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

June 26, 2023

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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Project Need

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 (NMDGF 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 roost is 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 Emory Cave in Texas and the Big Hatchet roost remains undefined. A record *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 (BCI 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 roost 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 nectivorous 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 of pooled fecal samples from the Big Hatchet roost, 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 nectivorous 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).

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 (Research Topic 22 of the NMDGF State Wildlife Action Plan). We will: 1) monitor for the continued presence of *L. nivalis* in the Big Hatchet roost using eDNA analysis of pooled fecal samples collected from 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. This work will aid in identifying the distributions and the potential range expansions of these SGCN species in the state and will help prioritize roost sites and foraging areas for protection and restoration. Our work will assist NMDGF and BLM in developing the best management strategies for these SGCN species and will greatly enhance the success of BCI's Agave Restoration Initiative in the state.

Project Activities

Bat Conservation International staff and Dr. Kathryn Stoner and Mallory Davies at Colorado State University will complete the following activities:

1) **Task 1: Monitor for the continued presence of the Mexican long-nosed bat (*Leptonycteris nivalis*) in the Big Hatchet roost** by testing for Mexican long-nosed bat environmental DNA (eDNA) in a pooled fecal sample. We will use a ground sheet, anchored with rocks with no ground disturbance and placed just inside the cave's entrance in 2022, to collect fecal samples as bats enter and exit the roost. We will collect the pooled fecal sample once in November 2023.

2) **Task 2: Monitor the distribution of the Mexican long-nosed bat, Lesser long-nosed bat (*L. yerbabuenae*), and Mexican long-tongued bat (*Choeronycteris mexicana*) outside of the Bootheel and identify these species' migratory corridor(s)** by deploying artificial nectar feeders, where possible on existing trees or structures, at a minimum of 7 residential sites in Grant and Hidalgo counties, including but not limited to sites in Cliff, Rodeo, and Silver City. We will fit feeders with PIT tag readers to detect and record the presence of any visiting nectar bats previously PIT-tagged by CSU researchers. We will mist net nectar bats near the feeders and collect pollen and fecal samples from the fur of captured nectar bats where feasible.

   As part of Task 2, we are using 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). We anticipate collecting approximately 164 eDNA samples from flowering agaves over 5 occasions at approximately 12 sites on Bureau of Land Management (BLM) Las Cruces District Office lands and at sites on New Mexico State Land Office (SLO) lands within areas of higher agave occurrence between May and October 2023. We will collect eDNA by swabbing open agave flowers with a polyester swab attached to a pole. We will use results from previous acoustic surveys, recommendations from Agency staff, and local phenology information from BLM staff and other local land managers to select eDNA sampling sites. We will develop a qPCR assay for the Mexican long-tongued bat and use this new assay, and two extant assays for the other two focal species, to analyze all eDNA samples for the presence of the three focal nectar bat species.

3) **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.** We will use pollen grain identification techniques to identify plant species in pollen and fecal samples. We will resuspend fecal pellets with 70% ethanol 24 hours prior to analysis and tease them apart under a dissecting scope. We will remove arthropod fragments; finely crush, separate, and subsample the remaining material; and create slides for pollen identification using a microscope, published identification guides, and a field-gathered reference collection.

Summary of Progress

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

Previous efforts in 2022

In May 2022, there was a joined effort between CSU, BLM, BCI, and the U.S. Fish and Wildlife Service to map out Big Hatchet Roost 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 since the team did not want to disturb the roosting Townsend's big-eared bat (*Corynorhinus townsendii*). In late October after all the bats were gone, the team attempted another exhibition 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 about a half hour collecting fecal samples from the rock wall by scrapping them off 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 from the past year, but this cannot be confirmed since the room has not been accessed in about 10 years. Mallory also collected two additional 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. Mallory suspects the samples collected from the tarps 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 so 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 (Northern Arizona University) 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 in the 2023 Field Season

The CSU team has returned to the Big Hatchet Roost and confirmed that the tarps are still deployed from the last visit. The BLM has confirmed continued support for the project and is working with the CSU team to plan for the next fecal collection this November 2023.

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

Development of Choeronycteris mexicana qPCR Assay

We are 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 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 nectivorous bats from eDNA left on agave flowers was established through a prior collaboration between Dr. Kristen Lear (BCI's Agave Restoration Program Manager) and Dr. Faith Walker (head of the Bat Ecology and Genetics Lab and Species From Feces lab at NAU) (Walker et al. 2022).

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 is supporting the development of a qPCR assay for this species so that all three pollinating bats can be detected from samples collected within the state and across the species' ranges.

As of this report (June 26, 2023), Dr. Walker's lab is working to generate baseline data from which to design primers for *C. mexicana*. This process has been 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, we expect primer design and testing to be completed in July 2023.

Deployment of Artificial Nectar Feeders

Mallory Davies and Kennedy Daniels (CSU) reported to the Stoner lab field site in Hachita, New Mexico on May 22, 2023, and Daniel Milton joined the field team on June 18, 2023. Since the start of the field season, we have deployed hummingbird feeders and trail cameras at a residential site in Silver City, New Mexico and at another residential site south of Rodeo, New Mexico. We are also in contact with one volunteer in Silver City and one in White Signal, New Mexico who are currently monitoring their hummingbird feeders with personal trail cameras for nectar bat activity. In spring 2023, Daniel successfully built a low-cost PIT tag reader that we can deploy on hummingbird feeders, and he is currently fine-tuning the antenna to optimize function and detectability. We intend to have seven sites established in New Mexico by the end of July 2023 with hummingbird feeders that have PIT tag readers attached and a trail camera deployed to monitor for activity. The CSU team

intends to perform bat capture surveys to collect pollen and fecal samples using mist nets at the feeder sites where the trail camera or PIT tag reader captures nectar bat activity.

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 (77% of flower cuts tested positive versus 70% of swabs), and is much less invasive. In addition, our field results indicate that both swabbing flowers and cutting flowers are 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, 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, as well as more permitting restrictions involved with 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 7-30 foot pole for taller agaves). Swabs are stored in 2 mL vials of RNALater, a non-toxic DNA preservative solution.

Rachel Burke's (former BLM staff) agave distribution models indicate the presence of suitable habitat for nectar bats in southern New Mexico, including the Brokeoff Mountains, Sacramento Mountains, and Guadalupe Mountains[16]. 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, but BCI conducted our first trip to the field in June 2023.

As of June 26, 2023, BCI has collected 49 samples from 49 agaves on BLM lands in Otero County between June 19-22, 2023. During the three-day survey, we recorded the locations of additional blooming agaves and non-blooming agave patches. We currently have two additional trips planned to return to these sites in June and July to re-sample these agaves in addition to sampling new agaves until the blooming period ends. The samples will be stored in a freezer until the end of the season, at which time all samples will be shipped to Dr. Walker's lab at NAU for analysis.

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

The CSU team has acquired a compound microscope and intends to use pollen grain identification techniques to identify plant species in pollen and fecal samples that will be collected this field season at our nectar feeder sites described in Task 2. Analysis of fecal samples will take place at the field house in fall 2023 and throughout the 2023-2024 winter.

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