DESCRIPTION AND IDENTIFICATION OF THE ECHOLOCATION CALLS OF *LEPTONYCTERIS NIVALIS* AND OTHER NECTAR FEEDING BATS IN MEXICO

A Thesis

by

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ABSTRACT

The conservation of nectar-feeding bats is critical for preserving arid, semi-arid and desert ecosystems, in addition to the genetic diversity of some ecologically and economically important plant species. In the northern region of Mexico, in particular, some of the species that have been affected by the disruption of the pollination dynamics are *Leptonycteris nivalis, Choeronycteris mexicana, Leptonycteris yerbabuenae,* and *Antrozous pallidus.*

In this study, I aim to identify and describe the echolocation call of the four active gleaning forager species with the purpose of providing a passive acoustic monitoring technique. I measured and assessed over 70 parameters and employed a PCA to determine the minimum number of variables that best described and allowed identification of species. A Kruskal-Wallis followed by pairwise comparisons was used to determine if there were statistically significant differences between the species and identify the most reliable descriptive variables for species segregation.

In addition to the description of the echolocation call sequences, I analyzed data of *A. pallidus* and *L. yerbauenae* collected from different localities in the northern portion of Mexico to measure potential geographical variation. To assess geographical variations, I employed a MANOVA for each species to assess multivariate differences among regions.

The results suggest that the nine predictor variables which best described the calls and segregated between species are the Bndwdth, HiFreq, LowFreq, CallDur, Fc, FreqKnee, DominantSlope, SteepestSlope, and LowestSlope of the calls. An LDA demonstrated that sufficient variability did exist among the groups to accurately discriminate calls among species. The multiple comparisons analysis results suggest that the discriminant variables that best segregated the calls by species were FreqKnee, HiFreq and LowFreq for *A. pallidus*; Bndwdth

and CallDur for *L. yerbabuenae;* DominantSlope, SteepestSlope and LowestSlope for *L. nivalis;* and LowestSlope for *C. mexicana.*

The results of the MANOVA comparing *L. yerbabuenae* calls among the different locations were not significant. Meanwhile for the species *A. pallidus* the results of the multivariate analysis of variance tests on the ten predictor variables comparing the call structure differences among the were significant for all logarithmic transformed variables. The changes in call structure followed a cline from West to East.

DEDICATION

To the first biologist I have ever met and inspired me the most, my mom, Ayleen.

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NOMENCLATURE

Antpal	*Antrozous pallidus*
BC	Baja California
Chomex	*Choeronycteris mexicana*
CF	Constant Frequency
DGO	Durango
E	Edge not over the water
FM	Frequency Modulated
IUCN	International Union for Conservation of Nature
LDA	Linear Discriminant Analysis
Lepniv	*Leptonycteris nivalis*
Lepyer	*Leptonycteris yerbabuenae*
O	Open space not over water
PCA	Principal Component Analysis
SO	Sonora
WNS	White-Nose Syndrome

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CHAPTER I

INTRODUCTION

The conservation of threatened species, exacerbated by the loss of biodiversity at an unprecedented rate, is one of the challenges most commonly faced by conservation biologists. Anthropogenic activities, the introduction of invasive species, land-use change, habitat loss, overexploitation of resources, and climate change are some of the main causes that have been identified as causes of population declines (Lacher & Roach, 2019). This rapid loss of biodiversity at the population scale can have detrimental effects on the ecosystem dynamics, altering and interrupting ecological services and their natural functionality (Lacher & Roach, 2019). Currently, 21.3% of the vertebrate species around the world are considered to be threatened by the International Union for Conservation of Nature (IUCN), a pattern mirrored by other major groups of organisms such as mammals, for which 17.4% of species assessed have been identified as threatened (IUCN Red List of Threatened Species, 2020).

Bats, in particular, are the most widely distributed terrestrial mammals on Earth and constitute nearly a fifth of mammalian biodiversity, with 1,395 species now recognized (Frick, Kingston, & Flanders, 2019). However, Chiroptera is one of the most threatened groups of mammals, with over half of the species (57%) catalogued as having unknown or decreasing population trends (IUCN Red List of Threatened Species, 2020). This order has life history and behavioral traits that make their populations vulnerable to factors that can result in population decline. In contrast to other mammals, bats have a low reproductive rate, and many species are specialists, so their food sources are limited by seasonality, and they require shelters that provide

microclimates with the particular conditions to raise young in summer or to hibernate during the winter (Wilson & Mittermeier, 2019). Under such conditions, bats colonies can be very vulnerable to environmental changes, food shortages due to overexploitation, disturbance and disruption by human activities, as well as to habitat degradation or modification.

All of these concerns remain as major threats to bat populations, but over time, new threats have also emerged. The mass die-offs of pteropodids bats in Australia and South Asia from heats waves (Welberngen et al., 2008), high rates of mortality at wind energy turbine installations (Frick et al., 2017), and the infectious fungal disease of bats, the white-nose syndrome (WNS) in North America (Frick, Kingston, & Flanders, 2019), are some examples of the risk factors that have been identified by researchers as recent drivers of population declines.

More recently, the outbreak of coronavirus disease 19 (COVID-19) that has been phylogenetically related to SARS-like bat viruses have also had a detrimental effect on bat populations. Although the zoonotic source of COVID-19 has yet been confirmed, various studies have suggested bats as the primary reservoir (Shereen, et.al., 2020). To make matters worse, public misinformation has led to a negative perception of bat species thus resulting in an increase in hostility towards bats worldwide.

Despite the fact that habitat loss, overexploitation and climate change are threats also faced by other groups of organisms, the conservation of bat populations is particularly challenging since there are certain aspects of bat ecology that remain unknown. While 15% of bats species are considered to be either Critically Endangered, Endangered, or Vulnerable by the IUCN, 18% of bat species are Data Deficient (Frick, Kingston, & Flanders, 2019). The data deficiency hinders accurate assessment of conservation status and the identification of main stressors, since not all bat species are threatened in the same magnitude or facing the same

threats. For example, for fruits bats and flying foxes the main threats faced are hunting, deforestation, introduction of invasive species and environmental disturbances, for New World species the major threats are habitat fragmentation, human destruction or disturbances of roosting caves and mines (Allen-Wardell, et al., 1998). Consequently, the criteria for prioritization will depend on the capacity, objectives, and desired outcomes and may differ even among groups who share similar values and goals (Frick, Kingston, & Flanders, 2019). Thus, assessing the main stressors of bat populations will be key to proposing actions that can decrease the risk of local or global extinctions and mitigate the drivers of species declines (Dirzo, et al., 2014).

In the case of pollinating bat populations, there is unequivocal evidence documenting dramatic declines for individual species (Allen-Wardell, et al., 1998). Habitat degradation, for example, has been strongly associated with the decline of many nectar-feeding bat species (Kunz, et. al., 2011). In the south-west United States and the northern region of Mexico, in particular, some of the species that have been affected by the disruption of the pollination dynamics are the Mexican long-nosed bat *(Leptonycteris nivalis,* Phyllostomidae*),* the Mexican long-tonged bat *(Choeronycteris mexicana*, Phyllostomidae*),* the Lesser long-nosed bat (*Leptonycteris yerbabuenae,* Phyllostomidae), and the Pallid bat *(Antrozous pallidus,* Vespertilionidae).

Leptonycteris nivalis, Choeronycteris mexicana and *Leptonycteris yerbabuenae*, are nectar-feeding bats species responsible for providing ecosystem services by pollinating plants and dispersing seeds in the northern region of Mexico. Nectar-feeding bat foraging strategies likely evolved from insectivorous ancestors that initially gleaned insects from plant surfaces (Gillete, 1975). As part of the evolutionary process, nectar-feeding species have developed

morphological adaptations such as elongated noses and tongues to extract nectar from flowers. The development of highly specialized morphological characteristics has created a strong dependency on nectar and pollen (Johnson & Steiner, 2000).

Antrozous pallidus, in contrast to *L. nivalis*, *C. mexicana*, and *L. yerbabuenae*, is considered as an insectivorous bat species as they feed primarily on insects in open habitats with relatively little vegetation at or near ground level (Bell, 1982). Notwithstanding, Pallid bats are opportunistic predators and nectarivores, and represent the first known case of nectarivorous habits in a New World bat outside of the family Phyllostomidae. The diet plasticity observed in this species amplifies the niche dimensions and generates competition with *L. yerbabuenae* in this region of Mexico where both distributions overlap.

Previously, it had been assumed that pallid bats visiting cardon flowers were gleaning insects attracted to the flowers, not consuming the nectar or at least doing so secondarily to foraging for insects (Frick, Heady III, & Hayes, 2009). Contrary to what was assumed, recent studies by Frick et. al. (2013), show that in the Sonoran Desert and the Baja California Peninsula regions pallid bats consumed enough pollen and nectar during the spring bloom to temporarily alter their stable isotope ratios to fall halfway between insectivorous and nectarivorous bats, like *L. yerbabuenae* which plays a fundamental role as the main pollinating species in this region. Due to the lack of morphological adaptations to successfully extract nectar from cacti flowers, pallid bats require greater maneuverability to access the nectar. Under these circumstances pallid bats are more effective pollinators of cardon cacti than the specialized nectar-feeding bat, *L. yerbabuenae*, delivering up to 8 times more pollen (Gervais, 2016). Even though, *A. pallidus* is not formally considered as a polinating species, the shared similarities in

the feeding strategies of *L. nivalis, C. mexicana, L. yerbabuenae* and *A. pallidus* allow us to classify them in the same ecological guild as substrate gleaners.

Some ecologically and economically important plant species in the south-western United States and the northern region of Mexico, including pitaya cactus (*Stenocereus* spp.), and agave (*Agave* spp.) have also evolved relying heavily on bat pollination and seed dispersal to propagate. Species of the family Agavaceae, for example, are considered keystone species with a fundamental role in semi-arid to arid regions. Agaves are semelparous organisms which reproduce once in a lifetime in a single reproductive act (Gomez-Ruiz, 2015a). As a consequence, these plants have developed different mechanisms to ensure their reproduction. They can reproduce asexually creating offspring genetically identical to the parent through clone propagation or sexually by seed dispersal. Insects, birds, and bats serve as a mobile link among plant populations facilitating pollen and gene flow over considerable distances (Gomez-Ruiz, 2015b). In the northern region of Mexico, agaves are used as raw materials for the production of beverages such as tequila, mezcal, agua miel and pulque. The prevention of soil erosion, the provisioning of material for the construction of fences, and providing food for livestock are other utilities of this group of plants. This wide variety of uses and services provided emphasize the ecological and socio-economic importance of the *Agave* ssp. in the northeast region of Mexico.

Unlike the agaves, the giant Mexican columnar cactus (cardon), *Pachycereus pringlei*, is not particularly known for its economic or commercial value. Nonetheless, it is a key species in the Sonoran Desert and the Baja California Peninsula regions that is also adapted for bat pollination. Its large white flowers are open during the night for less than 18 hours (opening at sunset and closing the next morning or midday) and produce copious amounts of nectar and

pollen (Fleming, Maurice, & Hamrick, 1998). Even though flowers are visited by a variety of birds and diurnal insects during the morning hours the majority of pollination activity occurs from nocturnal bat visits (Fleming, et. al., 2001). *P. pringlei* are trioecious, so the co-occurrence of male, female, and hermaphrodite individuals in high frequencies is observed within populations. As a consequence of nocturnal pollination adaptation, male and female individuals of *P. pringlei* are much more pollinator-dependent than hermaphrodites, making nectarivorous bats play an important role as pollinators for the species (Frick, Price, Heady III, & Kay, 2013).

Despite the fact that the food resources required by nectar-feeding bats are patchily distributed, the nectar is only seasonally available (Cole & Wilson, 2006). The strong correlation between the nectar-feeding bats' diets and the availability of local plant resources, promotes migratory behaviors (Cole & Wilson, 2006). Nectar feeding bats in nature exhibit a particular way to feed; they first visit several flowers and consume nectar until they satiate, then they rest in to digest their meal, then they visit flowers again. In this way they can visit up to 1,000 flowers per night (Tschapka and Dressler 2002). The pollen dispersal mechanism by these winged pollinators helps maintain genetic diversity within populations. This genetic diversity reduces threats by providing more flexibility to face environmental stress and by increasing the size of the gene pool, making the population less susceptible to inherited disorders. In contrast with other pollinators, bats are large-bodied and have the advantage of carrying larger pollen loads among distant individuals greatly augmenting gene flow (Fleming, Geiselman & Kress, 2009). As pollen and nectar mainly produced by Cactaceae and Agavaceae are the primary food source of pollinating bat species, a mutualistic relationship in which both organisms benefit has been established. However, this creates a co-dependence in which both parties can be affected if either of the two populations is threatened (Flores-Abreu, et al. 2019).

Unfortunately, the high demand and uncontrolled use of agave plants in Mexico, the collection of cacti in the United States, and habitat conversion for development, agriculture, and livestock grazing has led to the degradation of habitat and a decline in nectar-feeding bat populations. Farmers do not allow the flowering of several species of cultivated agaves by cutting the stem and the inflorescence. Consequently, the dynamics of pollination in many species is interrupted (Trejo-Salazar, Eguiarte, Suro-Piñeda, & Medellin, 2016).

The fact that many species of the family Phyllostomidae are food and habitat specialists, roost in caves, show migratory behavior, and are rare in numbers suggest that pollinating bats species are highly susceptible to extinction (Arita and Santos-Del-Prado, 1999). Information on the ecological and economic value of ecosystem services provided by bats can be used to protect or restore bat populations and associated habitats, as well as to improve public perception of bats (Kunz, Braun de Torrez, Bauer, Lobova, & Fleming, 2011). The data deficiency on the biology, abundance, and distribution patterns hinders our ability to accurate assess the conservation status of species that require immediate attention and impedes efforts to identify key sites and guide conservation actions. Clearly, more research is needed in order to establish effective conservation decision making and prioritization in the future.

Population monitoring techniques for pollinating bats, including studies of roosting behavior, foraging strategies, echolocation, and the importance of "nectar corridors" in migration are still in development (Gómez-Ruiz & Lacher, 2017), although technologies such as acoustic detectors offer many opportunities for study (Allen-Wardell, et al., 1998). As 80% of bats emit ultrasonic echolocation signals, acoustic monitoring can be used to conduct longitudinal studies to assess trends over time as well as compare across environmental gradients (e.g., land-use change) to provide a baseline understanding of bat habitat use and population trends (Frick,

Kingston, & Flanders, 2019). Information about the presence or absence of echolocating bats, foraging activity and the species of the detected bats can be assessed using echolocation calls. Passive acoustic sampling methods allow the recognition of key conservation sites and the assessment of anthropogenic impact and population trends by reducing the direct interaction with the individuals and not breaking the continuity of their foraging activities.

My thesis aims to identify, classify and describe, for the first time, the echolocation call structures of four narrow space active gleaning forager species *L. nivalis*, *C. mexicana*, *L. yerbabuenae* and *A. pallidus*, with the purpose of providing a passive acoustic monitoring method for these species. In the first study (Chapter II), I measured and assessed over 70 parameters, including temporal, amplitude and frequency-dependent variables, in addition to slope-related measurements for the description of the echolocation calls. I employed a principal component analysis (PCA) to determine the minimum number of variables that best described and allowed identification of species. After reducing the number of parameters to nine, I ran a Discriminant Analysis (DA) to assess interspecific variations among groups and determine the minimum number of dimensions needed to describe the possible differences between the echolocation call structures of the species. A non-parametric Kruskal-Wallis Analysis of Variance followed by pairwise comparisons was used to determine if there were statistically significant differences between the species and identify the most reliable descriptive variables for species segregation.

As many aspects of the calls depend on the foraging circumstances of the bat, the echolocation calls emitted are often subject to large intraspecific variability. Body size, habitat type, environmental conditions, and geographical location are some of the variables that may cause variation within and between populations on a local scale. If body size or condition varies

geographically, as occurs in some species, then call design may also vary geographically, for example with latitude (Barclay & Brigham, 2004).

The Neotropical leaf-nosed bats (Phyllostomidae), are often classified as "whispering bats" because of the low-intensity and high-frequency of the signals emitted. These characteristics make the recording process especially challenging, limiting our ability to properly describe the echolocation call structures for the species. However, field and laboratory studies have revealed that the structure of echolocation calls in phyllostomid bats is more variable at intra and interspecific levels than previously thought (Kalko, 2004). In addition to the description of the echolocation call sequences, in my second study (Chapter III), I also analyzed data of *A. pallidus* and *L. yerbauenae* collected from different localities in the northern portion of Mexico (Sonora, Baja California, Nuevo Leon, San Luis Potosi, and Durango) to measure potential geographical variation at an intraspecific level.

By analyzing the echolocation call structures of *Leptonycteris nivalis*, *Choeronycteris mexicana, Leptonycteris yerbabuenae* and *Antrozous pallidus* my goal is to provide a method to assess susceptibility of these species to the disruption of habitat, help fill information gaps in the biology of these species, and increase our ability to identify key conservation sites for better management and conservation practices.

CHAPTER II

DESCRIPTION AND IDENTIFICATION OF THE ECHOLOCATION CALLS OF *LEPTONYCTERIS NIVALIS, CHOERONYCTERIS MEXICANA, LEPTONYCTERIS YERBABUENAE* AND *ANTROZOUS PALLIDUS*

1. Synopsis

The current state of our knowledge regarding bat ecology is based largely on mist netting, radiotelemetry, and recent refinements in acoustic-detector technologies (Gannon & Sherwin, 2004). Over the past few decades, advances in acoustic technology have allowed the identification and a better understanding of the biology of foraging species. In this study, I described the echolocation calls for the nectar-feeding bats *Leptonycteris nivalis, Choeronycteris mexicana, Leptonycteris yerbabuenae* and the gleaning insectivorous bat *Antrozous pallidus*, a species that exhibits facultative nectar-feeding behavior.

I examined 74 temporal, amplitude and frequency-dependent variables, in addition to slope-related measurements to describe the echolocation call structures of these four species. The number of variables was reduced through an evaluation of intercorrelations and low weightings in an initial Principal Component Analysis (PCA) to determine a minimum of nine variables that best describe, and most contribute to the identification of the species. The description of the echolocation calls was mainly based on the calculated mean, range and standard deviations of the bandwidth, hi frequency, low frequency, call duration, characteristic frequency, frequency knee, dominant slope, steepest slope, and lowest slope of the calls. I used a PCA on the reduced variable set, followed by a Discriminant Analysis (DA) to determine the minimum number of dimensions needed to describe the differences between the echolocation call structure of the

species. A non-parametric Kruskal-Wallis Analysis of Variance, in conjunction with pairwise comparisons were used to determine if there were statistically significant differences between species distributions of the variables and identify the most reliable descriptive variables for species segregation.

The results suggest that the nine predictor variables which best described the calls and segregated between species are the Bndwdth, HiFreq, LowFreq, CallDuration, Fc, FreqKnee, Dominant slope, Steepest slope, and Lowest slope of the calls. Discriminant- Analysis demonstrated that sufficient variability did exist among the groups to accurately classify calls to the proper species. The multiple comparisons analysis results suggest that the discriminant variables that best segregated the calls by species were FreqKnee, HiFreq and LowFreq for *A. pallidus*; Bndwdth and CallDuration for *L. yerbabuenae;* Dominant slope, Steepest slope and Lowest slope for *L. nivalis;* and Lowest slope for *C. mexicana.*

This is one of the first studies to document the echolocation call structure of the three nectar-feeding bats *L. nivalis, C. mexicana*, *L. yerbabuenae* and the facultative nectar-feeding bat *A. pallidus*. It also provides guidelines about quantitative variables for the identification of the calls to a species level. This study also provides an acoustic population monitoring technique for the identification of key sites for pollinating bats.

2. Introduction

Healthy ecosystems are important in providing various regulatory processes (e.g., stabilization of soils, of diseases, regulation of climate, etc.); economic benefits (e.g., food, and beverages); and cultural benefits (e.g., aesthetic, educational, and recreational) that improve human well-being (Kunz, 1982). Bats, in particular, provide diverse ecosystem services as arthropod suppressors, seed dispersers, and pollinators (Kunz, 2002). The family

Phyllostomidae, commonly known as New World leaf-nosed bats, is comprised of 217 species, from which nearly 60 species are nectar-specialists (Carstens, et. al. 2002; Solari, et. al. 2019). These species from the Phyllostomidae family have developed morphological, physiological, and behavioral adaptations for a diet mainly based on floral nectar and pollen (Solari, et. al. 2019). Specializations by these species have resulted in a close association between bats and some groups of plants of great ecological and commercial value. Nectar-feeding bats play an important role as pollinators in tropical, sub-tropical and desert habitats (Lacher, et. al. 2019).

In the last decade, an alarming decline has been observed in different populations of nectar-feeding bat populations in North America (Frick, Kingston, & Flanders, 2019). In the intervening years, researchers have identified a series of ecological attributes that suggest that species in this group are more vulnerable to extinction than other groups of Neotropical bats (Arita & Santos del Prado, 1999). For example, the fact that the individuals that make up these species are specialists make them more susceptible to extinction than other groups of bats with general feeding habits. Many of these species require caves for roosting and form large concentrated aggregations (Gomez-Ruiz, et. al., 2015), exposing them to human intrusion, disturbance and habitat degradation due to vandalism, hunting and mining activities.

Nectar specialist bat species are an important component of the rich chiropteran fauna of Mexico (Arita & Santos-Del-Prado, 1999). Despite the fact that population trends and densities of nectar-feeding species are poorly documented due to a deficiency of data, in at least some parts of Mexico these populations are less abundant now than in the past years (Arita & Santos-Del-Prado, 1999). Four of the nectar-feeding bats species in the southwestern United States and the northern region of Mexico with evident population declines are the Mexican Long-Nosed bat (*Leptonycteris nivalis*, Phyllostomidae), the Lesser long-nosed bat (*Leptonycteris yerbabuenae*,

Phyllostomidae), the Mexican Long-Tonged bat (*Choeronycteris mexicana*, Phyllostomidae), and the Pallid bat (*Antrozous pallidus*, Vespertilionidae.

Although the decrease in these populations is largely due to the loss and degradation of their natural habitats (Arita & Santos del Prado, 1999), each of these species faces different threats and in different magnitudes. Even the threats faced by populations of the same species vary throughout their geographical range, thus complicating the assessment and identification of threats essential for the prioritization of conservation on a local scale. Below, I discuss in detail ecological and biological aspects, distribution patterns and current conservation status of each of the species included in this study to provide more context regarding the threats faced by these species and how the reduction of their populations can negatively impact the ecosystems in which they occur.

2.1. Leptonycteris nivalis

The migratory species, *L. nivalis*, is currently listed as endangered (EN) by the IUCN Red List of Threatened Species and by the US government (US Fish and Wildlife Service 1994), due to its rapid decline in the past 15 to 18 years (Medellín, 2016a). This nectar-feeding bat is a cave-dwelling species that migrates from south of the Big Bend National Park in southwestern Texas to the central region of Mexico (Figure 1). The species occurs in subtropical dry areas at high and medium elevations ranging from 500 – 3,000 meters (Medellin & Beardmore, 1994). Pregnant females migrate northward each spring following the blooming of *Agave* plants, their primary source of nectar in the northern portion of their range (Gómez-Ruiz, 2015b).

This food and habitat specialist species require underground sites such as caves and mines that are vital to the reproduction and survival of the species. In temperate countries, those sites may be used for breeding in summer and hibernation in winter, whereas in tropical

countries caves and mines may provide roosts for large colonies (Mickleburgh, Hutson, & Racey, 2002). Although it is a species that nests in colonies, the species seems to be rare throughout its range (Medellin & Beardmore, 1994).

L. nivalis is one of three species that have evolved into nectarivorous species adapted to desert conditions (Medellin & Beardmore, 1994). Their diet is mainly made up of nectar and pollen from agaves. The heavy reliance on agaves as the main food source has fostered a mutualistic relationship in which both groups of organisms benefit (Flores-Abreu, et. al. 2019). The *Agave* spp. are night-blooming plants that produce copious amounts of pollen at night, favoring the visit of night pollinators such as bats (Fleming, Maurice, & Hamrick, 1998). Despite having various reproductive mechanisms, whether through clones or seeds, agaves rely heavily on cross-pollination offered by nocturnal pollinators. Bats, in particular, play an important role in the reproduction of these plants since they have hairs and are comparably larger in size than other pollinators such as moths and hummingbirds, so they have the ability to transport larger amounts of pollen and travel long distances in one night (Fleming, Geiselman & Kress, 2009). This type of behavior encourages cross-pollination and prevents the spread of infectious diseases providing resistance and increasing their survival.

L. nivalis is considered a keystone species in maintaining habitats throughout its range, but the limited availability of roosting and foraging sites with suitable characteristics for the species threaten the survival of the species (Arita & Santos-del-Prado, 1999).

In addition to their ecological value, agaves have a variety of uses including the production of alcoholic beverages such as tequila and mezcal, the prevention of soil erosion, and food for livestock (Gomez-Ruiz et al., 2015b). This variety of uses has major social, economic,

and commercial implications that have led to over-exploitation of this resource, causing a decrease in food availability for *L. nivalis* and other nectar-feeding bats species.

L. nivalis is a species with a high degree of threat and a low potential for recovery (Medellin & Beardmore, 1994), a reason why in 1994 an alliance was created between the US Fish and Wildlife Service (USFWS) and Mexican local agencies for the development of a recovery plan. The recovery plan for *L. nivalis* published by USFWS in 1994 aimed to down list the species from endangered to threatened over a period of 10 years (Medellin & Beardmore, 1994). The plan also pointed out the urgency of the implementation of some major actions such as the following:

1. Development of effective roosting and foraging habitat protection.
2. Increase in public education.
3. Implementation of ecological studies applicable to recovery efforts.
4. Assessments of populations throughout their range.

Sadly, the plan has failed to meet its expectations as threats to the species such as disturbance and destruction of roost sites, the shortage of nectar sources, and habitat change for the use of agricultural activity are still a concern and further research and conservation action are clearly needed.

*2.2.*Choeronycteris mexicana

The monospecific genus *Choeronycteris* contains the pollen and nectar feeding bat *Choeronycteris mexicana*, which is currently considered near threatened (NT) by the IUCN Red List of Threatened Species (Solari, 2018). The distribution of the leaf-nosed bat, *C. mexicana* extends from southern California, southern Arizona, and New Mexico southward through much of northern and Central Mexico to El Salvador and Honduras (Wilson & Ruff 1999; Simmons

2005) (Figure 2). The species inhabits ecosystems of low humidity such as deciduous, semi-arid and pine-oak forests (Cryan & Bogan, 2003). Individuals of *C. mexicana* can be found from lowlands at 300m to 2,400m of elevation (Solari, 2018).

A medium size phyllostomid, it is often confused with *L. nivalis* due to its similar appearance. The diet mostly consists of fleshy fruits, nectar and pollen from cacti flowers. Unlike *L. nivalis, C. mexicana* is considered as an opportunistic species due to the absence of a specific type of roost structure (Cryan & Bogan, 2003). Ecologically, *C. mexicana* play a crucial role as a pollinator and seed disperser of diverse cacti, *Agave* ssp. and other flowering plants, such as the endemic columnar cacti *Neobuxbaumia tetetzo* (Godinez-Alvarez & Valiente-Banuet, 2000).

This elongated-rostrum bat is socially organized in small size colonies with no more than 12 individuals (Arrollo-Cabrales, Hollander, & Jones, 1987). Shallow caves, mines, or the entrance of extensive structures are some of the preferred roosting habitats. However, the conservation of *C. mexicana* is especially challenging due to their scarcity and rarity throughout their distribution. Currently, the population trends of *C. mexicana* remains unknown (Solari, 2018).

To date, the greatest threats faced by the population include residential and commercial development, energy production, mining, natural system modification due to fire suppression, habitat loss due agriculture and ranching, and human intrusion and disturbance. Currently C. mexicana is listed as Near Threatened by the IUCN (Solari, 2018) and is a candidate species under the U.S. Endangered Species Act (Federal Register Volume 59, Number 219, Pages 58982 - 59028, November 15, 1994). In 1994, the recovery plan published by the USFWS (Fleming,

1994) listed the species as endangered, however it is currently listed as a species of concern. To fulfill the objectives stipulated by the recovery plan the following major actions were called for:

1. The protection of roost sites and evaluation of the need for and implementation of protection for food plants.
2. Monitor all major roosts throughout their distribution in US and Mexico.
3. Assessment for additional roosts in the U.S. and Mexico
4. The conduct public education and information campaign

The anticipated recovery criteria for the year 2000 was not implemented. Consequently, many aspects of the physical requirements for roost, foraging ranges, reproduction and life history remain unknown. Notably, much work remains to be done, so critical research and the development of population census techniques is crucial for the development of conservation action planning.

2.3. Leptonycteris yerbabuenae

In the southwestern United States, *L. yerbabuenae* is found mainly in arid grasslands, scrublands, and oak forest (Arita, 1991). In central and southern Mexico, the preferred habitat includes arid grasslands, tropical thorn and deciduous forests, and pine-oak forests (Cole & Wilson, 2006). It is a relatively large (24-26 g) migratory nectar-feeding bat (Gonzalez-Terrazas, et. al., 2016), that depends on caves and forms large colonial aggregations of over 10,000 bats (Frick, Kingston, & Flanders, 2019). *L. yerbabuenae* migrates into northern Sonora and southern Arizona along two migration routes (Cole & Wilson, 2006) (Figure 3). Some populations of *L. yerbabuenae* are resident throughout the year in the tropical dry forest regions of central and western Mexico completing their life cycle without migrating (Fleming & Nassar, 2002).

Lesser long-nosed bats are opportunistic foragers and feed mainly on nectar and pollen of paniculate agave flowers and fruits of columnar cacti. The species can be distinguished from *C. mexicana* by the absence of conspicuous tail and the presence of brownish pelage (Cole & Wilson, 2006). Length of head and body of *L. yerbabuenae* averages as much as 10% shorter than *L. nivalis* individuals at comparable latitudes (Cole & Wilson, 2006). Most of the range of *L. nivalis* is included within the range of *L. yerbabuenae* (Arita and Humphrey 1988), but both species are spatially segregated along altitudinal and mean annual temperature gradients with *L. yerbabuenae* occupying lower and warmer areas than *L. nivalis* (Arita, 1991).

Currently, *L. yerbabuenae* is classified as near threatened (NT) by the IUCN Red List of Threatened Species (Medellín 2016b). However, the conservation status of this species is a particular case of interest, since recently *L. yerbabuenae* populations have recovered from an important decline thanks to the conservation efforts implemented in the northern region of Mexico. As a result of a bi-national consortium of researchers, NGOs, landowners, and government agencies which worked on local and range-wide conservation programs to protect the recovered populations of *L. yerbabuenae*, the species was removed from the Mexican endangered species list in 2013 and from the Endangered Species Act in the United States in 2018 (Frick, Kingston, & Flanders, 2019). Despite this great achievement, *L. yerbabuenae* current population trends are still decreasing and populations remain subject to major threats such as hunting, mining and the loss of habitat due to human disturbance and intrusion (IUCN, 2019).

2.4. Antrozous pallidus

The species *Antrozous pallidus* has a broad distribution that extends across western North America from central Mexico to southern British Columbia, Canada (Weyandt and Van Den Bussche, 2007) (Figure 4). Unlike the three nectar-feeding bats previously discussed, Pallid bats are known for hunting by passive listening, and glean large arthropods, such as scorpions or crickets, off the ground or plant surfaces (Frick, Heady III, & Hayes, 2009). However, *A. pallidus* represents the first known case of nectivorous habits in a New World bat outside the family Phyllostomidae (Frick, Heady III, & Hayes, 2009).

Feeding specialization is accompanied by morphological, physiological and behavioral adaptations (Datzmann, von Helversen, & Mayer, 2010). *A. pallidus* and *L. yerbabuenae* co-occur on the southern Baja California peninsula, where their ranges overlap with *P. pringlei*, and both bat species are common visitors to its flowers during the late March to early June flowering season (Frick, Heady III, & Hayes, 2009). The lack of morphological specializations in *A. pallidus* for nectar feeding, such as elongated noses and tongues requires greater maneuverability when it comes to extracting nectar and pollen, resulting in more contact with the flowers. These feeding patterns promote the movement of an amount of pollen eight times greater than that displaced by *L. yerbabuenae* in the same region (Frick, et. al., 2013). The facultative nectar feeding behaviors in *A. pallidus* makes the species play an important role as cacti pollinators in the northeastern region of Mexico (Frick, Heady III, & Hayes, 2009).

Although *A. pallidus* is considered a species of least concern (LC) with a population trend stable by the IUCN Red List of Threatened Species based on its widespread range and occurrence in protected areas (Arroyo-Cabrales & Grammont 2017). Gervais (2016) suggests there has been a population decline in some local populations. Direct loss of habitat from timber

harvest, land conversion, introduction of invasive species, climate change, mining, and development are major threats to the persistence of pallid bats (Gervais, 2016). Obviously, the conservation challenges faced by pallid bats throughout their distribution are highly variable and on many occasions are tied to their functions as providers of ecosystem services. This leads us to the need of assessing population trends and major threats on a local scale to direct conservation efforts for the species.

Conservation efforts for these species have been greatly affected by data deficiency with respect to biological aspects and lack of population census techniques. A commonly used way to monitor bats is through the detection of echolocation calls. Passive acoustic sensing has emerged as a powerful tool for quantifying anthropogenic impacts on biodiversity, especially for echolocating bat species (Aodha, et. al., 2018). Echolocating bats emit signals that help them build a map of their surroundings, navigate and locate their prey (Nelson, 2017). They can perceive the size, shape, direction, distance and velocity of objects by using acoustic images as a complement to vision (Lawrence & Simmons, 1982). Depending on their environments, echolocating bats use different information-gathering strategies for food acquisition (Simmons & Stein, 1980). Using echolocation calls, three types of information are potentially available: the presence or absence of echolocating bats, presence or absence of feeding activity, and species identification of detected bats (Thomas & West, 1989). With appropriate study design and implementation, monitoring programs using echolocation calls could help to identify potential drivers of population declines and feed directly into conservation decision making (Frick, Kingston, & Flanders, 2019).

Phyllostomid echolocation call structures are very similar among the species that constitute this group. The downward frequency-modulated (FM) of comparably low sound

pressure levels classifies phyllostomids as “whispering bats” (Macías, Mora, García, & Macías, 2006). Thus, the acoustic identification based on the shape of their echolocation calls is considered very difficult due to their less conspicuous and poorly detected pulses by bat acoustic detectors (Rodríguez-San Pedro & Allendes, 2017).

The main goal of this research study is to identify and describe the echolocation calls of the narrow space active gleaning species: *Leptonycteris nivalis, Choeronycteris mexicana, Leptonycteris yerbabuenae* and *Antrozous pallidus*. To describe the calls for each one of the species I used bioacoustics data collected by individuals in the northern region of Mexico to assess over 70 descriptive variables.

The study of echolocation provides an alternative technique of passive acoustic monitoring for the study of nectar-feeding bat species and can reduce biases associated with other methods such as those that require the sighting, identification, or capture of individuals. This increases our ability to identify key sites for conservation (Thomas & West, 1989). This study of echolocation calls structure of *L. nivalis, C. mexicana*, *L.yerbabuenae* and *A. pallidus*, in particular, in addition to providing a new technique for monitoring these populations, may also fill gaps of information on global patterns, identify common conservation problems, and guides conservation actions.

3. Methods

3.1. Ecological Sampling

I collected reference echolocation calls of the four different species through a combination of field work and provided material. The study of *L. nivalis* and *C. mexicana* was conducted in the state of Nuevo León, specifically at El Infierno cave (25°19’N, 100°12’W;

1,519 m elevation) and a smaller unnamed cave on a nearby trail from May 2018 to August 2018 (Figure 5). El Infierno is a limestone cave located in the municipality of Laguna de Sanchez and one of the maternity caves that has been identified for both species in the northeast region of Mexico. The cave has been described as 80 m deep with an entrance of 43.3 m by 20.5 m wide in an oak-pine forest (Moreno-Valdez, Honeycut & Grant, 2004). The site of my study was selected based on a previous study that shows the pattern followed by the *L. nivalis* colony at El Infierno cave. Several *C. mexicana* were captured exiting the smaller cave.

Individuals were captured placing mist nets at the cave entrance. Bats were identified to species level using field keys before being recorded. Sex, reproduction condition, weight, and length of forearm of each individual that was captured were assessed. To collect the acoustic recordings individually, a tunnel with a dimensional size of 0.5 m wide by 20.0 m long and 1.0 m tall was created. The base structure of the tunnel was built using PVC pipes and then covered with a mesh to minimize bounce and echo. The tunnel allows the collection of individual acoustic recordings of each specimen captured and previously identified in a limited range. Bioacoustics recordings were simultaneously taken using a Pettersson D500X device and two Wildlife Acoustics SM4 Bat - SF ultrasound detectors, which convert ultrasound waves into audible sound. The two Wildlife Acoustics SM4 Bat - SF detectors were installed at both ends of the tunnel and the Pettersson D500X was placed in the center (~10 m long). Files were saved in WAV format on flash cards. After the collection of measurements, the individuals were placed in the tunnel for about 2-3 minutes long and then they were released.

Full spectrum, real-time recordings from *L. yerbabuenae* and *A. pallidus* were donated by Veronica Gutierrez-Zamora, a colleague from the Centro Interdisciplinario de Investigación para el Desarrollo Integral, Regional (CIIDIR), Unidad Durango. The collection of *L. yerbabuenae*

and Pallid bats was done by placing mist nets in forest trails, habitat edges, streams, ponds, roosts, and other areas that we suspected would have concentrated bat activity. Data about sex, reproduction condition, weight, and length of forearm for each captured individual was collected. The acoustic data collection of *A. pallidus* and *L. yerbabuenae* were made under two conditions; hand released and attached to a zipline. Some of the individuals were recorded by hand release method after being properly identified. Using the hand release method, the individuals can be recorded during free flying when they were released directly from the hand. The other part of the acoustic sample was collected using the zip line method. In this case the individuals were recorded while flying tethered to a zip line of approximately 3-5 m length of elastic sewing thread by a loose-fitting fixed loop in the elastic pulled over the bat's head. The other end of the thread was attached to a 5 m long zip line about 1 m above the ground via a small keychain ring. Each individual was recorded for a period of 1-2 minutes and then released. A Pettersson D1000X detector with a sampling rate of 500 kHz was used for the bioacoustics recording of the calls.

Each of the methods mentioned above provides some advantages and disadvantages as forms of acoustic sampling. The advantage of using the zip line over the hand release method is that it allows the "controlled free flight" of the individual within a given range. This makes it possible to record calls at a predictable distance from the microphone and provides the opportunity for repeated flights to record good quality calls. On the other hand, zip-lined bats could be subject to experience higher levels of stress. However, the resulting recordings from the zip line method are considered a more accurate reflection of their standard calls than hand-released bat recordings (Ellison, Valdez, Cryan, O'Shea and Bogan, 2013).

The wide geographic range distribution of Pallid bats and *L. yerbabuenae* favored an extensive collection of data. Bats were recorded at different sites so intraspecific geographical and population variability are represented in the data set. Field work for data collection of *L. yerbabuenae* was conducted in the states of Sonora and San Luis de Potosi during the months of April and May, following the flowering peak of the main food source of the *L. yerbabuenae* and the most common specie of columnar cacti in the region, *Pachycereus pringlei*. Approximately 321 individuals were collected in the municipality of Rio Verde (21°52’N, 100°01’W) in San Luis Potosi. The cities of Quitovac (31°31’N, 112°45’W), Alamos (27°11’N, 109°05’W) and Los Norteños (31°04’N, 113°22’W), comprise the surveyed range in the Sonoran state (Figure 5).

A sample of acoustic data of *A. pallidus* was collected from five states in the northern portion of Mexico. Due to the fact that facultative nectarivorous feeding habits of Pallid bats have been documented in Sonoran Desert habitats, the largest sample was collected in the Sonora state in the cities of Hermosillo (29°22'N, 111°26'W), San Luis Rio Colorado (31°51'N, 114°38'W), Alamos (27°11'N, 109°05'W) and El Pinacate (31°33'N, 113°28'W). Another sample of bioacoustic data of *A. pallidus* was collected in the municipalities of San Fernando (29°58'N, 115°47'W), Mexicali (32°09'N, 115°47'W), and Tijuana (32°11'N, 109°05'W) in Baja California. Data from the state of Chihuahua were collected in Camargo (28°12'N, 104°34'W), Cumbres de Majalca (28°46'N, 106°29'W), and Coyamel del Sotol (29°38'N, 104°52'W). Acoustic surveys were conducted in the cities of Tlahualilo, Durango (26°38'N, 103°45'W), and Linares, Nuevo León (24°47'N, 99°31'W) to complete the sample of *A. pallidus* (Figure 5).

Even though the northern portion of Mexico can be characterized for having an arid to semi-arid climate, all the states included in the study present wide variations at the local scale.

The north region of Mexico is mainly covered by two deserts. The largest is the Chihuahuan desert which is centered between the Sierra Madre Occidental and Sierra Madre Oriental mountain ranges. Natural regions of Chihuahua are plateau and mountains with a dry to semi-arid climate. In the high plateau region and on the plains, native plants include lechuguilla (*Agave lechuguilla*, an evergreen succulent), mesquite (*Prosopis* spp., a common desert shrub), guayule (*Parthenium argentatum*, a rubber producing plant), and ocotillo (*Fouquieria splendens*, a desert succulent plant) (Valdez, Stuart, & Bogan, 1999).

The second desert that occurs in the northern region of Mexico is the Sonoran Desert and ranges from the majority of the Baja peninsula through the northwestern portion of the mainland. The Sonoran Desert geography is mostly broad, characterized by flat valleys with widely scattered, and small mountain ranges of mostly barren rock. Visually, two dominant life forms of plants can be observed in this area: legume trees and columnar cacti. The Baja Peninsula is characterized by having its low annual precipitation, a scarce vegetation cover and open spaces, but wide range of vegetation types including coastal chaparral, conifer forest, low desert scrub, and tropical deciduous forest. Geographically, it stands out for having a rough terrain, which includes many canyons, steep hills, and mesas.

The Tlahualilo municipality is a desert region that lacks water and stands out for its shrub land with bushes that do not exceed 4 m high and agaves. For its part, the city of Linares is characterized by a warmer and temperate climate since it annually receives a significant amount of rainfall. The ecosystem in this region is known as the Tamaulipan thorny scrubland, where vegetation is mostly dominated by xerophytes.

3.2. Data Preparation

I recorded a total of 1,795 echolocation pulses from 113 individuals: 298 echolocation pulses from 19 different individuals of *L. nivalis*; 8 pulses from 3 individuals of *C. mexicana*; 417 pulses from 27 individuals of *L. yerbabuenae* from two different localities; and1,072 pulses of 64 individuals of *A. pallidus* from five different localities. The total number of individuals, 113, was reduced to 109 for final analyses after 4 individuals of *L. nivalis* were removed for incomplete data.

All the echolocation calls were digitized by a computer using a bat call analysis software (SonoBat v. 4.0) at a sampling rate of 44.10 kHz with 16-bit resolution. Prior to the analysis, the calls sequences were visually inspected using the sound analysis software BatSound Pro (Version 3.31a© 1996-2001 by Pettersson Electronics AB) to remove approach-phase calls and terminal-phase calls from the spectrograms if present. Generally, a complete sequence of echolocation calls is made up of three phases that include the search phase, the approach phase and a terminal feeding buzz. Search-phase calls can be distinguished from approach and terminal-phase calls by a phase shift increase in interval and call duration, and a decrease in repetition rate of the pulses (Schnitzler & Kalko, 2001). The approach phase calls show shorter and faster pulses as the bat approaches the prey. Pulses emitted in shorter time intervals are more useful when the target is at a close range and provide more details regarding surroundings and prey position (Zamora-Gutierrez, 2019). The terminal feeding buzz are pulsations emitted just before prey capture and are characterized by the emission of a series of short signals at a remarkably high repetition rate (Pfalzer & Kusch, 2003). The constant and repetitive pattern of the search phase calls are more suitable for call structure description; thus, I employ call

sequences only consisting of pulses emitted during the search phase for the description of the call structures.

After removal of all the non-search-phase calls, I automatically extracted and parameterized the calls using the in-build algorithms of SonoBat v. 4.0. The sound analysis software extracted measurements for up to 74 quantitative variables of the calls. I assessed temporal, amplitude and frequency dependent variables, in addition to a series of measurements including slope-related measurements, exponential fit of the variables, time-amplitude trends and harmonic strengths ratios for the description of each echolocation calls recorded (See APPENDIX A.1-7 for variable descriptions). When there were multiple calls for an individual, the average of the variables was used, and subsequent analyses were performed on individuals. As echolocation calls emitted by phyllostomids are typically of low sound pressure levels and comparably high frequencies, to avoid faint calls and excessive noise, I removed all detected calls within the range of human hearing which is typically between 12 kHz and 20kHz (Armitage & Ober, 2010). A maximum of 100 calls with a 1.0 acceptable quality rating were considered per file in order to produce more reliable sequence identifications.

In order to proceed with subsequent analyses, I needed to reduce the number of variables to approximate the recommended minimum ratio of cases to variables of 10:1 to avoid computational difficulties (Osborne & Costello, 2004), so my target was nine echolocation variables for the call description. After I eliminated variables with constant or missing values, I employed multiple analyses to reduce the number of variables. I generated a correlation matrix and the number of variables was reduced through an evaluation of high intercorrelations among the 74 variables. I examined histograms to look for variables that were near constant or with highly non-normal distributions. Finally, I removed variable that had overall low weightings in

an initial Principal Component Analysis (PCA). Based upon this I selected nine variables that might best describe, and most contribute to the identification of species. The PCA provides information about the direction and magnitude of the variables which is very helpful in the decision making for future extractions. Several variables were constant for some species, others showed extreme and erratic variability, and other seemed to have little predictive capability based upon loadings on PC factors. All the variables with at least one strong loading value of the two PCA factors were retained. I also eliminated all those variables dependent on amplitude or decibel levels, due to their high variability, and the potential impact of different distances and methods for recording the bats. After eliminating all those variables that did not meet the established criteria, I came up with the following nine predictor variables for the echolocation call structure description:

1. ***CallDuration*** – Duration of the call.
2. ***Fc*** – Characteristic frequency of the call. Determined by finding the point in the final 40% of the call having the lowest slope or exhibiting the end of the main trend of the body of the call.
3. ***HiFreq*** – Highest apparent frequency of the call.
4. ***LowFreq*** - Lowest apparent frequency of the call.
5. ***Bndwdth*** - Total frequency spread of the call. Calculated from the difference between the highest and lowest frequency.
6. ***FreqKnee*** - Frequency at which the initial slope of the call most abruptly transitions to the slope of the body of the call.

7. ***Dominant Slope*** - Slope of the longest sustained trend in slope of the call. Determined by finding the segment of the call having the minimum residue for a linear regression of a segment of the call of 20% the duration of the call.
8. ***Steepest Slope*** – Steepest slope of the call calculated from a linear regression of a segment of 10% the duration of the call.
9. ***Lowest Slope*** - Lowest slope of the call calculated from a linear regression of a segment of 10% the duration of the call.

3.3. Data Analysis

I used a PCA on the reduced variable set to examine the pattern of dispersion of the points for the 109 bats and examined the directions of the variables in relation to the points. With a Principal Components Analysis, we can obtain information about the eigenvalues (which indicate the percent a variance explained by each eigenvector), scree plot, score plot, and the PCA loading table. This information is important for the description of the call structure since it helps us to identify the variables with the largest effect on the factors for group dispersion of the calls, indicates the percentage of explained variance of each of the variables, and graphs the scores of the second factor versus the first factor, which is ideal to detect clusters, outliers, and trends.

Multivariate statistical techniques like Discriminant Analysis are helpful to classify individual observations into non-overlapping groups using predictor variables (Armitage & Ober, 2010). As the main goal of this study is to quantitatively discriminate *L. nivalis*, *C. mexicana, L. yerbabuenae* and *A. pallidus* echolocation calls and group them by species, I employed RStudio v. 1.2.5042© 2009-2020 to run a Linear Discriminant Analysis to determine

if the species exhibit distinct groupings and assess the discriminatory strength of the nine predictor variables.

As *Leptonycteris nivalis, Choeronycteris mexicana,* and *Leptonycteris yerbabuenae* are nectar-feeding bat species from the same family, I expect the echolocation calls structures of this species to share some similarities, but we hypothesized them to be different enough to separate them by species. However, the low-intensity signals emitted by whispering bats in environments with dense vegetation where increased intensity produces more clutter echoes (Brinkløv, Kalko, & Surlykke, 2008), and can result in the misidentification of the calls. Consequently, to evaluate statistically significant differences among the groups by each selected variable, I used IBM SPSS v. 26 software to perform a Kruskal-Wallis Nonparametric test followed by pairwise comparisons for each variable independently, follow by a Bonferroni test. This is a very conservative post hoc test helpful to maintain the pairwise error rate and identify the potentially reliable descriptive variables for species segregation.

4. Results

4.1. Identifying the predictor variables for call structure parametrization

According to the criteria selected to reduce the quantitative variables, the most suitable predictor variables for the echolocation call structure description and the segregation of the species includes the following nine variables: HiFreq, LowFreq, CallDur, Bndwth, Fc, FreqKnee, Dominant slope, Steepest slope and the Slowest slope. The PCA results in Figure 6, suggest that the first three principal components explain 91.3% of the call variation, which is an acceptably high percentage.

To assess the quality of the variable, I used a correlation circle to evaluate the variables' contributions independently based on their cos2 values. The correlation circle reveals a good

representation on the principal component and a high correlation between the slope-related measurements (dominant, steepest and lowest slopes), but a negative correlation with call duration. Frequency-dependent variables as HiFreq, LowFreq, FreqKnee and Fc appear to be heavily correlated with each other, but inversely correlated with Bndwth. The distance between the origin and the variables indicates the contribution level of the predictor variables, indicating that the variables which least contribute to the species classification are Bndwdth and CallDuration (Figure 7).

4.2.Call structure species differentiation

I used the first two principal components (Dim1 = 50.2%, Dim2 = 18%) which explain a large percentage of the total variation to map the individuals, separate the groups and look for apparent patterns. Although some overlap can be observed between the species *L. nivalis, L. yerbabuenae* and *A. pallidus*, when all species are considered together the differentiation between the groups is evident (Figure 8). Across the four species evaluated, *L. nivalis* exhibited the largest variation in call structure. Although the amount of data available for *C. mexicana* was too limited to calculate the concentration ellipse, the individuals show a clear differentiation without overlapping with any of the species. As *L. nivalis* shows a large intraspecific variation, the slope-related measurements appear to be determinants or predictor variables for species differentiation.

Figure 9 shows the actual dispersion of each species sample delineated by a convex hull. Only when plotted together as Dim1 and Dim2, individuals of *L. nivalis,* are highly overlapped with *L. yerbabuenae* and *A. pallidus* individuals, even though the centroids of each species are very distant (points bigger in size).

The results of the Discriminant Analysis revealed similar results as the PCA with regard to the pattern of overlap among species (Figure 10) and the variables that most heavily influence the first two discriminant functions (Table 1).

I did a non-parametric Kruskal-Wallis Analysis of Variance, in conjunction with pairwise comparisons to determine statistical significances between the predictor variables among the species. The results for this test indicate significant differences among the groups for all the predictors ($p<0.001$). All pairwise significance values were adjusted with Bonferroni corrections. This conservative test suggests a good reliability in the statistical significance for the segregation of the samples. The box plots on the Figure 11, compare each predictor variable's means and standard deviations among the species included in the study.

The pair-wise comparisons in Table 2, were used to assessed statistical differences between the species groups on the nine predictor variables independently and the results show that all those species with the same letters are statistically indistinguishable. From the multiple comparisons, I can conclude that individuals of *L. nivalis* can be distinguished by the lowest and steepest slopes values. *A. pallidus* species can be differentiated by LowFreq, HiFreq, FreqKnee, and Fc variables. In the other hand, the best variables for the species differentiation of *L. yerbabuenae* appears to be Bndwdth and CallDuration, meanwhile for *L. nivalis* are Dominant, Steepest and Lowest slopes and Lowest Slope for *C. mexicana.*

4.3.Echolocation call structure description

The previous results define quantitative parameters for the description and segregation of the echolocation call structures. The spectrograms of the echolocation call structure, in conjunction to the descriptive statistics including the mean, standard deviation, range (maximum

value - minimum value), and the standard error of the mean of the ten predictor variables, were used for each of the species call structure parametrizations (Table 3).

As expected, the species *L. nivalis, C. mexicana,* and *L. yerbabuenae* echolocation calls show some similarities. These three species present typical phyllostomids call structures, emitting low-intensity, but high frequency broadband and downward frequency-modulated signals (FM). The emission of multiple-pulses clusters, in addition to multiple slightly overlapped harmonics, were observed in the spectrograms of the three nectar-feeding species.

L. nivalis individual's echolocation calls are mainly emitted in three-pulse clusters during the searching phase, although calls in pairs and groups of four have been also observed in the spectrograms (Figure 12). The descriptive statistics results of *L. nivalis* confirm the emission of highly frequency-modulated pulses with a highest apparent frequency at 85.8kHz. The bandwidth of the signals emitted by *L. nivalis* covered a wide range up to 27.3kHz with minimal and maximal frequencies at 22.3 - 49.3kHz, respectively. *L. nivalis* characteristic frequency of the call is around 53.7kHz and similar to others phyllostomids, presents short duration echolocation calls of approximately 2.5ms (Table 3). Up to two harmonics with some frequency overlap were recorded for the species, with the first one the most energetically intense and stable.

Regardless of the evident similarities observed in the downward frequency-modulated calls emitted by the three nectar-feeding bats species, the echolocation calls of *C. mexicana* can be distinguished for having a higher start frequency around the 97kHz and end frequency around the 88kHz (See Table 3). Mean frequency of the call bandwidth spread over approximately 14kHz of the spectrum. Calls are brief, usually around 2.0ms, and compared with the other two nectar-feeding bats species the calls are emitted with very low intensity. The low intensity display by the calls makes the start and end points, as well as the multiples harmonics hard to

discern. A pattern of paired calls can often be observed in the spectrograms, but occasionally, single calls and three-pulses clusters appearance makes the sequence unpredictable (Figure 13).

Two harmonics were recorded for the species *C. mexicana*. If we reduce the comparisons to two species the results of previous pair-wise multiple comparisons test suggest that Lowest slope is the best predictor for the differentiation between *C. mexicana* and *L. nivalis,* however Bndwdth is a good discriminant between *C. mexicana* and *L. yerbabuenae,* meanwhile Fc and LowFreq are for *C. mexicana* and *A. pallidus.*

In flight, *L. yerbabuenae* presents calls of a broad bandwidth range of 27kHz. The start frequency of the calls is usually at 83.25kHz and ends at 35.37 kHz. However high frequencies have a variable start frequency that can reach a maximum of approximately 97kHz. The downward frequency modulates calls of *L. yerbabuenae* last approximately 5.6ms and are more energetically intense than *L. nivalis* and *C. mexicana* calls. The characteristic frequency of the calls is around 41.67kHz (Table 3). Up to two harmonics were identified for the species, being the first harmonic the most intense and dominant (Figure 14).

In contrast to the three nectar-feeding bat species pallid bats present a simpler call structure (Figure 15). The sequence of echolocation calls consists of individual pulses ranging from a low frequency of 29.1kHz to a high frequency of approximately 65.9kHz with prolonged intervals of time between the pulses. The characteristic frequency of the species can be found at the beginning of the call around the 30.86 kHz (Table 3). Two harmonics were recorded for this species despite the fact they are not always visible. The first harmonic seems to be the most energetically intense. The calls of A. pallidus had a mean duration of 4.66ms, a broader band range of 14kHz, and are emitted a comparably high intensity levels in comparison to the calls emitted by phyllostomids.

5. Discussion

While many of the threats that bats face reflect the broader conservation challenges of our era, there are many aspects of bat ecology that present specific challenges and opportunities for conservation action (Frick, Kingston, & Flanders, 2019). This study contributes to the establishment of baseline knowledge about the echolocation call structure of three of the nectar-feeding bats *L. nivalis, C. mexicana, L. yerbabuenae,* and the facultative nectar-feeding bat *A. pallidus* and makes available a potential passive acoustic monitoring technique for the species.

Based on combined results from multiples analyzes including the examination of histograms, and low weightings in an initial Principal Component Analysis, I documented a minimum of ten predictor variables that describe the call structure differentiation of the four species. After the screening and reduction of the 74 initial variables, the quantitative parameters that best explain a high percent of the interspecific variance of the echolocation calls and segregated the species were HiFreq, LowFreq, CallDur, Bndwth, Fc, FreqKnee, Dominant slope, Steepest slope and the Slowest slope.

Results suggest that the echolocation calls emitted by the species *L. nivalis, C. mexicana* and *L. yerbabuenae* follow a typical Phyllostomid call structure (Kalko 2004). The mean values of the predictor variables are consistent with the spectrograms and suggest that the three species of nectar-feeding bats present downward frequency-modulated calls, a broad bandwidth range, short call duration and multiples harmonics. These patterns observed in the emission of the calls are mainly influenced by the individual's surroundings and the foraging ecology of the bat and are high-resolution characteristics of bats that forage close to vegetation in highly cluttered space (Schnitzler and Kalko, 1998). The short duration of the echolocation calls presented by this

group of species suggests that these species mainly forage opportunistically above the canopy for nectar, pollen and fruits but do not fly high above the canopy.

An interesting sequence of single-pulses, paired-pulses, and clusters of three to four-pulses patterns was observed in the species *L. nivalis, C. mexicana* and *L. yerbabuenae* spectrograms. This distinctive behavior has been documented for other phyllostomids, like *Brachyphylla nana,* which emits echolocation sequences of either single calls or in pairs (Macias, Mora, Garcia, & Macias, 2006). The emission of clustered pulses suggests a trade-off between call intensity and repetition rate (Jones, 1999).

The long-nosed bats species included in this study also present low duty cycles and low intensity calls. The apparent start frequency means for the nectar-feeding bats species are relatively high compared with other bats species, but *C. mexicana* was the long-nosed species with the highest apparent frequency range (HiFreq = 97kHz; LowFreq = 88kHz) and the lowest intensity of call emissions reported in this study. Both *L. nivalis* and *C. mexicana* emit echolocation pulses with short mean duration of 2.0 - 2.5ms approximately. These findings are consistent with previous studies by Jones (1999), which suggest that low-duty-cycle bats that call at high frequencies must therefore use short pulses to avoid pulse–echo overlap. *C. mexicana* have a comparably shorter bandwidth range of 14kHz, meanwhile *L. nivalis* and *L. yerbabuenae* present a broad bandwidth range of 27kHz. Although the species *L. nivalis* and *L. yerbabuenae* shares some similar mean values of predictor variables as HiFreq (Lepniv = 85.8kHz; Lepyer = 83.2kHz) and bandwidth range (both spp. = 27.0kHz), individuals of *L. yerbabuenae* present calls of longer duration (spp. mean = 5.4ms).

By comparison, the foraging behavior of the facultative nectar feeding bat *A. pallidus* from the Verspertillionidae family differs considerably from that used by nectivorous species.

Pallid bat diets are mainly composed by small invertebrates, taking the prey directly from the ground or low vegetation, consequently, I expected a greater degree of differentiation from the nectar-feeding bats species. However, there was some overlap between the echolocation call structure of this species with *L. yerbabuenae* and *L. nivalis*. Similar to the three-specialist nectar-feeding bats, *A pallidus* emits frequency-modulated signals, but consisting of mainly one prominent harmonic component and slightly sweep from 85kHz down to 49kHz. In addition, the time lapse between the pulses of *A. pallidus* are longer and emitted with a notably higher intensity. Pallid bats also present a broadband that covers a range of 22.5kHz and long have a mean call duration of approximately 4.66ms, characteristics which are presumed to provide some resistance to clutter.

Results show that there are significant differences between all the predictor variables. Discriminant-function analysis demonstrated that sufficient variability did exist among the groups to potentially classify calls to the proper species. The multiple comparisons analysis results suggest that the discriminant variables that best segregated the calls by species were FreqKnee, HiFreq and LowFreq for *A. pallidus*, bandwidth and call duration for *L. yerbabuenae*, and PcrntKneeDur for *C. mexicana*.

Search-phase calls of a species may be distinctive, but intraspecific variation can obscure differences among species and make identification problematic (Murray, Britzke, & Robbins, 2001). As the *L. nivalis* sample shows a high level of intraspecific variation and the distribution of the individuals are more dispersed overlapping with *L. yerbabuenae* and *A. pallidus* clusters, the slope related measurements become more relevant for species differentiation. Consequently, steepest and lowest slope values are good discrimination variables between the species *L. nivalis* and *L. yerbabuenae*, while dominant and steepest slopes are for *L. nivalis* and *A. pallidus*.

Although the sample size of *C. mexicana* was limited, obvious differences in call structure when measured as PC1 and PC2 are observed, positioning the mean centroid of the group very distant from the other species.

The temporal and frequency-dependent variables call duration, HiFreq, LowFreq and bandwidth are the only ones from the ten predictor variables selected for which values can be measured directly from the spectrograms making them more suitable for manual quantitative parametrization of the call structure. When acoustic surveys are carried out under natural conditions, the recordings are more exposed to high noise levels, overlapping of calls can be observed between different species and the variation between individuals can increase. In those cases, the slope-related measurements get greater reliability as discriminators between species, thus, multiple comparison analysis results should be taken into account when considering the identification of the calls to a species level or automatic identification.

Evidently, more research is needed to successfully guide conservation action on a local and global scale. The results included in this study can be substantially deepened by increasing the sample sizes and assessing, more in detail intraspecific variations due to sex, age, or across the geographic range of the individuals. Studies in controlled environments such as in-flight cages are recommended, even though echolocation calls recorded using hand release may be more similar to calls made during free flight than those recorded in flight cages (Jennings, et al., 2004). A monitoring technique that can improve the quantification of the variables is recording the calls during active foraging activity of bats by placing the microphones of the acoustics detectors on the agave inflorescence. Despite the fact that this technique, together with the use of video recordings, was initially implemented to collect the bioacoustics recordings of this study, the recordings were not of sufficient quality to provide quantitative guidelines of the call

structures. In addition, the identification of the species through video recordings was problematic.

Although the description of the call structures included in this study are mainly based on nine predictor variables, the descriptive statistics of the 74 initial parameters evaluated will be available for public access at the Texas A&M Data Repository *(Link: https://tamu.libguides.com/research-data-management/repositories)*. These data contribute to a clearer understanding of the call structures which makes them useful as a bat echolocation call assemblage library for further analysis and the utilization of automatic identification tools.

This is one the first studies to document the echolocation call structure of the three nectar-feeding bats *L. nivalis, C. mexicana, L. yerbabuenae* and the facultative nectar-feeding bat *A. pallidus*, in addition to providing guidelines of quantitative variables for the identification and differentiation of the calls to a species level. This study also contributes to our knowledge of bat ecology by providing guidelines for the improvement of an acoustic population monitoring technique for pollinating bats. Acoustic monitoring techniques with the appropriate experimental design and implementation, in conjunction with the advances in statistical modeling, open a door of new opportunities to monitoring schemes on a broad-scale and determine the status and trends of bat populations and guide conservation actions.

6. Figures

Figure 1. Potential distribution of *Leptonycteris nivalis.* The distribution has been adapted from the IUCN Red List of Threatened Species (Medellín 2016a).

Figure 2. Potential distribution of *Choeronycteris mexicana.* The distribution has been adapted from the IUCN Red List of Threatened Species (Solari 2018).

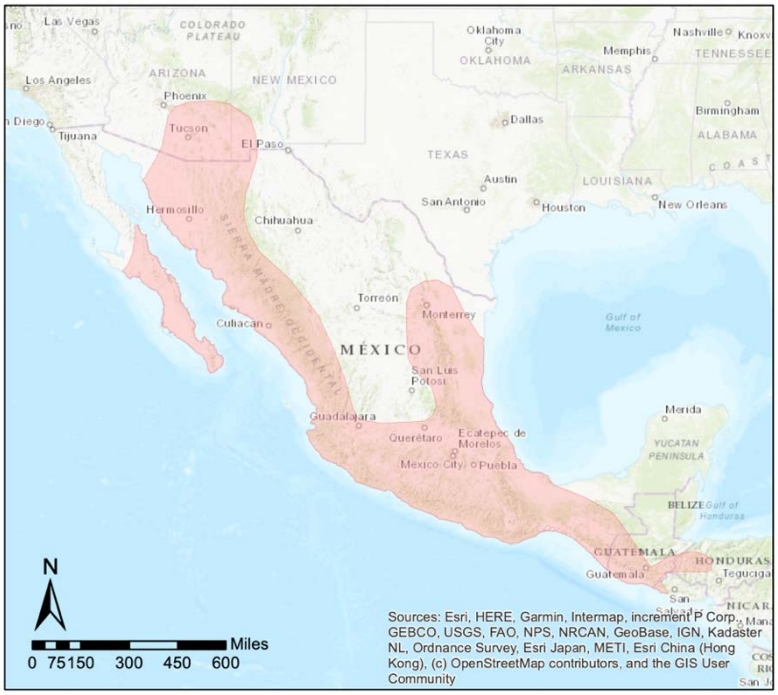

Figure 3. Potential distribution of *Leptonycteris yerbabuenae.* The distribution has been adapted from the IUCN Red List of Threatened Species (Medellín 2016b).

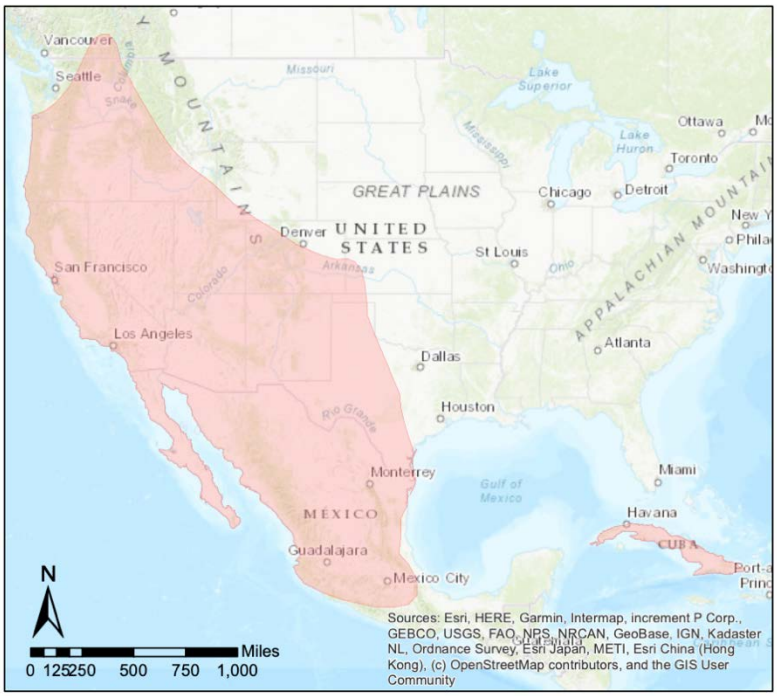

Figure 4. Potential distribution of *Antrozous pallidus.* The distribution has been adapted from the IUCN Red List of Threatened Species (Arroyo-Cabrales & Grammont 2017).

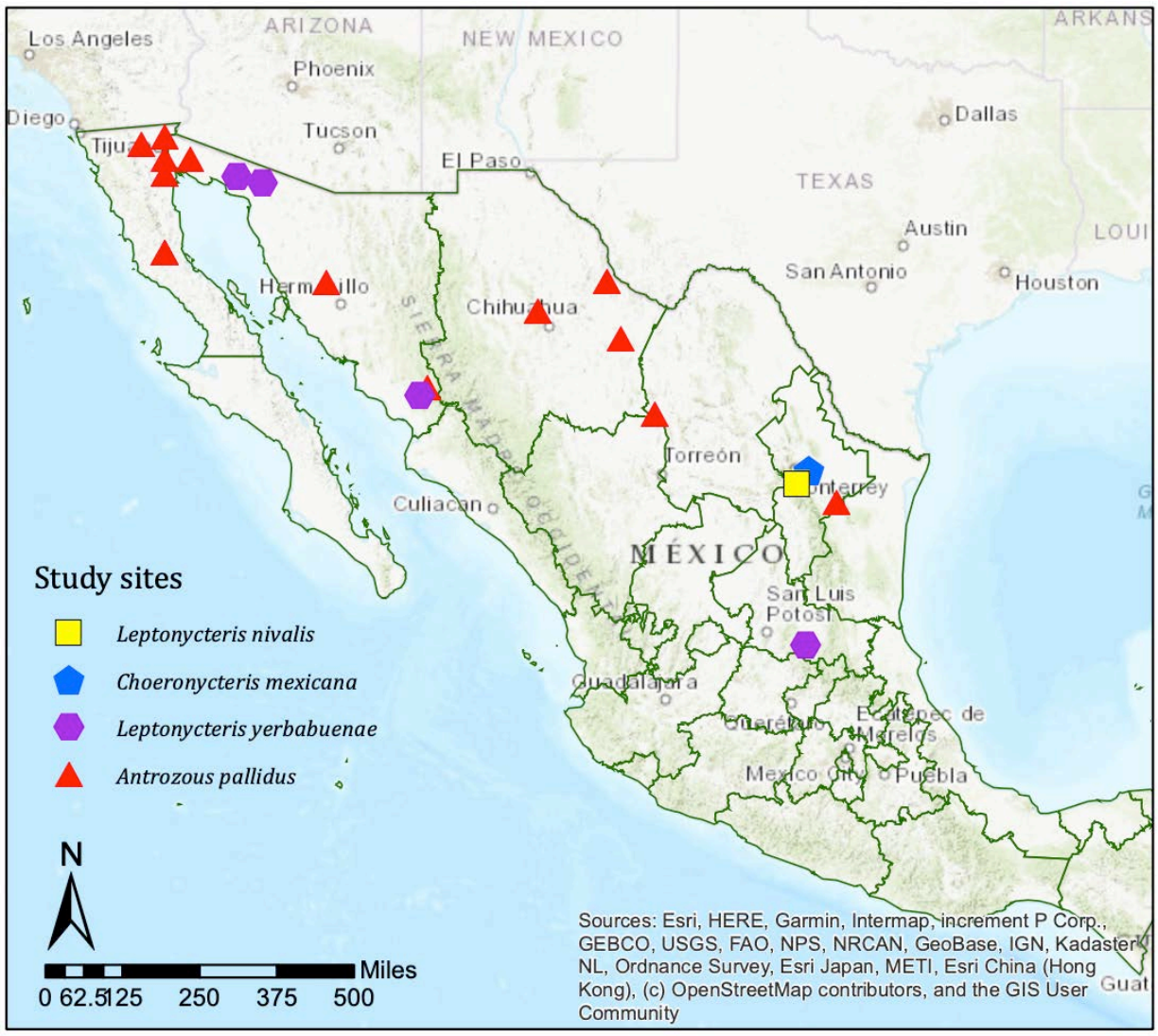

Figure 5. Sites of data collection in the northern region of Mexico. The map shows the exact localities were the data was collected for each species. *Leptonycteris nivalis* (yellow), *Choeronycteris mexicana* (blue), *Leptonycteris yerbabuenae* (purple), *Antrozous pallidus* (red).

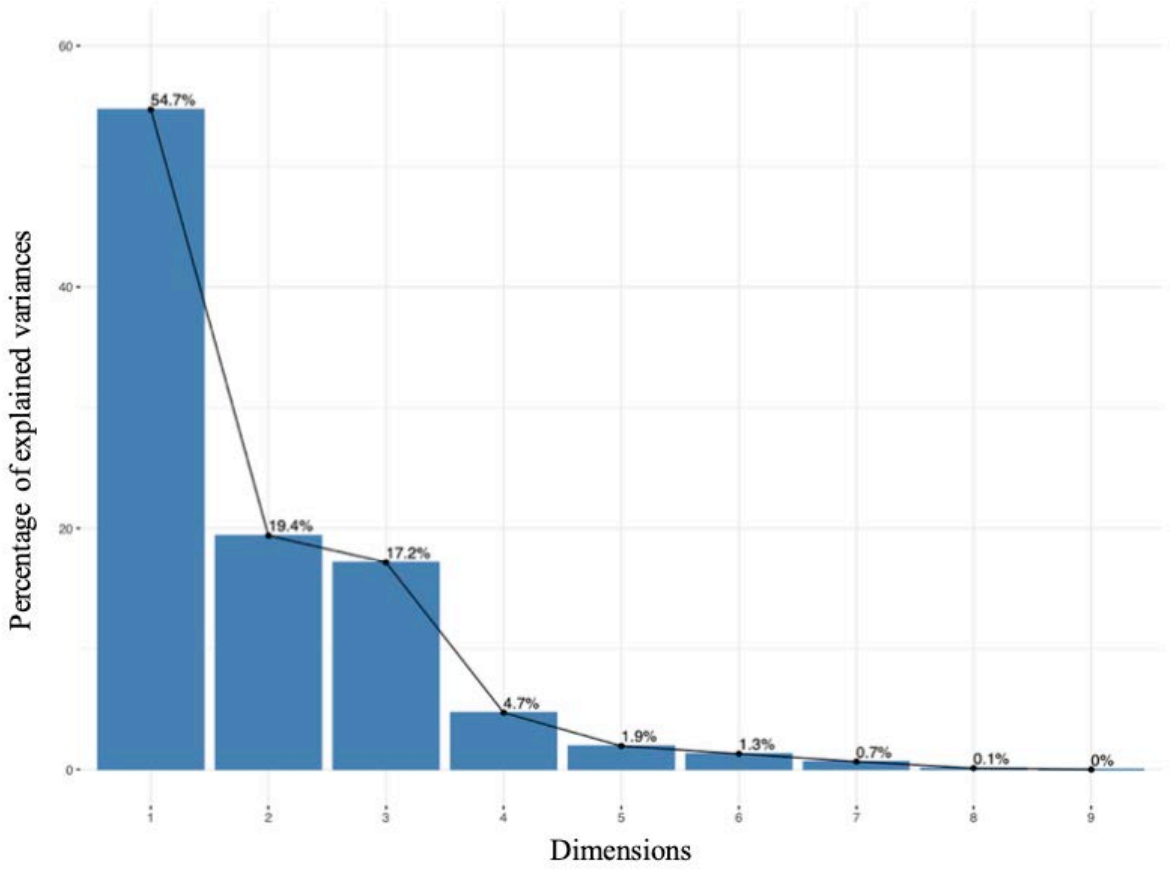

Figure 6. Scree plot and percentage of total variance accounted by each predictor variable. Data represent the proportion of variation explained by each predictor variable eigenvalue. The PCA results suggest that the first three principal components explain 91.3% of the call variation, which is a high percentage and explained most of the total variance of the calls structures by species.

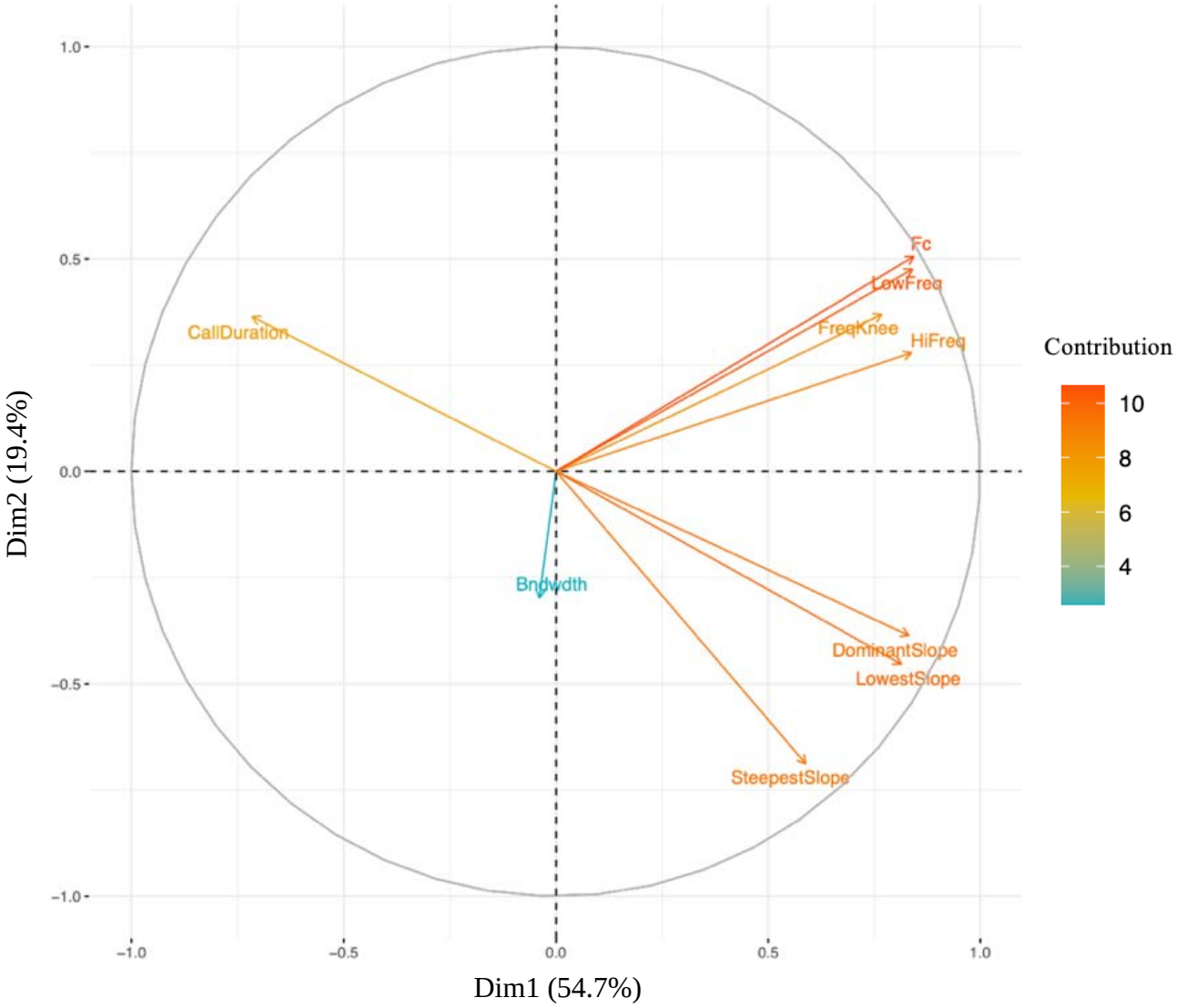

Figure 7. Contribution of each of the nine selected predictor variables to call structure identification. Variables with lines in similar directions are positively correlated, while those which point in opposite directions are negatively correlated. Distance between the variables and the origin measures the contribution of the variable to the species differentiation.

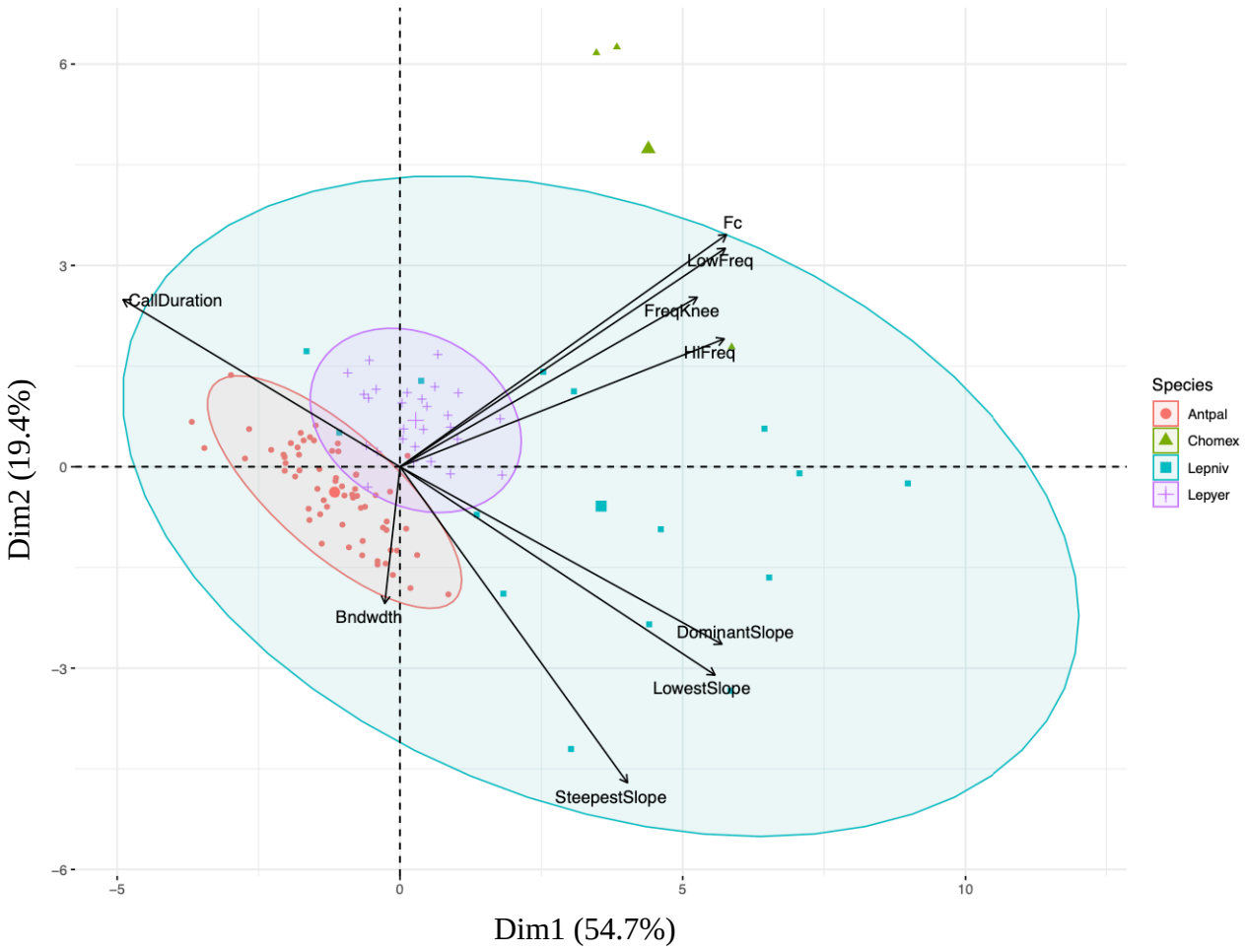

Figure 8. Species differentiation according to the call structure predictor variables, mean and 95% confidence ellipses of each groups. Arrangement of the *L. nivalis* (■), *C. mexicana* (▲), *L. yerbabuenae* (+), and *A. pallidus* (•) species along the axes of a principal component analysis (Dim1 vs. Dim2). The first two principal components (Dim1 = 54.7%, Dim2 = 19.4%) explain a large percentage of the total variation to map the individuals Circles represent 0.95 confidence ellipse. Data-deficiency of *C. mexicana* do not allowed the calculation of the 0.95 confidence ellipse. Large data points represent group means.

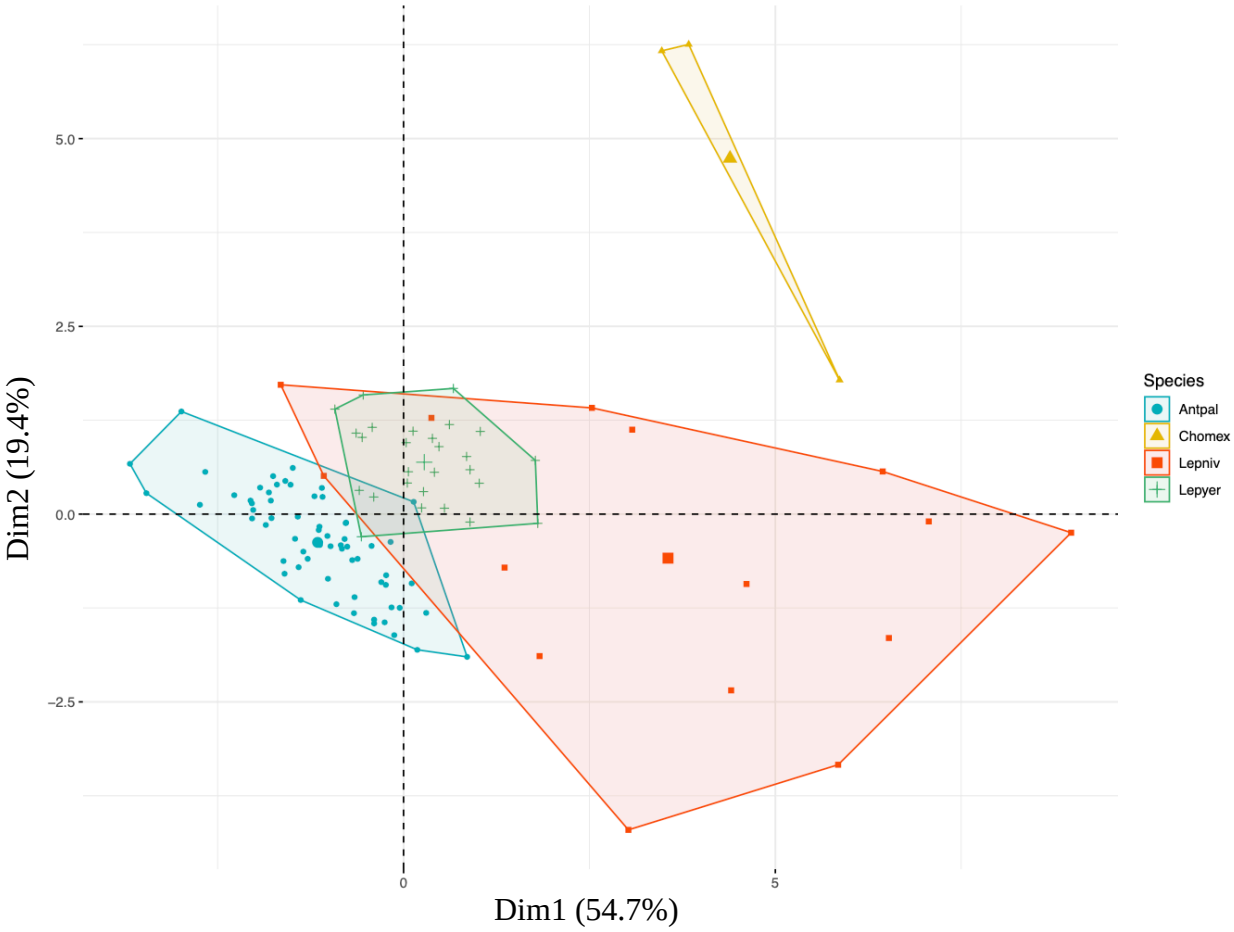

Figure 9. Actual dispersion of the individuals of *L. nivalis*, *C. mexicana*, *L. yerbabuenae*, and *A. pallidus* collected in the northern region of Mexico. The principal component analysis score plot of the first two principal components (Dim 1 and 2; 74.1%) show the differentiation between the species and dispersion of the individuals using a convex hull of the data points of *L. nivalis* (■), *C. mexicana* (▲), *L. yerbabuenae* (+), and *A. pallidus* (•) individuals. Large data points represent group means.

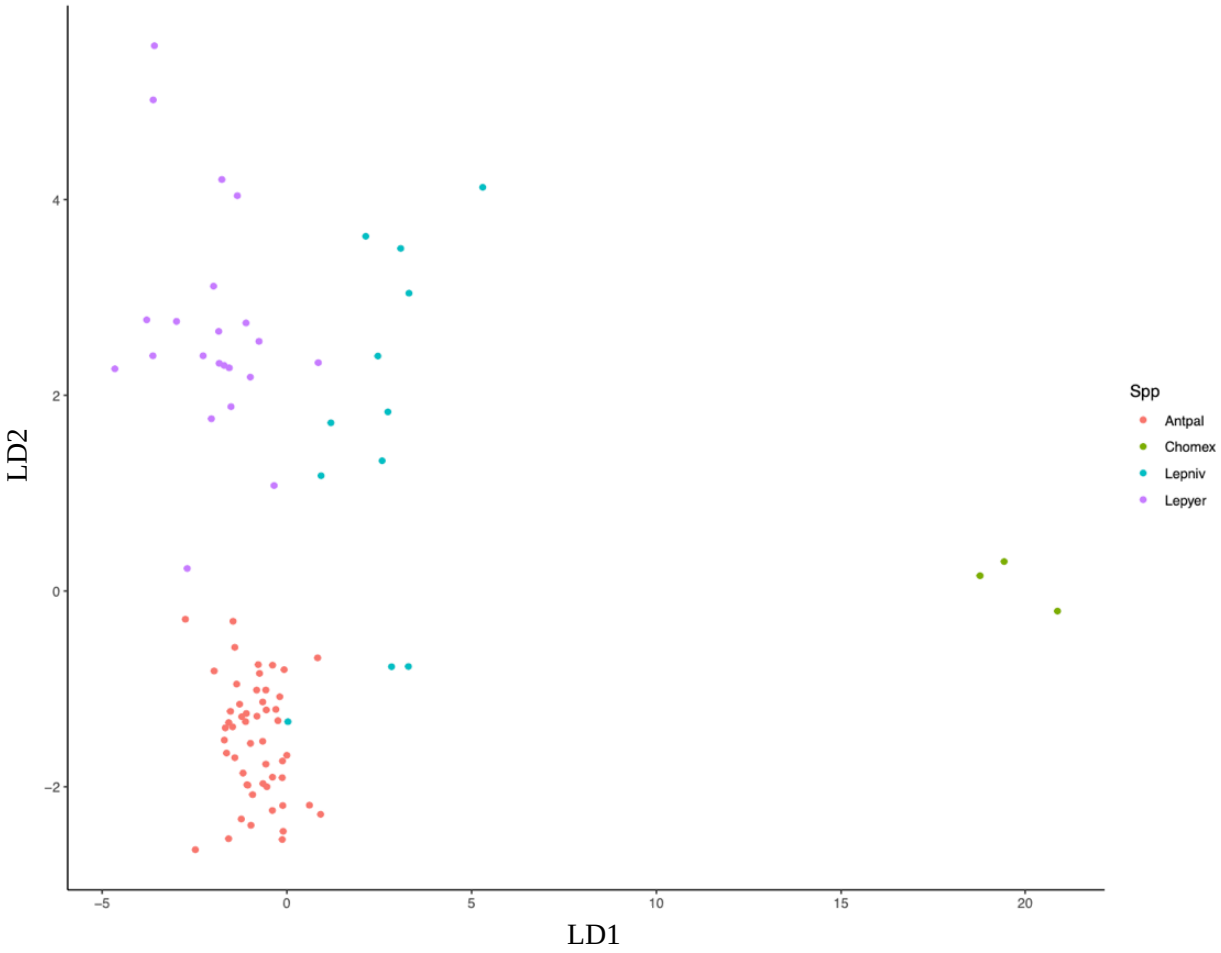

Figure 10. Plot of the group segregation based on the first two discriminant function of the linear discriminant analysis. The plot of the first two discriminant functions (Functions 1 and 2; 73.57%) show the separation based on the nine predictor variables of the species of *L. nivalis* (Spp:Lepniv), *C. mexicana* (Spp:Chomex), *L. yerbabuenae* (Spp:Lepyer), and *A. pallidus* (Spp:Antpal) individuals collected in the northern region of Mexico.

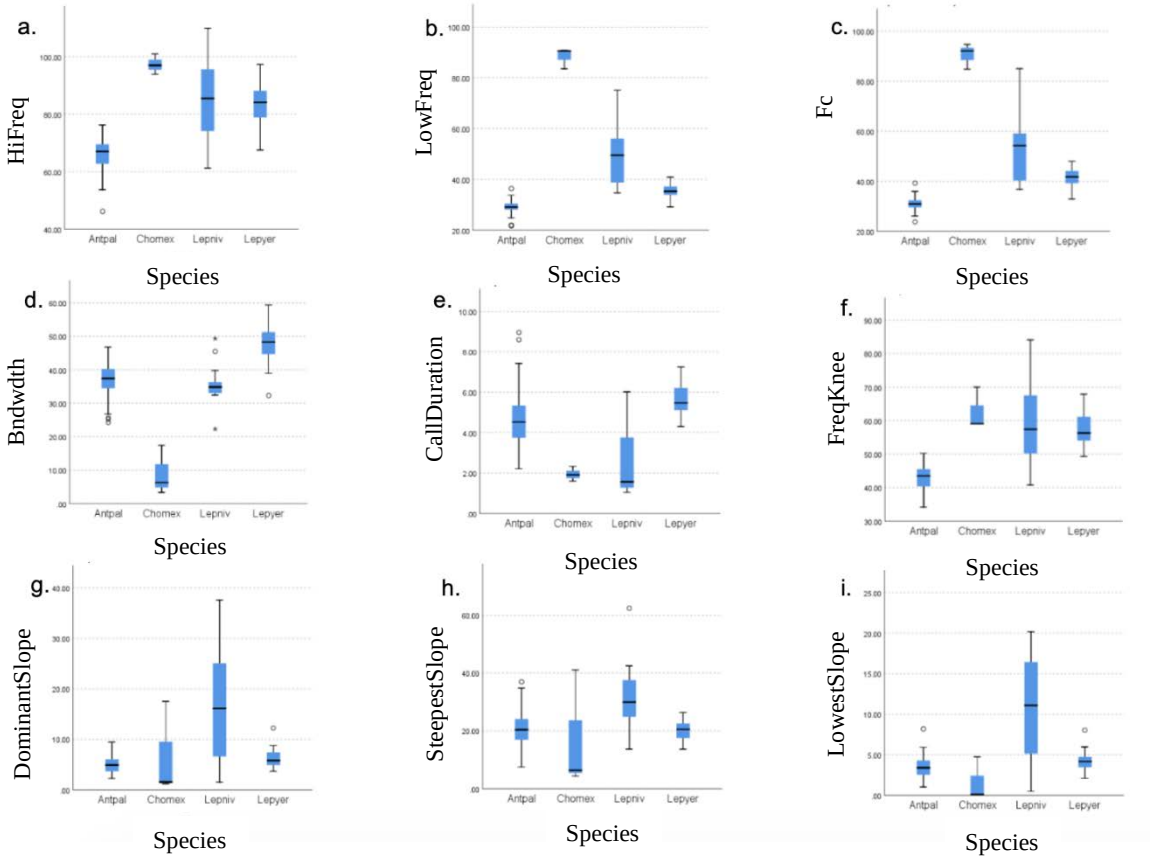

Figure 11. Results of Independent-sample Kruskal Wallis Analysis of Variance on the nine predictor variables. Box plot graph of the variables (a)HiFreq; (b)LowFreq; (c) Fc; (d) Bndwdth; (e) CallDuration; (f) FreqKnee; (g)Dominant slope; (h)Steepest slope; and (i)Lowest slope. Data points above or under the box plots reflect outliers, and the black dash show the median with the standard deviation of each group. The results for this test indicate significant differences among the groups for all the predictors ($p<0.001$). The significance level is 0.05. All pairwise significance values were adjusted with Bonferroni corrections.

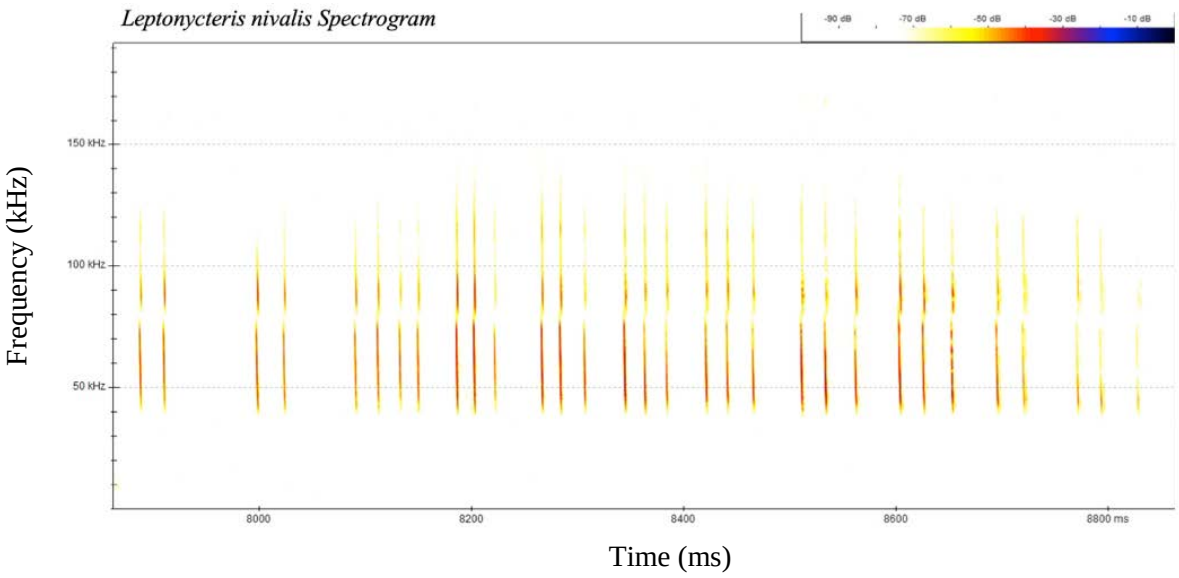

Figure 12. Searching-phase echolocation call sequence of *Leptonycteris nivalis.* The full-spectrum acoustic data was recorded at El Infierno cave using the tunnel sampling method with a Pettersson D500X and a Wildlife Acoustic Bat SM4 devices at a sampling rate of 44.10 kHz with 16-bit resolution. Spectrogram (BatSound FFT 1024, hanning window), shows the echolocation pulses emitted by one individual in a time interval of 800ms. The intensity of the calls is range from -70dB (yellow) to -10dB (blue).

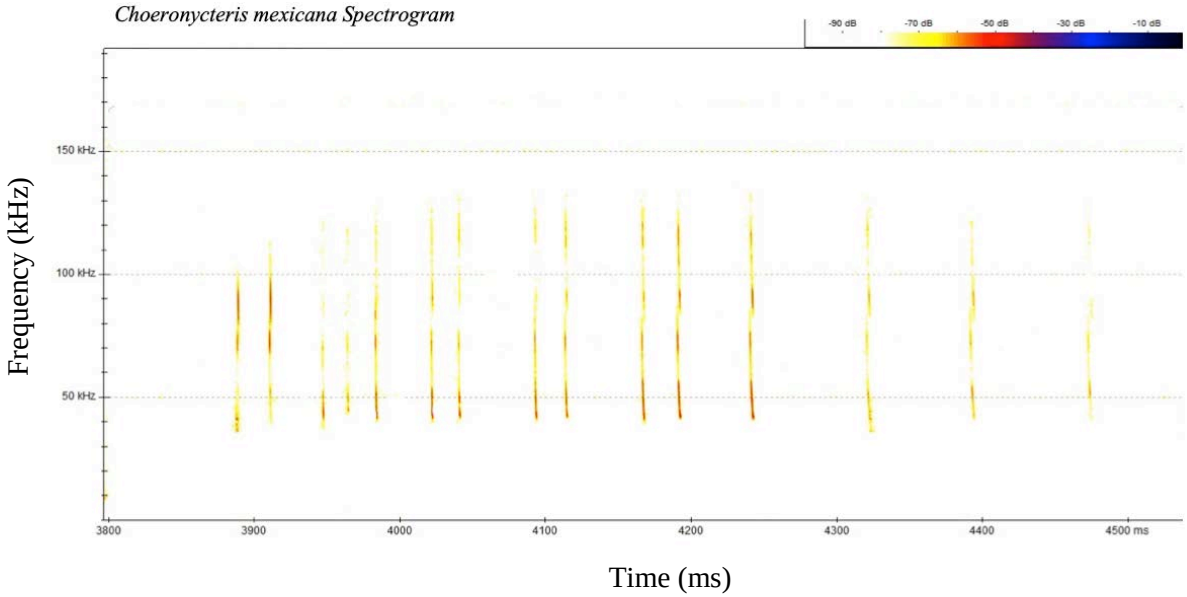

Figure 13. Searching-phase echolocation call sequence of *Choeronycteris mexicana.* The full-spectrum acoustic data was recorded near El Infierno cave using the tunnel sampling method with a Pettersson D500X and a Wildlife Acoustic Bat SM4 devices at a sampling rate of 44.10 kHz with 16-bit resolution. Spectrogram (BatSound FFT 1024, hanning window), shows the echolocation pulses emitted by one individual in a time interval of 700ms. The intensity of the calls is range from -70dB (yellow) to -10dB (blue).

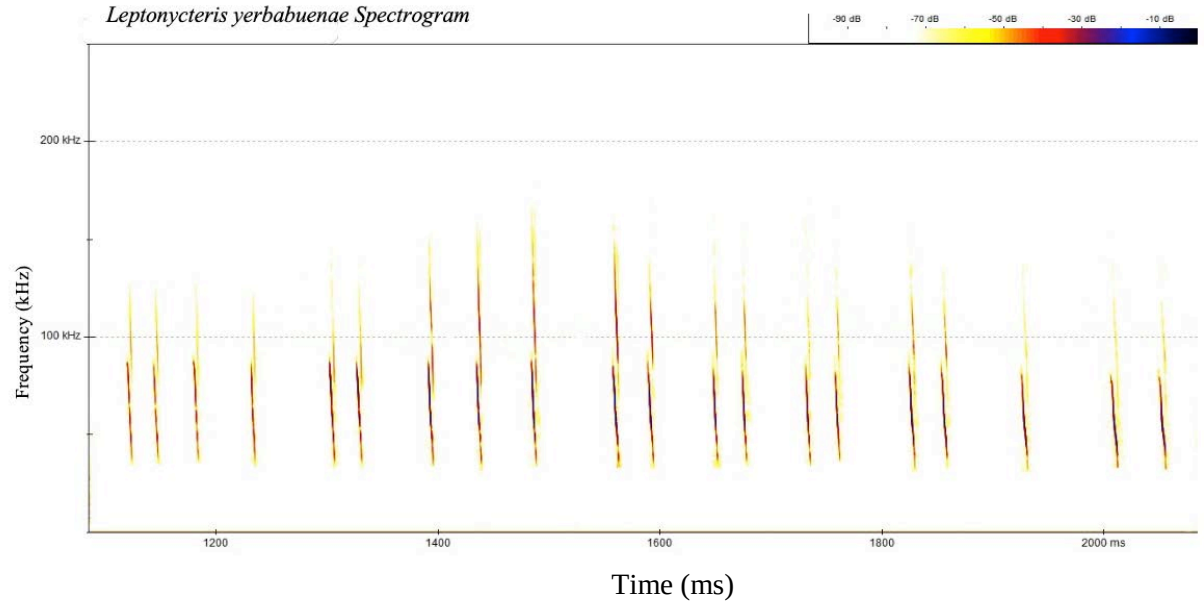

Figure 14. Searching-phase echolocation call sequence of *Leptonycteris yerbabuenae*. The full-spectrum acoustic data was recorded in the states of Sonora, and San Luis Potosí using the hand release method with a Pettersson D1000X device at a sampling rate of 44.10 kHz with 16-bit resolution. Spectrogram (BatSound FFT 1024, hanning window), shows the echolocation pulses emitted by one individual in a time interval of 800ms. The intensity of the calls is range from -70dB (yellow) to -10dB (blue).

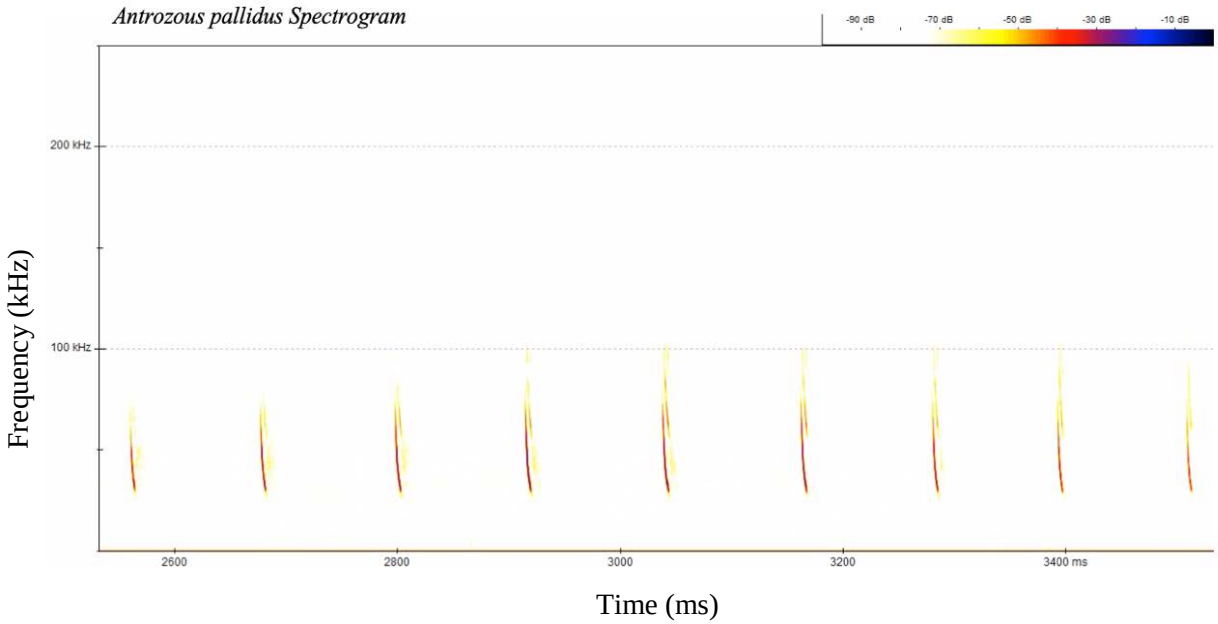

Figure 15. Searching-phase echolocation call sequence of *Antrozous pallidus*. The full-spectrum acoustic data was recorded in the states of Durango, Baja California, Nuevo Leon ans Sonora using the hand release and zipline method with a Pettersson D1000X device at a sampling rate of 44.10 kHz with 16-bit resolution. Spectrogram (BatSound FFT 1024, hanning window), shows the echolocation pulses emitted by one individual in a time interval of 800ms. The intensity of the calls is range from -70dB (yellow) to -10dB (blue).

7. Tables

Table 1. Coefficients of the linear discriminant variables that best separate the species *L. nivalis, C. mexicana, L. yerbabuenae,* and *A. pallidus*. Values suggest that high frequency and bandwidth variables best discriminate these species in multivariate space.

Variable	**LD1**	**LD2**	**LD3**
Bndwdth	187.35	139.01	88.21
CallDuration	-0.95	0.44	-1.20
FreqKnee	-2.10	0.90	-0.84
HiFreq	-259.51	-192.60	-120.50
LowFreq	280.50	197.12	127.15
Fc	-0.98	7.09	0.51
DominantSlope	-0.29	-0.30	0.11
SteepestSlope	-0.60	-0.01	-1.62
LowestSlope	-0.94	0.33	-0.96

Table 2. Kruskal-Wallis pairwise comparisons between the species *L. nivalis, C. mexicana, L. yerbabuenae,* and *A. pallidus*. All comparisons Bonferroni corrected. Species with the same letter are statistically indistinguishable.

Variable	***L. nivalis***	***C. mexicana***	***L. yerbabuenae***	***A. pallidus***
Bndwdth	a,b	a	c	a,b
CallDuration	a	a,b	c	b
FreqKnee	b	b	b	a
HiFreq	b	b	b	a
LowFreq	b	b	b	a
Fc	b	b	b	a
DominantSlope	b	a,b	a,b	a
SteepestSlope	b	a,b	a	a
LowestSlope	b	a	a	a,b

Table 3. Descriptive statistics of the nine predictor variables used for the call structure identification of the gleaning species *L. nivalis, C. mexicana, L. yerbabuenae,* and *A. pallidus*. Mean, standard deviation, range (maximum value - minimum value), and the standard error of the mean of the nine predictor variables for the species *L. nivalis* (n=15), *C. mexicana* (n=3), *L. yerbabuenae* (n=27) and *A. pallidus* (n=64).

		HiFreq	LowFreq	CallDur	Bndwth	Fc	FreqKnee	Dominant slope	Steepest slope	Slowest slope
mean	*Lepniv*	85.84	50.32	2.57	35.52	53.77	58.67	16.80	32.07	10.97
	Chomex	97.33	88.29	1.94	9.03	84.79	62.69	6.75	17.29	1.63
	Lepyer	83.25	35.37	5.64	47.88	41.67	57.63	6.16	20.00	4.16
	Antpal	65.95	29.08	4.66	36.87	30.86	42.94	5.16	7.44	0.99
Minimum	*Lepniv*	61.23	34.71	1.04	22.31	36.78	40.80	1.51	13.75	0.47
	Chomex	93.93	83.53	1.59	3.37	84.79	59.00	1.19	4.43	0.07
	Lepyer	67.58	29.19	4.30	32.31	32.88	49.31	3.64	13.64	2.09
	Antpal	46.20	21.66	2.20	24.25	23.74	34.18	2.20	7.44	0.99
Maximum	*Lepniv*	110.00	75.14	6.02	49.34	85.02	84.12	37.60	62.39	20.23
	Chomex	101.00	90.77	2.33	17.43	94.72	69.99	17.51	41.08	4.73
	Lepyer	97.33	40.85	7.25	59.31	47.97	67.90	12.24	26.32	8.03
	Antpal	76.27	36.36	8.96	46.80	39.23	50.22	9.47	36.97	8.18
Range	*Lepniv*	48.76	40.42	4.98	27.03	48.25	43.32	36.09	48.64	19.76
	Chomex	7.07	7.24	0.74	14.06	9.93	10.99	16.32	36.65	4.67
	Lepyer	29.75	11.66	2.95	27.00	15.09	18.59	8.60	12.68	5.94
	Antpal	30.07	14.70	6.75	22.55	15.49	16.04	7.27	29.53	7.19
Std. Deviation	*Lepniv*	14.50	13.28	1.77	6.11	15.34	11.80	11.62	11.68	6.62
	Chomex	3.54	4.12	0.36	7.42	94.72	6.32	9.32	20.63	2.69
	Lepyer	7.02	2.90	0.78	5.84	3.66	4.88	1.95	3.32	1.27
	Antpal	5.58	2.37	1.29	4.74	2.42	3.27	1.84	36.97	8.18
Std. Error of Mean	*Lepniv*	3.74	3.43	0.46	1.58	3.96	3.05	3.00	3.02	1.71
	Chomex	2.05	2.38	0.21	4.28	9.93	3.65	5.38	11.91	1.55
	Lepyer	1.35	0.56	0.15	1.12	0.70	0.94	0.37	0.64	0.24
	Antpal	0.69	0.29	0.16	0.59	0.30	0.41	0.23	29.53	7.19

CHAPTER III

ASSESSING GEOGRAPHIC VARIATION IN THE ECHOLOCATION CALLS OF *LEPTONYCTERIS YERBABUENAE* AND *ANTROZOUS PALLIDUS* IN THE NORTHERN REGION OF MEXICO

1. Synopsis

Acoustic monitoring methods represent a powerful approach to studying the distribution, ecology and behavior of microchiropteran bats (Rydell, Nyman, Eklöf, Jones, & Russo, 2017). However, the intraspecific call variation and interspecific overlap in call structures complicate the attempts to identify species by call structure. The aim of this study is to investigate whether there is significant geographical variation in the highest apparent frequency (HiFreq), the frequency of the end (LowFreq), the call duration (CallDuration), total frequency spread (Bndwdth), the characteristic frequency (Fc), frequency knee (FreqKnee), percent knee duration (PrcntKneeDur), dominant slope, the steepest slope and the slowest slope of *Leptonycteris yerbabuenae* and *Antrozous pallidus* among five states of Mexico.

To assess the extent of the geographical variations of *L. yerbabuenae* and *A. pallidus*, I used acoustic data collected in the states of Durango, Sonora, Nuevo Leon, Baja California, and San Luis Potosi. I employed a Multivariate Analysis of Variance (MANOVA) for each species to test for multivariate differences among regions; two sites for *L. yerbabuenae* and four sites *for A. pallidus*. Afterward, parametric and non-parametric univariate post-hoc comparisons were conducted for all non-transformed variables. For the species *L. yerbabuenae,* I used the Mann-Whitney U test to determine if there were statistically significant differences between the two regions in the distributions of the variables and identify the potentially reliable descriptive

variables associated with geographic variation. For *A. pallidus*, I employed a Kruskal-Wallis Analysis of Variance followed by post-hoc pairwise comparisons.

The results of the multivariate tests (MANOVA) on the ten predictor variables comparing *L. yerbabuenae* calls between the states of San Luis Potosi and Sonora were not significant. Meanwhile for the species *Antrozous pallidus* the results of the multivariate analysis of variance tests on the ten predictor variables comparing the call structure differences among the regions of Baja California, Sonora, and Durango were significant for the following logarithmic transformed variables: CallDuration, FreqKnee, HiFreq, LowFreq, Fc, Dominant slope, Steepest slope, Lowest slope. The changes in call structure followed a cline from West to East. The results of this study can contribute to a better understanding of the interpopulation variation in call structure of the species *L. yerbabuenae* and *A. pallidus*.

2. Introduction

All New World bats that have been studied to date emit echolocation calls at least for orientation, therefore methods for distinguishing species using the structure of their echolocation calls are exceptionally valuable (Jones, Vaughan, & Parsons, 2000). Acoustic data have been used to address both basic and applied issues, such as questions about the relative abundance of species in an area, the species diversity in a particular region, the ecological or morphological structure of bat communities, the presence of rare or endangered species, the use of foraging habitats by different species, and the determination of critical habitats (Bringham, et. al., 2002). The advancements of automated call classification software packages, in conjunction with multivariate statistical methods, favors the quantitative parameterization of bat echolocation calls to assess the composition of local assemblages through call structure identification of species. These analytical tools have improved the feasibility of using bioacoustic recordings for tracking

spatial-temporal changes in bat activity by species or species groups with a repeatable, automated data management flow (Frick, 2013).

The identification of search-phase echolocation calls of microchiropteran bats has proved extremely useful in the field identification of different species (Law, Reinhold, & Pennay, 2002) providing valuable information on the foraging ecology (Jones, 1999), and habitat use by bats (Frick, 2013). For example, based on the information available from bioacoustic recordings, it is reasonable to assume that sites with higher levels of bat activity experience greater use of the resources by bats than sites with less bat activity (Frick, 2013). It is also correct that call structures are related to function and morphology (Jones, et. al., 2000), as echolocation call parameters such as frequency, duration, and intervals between pulses are adapted to the acoustic constraints of food type and foraging environment (Brinkløv, Kalko, & Surlykke, 2008). The value of acoustic identification tools for conservation practices are evident with the demonstration that cryptic species that are difficult to discern by morphological criteria have been differentiated from their echolocation calls (Jones, et. al., 2000).

Even though the use of automated identification tools of echolocation calls has become very popular recently, the identification of species by call structure still has many uncertainties due to intraspecific call variation and interspecific overlap in call structure (Rydell, Nyman, Eklöf, Jones, & Russo, 2017). There is evidence of multiple bat species in the wild that exhibit situation-specific variation in structure of echolocation calls among individuals (Rydell, Nyman, Eklöf, Jones, & Russo, 2017). This means that individual pulses within a call sequence can change based on the size, age, sex, habitat structure, type of prey, obstacles and presence of conspecifics (Murray, Britzke, & Robbins, 2001), which increases the risk of misclassification when using automatic tools non critically.

Quantitative analyzes allow objective parametrization to ensure proper replication (Jones, et. al., 2000), although replication at the appropriate spatial and temporal scales is not always feasible as the techniques associated with acoustic monitoring of bat echolocation have limitations. To maintain objectivity and repeatability when assessing changes in the relative use of different habitats or bat population trends over time it is important to identify the factors that can introduce variability to the sample, such as the type of habitat and ecological traits of the species. Call pulses from a particular species, for example, are often higher in frequency, broader in frequency sweep, and shorter in duration as they get closer to more cluttered habitat (Barclay & Bringham, 2002); thus environmental clutter levels could mask prey detection echoes, resulting in differences in the call structure for the same species in different habitats. These situation- and habitat-specific differences affect the probability of detecting species in different habitats (Limpens & McCracken, 2002).

In geographically separated populations, differences in acoustic signals may be a result of genetic differentiation, learning or cultural drift, or adaptation to local environmental conditions (Jiang, et. al., 2010). However, the extent of geographic variation continues to remain controversial (Law, Reinhold, & Pennay, 2002). This represents a potential problem if call characteristics from one population are used to identify the calls of unknown individuals from another population, as most studies rely on a reference list of calls from unknown individuals (Law, Reinhold, & Pennay, 2002). Spatial and temporal variation among individuals of the same species may reduce the statistical power to detect biologically significant differences (Frick, 2013), and to establish the characteristics used to identify unknown bats recorded by monitoring systems (Barclay & Bringham, 2002).

Notably the differentiation and classification of different bat species remains a critical issue in improving confidence in the identification from their calls, and research is still needed to clarify the extent of geographic variation in the calls. In this study, I investigate whether there is significant geographical variation in ten different variables related to the call structures of *Leptonycteris yerbabuenae* and *Antrozous pallidus* among five states of Mexico.

These two species share similarities in foraging strategies and echolocation behaviors that allow us to classify them in the same ecological guild as a substrate gleaner. Substrate gleaning is a foraging strategy in which bats use echolocation, prey-generated sounds, and vision to localize and hunt surface-dwelling prey (Razak, 2018). Lesser long-nosed bats are opportunistic foragers and feed mainly on nectar and pollen of paniculate agave flowers and fruits of columnar cacti. Pallid bats are known to hunt by passive listening and glean large arthropods, such as scorpions or crickets, off the ground or plant surfaces (Frick, Heady III, & Hayes, 2009).

L. yerbabuenae, in particular, migrates into northern Sonora and southern Arizona along two migratory routes (Cole & Wilson, 2006). Some populations of *L. yerbabuenae* are resident throughout the year completing their life cycle without migrating, linked to food availability (Fleming & Nassar, 2002). *Antrozous pallidus* and *L. yerbabuenae* co-occur on the southern Baja California peninsula, where their ranges overlap with the important cactus nectar resource *Pachycereus pringlei*, and both bat species are common visitors to its flowers during the late March to early June flowering season (Frick, et. al., 2009). *A. pallidus* represents the first known case of nectarivorous habits in a New World bat outside the Phyllostomidae family (Frick, et. al., 2009).

Since biological, environmental, and technical factors can lead to discrepancies in the echolocation call structure patterns, I expect to observe significant differences in the mean

frequencies between the localities evaluated for *L. yerbabuenae* and *A. pallidus.* As the diet plasticity seems to have a direct impact on the foraging behavior of pallid bats, a change in the modulation of the frequencies emitted by the bats can be expected. Consequently, I also expect a higher level of variability in the call structure of *A. pallidus* individuals. The results of this study can contribute to a clearer understanding of the interpopulation variations of the species *L. yerbabuenae* and *A. pallidus*. The results can also help design acoustic surveys to infer bat populations densities, diversity, and vulnerability. Beyond academic research, these data can be used for the improvement of automated call identification practices and field acoustic monitoring techniques to identify species by call structure.

3. Methods

3.1.Ecological Sampling

I collected bioacoustic data samples from 2012 and 2013 through a combination of field work and provided material to assess geographic variation among populations of *L. yerbabuenae* and *A. pallidus*. The collection of individuals was entailed by placing mist nets in forest trails, habitat edges, streams, ponds, roosts, and other areas that we suspected would have concentrated bat activity to collect a representative sample. In addition to the acoustic recordings, data about sex, reproduction condition, weight, and length of forearm for each one of the individuals was also collected.

The collection of reference echolocation calls of *L. yerbabuenae* was made through the hand release method. This method allows the recording of the individuals during free flight when they were released directly from the hand. Meanwhile, individuals of *A. pallidus* were recorded under both, hand release and zip line methods. Using the zip line method, the individuals were recorded while flying tethered to a zip line of approximately 3-5 m length of elastic sewing

thread by a loose-fitting fixed loop in the elastic pulled over the bat's head. The other end of the thread was attached to the zip line via a small keychain ring bulk to a taut line of 5 m and about 1 m above the ground. Both of these methods provide some advantages and disadvantages as forms of acoustic sampling. When applying the zip line over the hand release method the "controlled free flight" of the individual within a given range is favored, which is advantageous for the collection of calls emitted under free-flight conditions. Also, this makes it possible to record calls at a predictable distance from the microphone and provides the opportunity for repeated flights to record good quality calls. On the other hand, zip-lined bats could be subject to experience higher levels of stress, although previous studies have agreed that the resulting recordings from the zip line method are a more accurate reflection of their standard calls than hand-released bat recordings (Ellison, Valdez, Cryan, O'Shea and Bogan, 2013).

To avoid high levels of stress, each individual was recorded for a period of 1-2 minutes and then released. A Pettersson D1000X detector with a sampling rate of 500 kHz was used for all the bioacoustics recording of the calls. Files were saved in WAV format on flash cards. Bats were identified to species level using field keys before being recorded. As previous studies have demonstrated that local conditions like temperature and humidity can affect call characteristics (Findlay & Barclay, 2020), the field work was concentrated during the months of July to October in 2012 and from March to May in 2013. In addition, during these months it is expected a higher abundance of *L. yerbabuenae* as the flowering peak of their main food source, *Pachycereus pringlei*, occurs during the months of April and May.

L. yerbabuenae and *A. pallidus* have a broad geographic range of distribution and their population trends are more stable than *L. nivalis* and *C. mexicana*, as a consequence bioacoustic samples with an acceptable level of interpopulation variation were available for the study.

Reference echolocation calls were recorded at different sites along their migratory route including the Mexican states of Baja California, Durango, Nuevo Leon, San Luis Potosí and Sonora, so intraspecific geographical and population variability are represented in the data set (Figure 16).

Field work for data collection of *L. yerbabuenae* was conducted through the states of Sonora and San Luis de Potosi, while *A. pallidus* acoustic samples include individuals collected in multiple locations from the states of Nuevo Leon, Sonora, Durango and Baja California (See Figure 16). All the echolocation calls were recorded on either edge or open surroundings not over the water (Table 4). Acoustic data from only one individual in the state of Nuevo Leon was available for the study, consequently the data collected in this area was not taken in consideration due to data deficiency.

3.2.Data Analysis

I assessed 1,489 reference echolocation call pulses from 27 individuals of *L. yerbabuenae* and 63 individuals of A. pallidus from five states of the northern region of Mexico. Full-spectrum, real-time recordings were provided by a collaboration with the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR), Unidad Durango, and Dr. Veronica Zamora-Gutierrez. All the echolocation calls were digitized by a computer using a bat call analysis software (SonoBat v. 4.0) at a sampling rate of 44.10 kHz with 16-bit resolution. Only search phase calls were analyzed for the call description. Before the analysis, the call sequences were visually inspected using the sound analysis software BatSound Pro (Version 3.31a© 1996-2001 by Pettersson Electronics AB) to remove approach-phase calls, terminal-phase calls and social calls from the spectrograms, if present. The echolocation call structure produced during the foraging activity changes during the search, approach, and terminal

stages by getting shorter and often over the time (Fenton, 1981). Consequently, calls emitted during the search-phase are the most constant in frequency-time structure, which make them ideal for characterization.

After removed all the non-search-phase calls, I automatically extracted and parameterized the calls using the in-build algorithms of SonoBat v. 4.0. Up to 74 temporal, amplitude, and frequency-dependent variables, in addition to slope-related measurements to describe the echolocation call structures of the species, were evaluated. When there were multiple calls for an individual, the average of the variables was used, and subsequent analyses were performed on individuals. I measured frequency and temporal variables for the description of each echolocation call recorded with a maximal intensity of more than 20 dB above the noise level. A maximum of 100 calls with a 1.0 acceptable quality rating were considered per file in order to produce more reliable sequence identifications.

I reduced the number of variables to approximate the ratio of cases to variables of 10:1. I employed the same predictor variables used in the previous study (Chapter II) in order to identify significant changes related to geographic variation. Using the same predictor variables, we can compare changes in call structures when the individuals are plotted together versus when they are plotted by geographic location. The screening of the variables was done by evaluating the intercorrelations of 74 variables, the examination of histograms, and the low weighting results from an initial Principal Component Analysis (PCA). In addition to the latitude and longitude, I made use of the following variables: bandwidth, hi frequency, low frequency, call duration, characteristic frequency, frequency knee, percent knee duration, dominant slope, steepest slope, and lowest slope (See APPENDIX A.1-7), to investigate geographic variation patterns in the call design of *L. yerbabuenae* and *A. pallidus.* The mean, range, and standard deviations of the ten

temporal, frequency-dependent, and slope related variables were calculated for all the individuals.

Before posterior analyses, all ten variables were converted to natural logarithm values to reduce skew in the distributions. Then, I ran a Multiple Analysis of Variance (MANOVA) for each species to test for multivariate differences among regions; two sites for *L. yerbabuenae* and four sites *for A. pallidus* (Table 4). Afterward, parametric univariate post-hoc comparisons were conducted for all non-transformed variables. For the species *L. yerbabuenae,* I used the Mann-Whitney U test, in conjunction with pairwise comparisons, to determine if there are statistically significant differences between regions in the distributions of the variables and identify the potentially reliable descriptive variables associated with geographic variation. In the case of *A. pallidus*, instead of used a Mann-Whitney U test, I employed a Kruskal-Wallis Analysis of Variance. All statistical tests were made in IBM SPSS v. 26 software or R Studio v. 1.2.5042© 2009-2020 using modules.

4. Results

Neither the MANOVA nor any of the ten predictor variables for the calls of *L. yerbabuenae* recorded in San Luis Potosi (N = 20) and Sonora (N = 7) were significant (MANOVA, P = 0.202, Table 5). There were non-significant differences for the parametric univariate comparisons. The results of the Mann-Whitney U tests on the non-transformed variables revealed that call duration (N = 27, Mann-Whitney U = 34.0, two-sided p = 0.048), and lowest slope (27, Mann-Whitney U = 112.0, two=sided p = 0.019) were the only two variables which differed significantly between sites for the species *L. yerbabuenae*. Overall, the support for significant geographic variation in the calls of *L. yerbabuenae* was weak.

On the other hand, for *Antrozous pallidus*, calls across the three sites (Baja California, N = 10; Sonora, N = 44; Durango, N = 9) showed significant differences both in multivariate (MANOVA, $p < 0.001$; Table 6) and in univariate comparisons. The parametric analysis of variance (ANOVA) conducted on the transformed variables were significant for the following predictors: lnCallDuration, lnFreqKnee, lnHiFreq, lnLowFreq, lnFc, lnDominant slope, lnSteepest slope, lnLowest slope. Pairwise comparison tests were performed based upon the results of a Kruskal-Wallis One-Way ANOVA on the non-transformed variables. The results of this test yielded six significant results: lnCallDuration, lnFreqKnee, lnHiFreq, lnLowFreq, lnFc, lnDominant slope, lnSteepest slope, lnLowest slope. The six significant variables revealed a consistent pattern across the evaluated regions (Durango, Sonora and Baja California).

The results for the Bonferroni adjusted pairwise comparisons of the variable call duration (CallDuration) (N=63, df = 2, KW Test statistic 20.09, $p < 0.001$) indicated a significant difference among all sites (Table 7, Figure 17).

The Bonferroni adjusted, pairwise comparisons, also indicated a significant difference between the mean of the frequency knee (FreqKnee) of the echolocation calls recorded in Baja California compared to the other two sites (Table 8, Figure18). However, there was no difference in the variable between Sonora and Durango.

In the case of the characteristic frequency (Fc) (N=63, df = 2, KW Test statistic 6.97, $p = 0.031$), the results of the pairwise multiple comparisons test (Bonferroni adjusted), indicated a significant difference only between Baja California and Durango with no differences between Baja California and Sonora or Sonora and Durango (Table 9, Figure 19).

Dominant slope (DominantSlope) (N=63, df = 2, KW Test statistic 14.76, $p = 0.001$) pairwise comparisons (Bonferroni adjusted) indicated a significant difference among individuals

collected in Baja compared to Sonora and Durango, however these latter two sites did not differ (Table 10, Figure 20).

For the steepest slope variable (SteepestSlope) (N=63, df = 2, KW Test statistic 14.68, p = 0.001) the Bonferroni adjusted pairwise multiple comparisons suggest a significant difference between the states of Baja California and Durango, and Sonora and Durango (Table 11, Figure 21).

Finally, the lowest slope (LowestSlope) mean values of the pairwise comparisons (Bonferroni adjusted) of the *A. pallidus* individuals (N=63, df = 2, KW Test statistic 14.46, p = 0.001) indicated a significant difference between the state of Baja California and and Durango as well as between Sonora and Durango (Table 12, Figure 22).

As I used the same ten predictor variables that were used for the description of the calls structure of *L. yerbabuenae* and *A. pallidus* in the previous study (Chapter II), in conjunction with the data associated with geographic variations (latitude and longitude), I used a principal components analysis (PCA) to visualize the distribution of the individuals and identify patterns of clusters by locality.

The correlation circle of the variables indicates that the variables that least contributed to the segregation of the species by locality were bandwidth and percent knee duration (Figure 23). The PCA results, also indicate that the first two principal components explain a 61.7% of the variance (Dim1 = 45.6%; Dim2 = 16.1%).

Due to the fact that all the individuals plotted are from the same species, I expected to have a large amount of overlap among the individuals from different regions. However, when all the pallid bats are plotted together a slight segregation between the individuals of the species by geographic area can be distinguish when visually inspected (Figure 24).

Overall the results reveal a strong geographical gradient in the call structure of *Antrozous pallidus* from west to east with values for all variables lower in Baja California with the exception of call duration, which was higher than all other sites. The variables in the eastern most site, Durango, were higher in all cases except for call duration, which was the lowest of all sites. Sonora was intermediate in all variables. In the case of *Leptonycteris yerbabuenae*, there was no geographic variation in the call structures due to geographic location.

5. Discussion

Growing evidence supports the hypothesis that echolocation pulses can encode information about a bat's identity, at a range of levels varying from geographical location through to colony, sex, body size, and age (Jones & Siemers, 2011). However, since the structure of the echolocation calls in microchiropteran bats can be used as indicators for species identification, the study of intraspecific variations has become more relevant for the use of acoustic monitoring techniques (Jiang, Wu, & Feng, 2015). In this study, I evaluated the differences in call structure of individuals of *L. yerbabuenae* and *A. pallidus* in four states of the northern region of Mexico to describe patterns associated with geographic variation.

As hypothesized, there were significant differences in the search-phase echolocation call characteristics of *A. pallidus* recorded in different locations. The analysis suggests a strong geographical gradient in the calls of pallid bats from west to east in the northern region of Mexico. Higher means for all the predictor variables, except for call duration, were recorded for the species in the state of Durango. All the individuals in this locality were recorded in the fairly open surroundings of Tamaulipan thorny scrubland, which would present relatively low levels of clutter. The call design of echolocating bats depends to a great extent on the nature of the habitat. Several publications suggest that calls of insect-eating bats are often higher in frequency, broader

in frequency sweep, and shorter in duration as the bat get closer to vegetation or as the habitat clutter increase (Schnitzler & Kalko, 2001; Barclay & Bringham, 2002). Although not considered a high clutter habitat, Tamaulipan scrub is more cluttered than the more open Sonoran Desert.

An inverse pattern was observed in the individuals recorded in the region of Baja California, where all variables present lower mean values in contrast with the other two localities, except for the call duration, which was the higher mean reported for the species in this study. As the sample collected in Baja California was also recorded in open desert habitat, relatively lower peak frequencies and higher call duration might be expected for several reasons. Schnitzler and Kalko (1998) hypothesized that low-frequency signals with long durations are suited to detect bigger insects at longer distances, moreover shorter signals with higher frequencies are adapted for the detection of smaller insects at closer range. Although it is unclear how gleaning species partition resources, current data suggest that niche distinction might be achieved through either difference in the mode and ability to find prey using echolocation pulses or other sensory systems (Santana, Geipel, Dumont, Kalka, & Kalko, 2011). The low frequency calls in the region might be related to a higher dependency on the use of terrestrial arthropods. In this region, I expect to observe seasonal sympatry with *L. yerbabuenae*. Differences in food items (diet plasticity) and the places where the resources are found can result in a modulation of call frequencies as a response of niche partitioning in this region. This pattern in the call structure may provide some advantages to avoid the call overlap with heterospecifics when performing different foraging tasks. That said, Chapter II shows that *A. pallidus* calls are at a consistently lower frequency than those of *L. yerbabuenae*, and not likely confused.

In Sonora, all the variables present intermediate mean values in contrast with the individuals from Durango and Baja California. The individuals included in these samples were recorded in both open and edge spaces. It is out of the scope of this study to specifically assess how the variability of the surroundings (open vs. cluttered) could affect the mean values of the call structure in the region, other than looking at broad trends. The PCA results shows a higher level of variance between the call structure of the individuals recorded in Sonora which can be related to the possibility that Sonora represents a gradual transition from the lower frequency calls of Baja to the higher frequency calls detected in Durango. The presence of the consistent cline in calls would suggest that they do reflect a geographical pattern, and additional research could indicate if his was indeed driven by broad scale differences in vegetation cover and foraging preferences.

Contrary to my hypothesis, there are no significant differences in the call structure for the species *L. yerbabuenae.* These results were unexpected as the broad geographic range of distribution and migratory behavior of *L. yerbabuenae* promotes a niche expansion or reduction according to the distribution of the resources and the presence of other species in the same location. These findings may not be representative of the whole *L. yerbabuenae* species, only a small range of their distribution was sampled. Roost-specific call signatures in bats can lead to geographic variation in echolocation calls of bats at small ($\leq$ 1 km) and continental scale (> 1000 km). However, the stronger dependence of *L. yerbabuenae* on nectar feeding across its range might also constrain geographic variability in the call structures.

The analysis of *A. pallidus* suggest that percent knee duration and bandwidth are the variables that least contributed to the discrimination of the individuals geographically. Each of the resulting ten predictor variables including, latitude and longitude, contributed 80% or more to

the model. The biplot reveals a lower variance in the echolocation pulses of the individuals recorded in Durango, intermediate variability in the call from Baja, and a large amount of variability in the call from Sonora, again perhaps related to it being a zone of transition in habitat structure between Baja and Durango.

The results of this study confirmed the presence of significant differences among regions, but it does not elucidate the functional meaning of this variability. Although acoustic divergence due to geographical variation occurred in *A. pallidus*, the generality of its causes needs further investigation by comparing sympatric and allopatric populations of species emitting similar pulses across gradients in habitat structure (Sun, et. al., 2013). In the case of *L. yerbabuenae*, further research is needed in order to properly evaluate the extent of geographic variation, including representative samples of their full distribution.

Studies of geographic variation in acoustic signals of animals may help to illuminate the diversified factors affecting the divergence and evolution of echolocation call structures (Jiang, Wu, & Feng, 2015). Interpopulation variations can be used to better infer call diversity and evaluate the vulnerability of bats to habitat change. The study of intraspecific variations can provide valuable information for the study of ecological and evolutionary traits in nectar-feeding bats and some facultative species, in addition to a novel insight for the development of acoustic monitoring techniques.

6. Figures

Figure 16. Map of *L. yerbabuenae* and *A. pallidus* data collection sites for the assessment of geographical variations in call structure. Data samples of individuals from *L. yerbabuenae* (▲), were collected in the states of Sonora and San Luis Potosí. Data samples of individuals from *A. pallidus* (■), were collected in the states of Sonora, Baja California, Durango, and Nuevo Leon. Final layout made in ArcGis 10.1 on Windows 7.

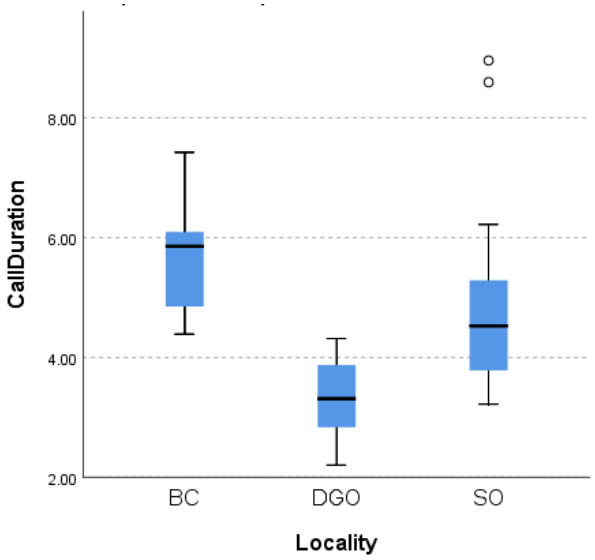

Figure 17. Results of Independent-sample Kruskal Wallis Analysis of Variance comparing the means of the predictor variable call duration of *A. pallidus* in the states of Baja California, Durango y Sonora. Box plot graph the call duration (CallDuration) variable among the different samples of *A. pallidus*. Data points above or under the box plots reflect sample outliers, and the black line show the median with the standard deviation of each group. The results for this test indicate significant differences among the states of Baja California (BC), Durango (DGO), and Sonora (SO) ($p<0.001$). The significance level is 0.05. All pairwise significance values were adjusted with Bonferroni corrections.

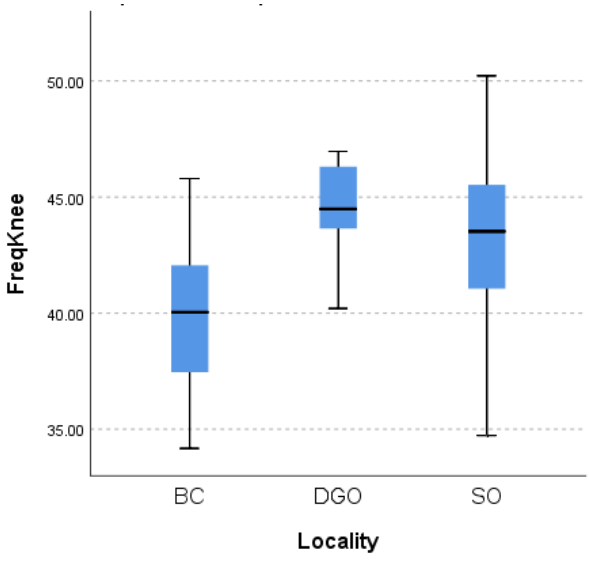

Figure 18. Results of Independent-sample Kruskal Wallis Analysis of Variance comparing the means of the predictor variable frequency knee of *A. pallidus* in the states of Baja California, Durango y Sonora. Box plot graph the frequency knee (FreqKnee) variable among the different samples of *A. pallidus*. Data points above or under the box plots reflect sample outliers, and the black line show the median with the standard deviation of each group. The results for this test indicate significant differences among the states of Baja California (BC), Durango (DGO), and Sonora (SO) ($p<0.001$). The significance level is 0.05. All pairwise significance values were adjusted with Bonferroni corrections.

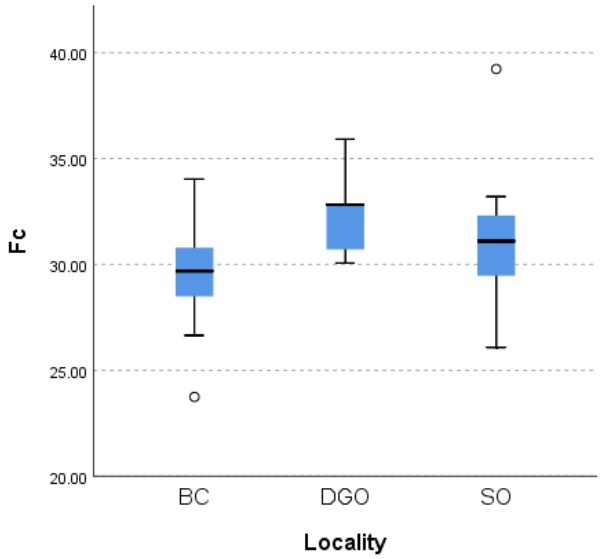

Figure 19. Results of Independent-sample Kruskal Wallis Analysis of Variance comparing the means of the predictor variable characteristic frequency of *A. pallidus* in the states of Baja California, Durango y Sonora. Box plot graph the characteristic frequency (Fc) variable among the different samples of *A. pallidus.* Data points above or under the box plots reflect sample outliers, and the black line show the median with the standard deviation of each group. The results for this test indicate significant differences among the states of Baja California (BC), Durango (DGO), and Sonora (SO) ($p<0.001$). The significance level is 0.05. All pairwise significance values were adjusted with Bonferroni corrections.

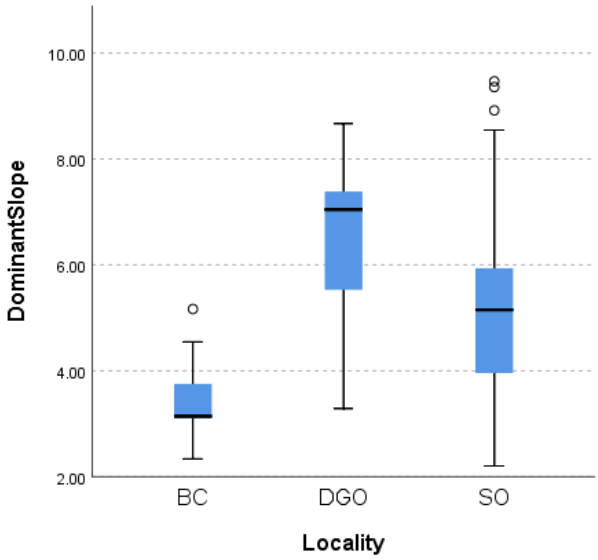

Figure 20. Results of Independent-sample Kruskal Wallis Analysis of Variance comparing the means of the predictor variable dominant slope of *A. pallidus* in the states of Baja California, Durango y Sonora. Box plot graph the dominant slope (DominantSlope) variable among the different samples of *A. pallidus.* Data points above or under the box plots reflect sample outliers, and the black line show the median with the standard deviation of each group. The results for this test indicate significant differences among the states of Baja California (BC), Durango (DGO), and Sonora (SO) ($p<0.001$). The significance level is 0.05. All pairwise significance values were adjusted with Bonferroni corrections.

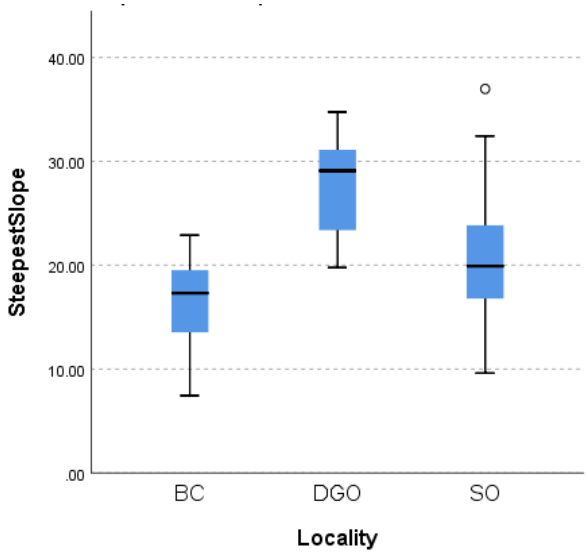

Figure 21. Results of Independent-sample Kruskal Wallis Analysis of Variance comparing the means of the predictor variable steepest slope of *A. pallidus* in the states of Baja California, Durango y Sonora. Box plot graph the steepest slope (SteepestSlope) variable among the different samples of *A. pallidus.* Data points above or under the box plots reflect sample outliers, and the black line show the median with the standard deviation of each group. The results for this test indicate significant differences among the states of Baja California (BC), Durango (DGO), and Sonora (SO) ($p<0.001$). The significance level is 0.05. All pairwise significance values were adjusted with Bonferroni corrections.

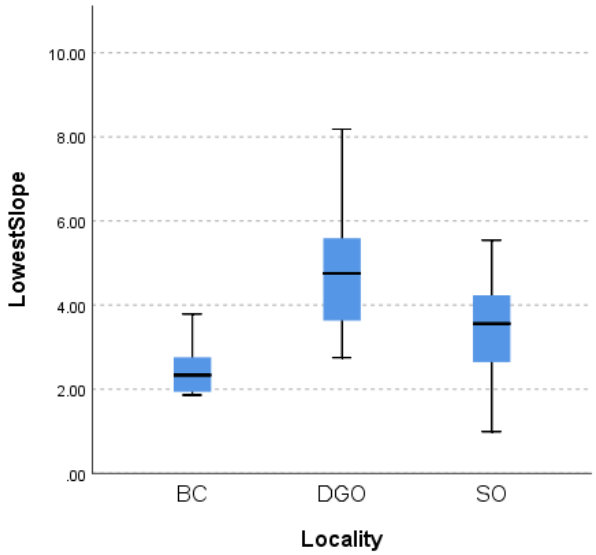

Figure 22. Results of Independent-sample Kruskal Wallis Analysis of Variance comparing the means of the predictor variable lowest slope of *A. pallidus* in the states of Baja California, Durango y Sonora. Box plot graph the lowest slope (LowestSlope) variable among the different samples of *A. pallidus.* Data points above or under the box plots reflect sample outliers, and the black line show the median with the standard deviation of each group. The results for this test indicate significant differences among the states of Baja California (BC), Durango (DGO), and Sonora (SO) ($p<0.001$). The significance level is 0.05. All pairwise significance values were adjusted with Bonferroni corrections.

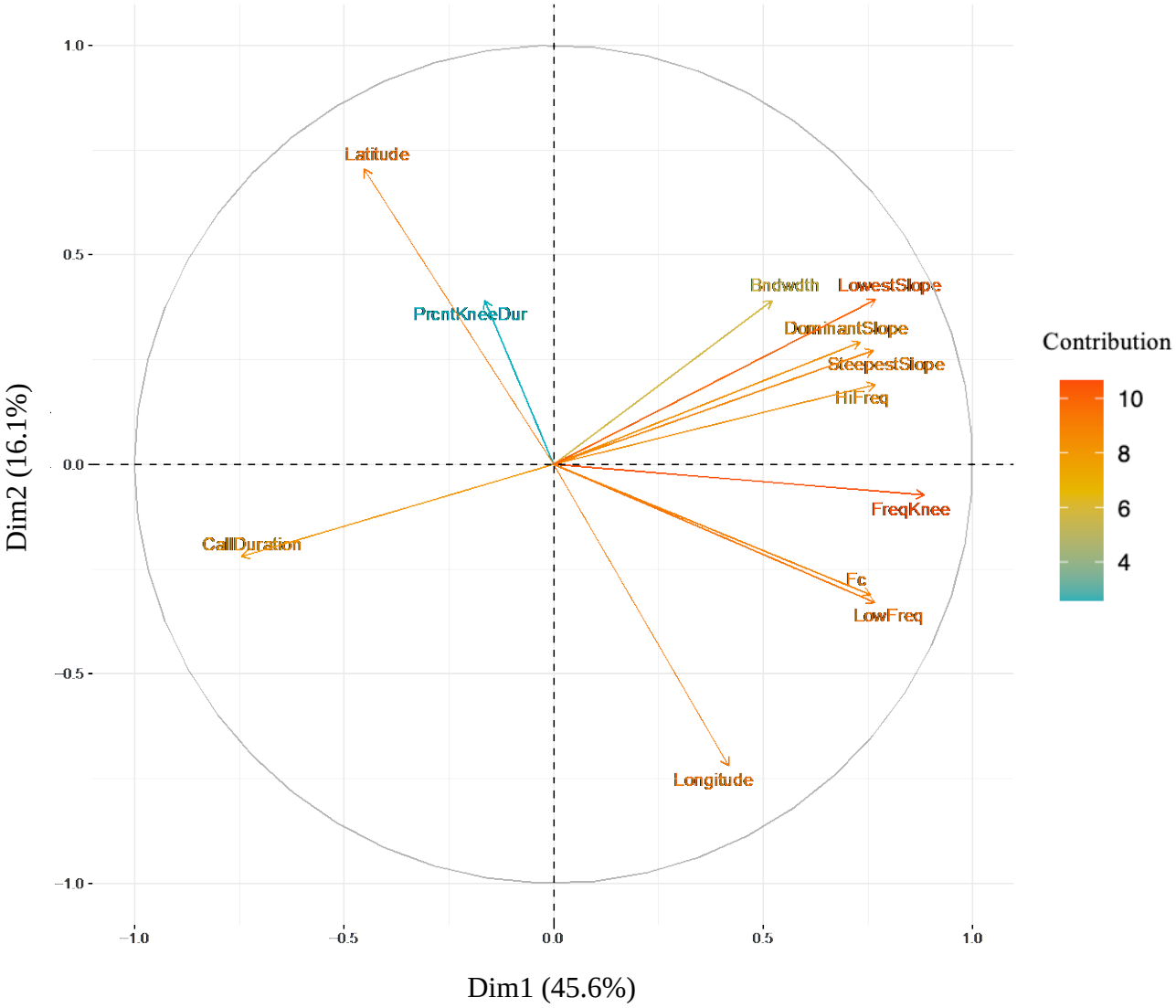

Figure 23. Contribution of the predictor variables to call structure differentiation of *A. pallidus* individuals by geographical location. Variables with lines in similar directions are positively correlated while those which point in opposite directions are negatively correlated. Distance between the variables and the origin measures the contribution of the variable to the species differentiation. The results suggest PrcntKneeDur and Bndwdth as the variables that less contribute to the geographical segregation of *A. pallidus* individuals, meanwhile frequency-dependent variables and slope related measurements are the predictor variables that better differentiate between the localities.

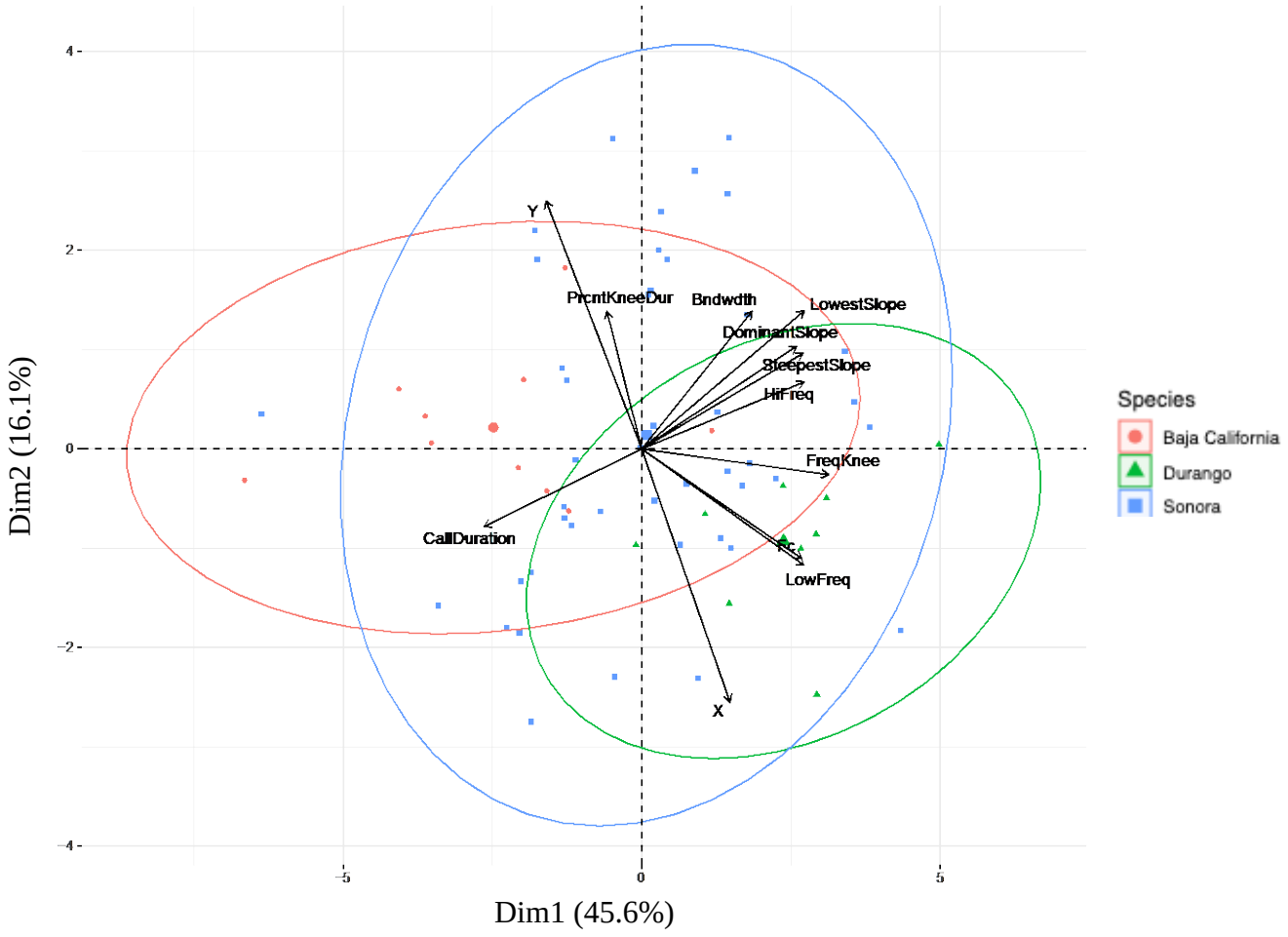

Figure 24: *A. pallidus* geographic variation according to the call structure predictor variables. Arrangement of *A. pallidus* individuals collected in the states of Sonora (■), Durango (▲), and Baja California (•) along the axes of a principal component analysis (Dim1 vs. Dim2). The first two principal components (Dim1 = 45.6%, Dim2 = 16.1%) explain a large percentage of the total variation to map the individuals. Circles represent 0.95 confidence ellipse. Large data points represent group means. Arrows represent the contribution of the predictor variables to call structure differentiation by geographical location. Variables with lines in similar directions are positively correlated while those which point in opposite directions are negatively correlated. Distance between the variables and the origin measures the contribution of the variable to the species differentiation. The results suggest PrcntKneeDur and Bndwdth as the variables that less contribute to the geographical segregation of *A. pallidus* individuals, meanwhile frequency-dependent variables and slope related measurements are the predictor variables that better differentiate between the localities.

7. Tables

Table 4. Exact location and habitat type of the collection sites of *L. yerbabuenae* and *A. pallidus* acoustic samples. Habitat type (E= edge not over water; O= open not over the water; E/O= recordings were collected under both circumstances) and exact localities of the acoustic data collection of *L. yerbabuenae* (Lepyer) and *A. pallidus* (Antpal). * Sample not included in the analysis.

Species	State	Specific Location		Habitat Type
		Latitude	Longitude	
Lepyer	San Luis Potosí	21.86806	-100.03220	E
	Sonora	27.19728	-109.09091	E
		31.51705	-112.75294	E/O
		31.66739	-113.38103	O
Antpal	Baja California	29.97055	-115.23796	O
		32.15387	-115.78878	O
	Durango	26.64361	-103.75809	O
	Nuevo Leon *	24.78822	-99.52435	O
	Sonora	27.19728	-109.09091	E
		29.37136	-111.44044	E/O
		31.85251	-114.63831	E/O
		32.15387	-115.78878	O

Table 5. Wilks' lambda test for parametric analysis of variance (MANOVA)of geographic variations of *L. yerbabuenae* among the Mexican states of San Luis Potosí and Sonora. Design: Intercept + Locality. (a = exact F value; N=27).

Effect	Test	Value	F	df	df Error	Significance
Intercept	Wilks' Lambda	>0.001	55621.498 [a]	10.00	16.00	> 0.001
Locality	Wilks' Lambda	0.504	1.573 [a]	10.00	16.00	0.202

Table 6. Wilks' lambda test for parametric analysis of variance (MANOVA)of geographic variations of *A. pallidus* among the Mexican states of Durango, Baja California and Sonora. Design: Intercept + Locality. (a = exact F value; N=63).

Effect	Test	Value	F	df	df Error	Significance
Intercept	Wilks' Lambda	>0.001	185345.158 [a]	10.00	51.00	> 0.001
Locality	Wilks' Lambda	0.416	2.812 [a]	20.00	102.00	> 0.001

Table 7. *A. pallidus* pairwise comparison results of the call duration variable (CallDuration) by locality including the states of Baja California, Durango and Sonora. Asymptotic significances (2-sided tests) are displayed. The significance level is .05. Significance values have been adjusted by the Bonferroni correction for multiple tests. N_0 = Sample 1 = Sample 2.

Sample 1 vs Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Significance	Adj. Sig.[a]
Baja California - Sonora	16.491	6.421	2.568	0.010	0.031
Baja California - Durango	37.678	8.422	4.474	>0.001	>0.001
Sonora - Durango	-21.187	6.706	-3.159	0.002	0.005

Table 8. *A. pallidus* pairwise comparison results of the frequency knee variable (FreqKnee) by locality including the states of Baja California, Durango and Sonora. Asymptotic significances (2-sided tests) are displayed. The significance level is .05. Significance values have been adjusted by the Bonferroni correction for multiple tests. N_0 = Sample 1 = Sample 2.

Sample 1 vs Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Significance	Adj. Sig.[a]
Baja California – Sonora	-16.891	6.421	-2.630	0.009	0.026
Baja California -Durango	-24.522	8.422	-2.912	0.004	0.011
Sonora – Durango	7.631	6.706	1.138	0.255	0.765

Table 9. *A. pallidus* pairwise comparison results of the frequency characteristic frequency (Fc) by locality including the states of Baja California, Durango and Sonora. Asymptotic significances (2-sided tests) are displayed. The significance level is .05. Significance values have been adjusted by the Bonferroni correction for multiple tests. N_0 = Sample 1 = Sample 2.

Sample 1 vs Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Significance	Adj. Sig.[a]
Baja California – Sonora	-9.359	6.421	-1.458	0.145	0.435
Baja California -Durango	-22.144	8.421	-2.630	0.009	0.026
Sonora – Durango	12.785	6.705	1.907	0.057	0.170

Table 10. *A. pallidus* pairwise comparison results of the dominant slope variable (DominantSlope) by locality including the states of Baja California, Durango and Sonora. Asymptotic significances (2-sided tests) are displayed. The significance level is .05. Significance values have been adjusted by the Bonferroni correction for multiple tests. N_0 = Sample 1 = Sample 2.

Sample 1 vs Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Significance	Adj. Sig.[a]
Baja California – Sonora	-20.977	6.422	-3.267	0.001	0.003
Baja California -Durango	-30.444	8.422	-3.615	0.000	0.001
Sonora – Durango	9.467	6.706	1.412	0.158	0.474

Table 11. *A. pallidus* pairwise comparison results of the steepest slope variable (SteepestSlope) by locality including the states of Baja California, Durango and Sonora. Asymptotic significances (2-sided tests) are displayed. The significance level is .05. Significance values have been adjusted by the Bonferroni correction for multiple tests. N_0 = Sample 1 = Sample 2.

Sample 1 vs Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Significance	Adj. Sig.[a]
Baja California – Sonora	-12.505	6.422	-1.947	0.052	0.155
Baja California -Durango	-31.967	8.422	-3.796	00.000	0.000
Sonora – Durango	19.462	6.706	2.902	.004	0.011

Table 12. *A. pallidus* pairwise comparison results of the lowest slope variable (LowestSlope) by locality including the states of Baja California, Durango and Sonora. Asymptotic significances (2-sided tests) are displayed. The significance level is .05. Significance values have been adjusted by the Bonferroni correction for multiple tests. N_0 = Sample 1 = Sample 2.

Sample 1 vs Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Significance	Adj. Sig.[a]
Baja California – Sonora	-17.864	6.421	-2.782	0.005	0.155
Baja California -Durango	-31.667	8.422	-3.760	>0.001	0.000
Sonora – Durango	13.803	6.706	2.058	0.040	0.011

CHAPTER IV

CONCLUSIONS

The unequivocal evidence documenting dramatic declines for individual species of pollinating bats mainly due to habitat degradation (Allen-Wardell, et. al., 1998) provoke concerns and an urgent necessity for the conservation of nectar-feeding bats in the south-west United States and the northern region of Mexico. However, the data deficiency about the population trends and certain aspect of their bat ecology hinders our efforts of accurate assessment the conservation status and the identification of the main stressors of the population. In this research study I aimed to contribute to the development of a quantitative guideline for the identification of the echolocation calls of the nectar-feeding bat species *Leptonycteris nivalis, Choeronycteris mexicana,* and *Leptonycteris yerbabuenae* and the facultative nectar-feeding bat *Antrozous pallidus,* to promote the use of acoustic monitoring techniques as a sampling method for the identification of key site for prioritization.

In the first study of my thesis (Chapter II), I provided a quantitative description for the identification of the echolocation call structure of *L. nivalis, C. mexicana, L. yerbabuenae* and *A. pallidus* using the mean, maximum, and minimum values of nine predictor variables including high frequency, low frequency, bandwidth, call duration, frequency knee, characteristic frequency, dominant slope, steepest slope and lowest slope. Also an assessment of the spectrograms was made to provide a qualitative description of the calls, as the type of signals (e.g. frequency modulated (FC), constant frequency (CF), etc.) or intensity of the calls (e.g. high, low, moderate), and corroborate temporal or frequency-dependent mean values including high frequency, low frequency, bandwidth and call duration.

As part of the analysis in Chapter II, I evaluated differences in mean values between the four species and identify the predictor variables that better discriminated between the species. I concluded that lowest slope was the predictor variable that better differentiate between *C. mexicana* and *L. nivalis*; bandwidth and call duration were the best discriminants for *L. yerbabuenae;* meanwhile frequency-dependent variables as high frequency, low frequency, frequency knee and characteristic frequency (Fc) were for *A. pallidus* and the slope related measurements (dominant, steepest and lowest slopes) for *L. nivalis.*

Acoustic data have been used to address both basic and applied issues, such as the ecological or morphological structure of bat communities, the presence of rare or endangered species, the use of foraging habitats by different species, and the determination of critical habitats (Barclay & Bringham, 2002), consequently one of the goals of this research study is to provide a baseline knowledge for the use of echolocation call structure as a sampling technique for the identification of pollinating bat species and key sites for conservation. However quantitative analyses need objective parametrization to ensure proper replication (Jones et al., 2000). To maintain objectivity and repeatability when assessing changes in the relative use of different habitats or bat population trends over time it is important to identify the factors that can introduce variability to the sample like type of habitat or geographical variation. In Chapter III, I investigated whether there is significant geographical variation in call structure between the individuals of *L. yerbabuenae* and *A. pallidus* among five states of the norther region of Mexico.

The results of the analyzes suggests a strong geographical gradient in the calls of pallid bats from west to east in the northern region of Mexico. Higher means for all the predictor variables, except for call duration, were recorded for the species in the state of Durango, while an inverse pattern was observed in the individuals recorded in the region of Baja California, where

all variables present lower mean values in contrast with the other two localities, except for the call duration, which was the higher mean reported for the species in this study. In the case of Sonora, all the variables present intermediate mean values in contrast with the individuals from Durango and Baja California. In the other hand, for the species *L. yerbabuenae* there are no significant differences in the call structure between the individuals recorded in the states of Sonora and San Luis Potosí.

It is beyond of the scope of this study to specifically assess how the variability of the surroundings (open vs. cluttered) could affect the mean values of the call structure in the region, other than looking at broad trends. However, several publications suggest that calls of insect-eating bats are often higher in frequency, broader in frequency sweep, and shorter in duration as the bat get closer to vegetation or as the habitat clutter increase (Schnitzler & Kalko, 2001; Barclay & Bringham, 2002). Consequently, in this study I made some assumptions on how the type of habitat, differences on the foraging task typically performed, the presence of heterospecifics and seasonal sympatry can have an effect of the frequency modulation of the calls. My findings support literature recommendations on considering geographical variations and type of habitat when using acoustic automatic denotification tools for the monitoring of bat species.

Noticeably, more research is needed to guide conservation action on a local and global scale for the protection of pollinating bats species. The results included in this study can be substantially deepened by increasing the sample sizes and assessing, more in detail intraspecific variations due to sex, age, or across the geographic range of the individuals. Studies in controlled environments such as in-flight cages are highly recommended, even though echolocation calls

recorded using hand release may be more similar to calls made during free flight than those recorded in flight cages (Jennings, et. al., 2004).

The assessment of echolocation calls using acoustics recordings during active foraging activity of pollinating bats can contribute to a better comprehension of their foraging behavior and usage of the resources. A monitoring technique that can improve the quantification of the variables is recording the calls during active foraging activity of bats by placing the microphones of the acoustics detectors on the agave inflorescence. Despite the fact that this technique, together with the use of video recordings, was initially implemented to collect the bioacoustics recordings of this study, the recordings were not of sufficient quality to provide quantitative guidelines of the call structures. In addition, the identification of the species through video recordings was problematic for the purposes of this study.

Although the description of the call structures included in this study are mainly based on ten predictor variables, the descriptive statistics of the 74 initial parameters evaluated will be available for public access at the Texas A&M Data Repository *(Link: https://tamu.libguides.com/research-data-management/repositories)*. These data contribute to a clearer understanding of the call structures which makes them useful as a bat echolocation call assemblage library for further analysis and the utilization of automatic identification tools.

This is one the first studies to document the echolocation call structure of the three pollinating bats in the northern region of Mexico, in addition to providing guidelines of quantitative variables for the identification and differentiation of the calls to a species level. This study also contributes to our knowledge of bat ecology by providing guidelines for the improvement of an acoustic population monitoring technique for pollinating bats.

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APPENDIX A

Appendix A: *Table of the description of the Sonobat parameters assessed for the identification of echolocation calls.*

A.1. Time-dependent variables

Parameter	Description	Units of Measurement
PrecedingIntrvl	Time between the current call and the previous call.	ms
CallsPerSec	Mean calls per second of the recording or section of recording displayed. The accuracy of the reported value depends both on the quality of the recording and the absence of other bats and other signals in the recording. Any other signal components that pass through the discrimination logic will be counted as calls and contribute to (and reduce the accuracy of) the calculation.	ms
CallDuration	Duration of the call.	ms
TimeFromMaxToFc	Time from the point at which the maximum amplitude occurs to the point in the call of the characteristic frequency	ms
LedgeDuration	Duration of the ledge, i.e., the most extended flattest slope section of the body of the call preceding the characteristic frequency.	ms
DurOf32dB	The duration of the call from the point of the call 32 dB below and before the point of maximum amplitude and the point of the call 32 dB below and after the point of maximum amplitude of the call.	ms
DurOf20dB	The duration of the call from the point of the call 20 dB below and before the point of maximum amplitude and the point of the call 32 dB below and after the point of maximum amplitude of the call.	ms
DurOf15dB	The duration of the call from the point of the call 15 dB below and before the point of maximum amplitude and the point of the call 32 dB below and after the point of maximum amplitude of the call.	ms
DurOf5dB	The duration of the call from the point of the call 5 dB below and before the point of maximum amplitude and the point of the call 32 dB below and after the point of maximum amplitude of the call.	ms

A.2. Frequency-dependent variables

Parameter	Description	Unit of Measurement
Fc	Characteristic frequency of the call. Determined by finding the point in the final 40% of the call having the lowest slope or exhibiting the end of the main trend of the body of the call.	kHz
HiFreq	Highest apparent frequency of the call.	kHz
LowFreq	Lowest apparent frequency of the call.	kHz
Bndwdth	Total frequency spread of the call. Calculated from the difference between the highest and lowest frequency.	kHz
FreqMaxPwr	The frequency of the maximum amplitude of the call.	kHz
FreqKnee	Frequency at which the initial slope of the call most abruptly transitions to the slope of the body of the call.	
PrcntKneeDur	Percentage of the entire call duration at which the knee occurs, i.e., the point at which the initial slope of the call most abruptly transitions to the slope of the body of the call.	**%**
StartF	Frequency of the start of the call. Typically, the same point as the highest frequency, but different if the call initially rises in frequency.	kHz
EndF	Frequency of the end of the call. Typically, the same point as the lowest frequency, but different if the call ends with a rise in frequency.	kHz
FreqLedge	Frequency of the ledge, i.e., the most abrupt transition to the most extended flattest slope section of the body of the call preceding the characteristic frequency, also referred to as the “ledge” of the call.	kHz
FreqCtr	Frequency at the center of the duration of the call.	kHz
Fbak32dB	Frequency of the call 32 dB below the point of maximum amplitude of the call, and preceding the point of maximum amplitude of the call.	kHz

FFwd32dB	Frequency of the call 32 dB below the point of maximum amplitude of the call, and after the point of maximum amplitude of the call.	kHz
Fbak20dB	Frequency of the call 20 dB below the point of maximum amplitude of the call and preceding the point of maximum amplitude of the call.	kHz
FFwd20dB	Frequency of the call 20 dB below the point of maximum amplitude of the call, and after the point of maximum amplitude of the call.	kHz
Fbak15dB	Frequency of the call 15 dB below the point of maximum amplitude of the call and preceding the point of maximum amplitude of the call.	kHz
FFwd15dB	Frequency of the call 15 dB below the point of maximum amplitude of the call, and after the point of maximum amplitude of the call.	kHz
Fbak5dB	Frequency of the call 5 dB below the point of maximum amplitude of the call and preceding the point of maximum amplitude of the call.	kHz
FFwd5dB	Frequency of the call 5 dB below the point of maximum amplitude of the call, and after the point of maximum amplitude of the call.	kHz
Bndw32dB	The total bandwidth covered from the point of the call 32 dB below and before the point of maximum amplitude and the point of the call 32 dB below and after the point of maximum amplitude of the call.	kHz
Bndw20dB	The total bandwidth covered from the point of the call 20 dB below and before the point of maximum amplitude and the point of the call 32 dB below and after the point of maximum amplitude of the call.	kHz
Bndw15dB	The total bandwidth covered from the point of the call 15 dB below and before the point of maximum amplitude and the point of the call 32 dB below and after the point of maximum amplitude of the call.	kHz
Bndw5dB	The total bandwidth covered from the point of the call 5 dB below and before the point of maximum amplitude and the	kHz

	point of the call 32 dB below and after the point of maximum amplitude of the call.	
HiFminusStartF	High frequency minus start frequency. This measure may be used as a quality control check to sort and reject improperly trended calls. For typical frequency modulated calls, a value greater than zero (i.e., start frequency less than high frequency) may indicate an improperly trended call.	kHz
FcMinusEndF	Characteristic frequency minus start frequency. This measure may be used as a quality control check to sort and reject improperly trended calls. Use as appropriate for different types of calls. For example, most calls from the genus Myotis should have a positive value for this measure indicating the end frequency is less than the characteristic frequency. A negative value might indicate an improper trend as the result of a poor signal or excessive echo obscuring the end of the call.	kHz

A.3. Amplitude-dependent variables

Parameter	***Description***	***Unit of Measurement***
PrcntMaxAmpDur	Percentage of the entire call duration at which the maximum amplitude occurs.	%
Amp1stQrtl	Total amplitude of the first quartile of the call.	relative units
Amp2ndQrtl	Total amplitude of the second quartile of the call.	relative units
Amp3rdQrtl	Total amplitude of the third quartile of the call.	relative units
Amp4thQrtl	Total amplitude of the fourth quartile of the call.	relative units
Amp1stMean	Mean of the first quartile amplitude.	relative units
Amp2ndMean	Mean of the second quartile amplitude.	relative units
Amp3rdMean	Mean of the third quartile amplitude	relative units
Amp4thMean	Mean of the fourth quartile amplitude.	relative units

A.4. Slope related measurements

Parameter	*Description*	*Unit of Measurement*
DominantSlope	Slope of the longest sustained trend in slope of the call. Determined by finding the segment of the call having the minimum residue for a linear regression of a segment of the call of 20% the duration of the call.	kHz/ms
SlopeAtFc	Instantaneous slope at the point of the characteristic frequency.	kHz/ms
StartSlope	Slope at the start of the call, calculated from the first 5% of the call duration.	kHz/ms
EndSlope	Slope at the end of the call, calculated from the final 5% of the call duration.	kHz/ms
SteepestSlope	Steepest slope of the call calculated from a linear regression of a segment of 10% the duration of the call.	kHz/ms
LowestSlope	Lowest slope of the call calculated from a linear regression of a segment of 10% the duration of the call.	kHz/ms
TotalSlope	Total slope of the call calculated from the difference in frequency and time from the point of highest frequency to the point of the characteristic frequency.	kHz/ms
HiFtoKnSlope	Slope of the call calculated from the difference in frequency and time from the point of highest frequency to the point of the knee.	kHz/ms
KneeToFcSlope	Slope of the call calculated from the difference in frequency and time from the point of the knee to the point of the characteristic frequency.	kHz/ms
CummNmlzdSlp	Average of the instantaneous slopes of the call (kHz/ms).	kHz/ms
AmpK@start	Slope of a logarithmic plot of the time-amplitude trend of the call from the start of the call to the point of maximum amplitude.	kHz/ms
AmpK@end	Slope of a logarithmic plot of the time-amplitude trend of the call from the point of maximum amplitude to the end of the call.	kHz/ms

A.5. Exponential fit of the variables

Parameter	*Description*	*Unit of Measurement*
HiFtoFcExpAmp	Amplitude parameter of an exponential fit of the call from the point of high frequency to the point if the characteristic frequency.	N/A
HiFtoFcDmp	Damping parameter of an exponential fit of the call from the point of high frequency to the point if the characteristic frequency.	N/A
KnToFcExpAmp	Amplitude parameter of an exponential fit of the call from the point of the knee to the point if the characteristic frequency.	N/A
KnToFcDmp	Damping parameter of an exponential fit of the call from the point of the knee to the point if the characteristic frequency.	N/A
HiFtoKnExpAmp	Amplitude parameter of an exponential fit of the call from the point of the high frequency to the point if the characteristic frequency.	N/A
HiFtoKnDmp	Damping parameter of an exponential fit of the call from the point of the high frequency to the point if the characteristic frequency.	N/A
HiFtoFcExpAmp	Amplitude parameter of an exponential fit of the call from the point of high frequency to the point if the characteristic frequency.	N/A
LnExpA_StartAmp	Amplitude parameter of an exponential fit of the time-amplitude trend of the call from the start of the call to the point of maximum amplitude.	N/A
LnExpB_StartAmp	Damping parameter of an exponential fit of the time-amplitude trend of the call from the start of the call to the point of maximum amplitude.	N/A
AmpStartLn60ExpC	Time parameter of an exponential fit of the time-amplitude trend of the call from the start of the call to the point of maximum amplitude.	N/A

LnExpA_EndAmp	Amplitude parameter of an exponential fit of the time-amplitude trend of the call from the point of maximum amplitude to the end of the call.	N/A
LnExpB_EndAmp	Damping parameter of an exponential fit of the time-amplitude trend of the call from the point of maximum amplitude to the end of the call.	N/A

A.6. Time-amplitude trends

Parameter	Description	Units of Measurement
AmpKurtosis	Kurtosis of the time-amplitude trend.	N/A
AmpSkew	Skew of the time-amplitude trend.	N/A
AmpVariance	Variance of the time-amplitude trend.	N/A
AmpMoment	Moment of the time-amplitude trend.	N/A
AmpGausR2	R-squared of a Gaussian fit of the time amplitude trend.	N/A

A.7. Harmonic strengths ratios

Parameter	**Description**	**Units of Measurement**
RelPwr2ndTo1st	Ratio of the strength of the harmonic that SonoBat trended (typically the first or primary harmonic) to the strength of the next higher harmonic (typically the second harmonic). A ratio of the 3rd harmonic that exceeds the 2nd harmonic's ratio typically indicates a saturated or "clipped" signal. Such calls will render inaccurate assessments of power distribution through the call, although the time-frequency trend will remain reliable.	N/A
RelPwr3rdTo1st	Ratio of the strength of the harmonic that SonoBat trended (typically the first or primary harmonic) to the strength of the second higher harmonic (typically the third harmonic). A ratio of the 3rd harmonic that exceeds the 2nd harmonic's ratio typically indicates a saturated or "clipped" signal. Such calls will render inaccurate assessments of power distribution	N/A

through the call, although the time-frequency trend will remain reliable.