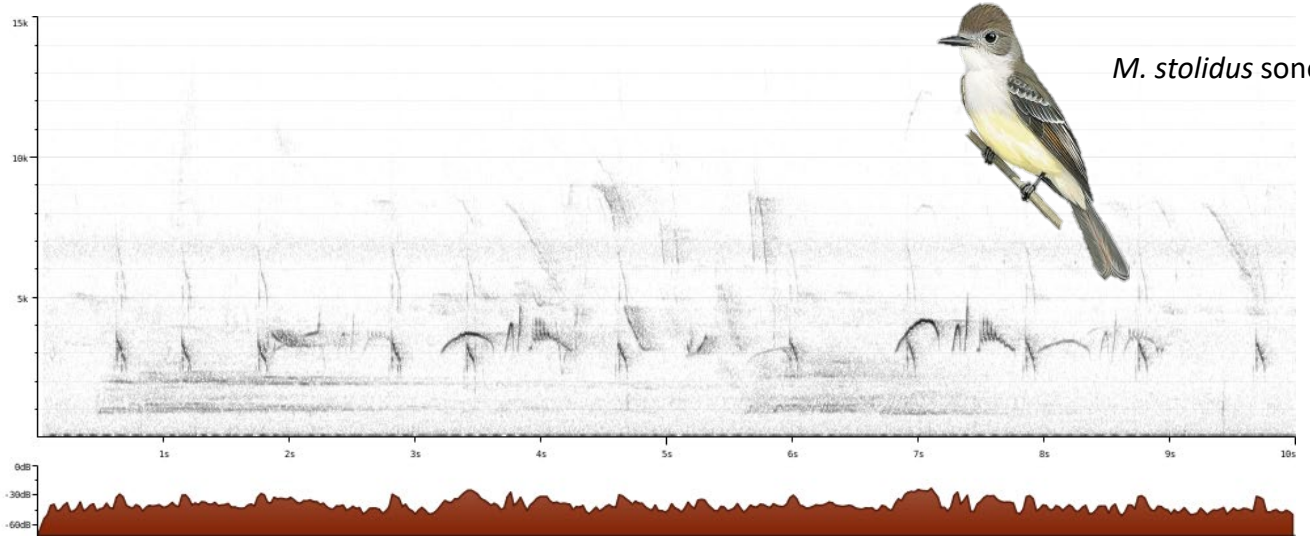
Upcoming Research [Vocal Variation]

- **Analysis of 2nd Phenotypic Character**
  - To assess vocal variation between *M. stolidus* and *M. sagrae*
    - Tyrant flycatchers are suboscine birds
    - We have obtained vocal records from the Macaulay Library at the Cornell Laboratory of Ornithology
      - Compare vocalizations from 6 different locations

The Cornell Lab of Ornithology
Macaulay Library



M. sagrae sonogram



M. stolidus sonogram

- **Macaulay Library at the Cornell Laboratory of Ornithology**
  - Audio recordings for sonogram analysis
    - Total song length
    - Syllable length
    - Frequency of maximum amplitude
    - Bandwidth
  - Discriminant function analysis

- **Vocal recordings localities**
  - Six locations spread throughout the Greater Antilles and Bahamas

Audio Recordings for Sonogram Analysis

The Cornell Lab of Ornithology
Macaulay Library

Upcoming Research [UCE Capture]

- **Ultra-conserved element (UCE) capture**
  - 7/14 sampling locations have collections separated by 130 years
    - Extracted DNA for a subset of samples (one/collection/location wherever possible)
      - Inform about any phylogenetic population changes that could have occurred over this interval

Final Thoughts

Final Thoughts

- **Current Goal**
  - Resolve taxonomic discrepancies and recover the distributional histories of *M. sagrae* and *M. stolidus*
    - Part of a comprehensive assessment of avian phylogeography in the West Indies
  - Further phylogenetic analysis would improve our phylogeographical understanding of these two species

UMSL

WHITNEY R. HARRIS WORLD ECOLOGY CENTER at the University of Missouri-St. Louis

The Cornell Lab of Ornithology Macaulay Library

FIELD MUSEUM

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