BAT CONSERVATION INTERNATIONAL

Final Report
New Mexico Department of Game and Fish, Share with Wildlife Program

April 16, 2024

Project Title:
Assessing the status, distribution, movement corridors, and foraging habitat requirements of nectar bats in New Mexico through eDNA, PIT tag detection, and diet analysis

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Background

The federally-endangered Mexican long-nosed bat (*Leptonycteris nivalis*; IUCN 'Endangered'), Lesser long-nosed bat (*Leptonycteris yerbabuenae*), and Mexican long-tongued bat (*Choeronycteris mexicana*) are identified as Species of Greatest Conservation Need (SGCN) in the New Mexico Department of Game and Fish (NMDGF) State Wildlife Action Plan (New Mexico Department of Game and Fish 2016). Each year, these nectarivores follow corridors of blooming columnar cacti and agaves to migrate from central Mexico to small portions of the southern United States, including Texas, Arizona, and New Mexico (Cockrum 1991, Fleming et al. 1993, Moreno-Valdez et al. 2000, Gómez-Ruiz and Lacher 2017). Although the geographic distributions of these three species overlap across much of their ranges in Mexico, they only co-occur in the U.S. in southwest New Mexico, where they seasonally share common roosts and food sources in the late summer and early fall (Bogan et al. 2006). *Leptonycteris yerbabuenae* has been documented in New Mexico in the Animas, Peloncillo, Big Hatchet, and Little Hatchet Mountains (Findley et al. 1975, Fleming et al. 2003, Bogan et al. 2006, Bogan et al. 2017), and was recently documented from northern Grant County along the Gila River on the southern edge of the Mogollon Plateau – indicating a 110 km northward expansion of its previously known range (Geluso and Geluso 2021). In New Mexico, *L. nivalis* has been documented in the Animas and Big Hatchet Mountains (Hidalgo County) (Bogan et al. 2017, Laverty and Stoner 2022), and *C. mexicana* has been documented in the Peloncillo and Animas Mountains of Hidalgo, Cibola, and Grant Counties (Cryan and Bogan 2003, Bentley and Eifler 2022, Davies and Stoner 2021). The only roost site in the United States in which the two species of *Leptonycteris* co-occur is found in the Big Hatchet Mountains in the Bootheel region of New Mexico.
The Big Hatchet Mountains are used annually by *L. yerbabuenae* as a late-summer transition roost; in addition, *L. nivalis* was detected here during a radio telemetry study 2005 (Bogan et al. 2017) and was detected with genetic analysis in 2016 (Stoner 2016). However, genetic analyses of fecal samples collected from 2019-2021 have failed to detect *L. nivalis* again at this site. Because of the difficulty of distinguishing the two *Leptonycteris* species via methods such as thermal camera censuses of emergences, it is important to conduct non-invasive genetic analyses of fecal samples to determine if *L. nivalis* is still using this roost as was reported in 2005 and 2016.

In addition, the current migratory corridor between Big Bend National Park in Texas and the Big Hatchet Mountains remains undefined. Records of *L. yerbabuenae* in El Paso County, Texas (Krejsa et al. 2020), and *L. nivalis* in the Chinati Mountains (Presidio County, Texas; Mollhagen 1973) suggest that these bats may be migrating through west Texas and southeast New Mexico. However, few surveys for these species have been conducted outside of southwest New Mexico, despite other research suggesting that this area provides important nectar bat foraging habitat (Burke et al. 2019, Burke and Stoner 2021). In 2009, BCI conducted seasonal bat surveys using mist netting and acoustic surveys of five areas on the Fort Bliss Military Reservation (Hueco Mountains, Organ Mountains, Otero Mesa, Sacramento Mountains, and Tularosa Basin), but no nectar bats were detected (Bat Conservation International 2010). However, the Fort Bliss Military Reservation has low habitat suitability for nectar bat food plants *Agave palmeri* and *Agave*

parryi (Burke et al. 2019, Burke and Stoner 2021), so it is not surprising that no nectar bats were detected. It is important that we target areas with high agave suitability and known agave populations for further surveys so that we can identify the bats' migratory corridors and ultimately protect these corridors.

It is urgent to better understand the use of the Big Hatchet Mountains and identify the bats' movement corridors as interest in large-scale development of wind energy on New Mexico land managed by the Bureau of Land Management (BLM) increases. Although the expansion of wind energy would provide environmental benefits through the production of alternative energy decreasing our reliance on fossil fuels, there are concerns about the known negative impacts of wind turbines on bat populations. Hundreds of thousands to millions of bat deaths are estimated to occur every year at wind facilities in the U.S. and Canada (Arnett and Baerwald 2013, Hayes 2013, Smallwood 2013). Dead *L. yerbabuenae* have been found at wind facilities in Mexico and Latin America during studies conducted between 2007 and 2014 (Agudelo et al. 2021), and a dead *L. yerbabuenae* was recorded at a wind facility in Willcox, Arizona in 2018 (Boudreau 2018). In order to conserve SGCN species like the nectar bats in our study during the large-scale development of wind energy in New Mexico, it is imperative to identify the species' distribution in addition to the nectar resources, roost sites, and movement corridors that should be protected.

Climate change models predict a northward range expansion of both *Leptonycteris* species, with areas of southern New Mexico playing a more significant role in the ecology of these bats in the future (Gómez-Ruiz and Lacher 2019, Cappelli et al. 2021). Recent captures of *L. yerbabuenae* in Grant County in 2021 and 2022 (Davies and Stoner 2021, Geluso and Geluso 2021, Laverty and Stoner 2022) are suggestive of this projected northward expansion. However, little work has been done to understand the bats' foraging habitat requirements in northern areas. Mexican long-nosed bats are believed to feed almost exclusively on the nectar of agaves while in the United States (Findley et al. 1975, Hevly 1979). In 2016 and 2017, traditional microscope analysis of pollen spores and next-generation sequencing (NGS) of pooled fecal samples from the Big Hatchet Mountains, conducted by Dr. Stoner's lab at Colorado State University (CSU), showed that agave were the most highly represented plants in the samples (Sellers et al. In Preparation). In 2019 and 2021, a similar study documenting individual variation in diet over their seasonal occupation at the roost again indicated that agave is the only plant food resource used by these bats in southwest New Mexico (Davies and Stoner 2021). However, peak flowering of the main paniculate agave species in New Mexico (*A. palmeri*) is typically in late July or early August, while long-nosed bats may begin to arrive earlier in June or July and peak in the end of July (Scott 2004). This suggests that long-nosed bats may also be using other food plants besides *A. palmeri* or the less common *A. parryi*, but very few detailed studies on the bats' diet have been done. In addition, the diet and foraging habitat requirements of *C. mexicana* in this area are virtually unknown. Because the loss of nectar plants across the bats' ranges is threatening the survival of these bats (U.S. Fish and Wildlife Service 2018), a better understanding of nectar bat resource use will allow us to better determine foraging habitat requirements for these species. This will, in turn, allow us to more effectively design conservation measures that address priority recovery actions, such as Bat Conservation International's (BCI) bi-national Agave Restoration Initiative that works to

restore and augment agaves in climate-resilient areas around known bat roosts and migratory corridors in Arizona, New Mexico, and northern Mexico (U.S. Fish and Wildlife Service 2022).

Objectives

This work builds upon the existing history of strong collaborations between nectar bat, agave, and eDNA experts at BCI, CSU, BLM, and Northern Arizona University (NAU). Our project focuses on three SGCN species (*L. nivalis*, *L. yerbabuenae*, and *C. mexicana*) to determine the species' presence, range distributions, and foraging habitat requirements (as described in Research Topic 22 of the Share with Wildlife Fiscal Year 2023 Call For Project Information). Through this project, we aimed to: 1) monitor for the continued presence of *L. nivalis* in the Big Hatchet Mountains using eDNA analysis of pooled fecal samples collected in the roost; 2) monitor the distribution of *L. nivalis*, *L. yerbabuenae*, and *C. mexicana* at the northern extent of their ranges and identify the bats' movement corridors using passive integrated transponder (PIT) tag monitoring and a novel, cost-effective, and non-invasive eDNA methodology to detect nectar bats from agave flowers and artificial feeders; and 3) determine the relative importance of agave in the bats' diet and determine if other nectar plants are utilized in the northernmost extent of the bats' ranges by using microscopic techniques to analyze pollen and fecal samples collected from captured bats at sites north of the Bootheel. The goal of this work was to aid in identifying the distributions and the potential range expansions of these SGCN species and to prioritize roost sites and foraging areas for protection and restoration. Our work will assist NMDGF and BLM in developing best management strategies for these species and will greatly enhance the success of BCI's Agave Restoration Initiative in the state.

Project Activities and Methods

To achieve our project's objectives, we identified several key tasks that are described in detail below. The 2022 work described below that was completed prior to initiation of the Share with Wildlife project in 2023 is included to provide further context for the Share with Wildlife project activities.

Task 1: Monitor for the continued presence of the Mexican long-nosed bat (*Leptonycteris nivalis*) in the Big Hatchet Mountains.

We tested for Mexican long-nosed bat environmental DNA (eDNA) using pooled fecal samples collected in the Big Hatchet Mountains in Hidalgo County, NM. On October 25th, 2022, plastic ground sheets were deployed to collect bat fecal material as bats entered and exited the roost. A total of four tarps were used, with one tarp placed beneath an alternate cave entrance called "The Crack" in the larger room, and another tarp placed in front of the entrance to the lowest room. In the lowest room two tarps were deployed. The first tarp was positioned at the base of the main wall, where the team was able to find fecal samples from *Leptonycteris spp*., and the second tarp was placed in an adjacent room. On May 28th, 2023, the CSU team visited the Big Hatchet Mountains to confirm that the ground sheets were still in place in the upper room.

On January 8th, 2024, Mallory Davies (CSU), Cody Howard (BLM), Meredith Davis (BLM), Lucas Castro (BLM), Jackson Bain (BCI subterranean team), and Myriam Bishop (BCI subterranean team) visited the Big Hatchet Mountains to collect fecal samples from the four deployed tarps and redeploy tarps in all rooms. The collected fecal samples were stored in sealed vials of RNAlater. The team collected a total of 7 vials: 4 from the larger room and 3 from the lowest room (Appendix 1 Table 1). The samples were then sent to Dr. Faith Walker's Bat Ecology and Genetics Lab at NAU for eDNA analysis to identify the bat species.

Figure 1. From left to right: Mallory Davies (CSU), Myriam Bishop (BCI subterranean team), Jackson Bain (BCI subterranean team), Cody Howard (BLM), Meredith Davis (BLM), and Lucas Castro (BLM) on January 8th, 2024, at the base of Big Hatchet. Photo: Mallory Davies.

Figure 2. Jackson Bain of BCI collecting fecal samples off the tarp in the lower room of the roost in the Big Hatchet Mountains, on January 8th, 2024. Photo: Mallory Davies.

Figure 3. Nectar bat fecal samples on tarp deployed in the Big Hatchet Mountains. Photo: Mallory Davies.

Task 2: Monitor the distribution of the Mexican long-nosed bat (*L. nivalis*), Lesser long-nosed bat (*L. yerbabuenae*), and Mexican long-tongued bat (*Choeronycteris mexicana*) outside of the Bootheel and identify these species' migratory corridor(s).

From July to September 2023, the CSU team deployed and maintained trail cameras on hummingbird feeders at three residential sites in Silver City, NM (Grant County), and maintained two sites at a ranch near Rodeo, NM (Hidalgo County). They also stayed in contact with two volunteers in Silver City and one in White Signal, NM (Grant County), who monitored their hummingbird feeders using personal trail cameras to observe nectar bat activity. The CSU team conducted bat capture surveys at four different residential sites seven times, collecting pollen and fecal samples using mist nets (Appendix 1 Tables 2-4).

The CSU team, along with undergraduate intern Daniel Milton, successfully built a functional low-cost PIT tag reader/antenna system specifically designed for nectar bats. This system can be easily attached to a hummingbird feeder and operates on a small battery pack. The construction of the PIT tag system followed the methods outlined by Bridge and Bonter (2011) and Bridge et al. (2019), with a modified hoop antenna that can be fitted to the outside of hummingbird feeders. To test the functionality of the setup, a pill bottle containing a 12mm PIT tag was used to simulate nectar bat feeding behavior. The hoop antenna was securely fastened around the feeder openings and adjusted at a slight angle, allowing bats to insert their heads through the antenna to access the nectar. This positioning ensured that the device successfully read the 12mm PIT tag. The performance of the PIT tag system was evaluated in early August 2023, a time when agave nectar availability decreased while nectar bat activity at hummingbird feeders increased. To assess the system's performance, we deployed it on hummingbird feeders at established residential bat capture sites. Simultaneously, we conducted bat capture surveys at nearby feeders to confirm the presence of bats.

As part of Task 2, we also used a non-invasive eDNA technique to survey for all three focal nectar bat species at flowering agaves in a potential migratory corridor in southeast New Mexico (Otero County).

BCI has been developing and deploying a novel, cost-effective, and non-invasive eDNA methodology to detect all three SGCN nectar bat species from agave flowers in a potential migratory corridor in southeast New Mexico where these bats likely occur at low densities. Traditional methods for surveying for the presence of these bats (e.g., mist netting; camera monitoring of food plants) often prove to be expensive, time-intensive, and unreliable, and acoustic techniques are unable to distinguish between the two *Leptonycteris* species. Due to recent advances, we are now able to collect organisms' DNA from their environment (i.e., environmental DNA, or eDNA). eDNA analysis can identify species interactions such as pollinator visits to plants. Proof-of-concept of the efficacy of detecting nectar bats from eDNA left on agave flowers was established through a prior collaboration between Dr. Kristen Lear (BCI's Agave Restoration Program Director) and Dr. Faith Walker (head of the Bat Ecology and Genetics Lab and Species From Feces lab at NAU; Walker et al. 2022).

Through our Share with Wildlife project, we successfully developed a quantitative

polymerase chain reaction (qPCR) assay for the Mexican long-tongued bat (*C. mexicana*), thus completing the assay development for all three nectar bat species. BCI staff collected 126 eDNA samples from blooming agaves on BLM lands in Otero County during three field trips between June 19 and July 13, 2023 (Appendix 1 Table 5).

Task 3: Use pollen and fecal samples gathered in Task 2 above to determine the relative importance of *Agave* spp. in nectar bat diets and determine if other nectar plants are utilized in the northernmost extent of the bats' ranges.

Pollen grain identification techniques were employed to identify plant species from the pollen samples collected during the 2023 season, as detailed in Task 2. Slides were prepared from these collected pollen samples and were then examined under a microscope. Pollen grains were identified using published identification guides (Kapp 1969) and reference collections gathered from the field. Due to limited funding and a small sample size, the team opted to send the fecal sample for DNA metabarcoding. This involved sending the fecal sample to Pisces Molecular Lab in Boulder, CO for NGS sequencing to identify plant and insect species that are in the individual bats' diets.

Figure 4. Bat 592, adult male Leptonycteris yerbabuenae covered in pollen. Captured in the Peloncillo Mountains, NM. Photo: Mallory Davies.

Results and Discussion

We have summarized our preliminary results below based on the Methods tasks described above.

Task 1: Monitor for the Continued Presence of Mexican Long-nosed Bat (*Leptonycteris nivalis*) in the Big Hatchet Mountains

Previous efforts in 2022 (*included to provide further context for Share with Wildlife project activities in 2023-2024)*

In May 2022, there was a joint effort between CSU, BLM, BCI, and the U.S. Fish and Wildlife Service to map out the Big Hatchet Mountains using LiDAR and survey the site for the endangered Mexican long-nosed bat (*L. nivalis*) using eDNA analysis of fecal samples. Mallory Davies (CSU) deployed fecal sheets at the base of the large room on May 3rd, 2022, but was unable to access the lower room because the team did not want to disturb the Townsend's big-eared bats (*Corynorhinus townsendii*) that were roosting there. In late October after all the bats had departed, the team attempted another expedition into the lower room. With the assistance of BCI's subterranean team, Mallory and Shawn Thomas (BCI) were able to rappel down into the lowest room and collect fecal samples. They spent approximately 30 minutes collecting fecal material from the rock wall by scraping samples into test tubes. In total, 30+ fecal samples were collected from the wall of the lower room and combined into one tube filled with RNAlater. The samples collected from this room appeared to be fresh (potentially having been deposited during the previous year), but this cannot be confirmed because the room had not been accessed during the previous approximately 10 years. Mallory also collected two tubes of fecal samples from tarps deployed at the base of the large room: one tube was collected near the entrance of the lower room and the second tube was collected from a trap deployed beneath the "crack" (a secondary entrance to the cave). Unfortunately, due to high precipitation during the 2022 season, the large room of the cave flooded multiple times and the fecal samples on these tarps seemed to have washed off; thus, Mallory suspects that the samples collected from the tarps in October 2022 represent the bat communities from later in the field season. It's important to note that there didn't appear to be any sign of water runoff in the lower room; therefore, fecal collection off tarps in that room should be successful in the future. Fresh tarps were deployed in the larger room and lower room for collection in 2023.

Findings from 2022 Efforts

Rachel Burke (BLM) sent the three test tubes to Faith Walker's lab at NAU for eDNA analysis. The two pooled samples collected from the larger room detected *L. yerbabuenae*, *C. townsendii*, Cave myotis (*Myotis velifer*), and Fringed-myotis (*Myotis thysanodes*). The pooled sample collected from the wall in the lower room only detected *L. nivalis*, suggesting that *L. nivalis* may not be using the cave incidentally but rather occupying their own physical space in the cave.

Progress from the 2023 Field Season

As of April 2024, the CSU team is awaiting eDNA results from Dr. Faith Walker's Bat Ecology and Genetics Lab at NAU.

Task 2: Monitor the Distribution of Mexican Long-nosed Bat (*L. nivalis*), Lesser Long-nosed Bat (*L. yerbabuenae*), and Mexican Long-tongued Bat (*Choeronycteris mexicana*) Outside of the Bootheel and Identify These Species' Migratory Corridor(s)

Deployment of Artificial Nectar Feeders and PIT Tag Detection Systems

From July to November 2023, the CSU team captured, and PIT tagged 11 *L. yerbabuenae* and 7 *C. mexicana* during bat capture surveys using mist nets (using BLM funds; Grant No. L22AC00505-00). They also recorded 8,053 videos and photos using trail cameras that were deployed on hummingbird feeders at residential sites. Currently, the team is processing the videos using Timelapse software to determine the activity levels of nectar bats at residential sites instead of relying solely on PIT tag results. During the upcoming field season, they hope to detect tag numbers of previously tagged bats using hummingbird feeder PIT tag detection systems.
To assess the performance of the PIT tag system and modified hoop antenna, the team deployed the system on hummingbird feeders at established residential bat capture sites. Simultaneously, they conducted bat capture surveys at nearby feeders to confirm the presence of bats.

The field team recorded videos of the deployed PIT tag systems using a Sony NightShot Camcorder with infrared lights to confirm nectar bat presence, activity, and use. The videos confirmed high activity around the hummingbird feeders equipped with the PIT tag systems, but little to no use of the feeders themselves. The team conducted tests on various diameters (7.6, 8.9, and 9.5 cm) and orientations (90, 45, and 0 degrees angled to the top of the hummingbird feeder) of the hoop antenna setup on five separate occasions from August to October 2023. They determined that the orientation of the antenna surrounding the opening of the feeder deters nectar bats from utilizing the feeder.

The team developed an alternative antenna design that would be less deterring to the bats, using a ferrite rod wrapped in copper wire that could be positioned near the opening to the feeder without encircling it. In March 2024, the team successfully built a ferrite rod antenna with an inductance of 1.2mH (milli Henrys), the necessary frequency to read 12mm BioMark PIT tags. The new ferrite rod antenna design is made using Binneker 30 AWG High Quality Enamel Wire (coated copper wire) windings, 33 mm in length, around a 0.5" diameter ferrite rod. When powered using a 5-volt battery, the antenna has a 9.0 cm read range. Because each device can run two antennas simultaneously, a 9.0 cm read range is sufficient for their purpose. However, the CSU team is still fine-tuning the antenna to optimize its performance, and plan to deploy the PIT tag system with the new ferrite rod antenna design in the 2024 field season.

Figure 5. Assessing the performance of the PIT tag system and modified hoop antenna. Photo: Mallory Davies.

Figure 6. PIT tag detection system and modified hoop antenna deployed in the field. Photo: Mallory Davies.

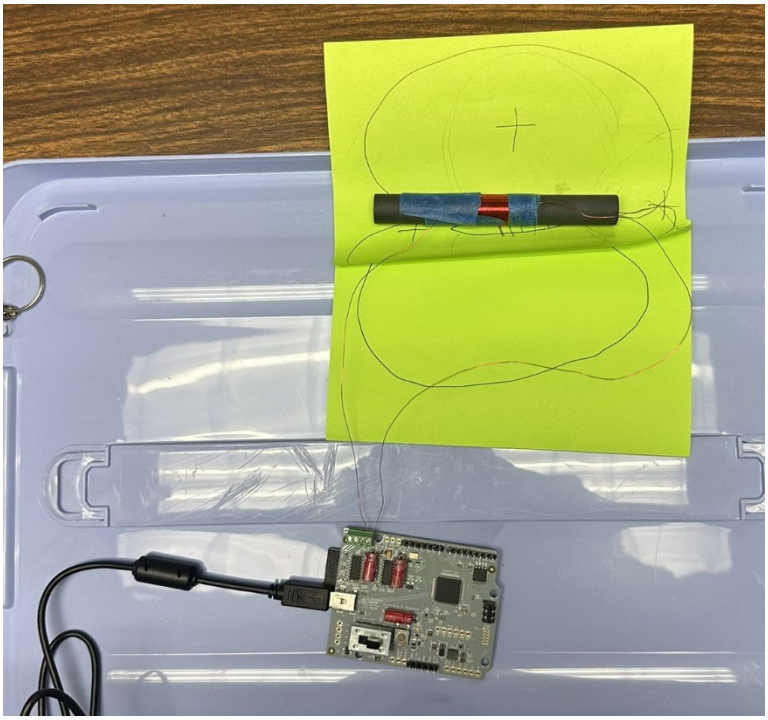


Figure 7. Prototype of the modified ferrite rod antenna design. the circles on the green paper represent the read range of the antenna. Photo: Mallory Davies.

Development of Choeronycteris mexicana qPCR Assay

Dr. Walker's lab successfully developed qPCR assays for *L. nivalis* and *L. yerbabuenae* in 2021 and 2022. Recognizing the importance of *C. mexicana* within New Mexico, our Share with Wildlife grant supported development of a qPCR assay for *C. mexicana* so that all three pollinating bats can be detected from samples collected within the state and across the species' ranges.

The process to design the primers for the *C. mexicana* qPCR assay was prolonged due to technical challenges, including issues with DNA acquisition, an inability to sequence using widely-used universal primers, and mistakes in public databases (e.g., one of the only reference sequences for *C. mexicana* is actually *L. yerbabuenae*). Despite these challenges, Dr. Walker's lab at NAU completed the assay in November 2023 and has been screening samples from 2023 field work for our Share with Wildlife project and additional field work conducted by BCI.

eDNA Surveys of Blooming Agaves in Southeast New Mexico

In the summers of 2021 and 2022, we piloted the use of the eDNA approach in the Trans-Pecos region of Texas and developed a field sampling protocol for surveying for nectar bats from blooming agaves. We determined that swabbing open agave flowers with polyester swabs attached to a pole was nearly as effective at collecting eDNA as removing flowers from the plant for analysis (70% of swabs versus 77% of cut flowers tested positive for eDNA), and is much less invasive. In addition, our field results indicate that swabbing flowers and cutting

flowers are both effective sampling methods for collecting *L. nivalis* DNA up to at least 24 hours after the bat's visit to the flower. After 48 hours post-visit, cutting flowers was more effective at collecting the DNA. However, given the increased negative impact on the plant of cutting flowers versus merely swabbing flowers, in addition to the increased permitting restrictions placed on removing plant material, we chose to sample flowers by swabbing them. Swabbing is done from the ground using a sterile polyester-tipped swab with a wood handle attached via a modified head attachment of PVC pipe connectors to a pole/telescoping pole; we used 8-foot-long lightweight garden stakes for shorter agaves and a telescoping 7- to 30-foot pole for taller agaves). Swabs are stored in 2 mL vials of RNALater, a non-toxic DNA preservative solution.

Rachel Burke (former BLM staff) developed agave distribution models that indicate the presence of suitable habitat for nectar bats in southern New Mexico, including the Brokeoff Mountains, Sacramento Mountains, and Guadalupe Mountains (Burke et al. 2019; Burke et al. 2021). For our Share with Wildlife grant project, we collaborated with Ms. Burke to identify priority sites on BLM lands administered by the Las Cruces District Office in Otero County for scouting and sampling in 2023. BLM staff were not able to collect opportunistic samples in spring 2023 due to staffing shortages and turnover. However, BCI staff collected 126 samples from blooming agaves on BLM lands in Otero County during three field trips between June 19 and July 13, 2023 (Appendix 1 Table 5; Figs. 8-10). By the third field trip (July 12-13), most of the agaves we sampled were almost finished blooming, with open flowers only at the upper parts of the flowering stalk. During our surveys, we also recorded the locations of additional blooming agaves (e.g., plants that were inaccessible on hill slopes or ridge tops) and non-blooming agave patches. Figure 11 shows locations of swabbed agaves and areas of high densities of blooming agaves; Figure 12 shows BCI's current priority areas for agave restoration activities in New Mexico and Arizona.

The eDNA samples were stored in a freezer until the end of the season, at which time all samples were shipped to Dr. Faith Walker's Bat Ecology and Genetics Lab at NAU. Dr. Walker's lab completed the *C. mexicana* qPCR assay in November 2023 and has begun screening our Share with Wildlife project samples and additional samples collected by BCI in summer 2023 for *L. nivalis*, *L. yerbabuenae*, and *C. mexicana*. We expect to have results back from the lab for all three nectar bat species by the end of April 2024.

From our field work, we developed instructions for an eDNA field sampling "kit" and protocol to collect samples from blooming agaves (draft of the protocol provided in Appendix 2).

Figure 8. BCI Restoration Specialist Brianna Mann swabbing a blooming agave on BLM land in Otero County to test for bat DNA using qPCR methods. Photo: Kristen Lear, Bat Conservation International.

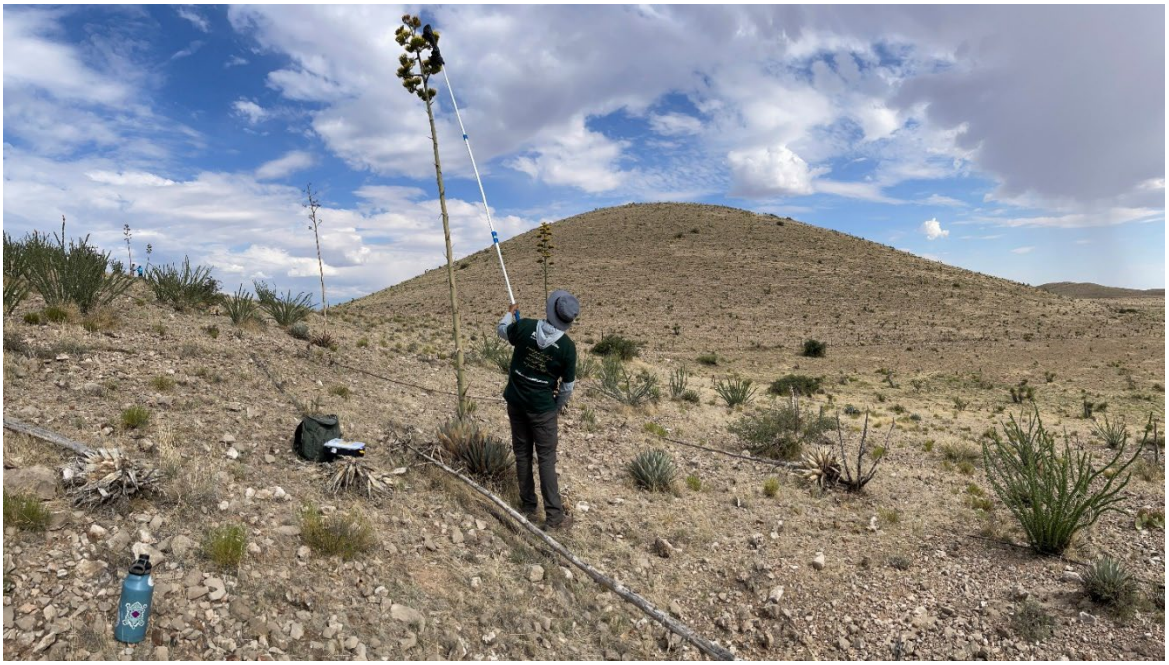


Figure 9. BCI Restoration Technician Devin Robbins swabbing a blooming agave on BLM land in Otero County to test for bat DNA using qPCR methods. Photo: Kristen Lear, Bat Conservation International.

Figure 10. A polyester swab and swabbing pole head after collecting an eDNA sample from a blooming agave. Photo: Kristen Lear, Bat Conservation International.

Task 3: Use Pollen and Fecal Samples Gathered in Task 2 to Determine the Relative Importance of *Agave spp.* in Nectar Bat Diets and Determine if Other Nectar Plants Are Utilized in the Northernmost Extent of the Bats' Ranges

In total, the team collected 11 *L. yerbabuenae* and 7 *C. mexicana* pollen samples, as well as 1 *C. mexicana* fecal sample from captured bats at residential sites in New Mexico (Appendix 1 Tables 3 and 4). The CSU team is developing a site-specific pollen identification key using microscopic images of pollen samples collected from known plants in southwestern New Mexico. Once completed, the team will use this key, along with other published pollen identification guides (e.g. Kapp 1969) to identify the plant species present in the individual pollen samples. The collected fecal samples were sent to Pisces Molecular Lab in Boulder, CO for next-generation sequencing (NGS) to identify the plant and insect species that are part of the individual bats' diets. We are waiting on results from Pisces Molecular Lab and expect to have all results back by August 2024.

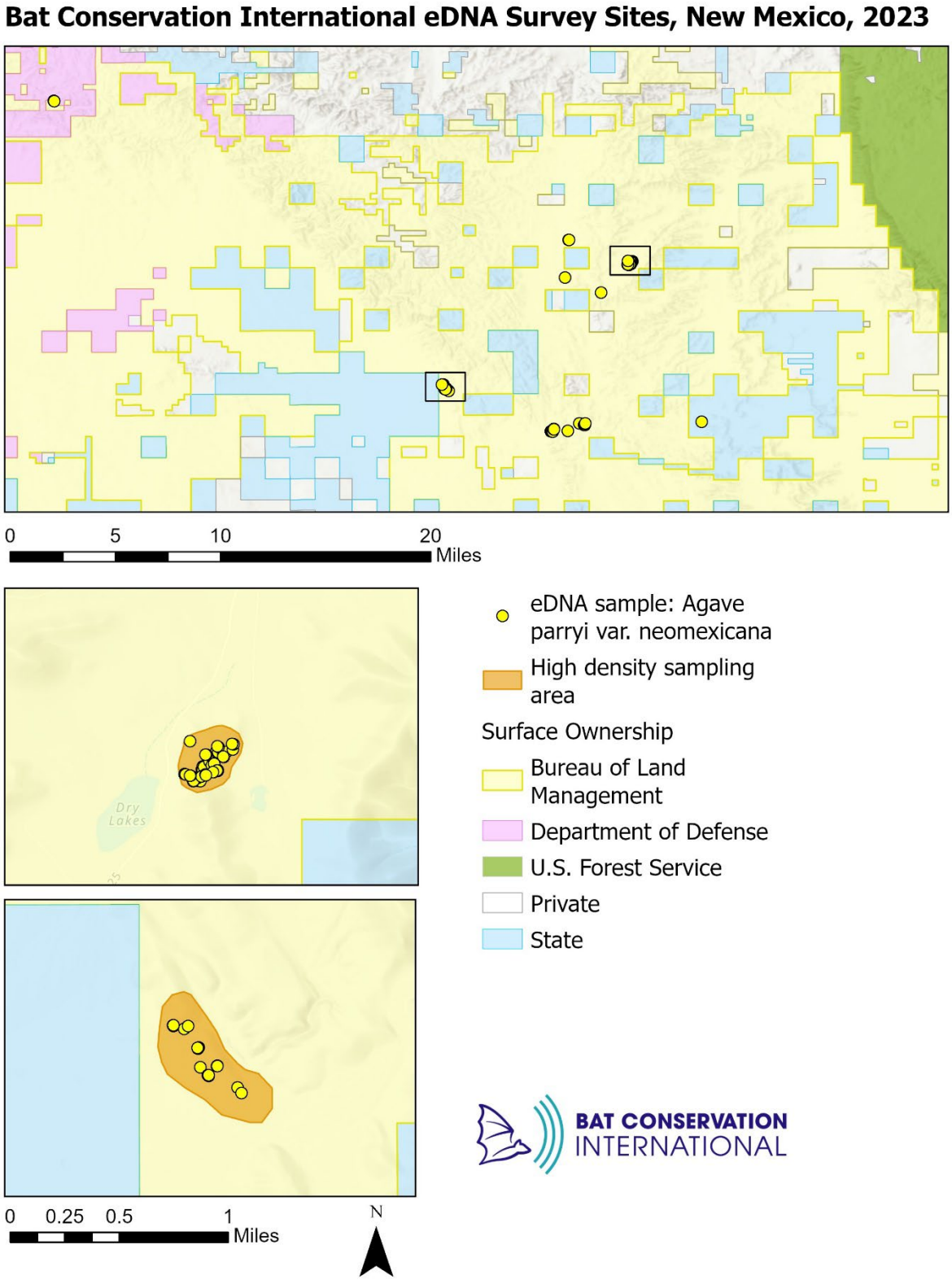
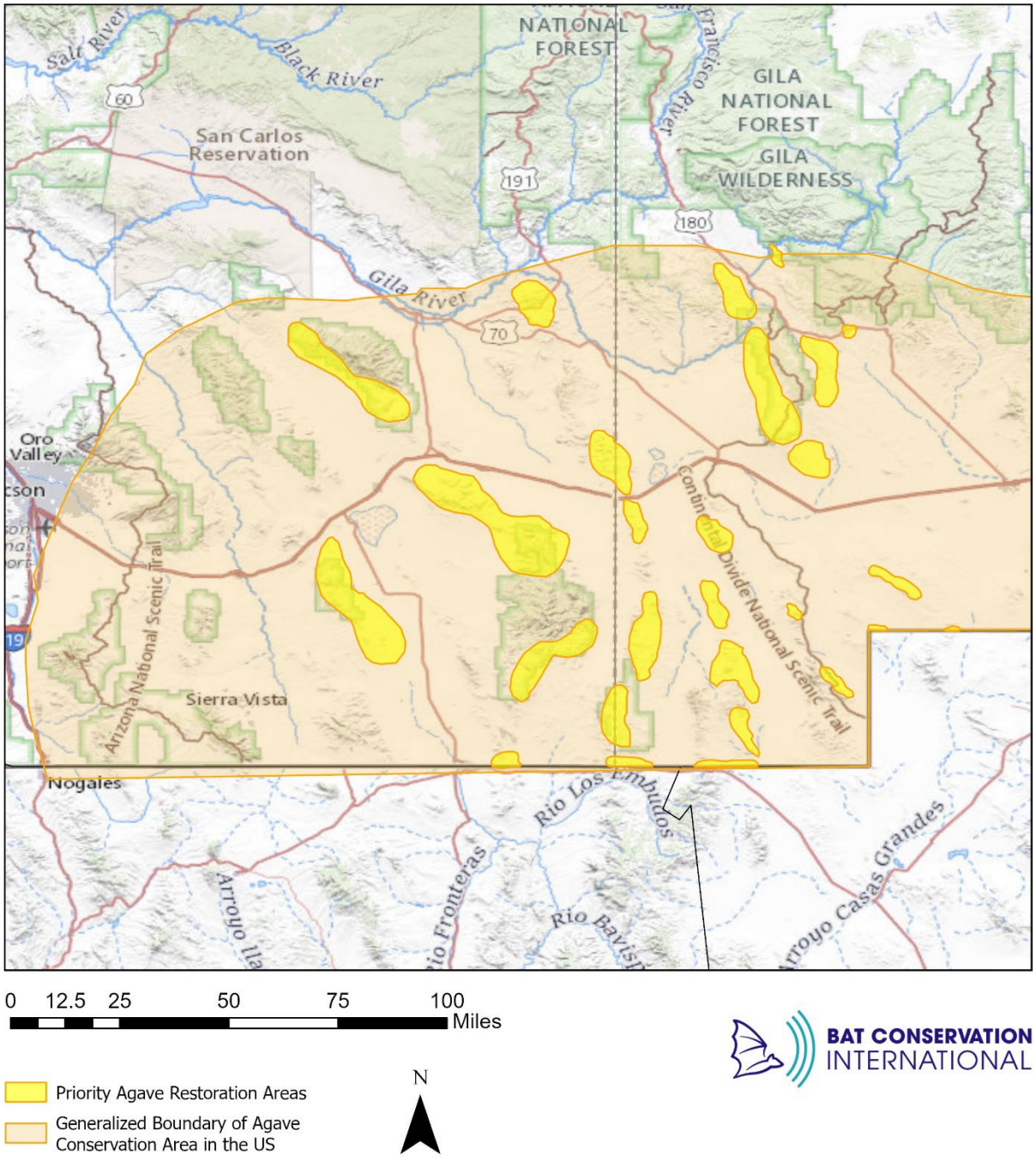


Figure 11. Map of eDNA survey locations from blooming agaves on BLM lands in Otero County, New Mexico; and areas of high densities of flowering agaves.

Bat Conservation International's
Agave Conservation and Restoration Focal Areas in Arizona and New Mexico



USGS The National Map: National Boundaries Dataset, 3DEP Elevation Program, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; USGS Global Ecosystems; U.S. Census Bureau TIGER/Line data; USFS Road data; Natural Earth Data; U.S. Department of State HIU; NOAA National Centers for Environmental Information

Figure 12. Priority areas for agave restoration activities in New Mexico and Arizona.

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Appendix 1: Data Tables

Table 1: Collection information for fecal samples from Big Hatchet Mountains, Hidalgo County, New Mexico, sent in for eDNA analysis to Dr. Faith Walker's Bat Ecology and Genetics Lab at NAU. All tarps deployed on 10/25/2022.

Date Collected	Sample ID	Tarp Location	Sample Notes	Sample Collection Description
1/8/2024	001	Entrance to Lower Room	High Quality Samples	Collected fecal samples that appeared to be nectar bat fecal splats, i.e., yellow, round or elongated shape, and flat on tarp.
1/8/2024	002	Entrance to Lower Room	Low Quality Samples	Fecal samples collected from pooled locations on tarp due to water collection.
1/8/2024	003	Cave Crack	High Quality Samples	Collected fecal samples that appeared to be nectar bat fecal splats and not water drops, i.e., yellow, round or elongated shape, and flat on tarp.
1/8/2024	004	Cave Crack	Low Quality Samples	Collected fecal samples that were dusty but appeared to be nectar bat fecal splats and not water drops, i.e., yellow, round or elongated shape, and flat on tarp.
1/8/2024	005	Lower Room, Tarp 1	High Quality Samples	Collected fecal samples with no visible dust that appeared to be nectar bat fecal splats, i.e., yellow, round, or elongated shape, and flat on tarp. 10 individual splats total. No signs of water contamination on tarp.
1/8/2024	006	Lower Room, Tarp 1	Low Quality Samples	Collected fecal samples that may have dust but appeared to be nectar bat fecal splats, i.e., yellow, round or elongated shape, and flat on tarp. 65 individual splats total. No signs of water contamination on tarp.
1/8/2024	007	Lower Room, Tarp 2	All Samples Present	Collected fecal samples that appeared to be nectar bat fecal splats, i.e., yellow, round or elongated shape, and flat on tarp. 30 individual splats total. No signs of water contamination on tarp.

Table 2: Fecal samples collected from captured bats in New Mexico that were sent to Pisces Molecular Lab in Boulder, CO for NGS sequencing.

Date Collected	Sample ID	Source	Location	County	Species	Pisces Molecular Lab Service
7/18/2023	001	captured bat #567	Big Hatchet Mountains	Hidalgo County	*L. yerbabuenae*	NGS sequencing to identify micro-or macrobiome in a sample. Identifying plant and insect species
8/10/2023	008	captured bat #594	Peloncillo	Hidalgo County	*L. yerbabuenae*	NGS sequencing to identify micro-or macrobiome in a sample. Identifying plant and insect species
8/11/2023	009	captured bat #597	Peloncillo	Hidalgo County	*C. mexicana*	NGS sequencing to identify micro-or macrobiome in a sample. Identifying plant and insect species
8/16/2023	011	captured bat #607	Big Hatchet Mountains	Hidalgo County	*L. yerbabuenae*	NGS sequencing to identify micro-or macrobiome in a sample. Identifying plant and insect species
8/27/2023	012	captured bat #608	Big Hatchet Mountains	Hidalgo County	*L. yerbabuenae*	NGS sequencing to identify micro-or macrobiome in a sample. Identifying plant and insect species
10/20/2023	021	captured bat #666	Silver City, NM	Grant County	*L. yerbabuenae*	NGS sequencing to identify micro-or macrobiome in a sample. Identifying plant and insect species

Table 3: New Mexico bat capture site and visit information. Includes data collected in the Mountains which was funded independently by the BLM.

Date	County	Elev. (m)	Weather (degrees F)	No. Nets	Net Sizes (m)	Time Opened	Time Closed
6/14/23	Hidalgo	1760	65 degrees, 0% clouds, 15-20 mph wind	1	12	21:13	0:13
6/15/23	Hidalgo	1760	65 degrees, 0% clouds, slowed to 5-10mph wind	1	12	5:28	6:21
6/30/23	Hidalgo	1760	70 degrees, 0% clouds, 7 mph gusts of wind	1	12	21:01	23:01
7/1/23	Hidalgo	1760	70 degrees, 0% clouds, 7 mph gusts of wind	1	12	4:51	6:16
7/5/23	Hidalgo	1760	85 degrees, 0% clouds, 15 mph gusts of wind	1	12	20:52	23:31
7/18/23	Hidalgo	1760	80 degrees, 5% clouds, 10-20 mph wind	1	12	21:04	2:10
8/4/23	Hidalgo	1760	88 degrees, 0% clouds, 1-2 mph gusts of wind	1	12	20:24	2:02
8/10/23	Hidalgo	1650	80 degrees, 85% clouds, 3mph gusts of wind	3	2,9,4	20:50	0:44
8/11/23	Grant	1921	70 degrees, 50% clouds, 5 mph wind	2	6,9	20:30	22:50
8/15/23	Hidalgo	1760	81 degrees, 25% clouds, 3 mph wind	1	12	20:32	23:34
8/16/23	Hidalgo	1760	72 degrees, 0% clouds, 0 mph wind	1	12	4:48	6:40
9/7/23	Hidalgo	1760	84 degrees, 75% clouds, 6mph wind	1	12	20:25	22:45
9/8/23	Hidalgo	1760	76 degrees, 10% clouds, 4mph winds	1	12	4:55	6:50
9/10/23	Grant	1866	76 degrees, 50% clouds, 3-13mph wind	2	2.6,6	21:43	1:15
9/24/23	Grant	1866	76 degrees, 0% clouds, 0-1 mph wind	0	N/A	Owl present did not open nets 18:30	Monitored w/ Trail camera, no bat activity 23:59
10/20/23	Grant	1866	opening: 71 degrees, 0% clouds, 2mph wind. closing: 60 degrees, 0% clouds, 1mph wind	2	6,4	22:35	0:30

Table 4: New Mexico bat capture data by site. Includes data collected in the Big Hatchet Mountains and PIT tag numbers for bats which was funded independently by the BLM.

Date	BatNo.	Species	Age	Sex	Reproductive status	Tag number	Pollen sample? (Yes/No)	Fecal sample? (Yes/No)
6/14/2023	558	*Corynorhinus townsendii*	adult	female	lactating	N/A	N/A	N/A
6/14/2023	559	*Corynorhinus townsendii*	adult	female	lactating	N/A	N/A	N/A
7/18/2023	560	*Corynorhinus townsendii*	juvenile	male	non-scrotal	N/A	N/A	N/A
7/18/2023	561	*Corynorhinus townsendii*	N/A	N/A	N/A	N/A	N/A	N/A
7/18/2023	562	*Corynorhinus townsendii*	juvenile	male	non-scrotal	N/A	N/A	N/A
7/18/2023	563	*Corynorhinus townsendii*	juvenile	female	non-reproductive	N/A	N/A	N/A
7/18/2023	564	*Corynorhinus townsendii*	juvenile	female	non-reproductive	N/A	N/A	N/A
7/18/2023	565	*Corynorhinus townsendii*	juvenile	female	non-reproductive	N/A	N/A	N/A
7/18/2023	566	*Corynorhinus townsendii*	juvenile	male	non-scrotal	N/A	N/A	N/A
7/18/2023	**567**	***Leptonycteris yerbabuenae***	**adult**	**male**	**scrotal**	**989.001041205077**	**Yes**	**Yes**
7/19/2023	568	*Corynorhinus townsendii*	juvenile	female	non-reproductive	N/A	N/A	N/A
7/19/2023	569	*Myotis ciliolabrum*	adult	male	non-scrotal	N/A	N/A	N/A
8/4/2023	570	*Corynorhinus townsendii*	juvenile	female	non-reproductive	N/A	N/A	N/A
8/4/2023	571	*Corynorhinus townsendii*	juvenile	male	non-scrotal	N/A	N/A	N/A
8/4/2023	572	*Corynorhinus townsendii*	juvenile	male	non-scrotal	N/A	N/A	N/A
8/4/2023	573	*Corynorhinus townsendii*	N/A	N/A	N/A	N/A	N/A	N/A
8/4/2023	574	*Myotis ciliolabrum*	adult	male	non-scrotal	N/A	N/A	N/A
8/4/2023	575	*Myotis ciliolabrum*	juvenile	male	non-scrotal	N/A	N/A	N/A
8/4/2023	576	*Myotis ciliolabrum*	juvenile	male	non-scrotal	N/A	N/A	N/A
8/4/2023	577	*Myotis velifer*	adult	male	non-scrotal	N/A	N/A	N/A
8/5/2023	578	*Corynorhinus townsendii*	adult	female	non-reproductive	N/A	N/A	N/A

8/10/2023	585	*Myotis ciliolabrum*	adult	female	non-reproductive	N/A	N/A	N/A
8/10/2023	586	*Myotis ciliolabrum*	juvenile	male	non-scrotal	N/A	N/A	N/A
8/10/2023	**587**	***Leptonycteris yerbabuenae***	**juvenile**	**female**	**non-reproductive**	**989.001041205086**	**Yes**	**No**
8/10/2023	**588**	***Leptonycteris yerbabuenae***	**juvenile**	**female**	**non-reproductive**	**989.001041205039**	**Yes**	**No**
8/10/2023	589	*Myotis thysanodes*	juvenile	female	non-reproductive	N/A	N/A	N/A
8/10/2023	590	*Myotis thysanodes*	adult	female	lactating	N/A	N/A	N/A
8/10/2023	591	*Myotis ciliolabrum*	juvenile	male	non-scrotal	N/A	N/A	N/A
8/10/2023	**592**	***Leptonycteris yerbabuenae***	**adult**	**male**	**scrotal**	**989.001041205043**	**Yes**	**No**
8/10/2023	**593**	***Leptonycteris yerbabuenae***	**adult**	**female**	**non-reproductive**	**989.001041205034**	**Yes**	**No**
8/10/2023	**594**	***Leptonycteris yerbabuenae***	**adult**	**female**	**post-lactating**	**989.001041205004**	**Yes**	**Yes**
8/10/2023	**595**	***Choeronycteris mexicana***	**adult**	**female**	**non-reproductive**	**989.001041205062**	**Yes**	**No**
8/11/2023	596	*Myotis ciliolabrum*	juvenile	female	non-reproductive	N/A	N/A	N/A
8/11/2023	**597**	***Choeronycteris mexicana***	**adult**	**female**	**lactating**	**989.001041205003**	**Yes**	**Yes**
8/15/2023	602	*Corynorhinus townsendii*	adult	female	non-reproductive	N/A	N/A	N/A
8/15/2023	603	*Myotis thysanodes*	juvenile	male	non-scrotal	N/A	N/A	N/A
8/15/2023	**604**	***Leptonycteris yerbabuenae***	**adult**	**female**	**post-lactating**	**989.001041205000**	**Yes**	**No**
8/15/2023	605	*Myotis ciliolabrum*	adult	male	non-scrotal	N/A	N/A	N/A
8/15/2023	606	*Myotis ciliolabrum*	adult	female	non-reproductive	N/A	N/A	N/A
8/16/2023	**607**	***Leptonycteris yerbabuenae***	**juvenile**	**female**	**non-reproductive**	**989.001041205081**	**Yes**	**Yes**

8/16/2023	**608**	***Leptonycteris yerbabuenae***	**sub-adult**	**male**	**non-scrotal**	**989.001041205031**	**Yes**	**Yes**
8/16/2023	609	*Corynorhinus townsendii*	juvenile	male	non-scrotal	N/A	N/A	N/A
9/8/2023	632	*Corynorhinus townsendii*	adult	female	non-reproductive	N/A	N/A	N/A
9/10/2023	**633**	***Leptonycteris yerbabuenae***	**sub-adult**	**female**	**non-reproductive**	**989.001045162705**	**Yes**	**No**
9/11/2023	**634**	***Leptonycteris yerbabuenae***	**sub-adult**	**male**	**non-scrotal**	**989.001045162684**	**Yes**	**No**
10/20/2023	**665**	***Leptonycteris yerbabuenae***	**adult**	**female**	**non-reproductive**	**989.001045162640**	**Yes**	**No**
10/20/2023	**666**	***Leptonycteris yerbabuenae***	**adult**	**female**	**post-lactating**	**989.001045162700**	**Yes**	**Yes**
10/20/2023	**667**	***Leptonycteris yerbabuenae***	**sub-adult**	**male**	**non-scrotal**	**989.001045162666**	**Yes**	**No**

Table 5: Samples from eDNA surveys of blooming agaves on BLM lands in southeast New Mexico (Otero County).

Date	Site Name	Agave ID	Lat	Long	Sample Name	Number of Swabs	Agave Species
6/19/2023	RR 506 NM	SWW1	32.322582590000025	-105.2445403	KL23A0034	1	*Agave parryi neomexicana*
6/19/2023	RR 506-2 NM	SWW2	32.42171176000005	-105.33851	KL23A0001	2	*Agave parryi neomexicana*
6/20/2023	BLM 3 NM	SWW4	32.54313821000005	-105.6898021	KL23A0027	1	*Agave parryi neomexicana*
6/20/2023	BLM 3 NM	SWW5	32.542726850000065	-105.6897421	KL23A0033	2	*Agave parryi neomexicana*
6/20/2023	BLM 2 NM	SWW6	32.56787894000007	-105.7286465	KL23A0009	1	*Agave parryi neomexicana*
6/20/2023	BLM 2 NM	SWW7	32.567703160000065	-105.7288598	KL23A0013	1	*Agave parryi neomexicana*
6/20/2023	BLM 2 NM	SWW8	32.567508040000064	-105.7293035	KL23A0021	2	*Agave parryi neomexicana*
6/20/2023	BLM 2 NM	SWW9	32.56764057000004	-105.7291555	KL23A0029	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Jackpot	SWW11	32.346560440000076	-105.4214813	KL23A0020	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Jackpot	SWW12	32.34398863000007	-105.4188644	KL23A0032	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Jackpot	SWW13	32.34360108000004	-105.4186047	KL23A0031	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Jackpot	SWW14	32.34803470000003	-105.4221453	KL23A0017	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Jackpot	SWW15	32.345321230000025	-105.4213445	KL23A0022	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Cornuda 1	SWW16	32.05721597000007	-105.518467	KL23A0007	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Cornuda 1	SWW17	32.05851806000004	-105.5182867	KL23A0014	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Cornuda 1	SWW18	32.05857038000005	-105.5180686	KL23A0016	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Cornuda 1	SWW19	32.05821308000003	-105.5176712	KL23A0019	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Cornuda 1	SWW20	32.05666226000005	-105.5184519	KL23A0011	2	*Agave parryi neomexicana*
6/21/2023	BLM NM Cornuda 1	SWW21	32.05834290000007	-105.5183595	KL23A0006	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Cornuda 1	SWW22	32.05817052000003	-105.5176827	KL23A0023	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Cornuda 1	SWW23	32.34540885000007	-105.4202053	KL23A0005	2	*Agave parryi neomexicana*
6/21/2023	BLM NM Jackpot	SWW24	32.34805785000003	-105.4231281	KL23A0028	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Jackpot	SWW25	32.34786260000004	-105.4224098	KL23A0026	1	*Agave parryi neomexicana*
6/21/2023	BLM NM Jackpot	SWW26	32.34472910000005	-105.4207901	KL23A0018	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 9	SWW27	32.321315800000036	-105.3286825	KL23A0024	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 8	SWW28	32.31628297000003	-105.3363896	KL23A0008	1	*Agave parryi neomexicana*
6/22/2023	BLM NM Boatload	SWW29	32.43152618000005	-105.2939475	KL23A0146	1	*Agave parryi neomexicana*

6/22/2023	BLM NM Boatload	SWW30	32.43040184000006	-105.2949479	KL23A0136	1	*Agave parryi neomexicana*
6/22/2023	BLM NM Boatload	SWW31	32.43052171000005	-105.2942615	KL23A0215	1	*Agave parryi neomexicana*
6/22/2023	BLM NM Boatload	SWW32	32.43106024000008	-105.2939916	KL23A0166	1	*Agave parryi neomexicana*
6/22/2023	BLM NM Boatload	SWW33	32.430828650000024	-105.2941418	KL23A0185	1	*Agave parryi neomexicana*
6/22/2023	BLM NM Boatload	SWW34	32.430256660000055	-105.2946137	KL23A0225	1	*Agave parryi neomexicana*
6/22/2023	BLM NM Boatload	SWW35	32.43032254000008	-105.2949028	KL23A0176	1	*Agave parryi neomexicana*
6/22/2023	BLM NM Boatload	SWW36	32.43070957000003	-105.2954791	KL23A0156	1	*Agave parryi neomexicana*
6/22/2023	BLM NM Boatload	SWW37	32.43024314000007	-105.2948978	KL23A0186	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 10	SWW38	32.32102796000004	-105.3244777	KL23A0135	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 10	SWW39	32.320337530000074	-105.3249284	KL23A0145	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 10	SWW40	32.321361910000064	-105.3244056	KL23A0175	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 10	SWW41	32.32019929000006	-105.3252661	KL23A0015	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 11	SWW42	32.44733647000004	-105.3358693	KL23A0155	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 12	SWW43	32.41117622000007	-105.3136073	KL23A0165	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 7	SWW44	32.317101140000034	-105.3463233	KL23A0003	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 7	SWW45	32.31767938000007	-105.3461076	KL23A0010	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 7	SWW46	32.315958470000055	-105.3482621	KL23A0012	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 7	SWW47	32.31570861000006	-105.3469867	KL23A0002	1	*Agave parryi neomexicana*
6/22/2023	BLM NM 7	SWW48	32.31572306000004	-105.3469828	KL23A0004	1	*Agave parryi neomexicana*
6/29/2023	BLM NM	SWW8	32.567508040000064	-105.7293035	KL23A0144	2	*Agave parryi neomexicana*
6/29/2023	BLM NM 10	SWW41	32.32019929000006	-105.3252661	KL23A0207	2	*Agave parryi neomexicana*
6/29/2023	BLM NM 10	SWW49	32.32140286900005	-105.3244133	KL23A0196	2	*Agave parryi neomexicana*
6/29/2023	BLM NM 10	SWW50	32.32051454800006	-105.325144	KL23A0205	2	*Agave parryi neomexicana*
6/29/2023	BLM NM 10	SWW51	32.32105228700004	-105.3244929	KL23A0218	2	*Agave parryi neomexicana*
6/29/2023	BLM NM 10	SWW39	32.320337530000074	-105.3249284	KL23A0157	2	*Agave parryi neomexicana*
6/29/2023	BLM NM 3	SWW5	32.542726850000065	-105.6897421	KL23A0154	2	*Agave parryi neomexicana*
6/29/2023	BLM NM 7	SWW52	32.315936633000035	-105.3476114	KL23A0214	2	*Agave parryi neomexicana*
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6/29/2023	BLM NM 7	SWW44	32.317101140000034	-105.3463233	KL23A0177	2	*Agave parryi neomexicana*
6/29/2023	BLM NM 7	SWW53	32.31730691800004	-105.3459471	KL23A0147	2	*Agave parryi neomexicana*

6/29/2023	BLM NM Boatload	SWW54	32.43199626300003	-105.2928852	KL23A0168	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW55	32.43087950800003	-105.2936465	KL23A0188	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW56	32.430973031000065	-105.2932844	KL23A0208	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW57	32.43052697500008	-105.2945073	KL23A0153	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW58	32.43044629800005	-105.294617	KL23A0143	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW31	32.43052171000005	-105.2942615	KL23A0167	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW59			KL23A0228		*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW60	32.431894019000026	-105.2928854	KL23A0178	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW61	32.43113719200005	-105.2939791	KL23A0183	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW37	32.43024314000007	-105.2948978	KL23A0198	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW62	32.43024528700005	-105.2944405	KL23A0224	2	*Agave parryi neomexicana*
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6/29/2023	BLM NM Boatload	SWW64	32.43069736000007	-105.2954165	KL23A0234	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW65	32.431327897000074	-105.2936988	KL23A0193	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW66	32.43068542800006	-105.2941045	KL23A0137	2	*Agave parryi neomexicana*
6/29/2023	BLM NM Boatload	SWW67	32.43102733500007	-105.2943347	KL23A0163	2	*Agave parryi neomexicana*
6/29/2023	Jackpot	SWW23	32.34540885000007	-105.4202053	KL23A0174	2	*Agave parryi neomexicana*
6/29/2023	Jackpot	SWW11	32.346560440000076	-105.4214813	KL23A0164	2	*Agave parryi neomexicana*
6/29/2023	Jackpot	SWW24	32.34805785000003	-105.4231281	KL23A0194	2	*Agave parryi neomexicana*
6/29/2023	Jackpot	SWW26	32.34472910000005	-105.4207901	KL23A0184	2	*Agave parryi neomexicana*
6/30/2023	BLM NM	SWW68	32.567554802000075	-105.7291791	KL23A0141	2	*Agave parryi neomexicana*
6/30/2023	BLM NM 3	SWW5	32.542726850000065	-105.6897421	KL23A0160	2	*Agave parryi neomexicana*
6/30/2023	BLM NM 7	SWW69	32.31581598300005	-105.3470078	KL23A0192	2	*Agave parryi neomexicana*
6/30/2023	BLM NM 7	SWW70	32.317309745000045	-105.3461996	KL23A0139	2	*Agave parryi neomexicana*
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6/30/2023	BLM NM Boatload	SWW75	32.432682202000024	-105.2922334	KL23A0179	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW56	32.430973031000065	-105.2932844	KL23A0138	2	*Agave parryi neomexicana*

6/30/2023	BLM NM Boatload	SWW76	32.43195515000008	-105.2929058	KL23A0219	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW77	32.43186789500004	-105.2928816	KL23A0229	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW78	32.431296674000066	-105.2936621	KL23A0158	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW79	32.432717471000046	-105.2923417	KL23A0169	2	*Agave parryi neomexicana*
6/30/2023	BLM NM 11	SWW42	32.44733647000004	-105.3358693	KL23A0159	2	*Agave parryi neomexicana*
6/30/2023	BLM NM 10	SWW38	32.32102796000004	-105.3244777	KL23A0200	2	*Agave parryi neomexicana*
6/30/2023	BLM NM 10	SWW40	32.321361910000064	-105.3244056	KL23A0149	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW80	32.43235094600004	-105.2922883	KL23A0189	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW81	32.43200824000007	-105.294051	KL23A0233	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW82	32.43098164200006	-105.2942653	KL23A0187	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW83	32.43138423000005	-105.2935171	KL23A0148	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW84			KL23A0195		*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW85			KL23A0142		*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW86	32.43155304000004	-105.2938397	KL23A0213	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW87	32.431185596000034	-105.2942036	KL23A0203	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW88	32.432160242000066	-105.2932628	KL23A0152	2	*Agave parryi neomexicana*
6/30/2023	RR506-2	SWW2	32.42171176000005	-105.33851	KL23A0162	2	*Agave parryi neomexicana*
6/30/2023	BLM NM 10	SWW89	32.32032717800007	-105.3249381	KL23a0172	2	*Agave parryi neomexicana*
6/30/2023	BLM NM 10	SWW90	32.32049197400005	-105.3251421	KL23a0182	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Boatload	SWW91	32.43225442700003	-105.2933559	KL23A0223	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Jackpot	SWW24	32.34805785000003	-105.4231281	KL23A0230	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Jackpot	SWW92	32.34661991200005	-105.4214506	KL23A0232	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Jackpot	SWW93	32.34539958400006	-105.4202239	KL23A0222	2	*Agave parryi neomexicana*
6/30/2023	BLM NM Jackpot	SWW94	32.34479735700006	-105.4208291	KL23A0212	2	*Agave parryi neomexicana*
7/12/2023	BLM NM Boatload	SWW95	32.430841446000045	-105.293551	KL23A0073	2	*Agave parryi neomexicana*
7/12/2023	BLM NM Boatload	SWW96	32.432005969000045	-105.2940715	KL23A0070	2	*Agave parryi neomexicana*
7/12/2023	BLM NM Boatload	SWW97			KL23A0081	2	*Agave parryi neomexicana*
7/12/2023	BLM NM Boatload	SWW98	32.43184913500005	-105.292879	KL23A0078	2	*Agave parryi neomexicana*
7/12/2023	BLM NM Boatload	SWW99	32.43060996100007	-105.2951035	KL23A0064	2	*Agave parryi neomexicana*
7/12/2023	BLM NM Boatload	SWW100	32.43250672400006	-105.2932353	KL23A0067	2	*Agave parryi neomexicana*

7/12/2023	BLM NM Boatload	SWW101	32.43095565900006	-105.29328	KL23A0080	2	*Agave parryi neomexicana*
7/13/2023	BLM NM Boatload	SWW102	32.432562990000065	-105.2933098	KL23A0181	2	*Agave parryi neomexicana*
7/13/2023	BLM NM Boatload	SWW103	32.43200582500003	-105.2940966	KL23A0075	2	*Agave parryi neomexicana*
7/13/2023	BLM NM Boatload	SWW104	32.43185832300003	-105.2929185	KL23A0043	2	*Agave parryi neomexicana*
7/13/2023	BLM NM Boatload	SWW105	32.43083979800008	-105.2935855	KL23A0231	2	*Agave parryi neomexicana*
7/13/2023	BLM NM Boatload	SWW106	32.43064096100005	-105.2940774	KL23A0140	2	*Agave parryi neomexicana*
7/13/2023	BLM NM Boatload	SWW107	32.43095460400008	-105.293336	KL23A0076	2	*Agave parryi neomexicana*
7/13/2023	BLM NM Boatload	SWW108	32.432898375000036	-105.2951192	KL23A0171	2	*Agave parryi neomexicana*
7/13/2023	BLM NM Boatload	SWW109	32.430602763000024	-105.2951175	KL23A0066	2	*Agave parryi neomexicana*

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Appendix 2: Draft instructions for an eDNA field sampling "kit" and eDNA sampling protocol from blooming agaves

Nectar Bat eDNA from Agaves Collection Protocol

This protocol describes the collection of eDNA (environmental DNA) from blooming agaves using qPCR (quantitative polymerase chain reaction) to detect foraging patches and migratory corridors of nectar-feeding bat species.

Traditional methods for surveying for the presence of nectar-feeding bats (e.g., roost surveys; acoustic monitoring or camera monitoring of bat visits to foraging resources) often prove expensive, time-intensive, and unreliable. Due to advances in science and technology, we are now able to collect organisms' DNA from their environment (called environmental DNA, or eDNA). eDNA analysis is a rapid, cost-effective, non-invasive biodiversity monitoring tool that uses DNA left behind in the environment by organisms for species detection.

Proof-of-concept of the efficacy of detecting nectar-feeding bats from eDNA left on agave flowers was published in 2022 (Walker, F. M., Sanchez, D. E., Froehlich, E. M., Federman, E. L., Lyman, J. A., Owens, M., & Lear, K. (2022). Endangered nectar-feeding bat detected by environmental DNA on flowers. Animals, 12(22), 3075).

This procedure defines the steps to: identify target agaves; collect samples; exercise contamination control; document/mark/store samples; and ship samples for analysis.

The three nectar-feeding bat species currently being identified are: Mexican long-nosed bat (*Leptonycteris nivalis*); Lesser long-nosed bat (*Leptonycteris yerbabuenae*); and Mexican long-tongued bat (*Choeronycteris mexicana*).

Target agaves for sampling are paniculate (branching) blooming agaves in the Trans-Pecos of Texas; southwestern New Mexico; and southeastern Arizona.

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General

Things to keep in mind:

- If possible, swab in the early morning to minimize the time that any eDNA has had to degrade after deposition and to help workers avoid the midday heat.
- Contamination control protects both the bats and the eDNA's quality.
- Documentation and identification are as important as the sample itself.
- Pay attention to your surroundings and your safety; you are what makes this research possible.

Contamination control note:

Due to the variability of field conditions, sample contamination control is addressed through layers of protection. Washing one's hands often and laundering clothing such as safety vests on a regular basis assists with this endeavor. Taking care in the sequence of operations and promptly closing containers of clean sampling supplies after use helps prevent the introduction of stray material and other contaminants.

Documentation and identification note:

In order for the samples to be useful they must be identifiable to a specific location, with additional data providing more site-specific and date information. Clearly mark samples and maintain the precision of geotagging and address references.

Expected preferences of sampling selection:

The probability of detecting the target bats is primarily improved by maximizing the number of samples collected during agave blooming season. The UV exposure resulting from long days, clear skies, and high elevation will limit the life of eDNA left on blooms. It is unknown whether the probability of detection is more effective with repetitive sampling of a limited group of plants or by sampling more widely-distributed plants just once. The primary variable in increasing the probability of detection is the number of samples. In general, a sampling scheme that uses both sampling methods will enable the maximum collection of samples and is thus preferred.

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Materials and Supplies Needed

Pole

- Telescoping 6-24” DocaPole with 2 angle adapters and flexible foam or rubber tip. This reduces bulk and provides for angle adjustment of the sampling head. Other telescoping poles are available, but we have found the DocaPole to be less prone to damage. (Alternative: If shorter agaves are predominant, an 8-foot-long lightweight garden stake may be used. If angle adapters are not available, PVC plumbing pieces may be used to configure the head with duct tape and a chair leg’s punctured rubber cap can hold swabs – see below.)

Contamination Control

- Disposable sanitary gloves per site, Latex/Nitrile/Polypropylene acceptable as convenient and tolerable for user. Use adequate size for convenient use; larger gloves allow easier donning and removal.
- Alcohol wipes
- Distilled water in flip-top squeeze bottle, multiple 1 oz are convenient.
- Hand sanitizer
- Poly tubing 6”W x ≥36”L (e.g., ULINE S-5765) or Large Obstetric Veterinary gloves, for covering top of pole during sampling. (Alternative: Trash bags cinched with rubber bands or painters tape. Primary materials are preferable for ease of use and reduced wind effect.)

Sampling Materials

- Swabs
- Vials preloaded with RNALater
- Vial labels
- Parafilm cut into strips for sealing vials
- Ziploc bags (snack, quart, and gallon)
- Sharpie permanent markers (fine & ultra-fine)
- A small binder to keep paperwork and labels together, especially in windy conditions
- Garbage bucket 2-5 gal (Squatty 2 gal tends to be convenient and stable)
- Trash liners
- Rubber bands
- Soft cooler (ice pack/blue ice/freezable cooler)
- Plastic organizer box to reduce materials loss, keep small bits organized, and simplify inventory. Linear packing reinforces the sampling procedure. Packed properly, this gives a grab-and-run kit. Stanley Sortmaster and Sortmaster Juniors are effective. Ensure secure closure and section segregation.
- Backpack or larger case. (Sometimes helpful if working away from home or vehicle, also contains bulkier items. Tackle box or tool box for larger field operations)

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Recommended Tools

- Folding utility knife or folding knife
- Screwdriver appropriate to DocaPole for adjustment of clamps
- Small straight blade screwdriver to assist label lifting and parafilm separation
- Alternate: multitool

Safety Items

- Safety vest (with large pockets)
- Business cards of BCI handler, currently Kristen Lear
- Optional "AGAVE SURVEY" sign and/or flashing yellow light for vehicles. These are useful in high-traffic or metropolitan areas. Signs and lights provide some clarity for public works and law enforcement personnel, and may also foster conversation with passersby on the project. Additionally, local public contacts (i.e., public works) may allow notification of projects in city social media, which can provide additional public validity for sampling activities.

Examples:

Your configuration should be as convenient as possible for your collection location and means of transportation. 2023 collections ranged from driving city streets to stepping out in the back yard to loading up a mule and riding out to the back country. The procedure establishes the requirements, so how you set up your own equipment is at your convenience.

In-town car set up. Field Box: All packs into large box, small pieces in Stanley box, Binder holds procedures, notes, and holds down labels in wind. Photos: Lindsey Bredemeyer.

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Selecting Agave for Sampling

Target agaves:

The target agaves are paniculate species. In the United States these are: *Agave palmeri*; *Agave parryi parryi*; *Agave parryi neomexicana*; *Agave havardiana*; *Agave glomeruliflora*; *Agave americana*; and *Agave salmiana*. These have branching bloom clusters. While *Agave lechuguilla* is an agave, it does not have a bloom configuration benefiting the nectar-feeding bats in the United States. Yuccas and sotols are also not known as food sources in the United States. In the Sonoran Desert, *Leptonycteris yerbabuenae* is known to also feed from columnar cacti. Within the Chihuahuan Desert, alternate food sources are not known. If there are significant plant populations with a high nectar load in bloom at the same time as the agave, it may be useful to sample a few of these on occasion.

Assessing the Sampling Site:

Upon arrival at blooming agave, assess site for safe and appropriate sampling. Look for adverse conditions or barriers such as traffic, power lines, weather conditions, property limits, wildlife/livestock, and legal access. State and national parks require official approvals for sampling. Ensure that public areas do not have similar restrictions.

Assessing blooms:

Ensure that the blooms are properly open. Ensure that the blooms have not dried out. In some cases, there may be a smell of nectar or even obvious dripping from the blooms. Depending on available blooms at the beginning and end of the season, it may be worthwhile to sample blooms for which the entire cluster is not yet open or for which some flowers are starting to wither. The limited feeding opportunities outside of peak bloom may help channel bats to these locations.

Locations:

If working intermittently in an open field location or you have limited tim, attempt to sample as many of the plants with higher numbers of open flowers.

If repetitively sampling an area with many plants (e.g., a municipality), attempt to sample different plants each day. In an artificial environment, the different species of plants may provide a much longer total bloom period than natural agaves.

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Recommended Data Entry:

Lead surveyor name*
initials-name. e.g., "JFD-John Doe." Initials provide indexing for later entries.

Other surveyors
Not necessary with most sampling, even if someone is helping with same samples. Only needed for crew type operations.

State*

Site Name*
This is primarily for surveyor reference. It can be a quick reference or a supporting location to the point*. On a street with a large number of agaves "E Ave A 306" will clarify the location. Also, specific sites such as TxDOT or motel. If sampling the same site over time and site name is not repeated exactly, don't worry, Point* will clarify.

Landowner Name
This is relevant if specific permission is required such as private landowner or agency property (e.g. DMSP, TPWD, or DMP TNC). It can also give general information such as streetside or city park.

Coordinates*

Date and time of sample collection*

Sample name*
Unique to each agave and sample. The Sample name should be labeled on the sample vial exactly as defined. The day's collection bag should be marked with surveyor and date (e.g. "-JFD-2023-01-23") to provide additional identification if marking is damaged.

Number of swabs in sample*
Preferably 2 swabs per vial, from the same agave.

Agave species
Identify if possible. In domestic areas, there may be a range of non-local species. Good photos address this if species identification is problematic.

Site photo
Photo to show multiple plants in the area, road/light, and other proximity variables

Agave photo
Photo of full height of plant

Rosette photo
Photo of the rosette only (this is significant if confirmation of species is required)

Additional notes
Anything that may better define habitat: grove conditions, scattered agave, nearby tree coverage, high traffic, artificial night lighting, etc.

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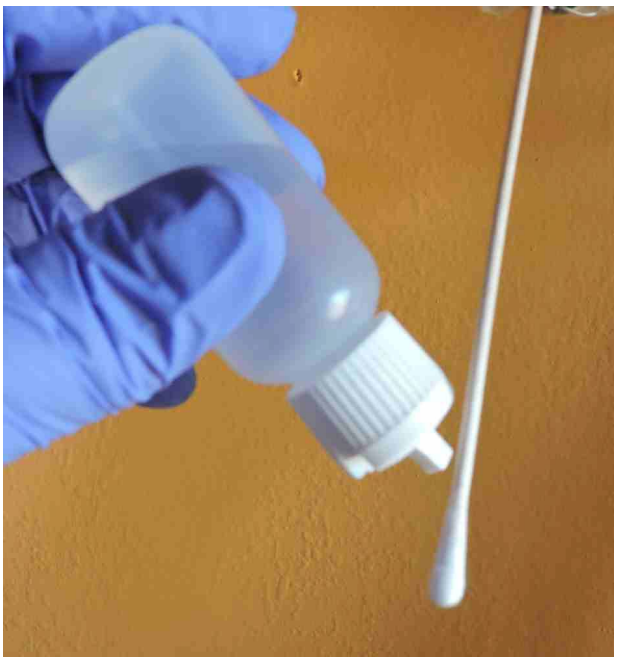
Equipment Preparation

Use caution when handling materials to prevent accidental contact of sampling swabs with non-target surfaces.

- Select pole as appropriate for height of the agave. It may be easier to adjust the pole height before preparation or after preparation. Adjust head angle as necessary to present swab straight into blooms.
- Secure trash liner in trash bucket or ready a trash bag as preferred.
- Put on disposable gloves, avoiding extraneous contact as much as possible.
- Prepare label for vial with permanent marker. Use the EXACT sample name entered in the data sheet. Wrap label around vial so that sample name is clearly presented. Use caution not to unscrew the vial's lid when applying label. Place vial in clean accessible position.

- Cover upper pole with poly tubing or vet glove. (Alternative: Use garbage bags secured in place with painters' tape or rubber bands. Snugly secure bag to prevent flapping and blowing around in the wind. Using garbage bags isn't preferred because they can be blown around in the wind and the amount of effort required to mount and remove.)
- Open the stick end of swab, then insert the swab into the swab holder attached to the pole, puncturing the plastic pole covering.
- Pull off swab cover, drip distilled water on swab end. Flip-top bottles can be opened with one hand. Drip water onto swab, avoiding direct contact. Dispose of swab wrapper in the garbage bucket.
- Use two swabs per plant. If there are many open umbels, you can use one swab on some umbels and the second swab on the other umbels. If there are only a few umbels open, you can use both swabs on the same umbels. Put both swabs into the same labeled vial.



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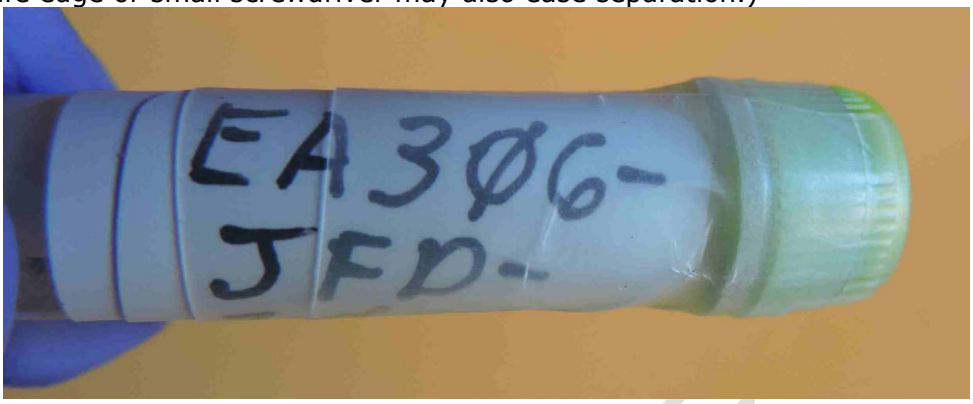
Swabbing the Agave

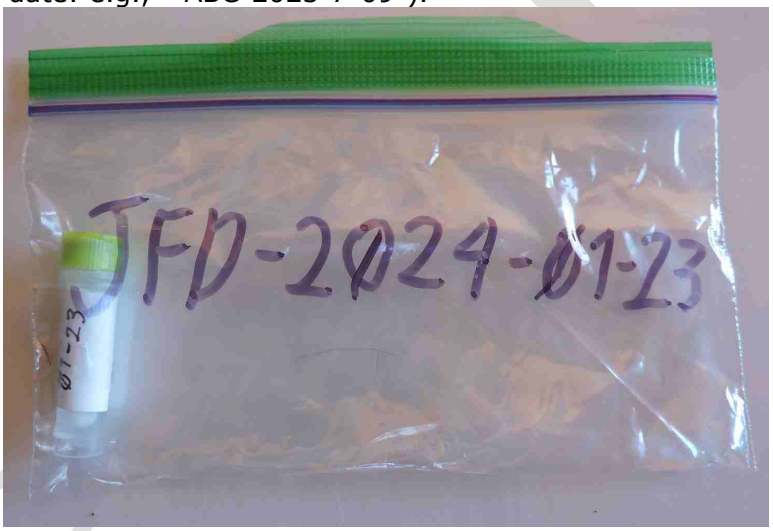
- Extend the pole as necessary.
- Swab open flower clusters (branchlets, or umbels) for 15 seconds. If there are many open umbels, you can use one swab on some umbels and the second swab on the other umbels. If there are only a few umbels open, you can use both swabs on the same umbels. Both swabs will be put into the same labeled vial.
- Bring down pole as convenient, minimizing swab contamination through extraneous contact with non-target surfaces.

- Open the vial, keeping the vial upright.
- Remove swab by holding upper end of stick. Place swab into vial and snap stick at top of vial.
- Replace cap on vial.

- Repeat prior procedure with second swab and swab a second bloom on the plant.
- Remove swab and place in the same vial as before.
- Close the vial by screwing the cap on tightly . Stretch parafilm around the cap of the vial several times to secure it from leaks. Take care not to loosen cap while wrapping the parafilm. (Note: Parafilm does not contain adhesive, but rolling or flicking the edge may assist separating the film from the backing. A knife edge or small screwdriver may also ease separation.)



- Place the vial in a snack size Ziploc bag. Each bag should be marked with simplified sample information (sampler-date: e.g., "-ABC-2023-7-09").



- Place bag in cooler, preferably with the vial caps facing up to ensure sample saturation with RNALater.
- In the event of delayed return or loss of cold storage, store the samples in conditions as dark, cool, and sanitary as possible. Placing all sample vials in an additional bag barrier, keeping vials upright, and wrapping the bag holding the sample vials in a wet towel will provide significant protection under desert conditions.
- If sampling another plant in the immediate area, repeating the process with the same pole cover and gloves is acceptable unless contamination is suspected.
- Sample plants as necessary in the site, then decontaminate the sampling gear (e.g., pole).
- After finishing sampling the agaves within a site, place all individual Ziploc bags into a gallon Ziploc bag, and label the gallon Ziploc bag with the Site Name. This provides some redundancy in marking and additional isolation/containment for samples from unique areas.

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Decontaminate Equipment

- Remove the sleeve from the pole, strip inside out if possible, being as careful as possible to avoid touching the outside of the bags to the pole. Dispose of sleeve.
- Remove your gloves (reference current medical method of glove removal to minimize contaminated contact) and dispose of gloves.
- Use alcohol wipes to wipe the pole from top to bottom, being sure not to touch any of the already sanitized surface with your bare hands. Dispose of the dirty wipe.
- Sanitize your hands.
- Collapse the pole if needed and put it away.
- Load supplies into vehicle as necessary.

Sample Storage

- Remove the Ziploc bags of samples for the day and place them in a refrigerator, making sure the tops of vials face upwards. For the sampling season, refrigerator storage is adequate for protection of eDNA. If you will be storing samples for months before shipping for analysis, place vials in a freezer (ensure that it's not a self-defrosting freezer that will go through freeze-thaw cycles).
- Wash the soft cooler and ice packs. Sanitize both with alcohol wipes after removing the samples.
- Place the cooler and ice packs back in the freezer to cool for the next day's collection effort, if applicable.

Shipping the Samples for Analysis

- Shipping direct may be necessary for some remote surveyors. If working with a particular local agency on sampling, they may provide consolidation and more experience in handling biological samples.
- Ice packs with next day or 2nd day delivery is fine for most samples. If samples are shipped frozen, package with dry ice to maintain frozen condition. Commercial cold ship packaging is adequate.
- If sending to Dr. Faith Walker at Northern Arizona University directly, include a hard copy and digital Excel spreadsheet of sample information.

Bat Conservation International works with Dr. Faith Walker (Northern Arizona University, Faith.Walker@nau.edu) for sample analysis. If sending to Dr. Walker directly, FedEx the samples to the following address:
Dr. Faith Walker
Northern Arizona University
Applied Research and Development Bldg 56
PMI 2nd Floor
Knoles Dr.
Flagstaff, AZ
86011-4073
USA
Mark packages as "**TEMPERATURE AND TIME SENSITIVE**"

Upon receipt, Dr. Walker's lab will receive and properly store the samples.
The qPCR method favors batch processing for each species. The complete analysis of all samples and species may be delayed.

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Appendix 3: eDNA results from Big Hatchet Mountains monitoring for Task 1

Task 1 Results: Monitor for the Continued Presence of Mexican Long-nosed Bat (*Leptonycteris nivalis*) in the Big Hatchet Mountains

Combined Trip with CSU, BCI, and BLM - Big Hatchet Mountains Fecal eDNA Collection
Date: January 8th, 2024
Initial Tarp Deployment: On October 25th, 2022, plastic ground sheets were deployed to collect bat fecal material as bats entered and exited the roost. A total of four tarps were used, with one tarp placed beneath an alternate cave entrance called "The Crack" in the larger room, and another tarp placed in front of the entrance to the lowest room. In the lowest room two tarps were deployed. The first tarp was positioned at the base of the main wall, where the team was able to find fecal samples from Leptonycteris spp., and the second tarp was placed in an adjacent room. On May 28th, 2023, the CSU team visited the roost to confirm that the ground sheets were still in place in the upper room.
Trip Notes: On January 8th, 2024, Mallory Davies (CSU), Cody Howard (BLM), Meredith Davis (BLM), Lucas Castro (BLM), Jackson Bain (BCI subterranean team), and Myriam Bishop (BCI subterranean team) visited the roost to collect fecal samples from the four deployed tarps and redeploy tarps in all rooms. The collected fecal samples were stored in sealed vials of RNAlater. The team collected a total of 7 vials: 4 from the larger room and 3 from the lowest room. The samples were then sent to Dr. Faith Walker's Bat Ecology and Genetics Lab at NAU for eDNA analysis to identify the bat species.
Additional Notes: Significant rain event in October may have compromised samples in in the upper rooms. High quality samples were collected separately from low quality samples to control for contamination.

Figure 1: Big Hatchet Mountains eDNA collection trip notes.

Table 1: Collection Information for Fecal Samples from Big Hatchet Mountains.
All tarps were deployed on 10/25/2022, and all samples were collected on 1/8/2024. The collected fecal samples were stored in sealed vials of RNAlater and shipped to Dr. Faith Walker's Bat Ecology and Genetics Lab at NAU for eDNA analysis.

Sample ID	Collection Location	Sample Notes	Collection Description
001	Entrance to Lower Room	High Quality Samples	Collected fecal samples that appeared to be nectar bat fecal splats, i.e., yellow, round or elongated shape, and flat on tarp.
002	Entrance to Lower Room	Low Quality Samples	Fecal samples collected from pooled locations on tarp due to water collection.
003	Cave Crack	High Quality Samples	Collected fecal samples that appeared to be nectar bat fecal splats and not water drops, i.e., yellow, round or elongated shape, and flat on tarp.
004	Cave Crack	Low Quality Samples	Collected fecal samples that were dusty but appeared to be nectar bat fecal splats and not water drops, i.e., yellow, round or elongated shape, and flat on tarp.
005	Lower Room, Tarp 1	High Quality Samples	Collected fecal samples with no visible dust that appeared to be nectar bat fecal splats, i.e., yellow, round or elongated shape, and flat on tarp. 10 individual splats total. No signs of water contamination on tarp.
006	Lower Room, Tarp 1	Low Quality Samples	Collected fecal samples that may have dust but appeared to be nectar bat fecal splats, i.e., yellow, round or elongated shape, and flat on tarp. 65 individual splats total. No signs of water contamination on tarp.
007	Lower Room, Tarp 2	All Samples Present	Collected fecal samples that appeared to be nectar bat fecal splats, i.e., yellow, round or elongated shape, and flat on tarp. 30 individual splats total. No signs of water contamination on tarp.

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Table 2: Metabarcoding bat ID (multi-species) results for fecal samples collected from Big Hatchet Mountains on 1/8/2024. All samples were preserved in RNAlater using the Species from Feces sampling kit and extracted with the Qiagen Fast Stool Mini prep. Out of seven samples, six passed sequencing but sample 005 failed and has no results. The lab noted a slight leak in the sample bag upon receipt of the samples, which may have caused contamination or loss of sample material in the bag, thus affecting the ability to successfully run sample analyses (for example, leading to insufficient sample material for analysis or lower numbers of reads than would have occurred otherwise). Six out of seven samples had positive reads for *L. yerbabuenae* and *L. nivalis* was not detected in any of the samples. However, there were low numbers of *L. yerbabuenae* reads in samples 006 and 007 despite these samples being collected from 65+ and 30+ individual yellow splats in the roost (which, based on our previous work, should have yielded much higher numbers of reads). This suggests that the bag leak led to loss of sample material in the bag and therefore artificially low reads for *L. yerbabuenae*. Because of the low reads for *L. yerbabuenae* (which historically occur at higher numbers than *L. nivalis* in the site), we also suggest that the negative results for *L. nivalis* be considered inconclusive.

Sample ID	Status	Species	Reads	Marker	Classifier	Laboratory comments
001	Pass	*Myotis yumanensis* OR *ciliolabrum* OR *californicus*	18041	SFF	BLAST.MEGAN. LCA.97	Slight leak in bag; may not have enough to subsample
001	Pass	***Leptonycteris yerbabuenae***	12281	SFF	NB	Slight leak in bag; may not have enough to subsample
002	Pass	***Leptonycteris yerbabuenae***	23321	SFF	NB	Slight leak in bag
002	Pass	*Corynorhinus townsendii*	2099	SFF	NB	Slight leak in bag
002	Pass	*Peromyscus eremicus*	1866	SFF	BLAST. MEGAN. LCA.97	Slight leak in bag
002	Pass	*Myotis evotis* OR *thysanodes*	762	SFF	NB	Slight leak in bag
002	Pass	*Homo sapiens (Human)*	326	SFF	BLAST. MEGAN. LCA.97	Slight leak in bag
002	Pass	*Myotis velifer*	146	SFF	NB	Slight leak in bag
003	Pass	***Leptonycteris yerbabuenae***	31454	SFF	NB	Slight leak in bag; may not have enough to subsample
004	Pass	*Myotis yumanensis* OR *ciliolabrum* OR *californicus*	8666	SFF	BLAST. MEGAN. LCA.97	May not have enough to subsample
004	Pass	*Corynorhinus townsendii*	7722	SFF	NB	May not have enough to subsample
004	Pass	***Leptonycteris yerbabuenae***	6606	SFF	NB	May not have enough to subsample
004	Pass	*Peromyscus eremicus*	5871	SFF	BLAST. MEGAN. LCA.97	May not have enough to subsample
005	Fail					May not have enough to subsample
006	Pass	*Corynorhinus townsendii*	19384	SFF	NB	May not have enough to subsample
006	Pass	***Leptonycteris yerbabuenae***	10	SFF	NB	May not have enough to subsample

007	Pass	*Myotis evotis* OR *thysanodes*	11183	SFF	BLAST. MEGAN. LCA.97	Slight leak in bag; may not have enough to subsample
007	Pass	*Corynorhinus townsendii*	1424	SFF	NB	Slight leak in bag; may not have enough to subsample

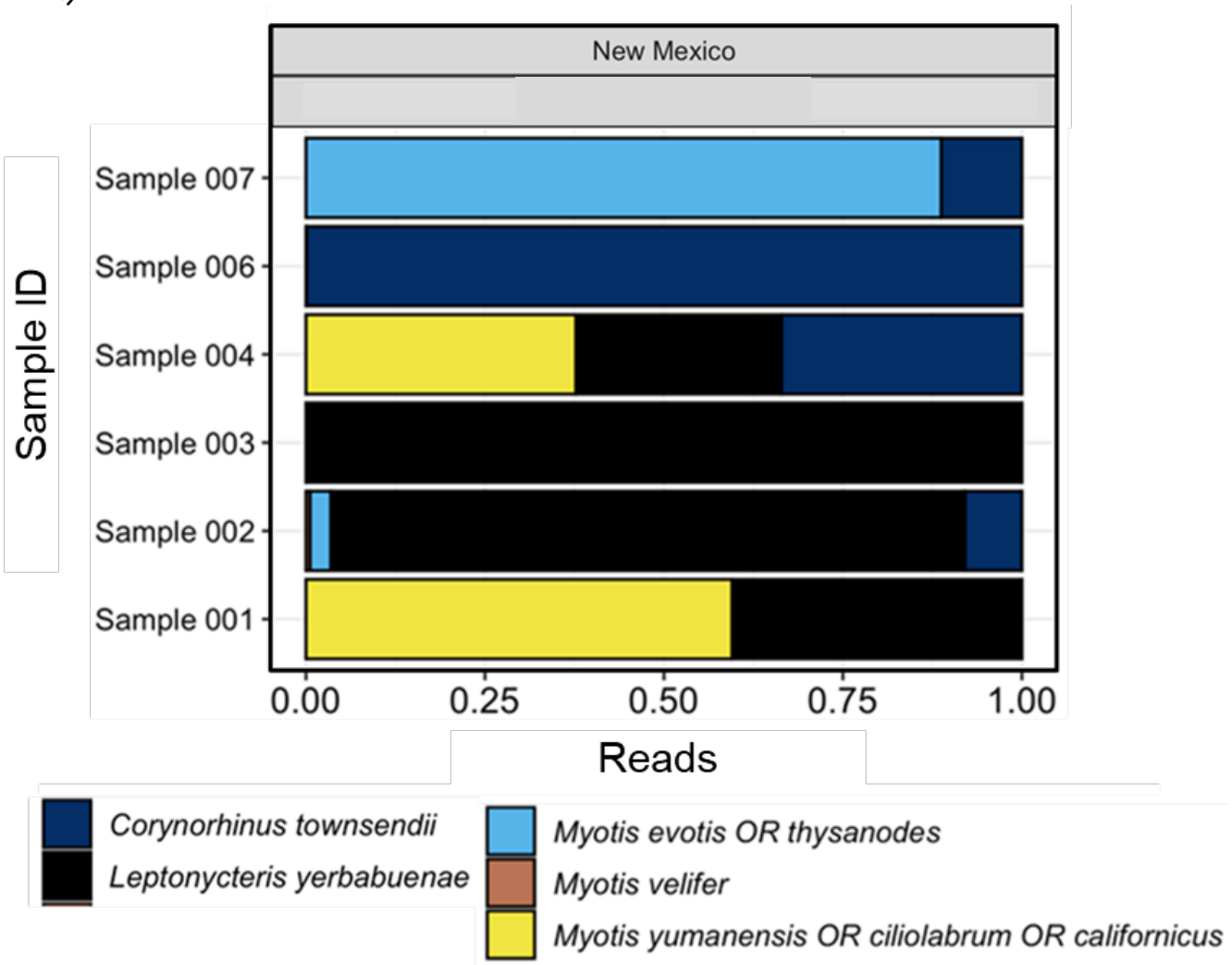

Figure 2: Detection plot for proportion of species reads by sample ID. *Leptonycteris yerbabuenae* makes up more than 25% of the reads in samples 001 - 004. Sample 006 shows dominant *Corynorhinus townsendii* reads and less than 1% *L. yerbabuenae* reads. Sample 007 has no *L. yerbabuenae* reads.

Interpretation of Big Hatchet Mountains findings for the continued presence of Mexican Long-nosed Bat (*Leptonycteris nivalis*)

Six out of seven samples collected from the Big Hatchet Mountains had positive reads for *L. yerbabuenae*, while *L. nivalis* was not detected in any of the samples. However, there were low numbers of *L. yerbabuenae* reads in samples 006 and 007 despite these samples being collected from 65+ and 30+ individual yellow splats in the roost (which, based on our previous work, should have yielded much higher numbers of reads). Upon receipt of the samples, the lab noted a slight leak in the sample bag which could have led to loss of sample material and therefore artificially low reads for *L. yerbabuenae*. Because of the low reads for *L. yerbabuenae* (which historically occur at higher numbers than *L. nivalis* in the site), we also suggest that the negative results for *L. nivalis* be considered inconclusive, and that further monitoring of the site for *L. nivalis* should be conducted.

Appendix 4: eDNA results from eastern New Mexico agave surveys for Task 2

qPCR analysis of the 126 eDNA samples collected from blooming agaves on BLM lands in Otero County did not yield any detections/potential detections for *L. nivalis*, *L. yerbabuenae*, or *C. mexicana*. These results suggest that this area of southeastern New Mexico is not a migratory corridor at this time. However, additional surveys for nectar bats (eDNA surveys and acoustic surveys) in this area are recommended over additional years to better assess whether this area may be a migratory corridor.

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Appendix 5: Diet analysis results for Task 3

Task 3 Results: Use pollen and fecal samples gathered in task 2 to determine the relative importance of *Agave* spp. in nectar bat diets and determine if other nectar plants are utilized in the northernmost extent of the bats' ranges

Table 1: Sample information for samples sent to Pisces Molecular lab in Boulder, CO.
Service: NGS sequencing to identify micro- or macro-biome in a sample. Identifying plant and insect species.

Date Collected	Sample ID	Location	Species	Bat #	Notes on Capture Site & individual
7/18/2023	**001**	Big Hatchet Mts, NM	*Leptonycteris yerbabuenae*	567	Active roost site located at a cave in a BLM wilderness study area. Reproductive adult male caught in the PM during emergence.
8/10/2023	**008**	Peloncillo Mountains, NM	*Leptonycteris yerbabuenae*	594	Residential site located on the edge of BLM land. Post lactating adult female caught in the PM at a hummingbird feeder. Lack of plants and insects detected in diet may indicate a reliance on sugar water from hummingbird feeders.
8/11/2023	**009**	Peloncillo Mountains, NM	*Choeronycteris mexicana*	597	Residential site located on the edge of BLM land. Lactating adult female caught in the PM at a hummingbird feeder.
8/16/2023	**011**	Big Hatchet Mts., NM	*Leptonycteris yerbabuenae*	607	Active roost site located at a cave in a BLM wilderness study area. Juvenile female caught in the AM during return.
8/27/2023	**012**	Big Hatchet Mts., NM	*Leptonycteris yerbabuenae*	608	Active roost site located at a cave in a BLM wilderness study area. Juvenile male caught in the AM during return.
10/20/2023	**021**	Silver City, NM	*Leptonycteris yerbabuenae*	666	Residential site located in Silver City, NM. Post lactating adult female caught in the PM at a hummingbird feeder.

Table 2: Plant taxa found by trnL metabarcoding (short barcode region generated by c and h primers; Taberlet et al. 2007). Numbers represent counts of NGS reads per taxon per sample. Five of six samples contained reads for Agave *spp.* In total, 8 plant families were detected that have species that are pollinated primarily by insects or bats. Notable detections include the four genera of the Fabaceae family *(Erythrostemon* spp.*, Eysenhardtia texana, Medicago* spp.*, Vicia bungei),* Oleaceae *(Fontanesia phillíraeoides),* Onagraceae *(Chamaenerion angustifolium),* Solanaceae *(Solanum* spp.*),* Musaceae *(Musa* spp.*)*, and Rosaceae family. Sample 8 did not contain any Asparagaceae reads but did contain reads for the Fabaceae family, *Vicia bungei,* and the Rosaceae family.

Customer Sample ID:	**1**	**8**	**9**	**11**	**12**	**21**
Pisces ID:	173543	173548	173549	173553	173554	173563
Taxon	**S089987**	**S089937**	**S089928**	**S089966**	**S089956**	**S089945**
	0	0	0	0	35	0
Eukaryota; Streptophyta; Magnoliopsida						
Asparagales; Asparagaceae;	**0**	**0**	**216**	**0**	**0**	**0**
Agave spp.	**28491**	**0**	**3486**	**7493**	**16858**	**1556**
Asterales; Asteraceae;	0	0	0	0	0	8741
Fabales; Fabaceae;	0	1368	341	0	0	0
Erythrostemon spp.	0	0	944	0	0	0
Eysenhardtia texana	0	0	265	0	0	0
Medicago spp.	0	0	0	0	0	190
Vicia bungei	0	1411	0	0	0	0
Lamiales; Oleaceae;	0	0	3079	0	0	0
Fontanesia phillíraeoides	0	0	38	0	0	0
Malpighiales; Salicaceae; *Populus* spp.	0	0	3207	0	0	0
Myrtales; Onagraceae; *Chamaenerion* spp.	0	0	0	100	0	0
Chamaenerion angustifolium	0	0	0	173	0	0
Rosales; Rosaceae;	177	2234	0	0	0	0
Solanales; Solanaceae; *Solanum* spp.	0	0	579	0	0	0
Zingiberales; Musaceae; *Musa* spp.	0	0	0	0	0	1714
Eukaryota; Streptophyta; Pinopsida						
Cupressales; Cupressaceae;	0	0	620	0	0	0
Hesperocyparis spp.	0	0	5402	0	0	0
Juniperus spp.	0	0	279	0	0	0
Pinales; Pinaceae; *Pinus* spp.	0	0	0	0	0	8712
TOTAL READS PER SAMPLE	**28668**	**5013**	**18456**	**7766**	**16893**	**20913**

Table 3: Arthropod taxa found by COI metabarcoding (short barcode region generated by ZBJ-ArtF1c and ZBJ-ArtR2c primers [Zeale et al. 2011]). Numbers in the table are counts of NGS reads per taxon per sample. Four out of the six samples contained reads for arthropods. The majority of the reads were from the Coleoptera, Diptera, and Lepidoptera orders. Samples 8 and 12 contained no arthropod reads.

Customer Sample ID:	**1**	**8**	**9**	**11**	**12**	**21**
Pisces ID:	173543	173548	173549	173553	173554	173563
Taxon	**S089987**	**S089937**	**S089928**	**S089966**	**S089956**	**S089945**
Eukaryota; Arthropoda; Arachnida; Araneae; Araneidae; *Neoscona crucifera*	0	0	31	0	0	0
Eukaryota; Arthropoda; Insecta;						
Coleoptera						
Dytiscidae; *Hygrotus* spp.	0	0	37	0	0	0
Elateridae;	0	0	339	0	0	0
Diptera;	0	0	0	1849	0	0
Sciomyzidae; Dictya;	0	0	0	27	0	0
Streblidae; *Trichobius dugesii*	0	0	0	0	0	355
Trichobius sphaeronotus	0	0	0	0	0	948
Lepidoptera						
Erebidae; *Matigramma emmilta*	65	0	0	0	0	0
Geometridae; *Digrammia* spp.	0	0	3820	0	0	0
Noctuidae; *Protorthodes ustulata*	0	0	0	0	0	11
Eukaryota; Chlorophyta; Chlorodendrophyceae; Chlorodendrales; Chlorodendraceae; *Scherffelia dubia*	0	0	80	0	0	0
Eukaryota; Oomycota; unk_class; Pythiales; Pythiaceae;	0	0	0	0	46	0
Eukaryota; Rotifera; Eurotatoria; Ploima; Lecanidae; Lecane; *Lecane hamata*	0	0	0	0	96	0
TOTAL READS PER SAMPLE	**65**	**0**	**4307**	**1876**	**142**	**1314**
		≤ 10 Arth CO1 reads				

Interpretation of diet findings:

Fecal samples 8, 9, and 21 were collected from nectar bats captured at hummingbird feeder sites. Sample 8 contained no agave reads and no insect reads, suggesting that the individual was feeding on non-plant and non-insect food resources such as sugar water from the hummingbird feeders. All samples collected in the Big Hatchet Mountains contained positive reads for *Agave* spp. and less than 4% reads for non-Asparagaceae plants; samples collected at residential sites with hummingbird feeders had at least 80% of their plant reads from non-Asparagaceae plants. Some notable detections of plants that are primarily pollinated through biotic factors and therefore have a lower possibility of being consumed incidentally (i.e., bats grooming themselves and incidentally consuming wind-dispersed pollen that was collected on their fur) include four genera of the Fabaceae family (*Erythrostemon* spp., *Eysenhardtia texana, Medicago* spp., *Vicia bungei*), Oleaceae (*Fontanesia phillиraeoides*), Onagraceae (*Chamaenerion angustifolium*), Solanaceae (*Solanum* spp.), and Musaceae (*Musa* spp.). Cross-contamination of pollen from other nectarivores (i.e., bees, birds, beetles) from non-agave plants to agave plants and cross-contamination from insect pollinators consumed by the bats after the insects have fed on non-agave plants may occur and should be considered. However, the notable plants that were detected possess traits that are common with chiropterophilous flowers such as light-colored upward-facing inflorescences, nocturnal nectar release, wide-mouthed and elongated flower shape, etc. In addition, some of the plants detected in the diet analysis are food plants for these nectar bats in other parts of their ranges.

Choeronycteris mexicana sample #9 contains a wider diversity of food resources than do the *L. yerbabuenae* samples. More data are necessary to decisively characterize the diet of *C. mexicana*; however, it is possible that this species is more of a dietary generalist than is *L. yerbabuenae.*

Our findings suggest that lesser long-nosed bats in New Mexico supplement their diet with non-agave plant food sources such as other plant nectar resources and sugar water from hummingbird feeders when agave nectar is less available. The CSU team intends to further investigate the diet of *L. yerbabuenae* by incorporating these results into a larger data set to look at how the bats' diets change within seasons and across years in relation to the relative availabilities of agave nectar and sugar water from hummingbird feeders.