

Environmental DNA (eDNA) applications for detecting bat species



 **Daniel E. Sanchez**



Who we serve

We use the newest genetic technologies to assist wildlife managers and researchers.

IN > Bat Ecology and Genetics Lab > Species from Feces



SPECIES FROM FECES



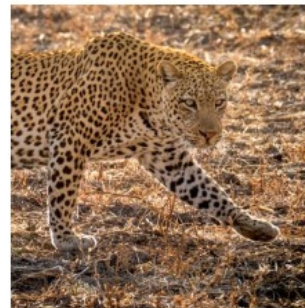
Our Genetic ID services:

Species from Feces

Bat Carcass ID for wind farms

Species detection via environmental DNA

We work with any taxon



Overview

DNA metabarcoding

eDNA assays

Consultation

Interpretation of results

>400 completed projects for agencies, consultants, universities, and NGO's

Broad taxonomic scope

>10 SFF publications

Who we are: wildlife geneticists, ecologists, and bioinformaticians

Our mission: to offer species identification services to further wildlife research, management, and conservation globally



Emma Federman



Savannah Marriott



Dr. Daniel Sanchez



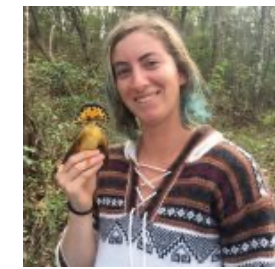
Dr. Faith Walker



Dr. Carol Chambers



Ellen Liebig



Morgan Hughes



Emma Froehlich



Salix Scoresby



SPECIES FROM FECES



Anna Riley



Colin Sobek



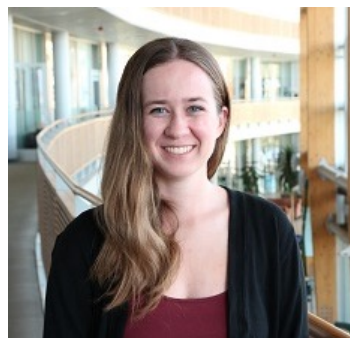
Austin Dikeman



Jacque Lyman



Meagan Owens



Samantha Hershauer



Abby Tobin



Dr. José Gabriel Martinez-Fonseca



Jordyn Upton



Kiera Majzner



Regina Go

Outline

- **Field of eDNA**
 - Foundations and history
 - Concepts and considerations
- **eDNA methods for bat detection**
 - Where can we get it from?

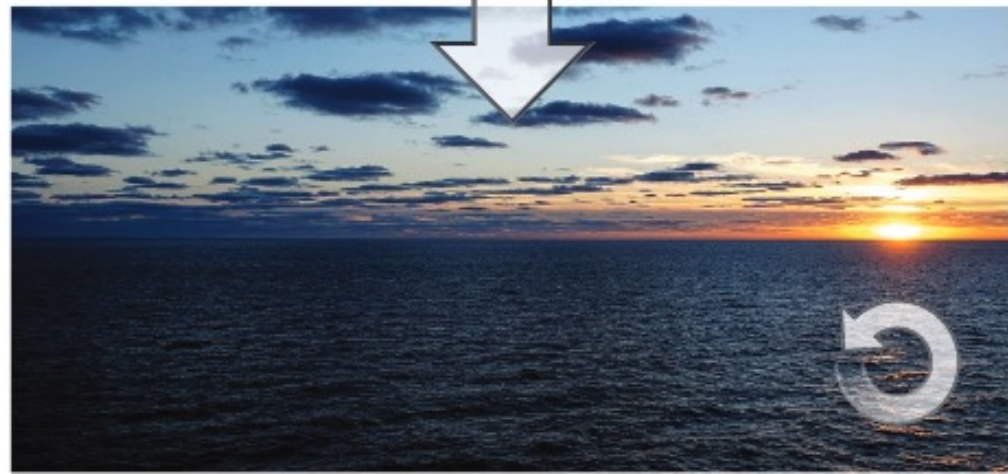
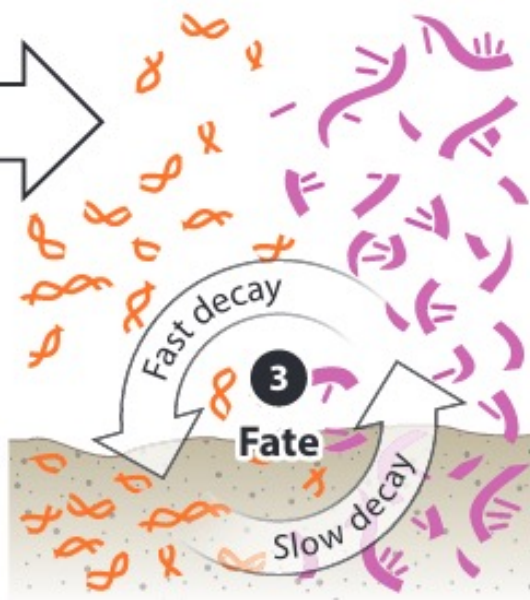
Terrestrial



Freshwater



Marine



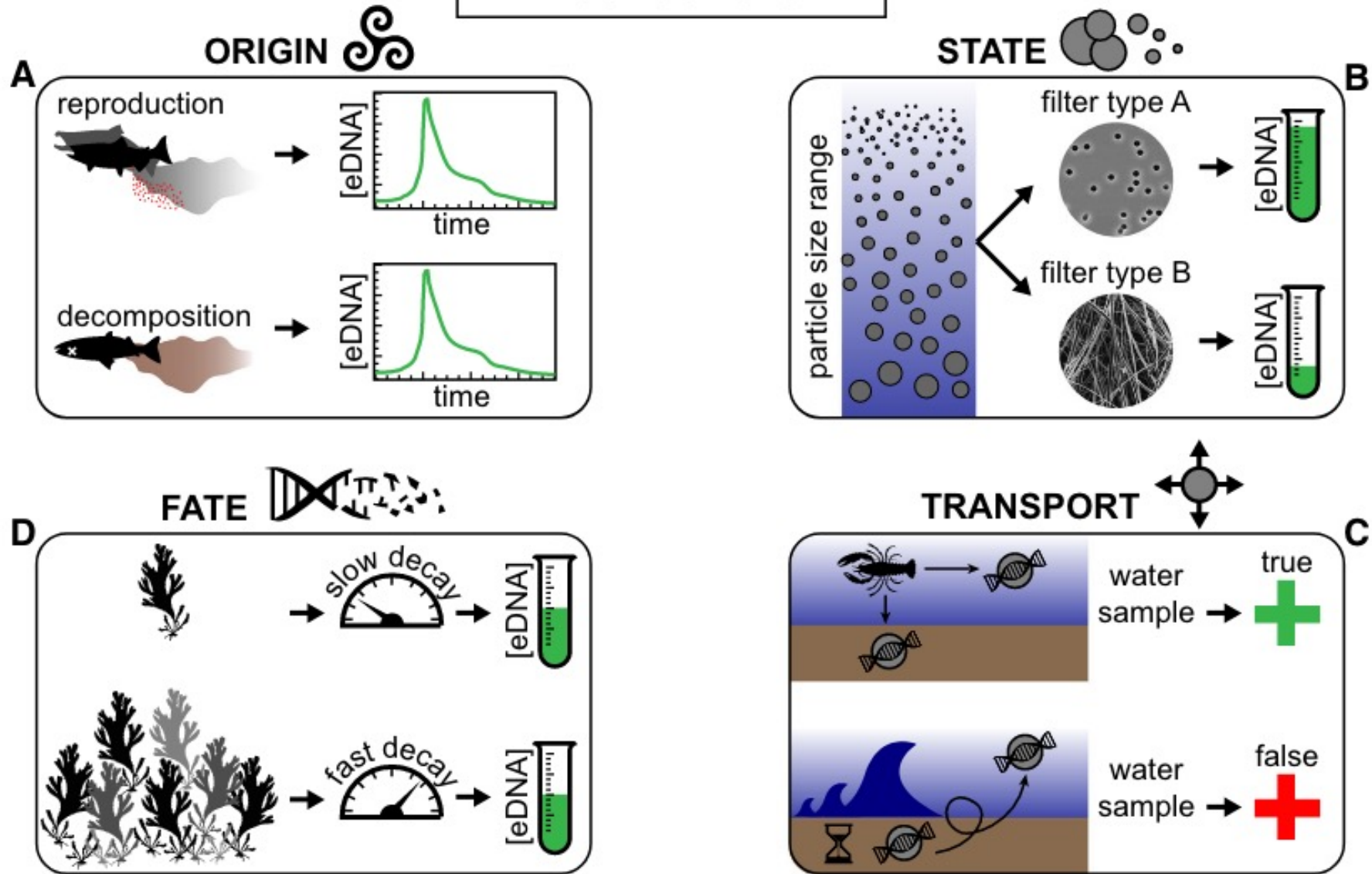
eDNA

Cellular

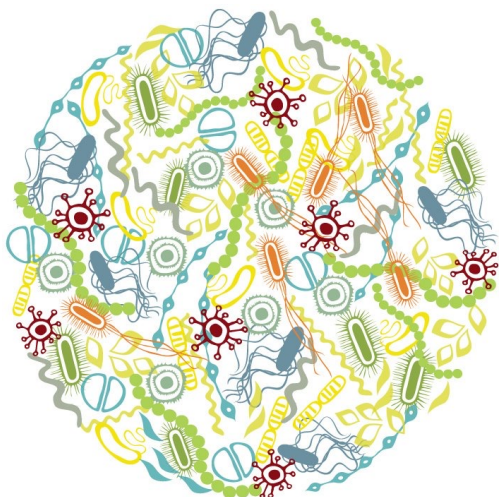
Extracellular

Cristescu & Hebert (2018)

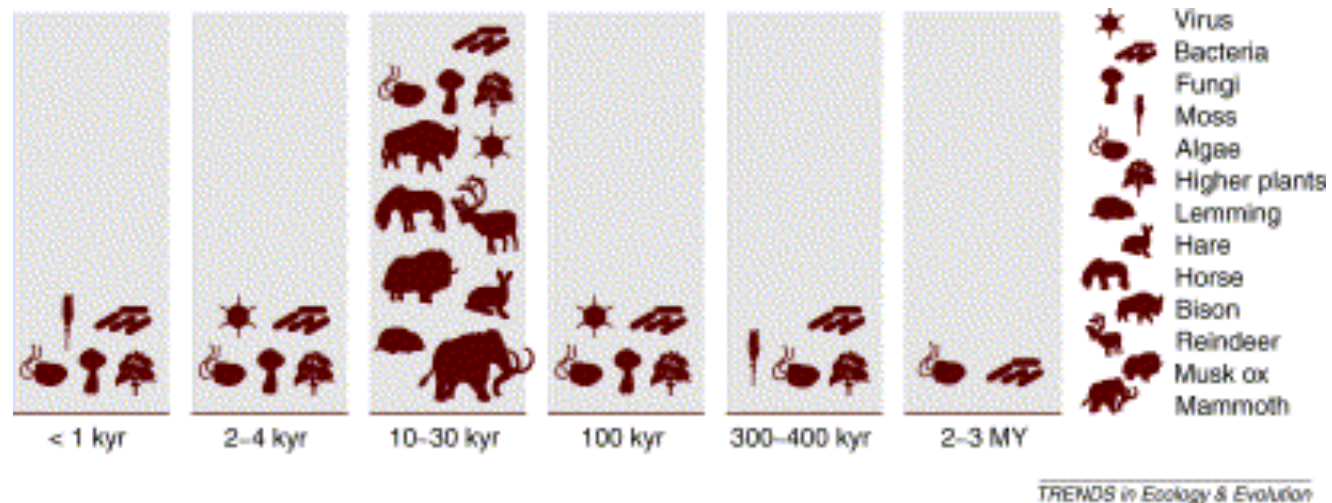
THE ECOLOGY of eDNA



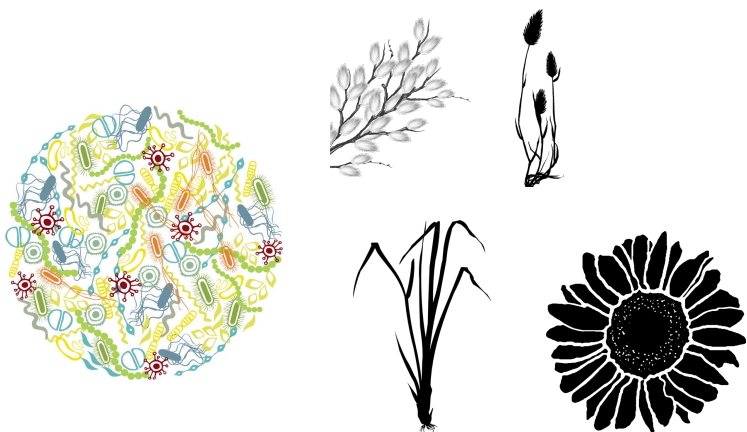
Microbial eDNA (1983 – 1991)



Macrobial eDNA from ancient permafrost (Willerslev et al. 2004)



Community eDNA: the NGS-era (2006-2010)



Targeted eDNA: invasive species (Ficetola et al. 2008)



American bullfrog

First eDNA reviews (2012-2014)



eDNA: an exciting and developing field

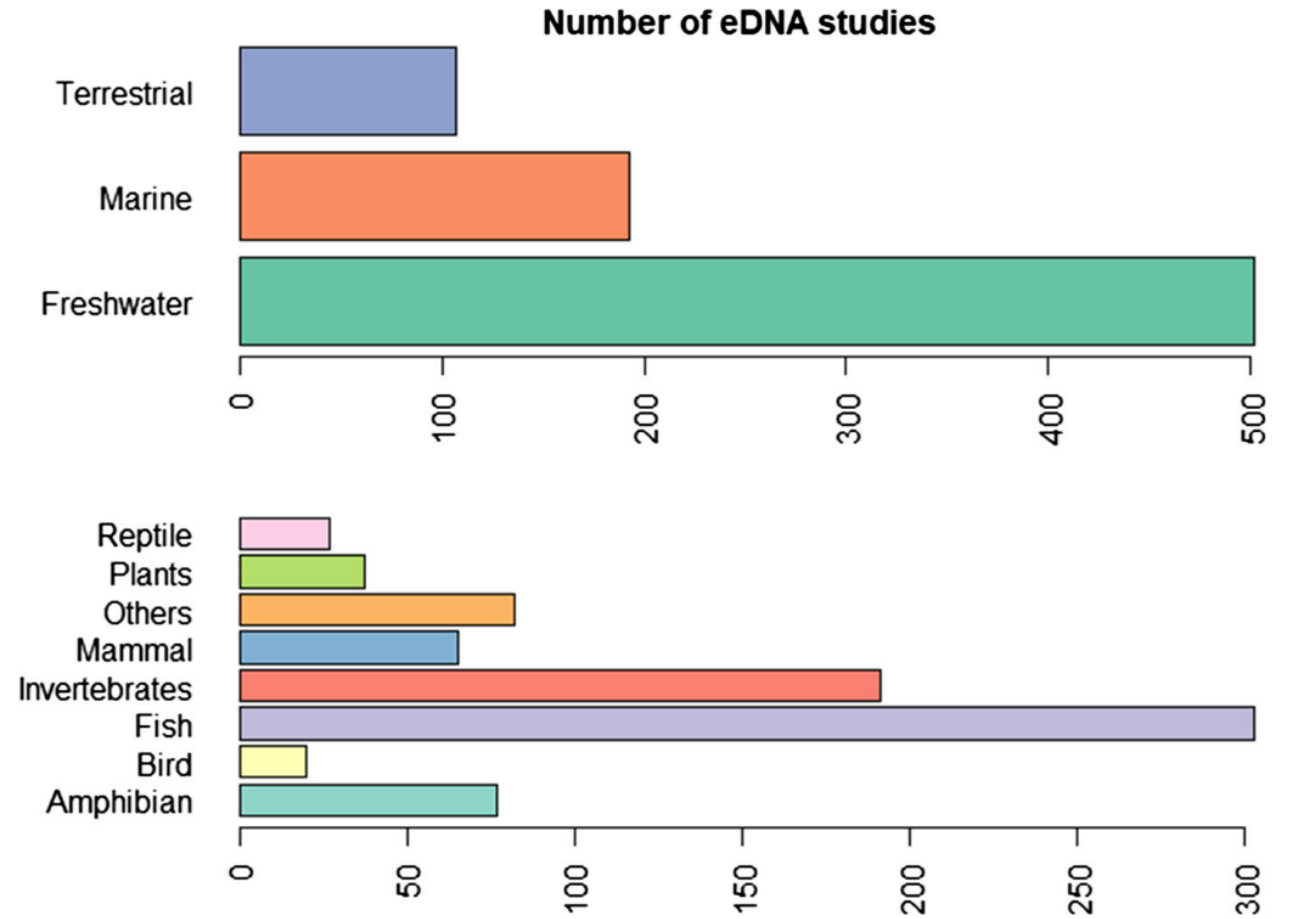
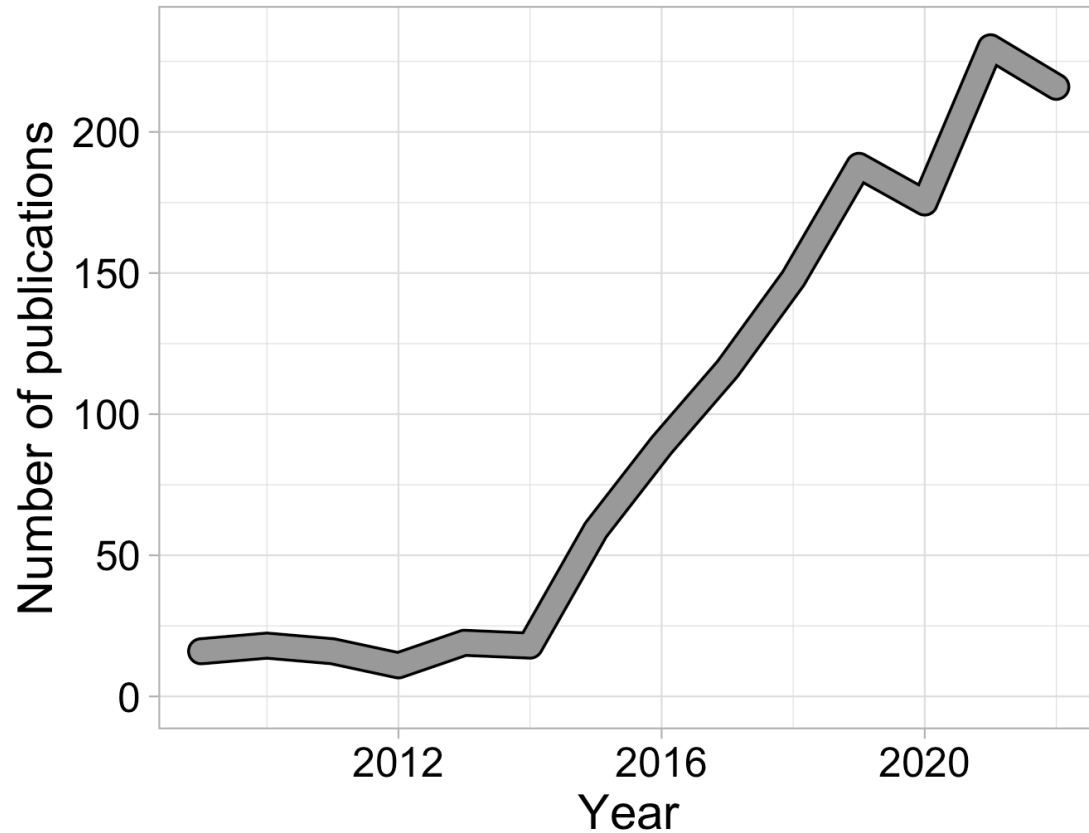


Fig. 2 Number of studies using environmental DNA (eDNA) recovered from a literature search with the words 'environmental DNA' OR 'eDNA' for the period between 1 January 2008 and 31 December 2019 that utilized a different organismal group and ecosystem

Beng & Corlett (2020)

eDNA: opportunities, challenges, solutions

Application	Promises	Challenges	Existing/promising solutions
Ecology & Biomonitoring	<p>Quantify biodiversity</p> <p>Cost-effective</p> <p>Establish baselines</p> <p>Understand biotic and abiotic influences (turn-over, disturbance)</p>	<p>Abundance estimation</p> <p>Incomplete reference libraries</p> <p>Spatial and temporal acuity</p> <p>False negatives</p>	<p>Shotgun metagenomics</p> <p>OTUs</p> <p>Continue to build and curate DNA reference libraries</p> <p>Study origin, state, transport, and fate</p>
Conservation biology	<p>Cost-effective</p> <p>Non-invasive sampling</p>	<p>Population viability</p> <p>demography</p> <p>life-stage</p> <p>False positives</p> <p>Skepticism</p> <p>Decision-making frameworks</p> <p>Cost-effective ≠ inexpensive</p>	<p>eRNA</p> <p>Standardized protocols and guidelines from planning to field to lab to analysis</p> <p>Complement traditional survey</p>
Invasion biology	<p>Early surveillance</p> <p>Cost-effective</p> <p>Detect from low abundance</p>	<p>Data turn-over</p>	<p>Collaboration at every step (manager, laboratory, bioinformatician, statistician)</p> <p>Allow process to be iterative</p>



WORKFLOW

Study design

In the field

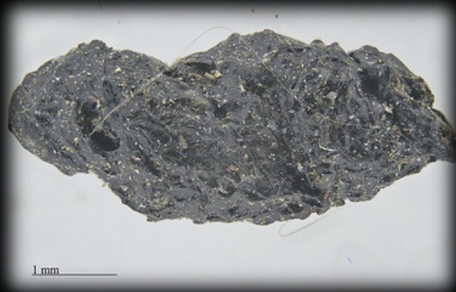
In the laboratory

At the keyboard

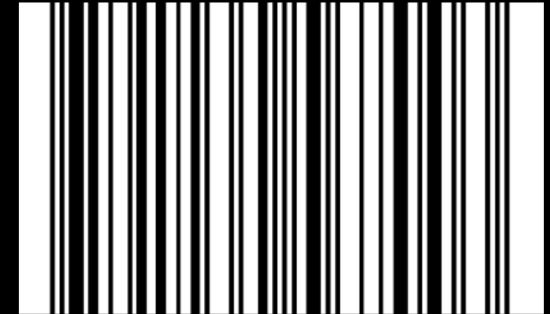
Common markers for DNA barcoding

Organism Group	Marker Gene
Animals	COI, Cyt B, 12S rRNA, 16S rRNA
Plants	ITS2, rbcL
Bacteria	16S rRNA (e.g., microbiomes)
Fungi	ITS1, ITS2

What bat species is this?



```
TTTCTTCATGGTCATACCTATTATAATC
GGAGGCTTCGGAAACTGATTAGTCCCC
TTAATGATTGGAGCTCCCGATATAGCT
TTCCCCCGAATGAATAATATGAGTTTC
TGACTCCTTCCACCCTCCTTCTACTA
CTCTTGGCCTCTTCCACAGTAGAAGCC
GGGGCAGGTACTGGATGGACAGTATAC
CCCCCTTAGCT
```



Query: unlabeled_sequence

Top Hit: Chordata, Mammalia, Chiroptera, Vespertilionidae, Corynorhinus, *Corynorhinus townsendii* (100%)

Search Result:

The submitted sequence has been matched to *Corynorhinus townsendii*. This identification is solid unless there is a very closely allied congeneric species that has not yet been analyzed. Such cases are rare.

A species page is available for this taxon:

[SPECIES PAGE](#)

Closest matching BIN (within 3%):

[BIN PAGE](#)

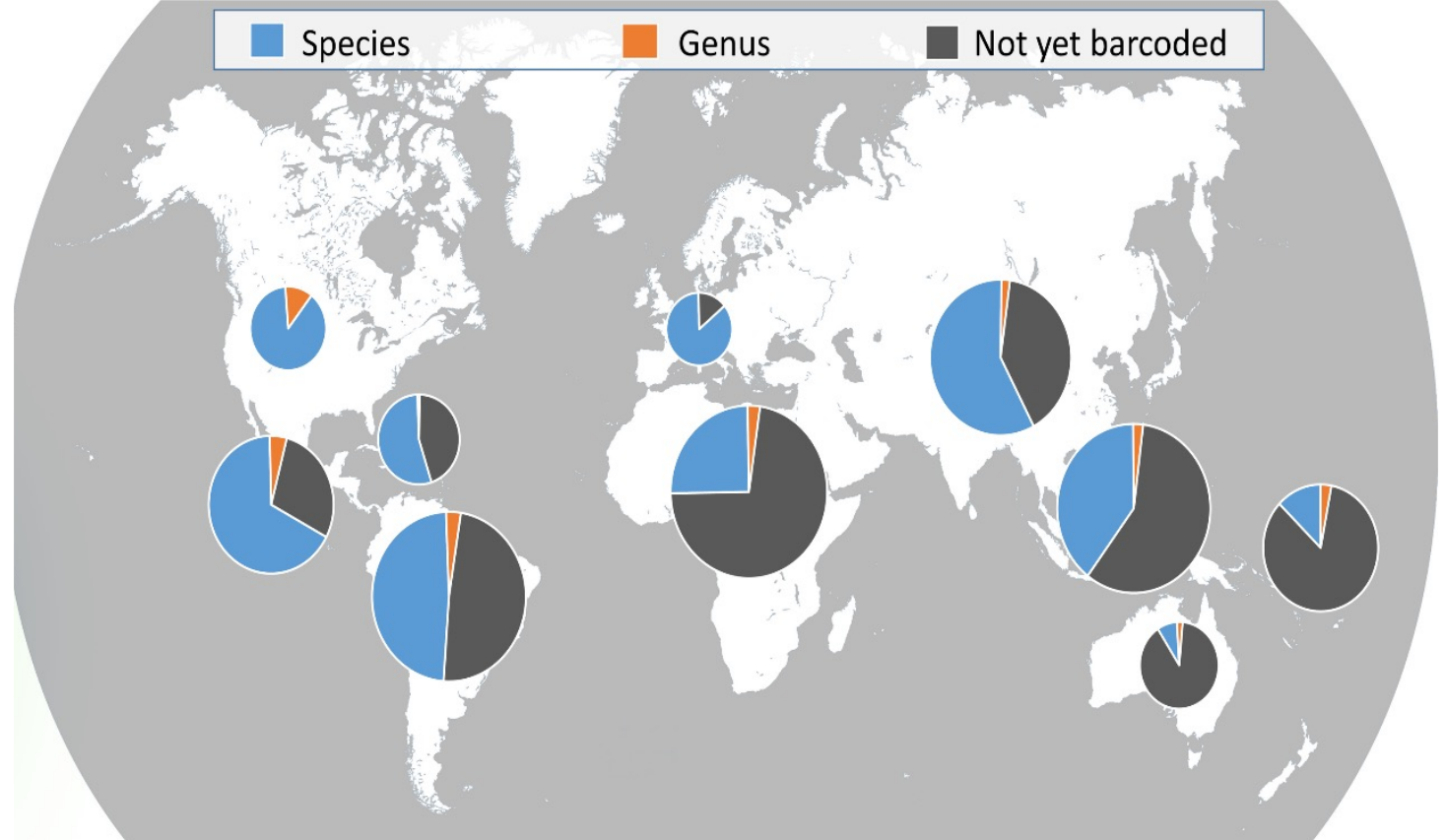
For a hierarchical placement - a neighbor-joining tree is provided:

[TREE BASED IDENTIFICATION](#)

BOLDSYSTEMS

Species From Feces: Order-Wide Identification of Chiroptera From Guano and Other Non-Invasive Genetic Samples

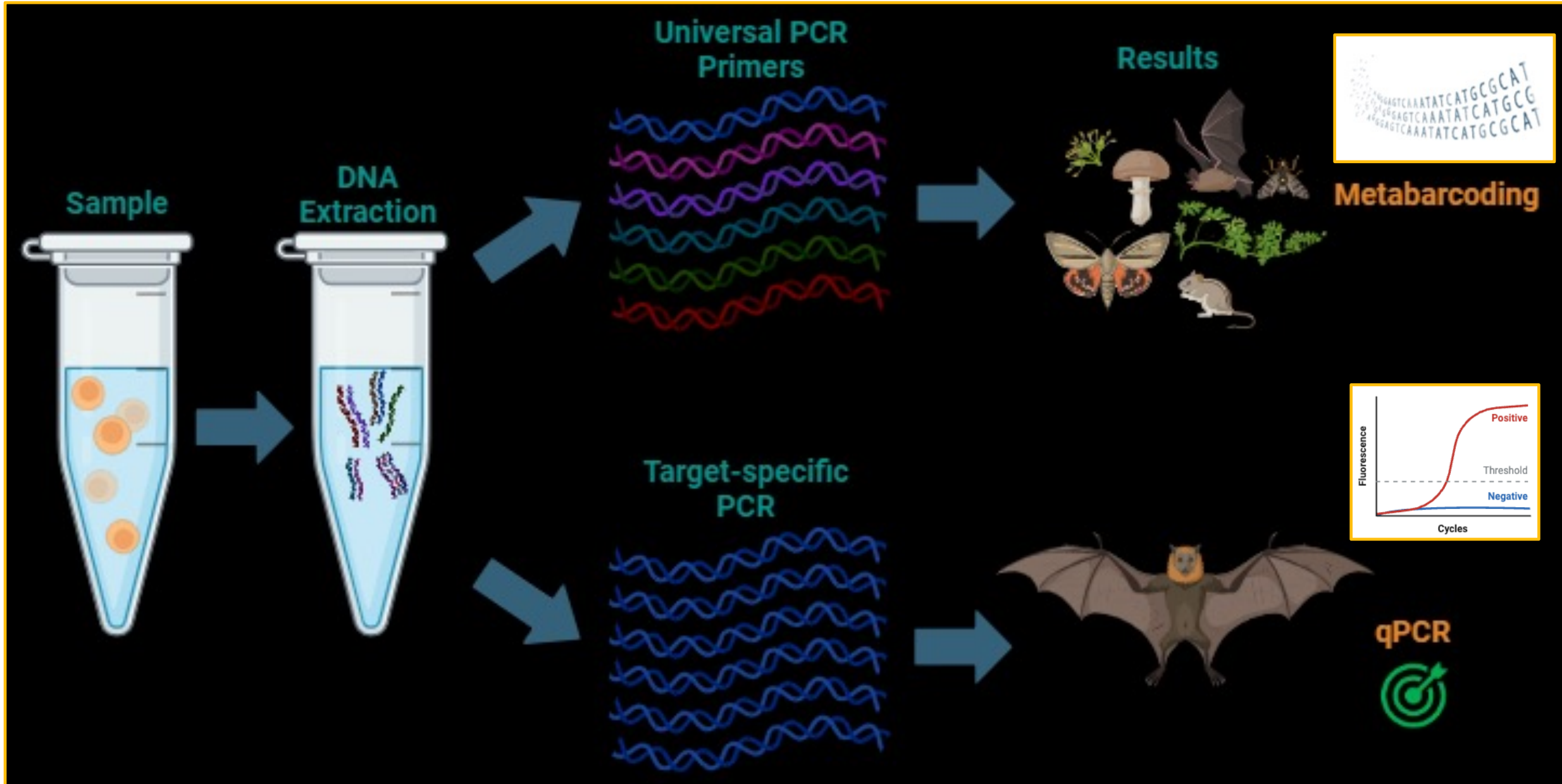
Faith M. Walker^{1,2*}, Charles H. D. Williamson², Daniel E. Sanchez^{1,2}, Colin J. Sobek^{1,2}, Carol L. Chambers¹






92%

Common eDNA detection methods

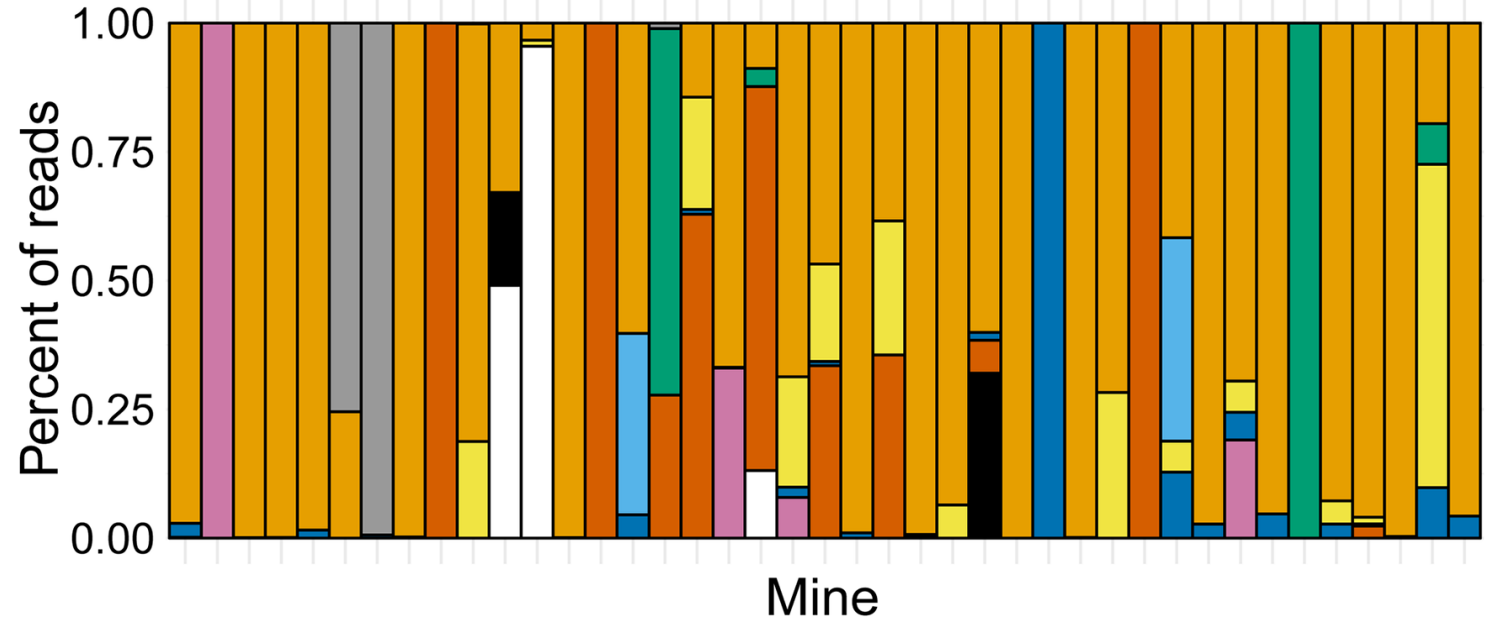
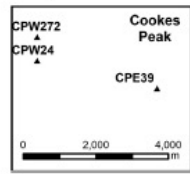
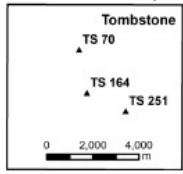
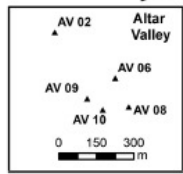
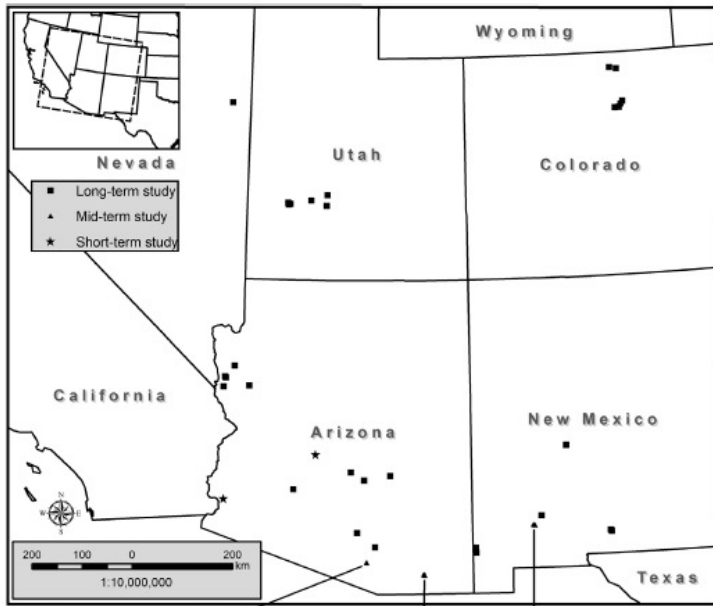








A fecal sequel: Testing the limits of a genetic assay for bat species identification

Faith M. Walker ^{1,2}, Abby Tobin¹, Nancy B. Simmons³, Colin J. Sobek^{1,2}, Daniel E. Sanchez^{1,2}, Carol L. Chambers¹, Viacheslav Y. Fofanov⁴

Published: November 14, 2019





- | | |
|-----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
|  <i>Antrozous pallidus</i> |  <i>Myotis leibii/californicus/ciliolabrum</i> |
|  <i>Corynorhinus townsendii</i> |  <i>Myotis velifer</i> |
|  <i>Idionycteris phyllotis</i> |  <i>Myotis volans</i> |
|  <i>Macrotus californicus</i> |  <i>Parastrellus hesperus</i> |
|  <i>Myotis evotis/thysanodes</i> |  <i>Tadarida brasiliensis</i> |

First eDNA detection of bats from sediment (soil, sand, and rocks)



Common vampire bat

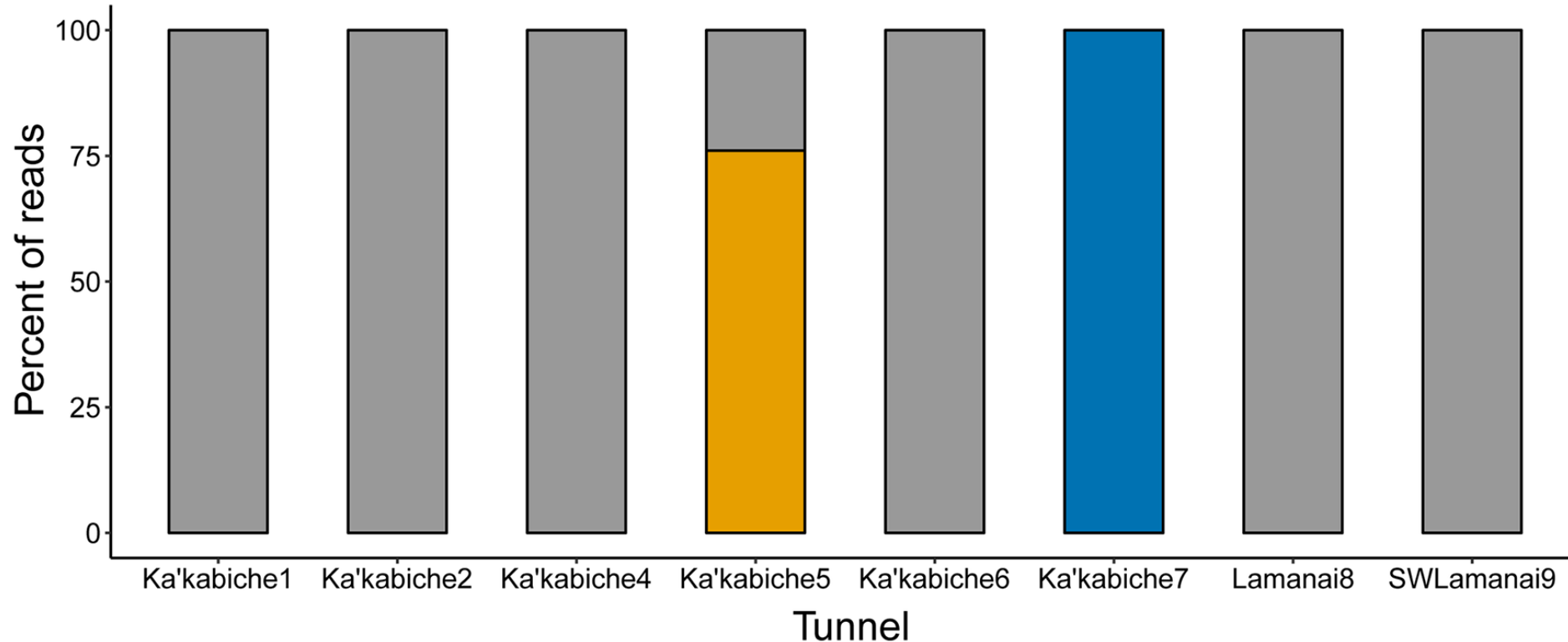
Big-eared woolly bat

Mexican greater funnel-eared bat



Inconspicuous eDNA sources?

Walker et al. (2019)



First eDNA detections of bats from water, soil, and air

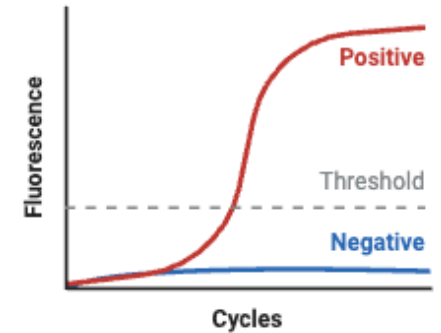
Molecular genetic analysis of air, water, and soil to detect big brown bats in North America

Natasha R. Serrao^{a,b}, Julie K. Weckworth^{a,b,*}, Kevin S. McKelvey^a, Joseph C. Dysthe^a, Michael K. Schwartz^a

^a National Genomics Center for Wildlife and Fish Conservation, Rocky Mountain Research Station, United States Forest Service, Missoula, MT, United States of America

^b Wildlife Biology Program, Department of Ecosystem and Conservation Sciences, W. A. Franke College of Forestry and Conservation, University of Montana, Missoula, MT, United States of America

Biological Conservation 261 (2021) 109252



Known occupation



1/4 samples: 1 site Natural site



1/5 samples: 1 site Natural site



6/7 samples: 1 site Captive colony

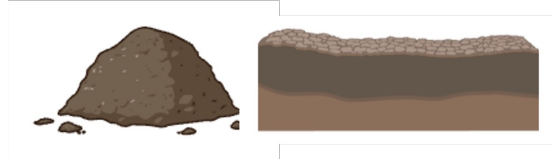
Eptesicus fuscus





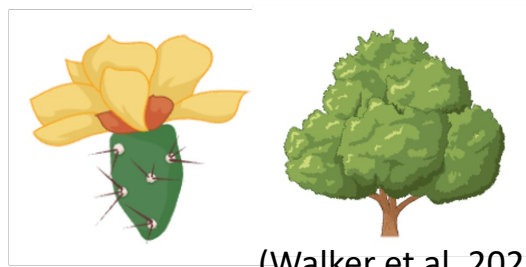
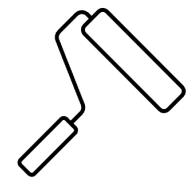
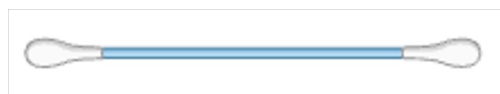
WATER

(Serrao et al. 2021; Marshall et al. 2022)



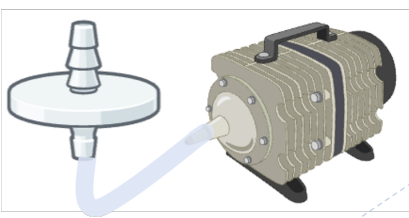
SOIL/SEDIMENT

(Walker et al. 2019; Serrao et al. 2021; Allen et al. 2023)



RESOURCES/HABITAT

(Walker et al. 2022; Allen et al. 2023)



Active

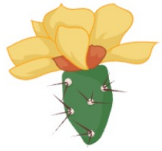
Passive?...
Stay tuned



AIR

(Garrett et al. 2022; Garrett et al. 2023)





RESOURCES/HABITAT

Can we detect nectar-feeding bats from agave flowers?



Open Access Article

Endangered Nectar-Feeding Bat Detected by Environmental DNA on Flowers

by Faith M. Walker^{1,2,*} Daniel E. Sanchez^{1,2}, Emma M. Froehlich^{1,2}, Emma L. Federman^{1,2}, Jacque A. Lyman^{1,2}, Meagan Owens^{1,2} and Kristen Lear^{3,4}



animals

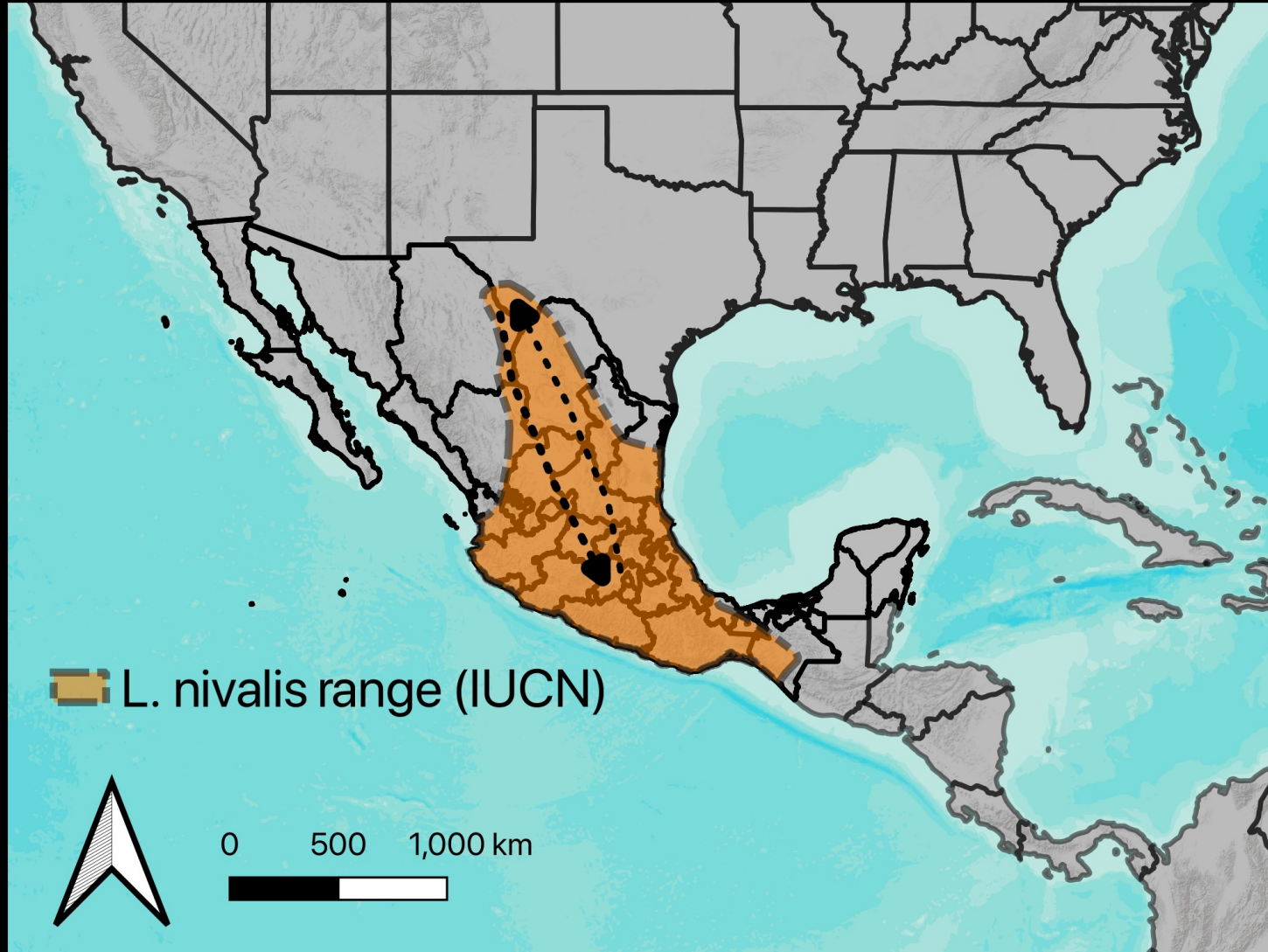


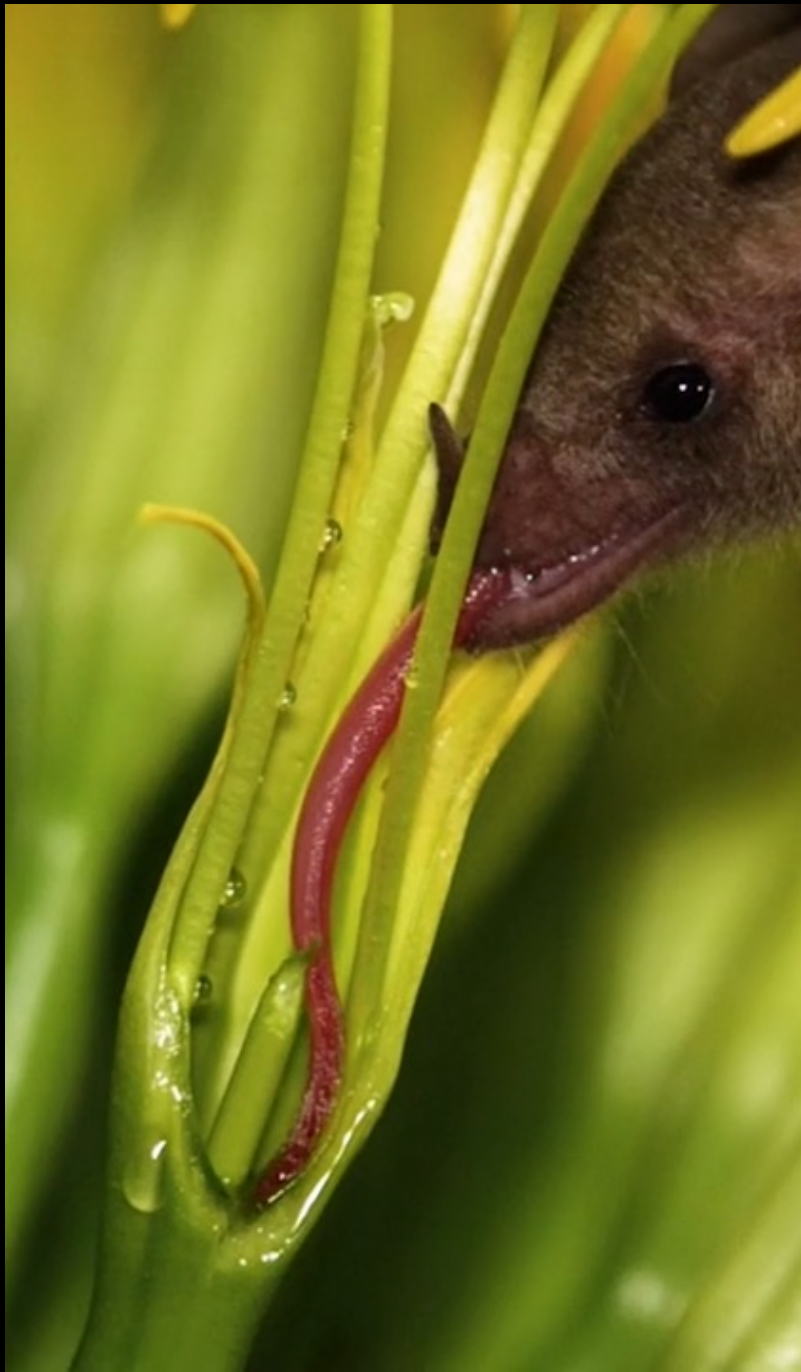
Kristen Lear



Mexican long-nosed bat (*Leptonycteris nivalis*)

- Unknown migration corridors





1001

Swabs

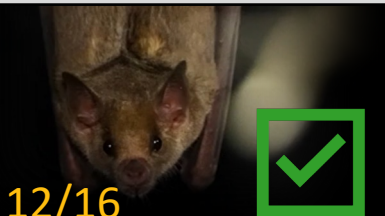


eDNA: Metabarcoding

Capturing a desert community from 2 agave plants

Mammals

Mexican long-nosed bat



12/16

Gray fox



Ringtail



Deer



6 taxa

Arthropods

4 bee species



Western flower thrip



Crab spider



Leaf-footed bug



62 taxa
3 classes
15 orders

Plants

Agave



Scarlet bouvardia



Evening primrose



Alkali sacaton

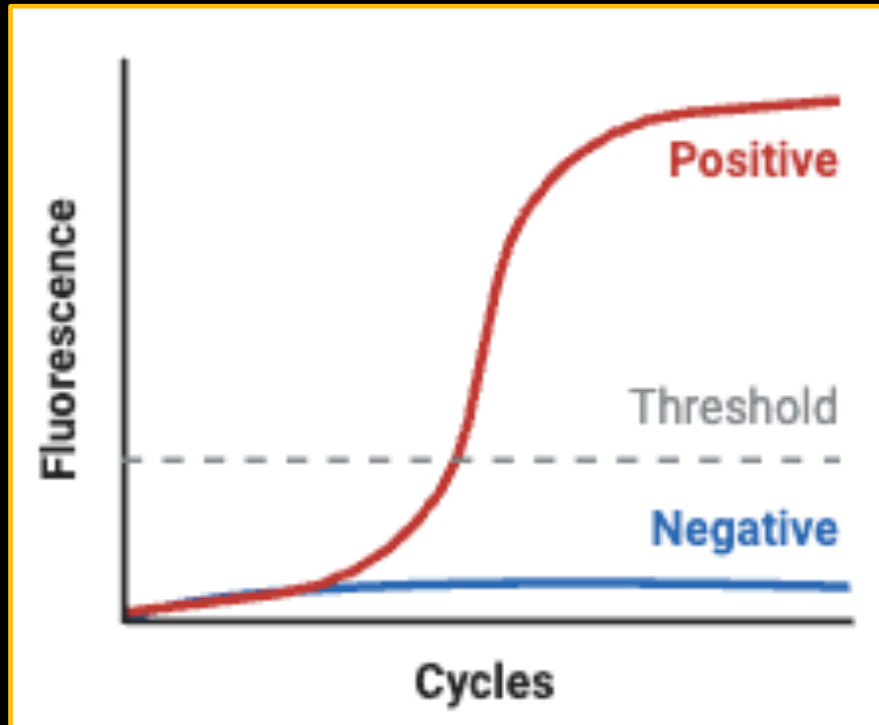


23 taxa
12 families



eDNA: qPCR

Agave a promising eDNA source of nectar feeding bats



13/16 samples



qPCR: Cheaper, faster, likely more sensitive

Also developed new qPCR assays for other nectar feeding bats

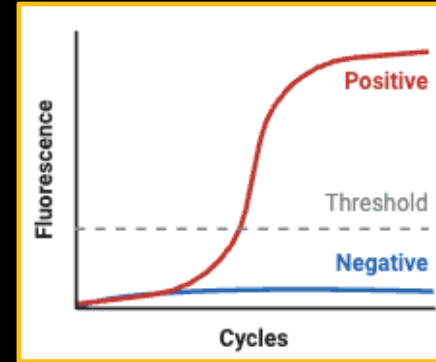


Anna Riley



Mexican long-tongued bat
(*Choeronycteris Mexicana*: CHME)

eDNA: qPCR 



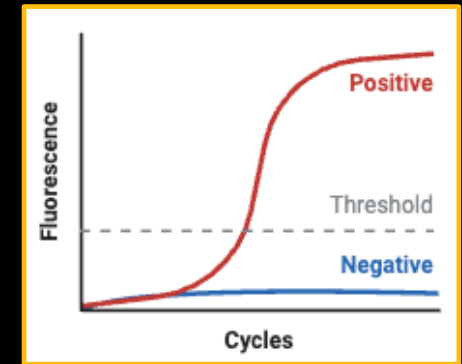
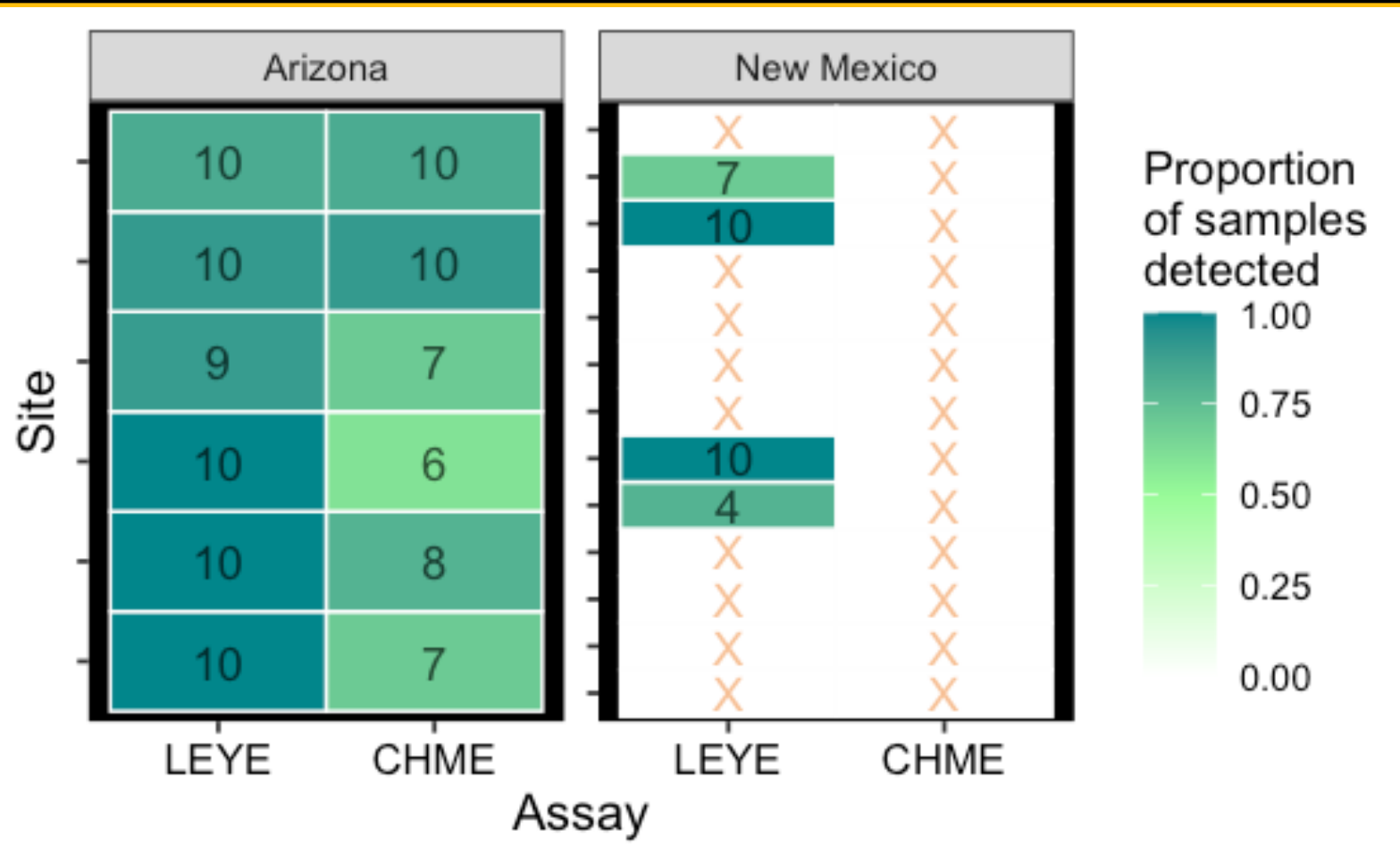
Lesser long-nosed bats at a hummingbird feeder in Arizona. Photo: Ted Fleming



Lesser long-nosed bat
(*Leptonycteris yerbabuenae*: LEYE)

eDNA: qPCR

Opportunities for citizen science



Lesser long-nosed bats at a hummingbird feeder in Arizona. Photo: Ted Fleming

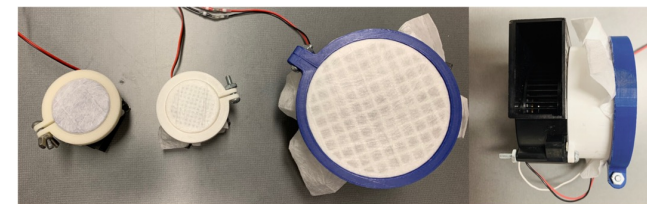
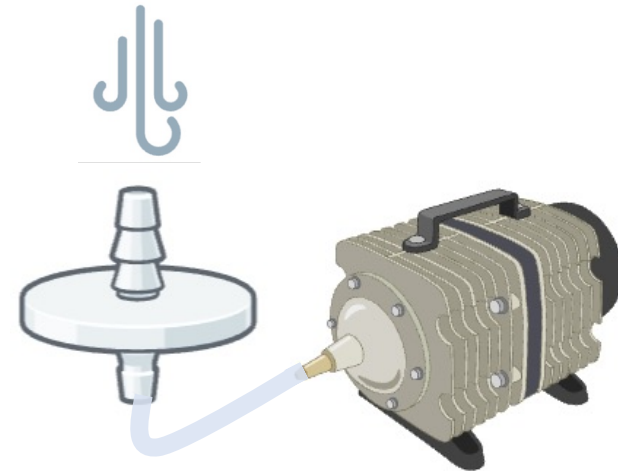


AIR



Out of thin air: surveying tropical bat roosts through air sampling of eDNA

Nina R. Garrett¹, Jonathan Watkins², Charles M. Francis³,
Nancy B. Simmons⁴, Natalia Ivanova⁵, Amanda Naaum⁵,
Andrew Briscoe⁶, Rosie Drinkwater⁷ and Elizabeth L. Clare¹



PeerJ

Published 26 April 2023



AIR



**Can we detect bat
eDNA passively?**

- **No pump/vacuum**

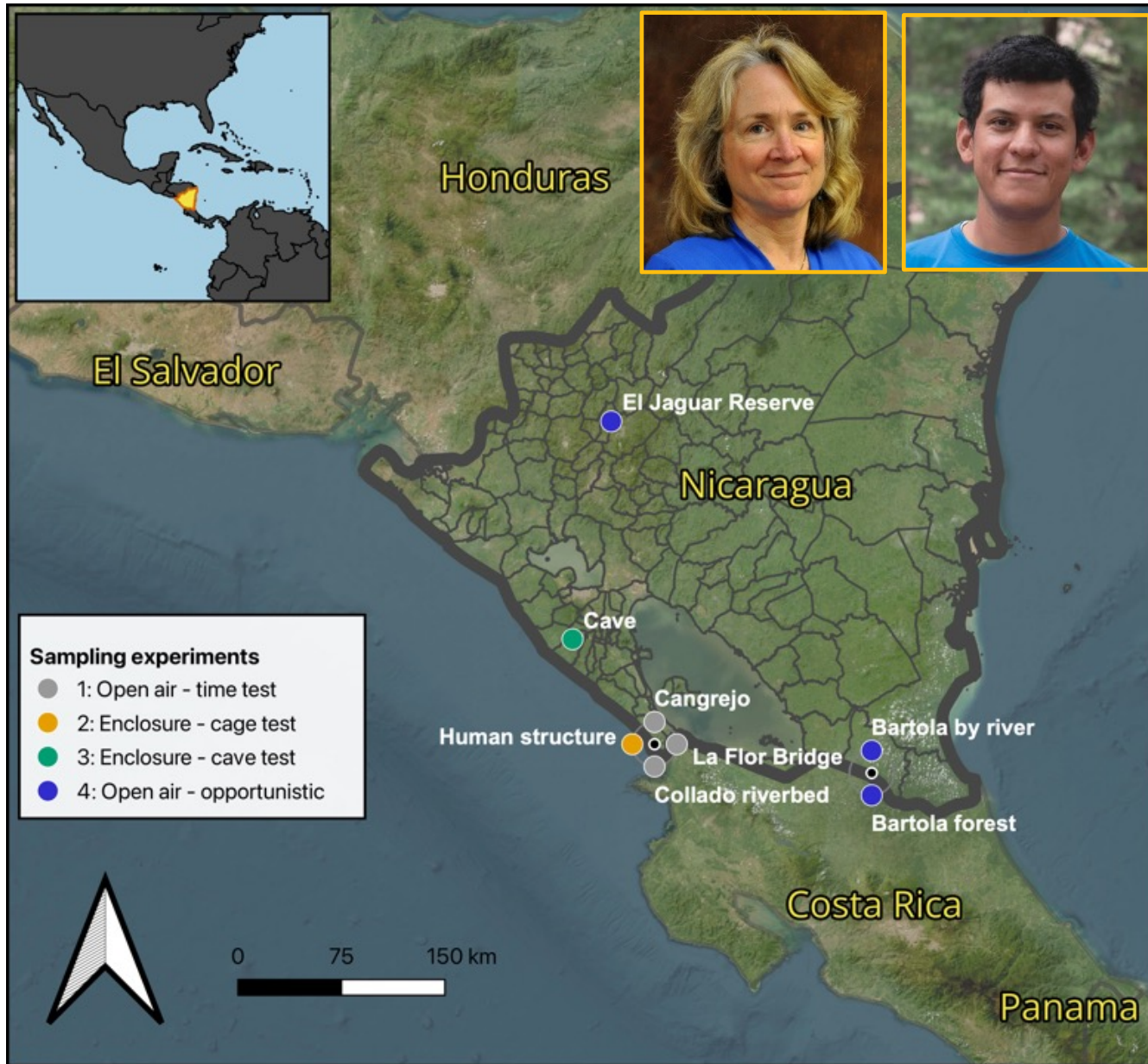
**Can we detect from the
open air?**



Cheesecloths? (see Frere et al. 2023)

Dr. Carol Chambers

Dr. José Gabriel Martínez-Fonseca



Air samplers: Cheesecloth

Open air: netting sites



Enclosure: cave roost



Detection method: Metabarcoding 



Open air eDNA detections

Air samplers: Cheesecloth

Detection method: Metabarcoding 



Open air - time test		
Cangrejo		
	Sample detections	At site
<i>Artibeus jamaicensis</i>		60
<i>Artibeus lituratus</i>		6
<i>Artibeus phaeotis</i>		8
<i>Carollia perspicillata</i>		32
<i>Centurio senex</i>		1
<i>Desmodus rotundus</i>		18
<i>Eptesicus furinalis</i>		3
<i>Glossophaga commissarisi</i>		3
<i>Glossophaga mutica</i>	1	7
<i>Lichonycteris obscura</i>		1
<i>Lophostoma brasiliensis</i>		2
<i>Micronycteris microtis</i>		2
<i>Phyllostomus discolor</i>		12
<i>Pteronotus mesoamericanus</i>		1
<i>Rhogeessa bickhami</i>		2
<i>Sturnira parvidens</i>	1	10
<i>Trinycteris nicefori</i>		2

Open air - time test		
Collado Riverbed		
	Sample detections	At site
<i>Artibeus jamaicensis</i>	0.5	31
<i>Artibeus lituratus</i>	0.5	2
<i>Artibeus phaeotis</i>		3
<i>Carollia perspicillata</i>		19
<i>Carollia subrufa</i>		1
<i>Desmodus rotundus</i>		1
<i>Eptesicus furinalis</i>		1
<i>Glossophaga commissarisi</i>		4
<i>Glossophaga mutica</i>		2
<i>Lophostoma silvicolum</i>		1
<i>Micronycteris microtis</i>		1
<i>Phyllostomus discolor</i>		8
<i>Pteronotus gymnonotus</i>		1
<i>Pteronotus mesoamericanus</i>		3
<i>Saccopteryx bilineata</i>		1
<i>Sturnira parvidens</i>		5
<i>Tonatia bakeri</i>		3

Open air - time test		
La Flor Bridge		
	Sample detections	At site
<i>Artibeus jamaicensis</i>	1	22
<i>Artibeus lituratus</i>		3
<i>Carollia perspicillata</i>		16
<i>Carollia subrufa</i>		2
<i>Desmodus rotundus</i>		4
<i>Glossophaga commissarisi</i>		1
<i>Glossophaga mutica</i>		1
<i>Noctilio leporinus</i>		5
<i>Pteronotus davyi</i>		1
<i>Pteronotus gymnonotus</i>		1
<i>Pteronotus personatus</i>		3
<i>Saccopteryx bilineata</i>		1
<i>Sturnira parvidens</i>		1



eDNA detections in a cave roost

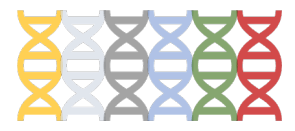
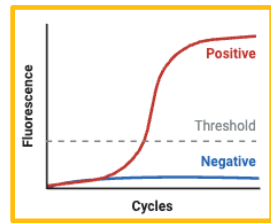
Air samplers: Cheesecloth

Detection method: Metabarcoding 



	Enclosure - cave test	
	Cave	
	Sample detections	At site
<i>Carollia perspicillata</i>	1	
<i>Desmodus rotundus</i>	1	~40
<i>Diphylla ecaudata</i>		2
<i>Glossophaga mutica</i>	3	
<i>Phyllostomus discolor</i>	1	
<i>Pteronotus mesoamericanus</i>	3	





Environmental DNA (eDNA) applications for detecting bat species

- **Bat eDNA can be detected from numerous environmental sources**
 - **Conspicuous**
 - Guano
 - **Inconspicuous**
 - Water
 - Soil/sediment
 - Resources/habitat
 - Air



Daniel.Sanchez@nau.edu

