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FILOGEOGRAFÍA Y CONSERVACIÓN DEL MURCIÉLAGO MAGUEYERO MENOR, *LEPTONYCTERIS YERBABUENAE* (MARTÍNEZ Y VILLA 1940)

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Junapú y Xbalamqué se metieron dentro
de sus cerbatanas a dormir y
aunque los murciélagos revoloteaban a su alrededor,
no pudieron morderlos.
Junajpú quiso ver si había amanecido
y al sacar la cabeza para certificarlo
se la cortó Camazotz, el Murciélago,
quedando sólo su cuerpo.
Los murciélagos fueron a poner
la cabeza de Junajpú
al atrio donde se jugaba a la pelota.

Popol Vuh.

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Resumen

El murciélago magueyero menor (lesser long-nosed bat, murciélago tequilero), *Leptonycteris yerbabuenae* es un quiróptero que consume principalmente recursos florales (néctar-polen) para cubrir sus requerimientos energéticos. Este murciélago cumple el rol de polinizador primario de uno de los grupos más emblemáticos y mejor representados, ecológicamente hablando para México, el género *Agave*. Uno de los problemas de conservación en torno de este murciélago es la pérdida de refugios y zonas de forrajeo. Aunado a esto, la falta de conocimiento en algunas comunidades provoca confusión y falsas alertas, lo cual orilla a la gente a vandalizar los refugios, no sólo de esta especie sino de muchos más quirópteros que habitan en México.

Una de las características ecológicas más llamativas del murciélago tequilero es el comportamiento migratorio que presenta una parte de la población de hembras, el cual consiste en formar cuevas de maternidad al norte de su distribución durante las estaciones más cálidas (primavera-verano), mientras que durante la época fría (otoño-invierno) se refugian al centro-sur de México. En este trabajo se determinó, con base análisis filogeográficos, la distribución geográfica de linajes diferenciados a lo largo de su área de distribución. También, basados en análisis filogenéticos se calculó el tiempo de divergencia y se infirió el centro de origen de la especie. Finalmente, se propuso un plan de manejo que ayuda a la conservación del murciélago con base en la relación ecológica más importante que ha establecido como polinizador principal del género *Agave*.

Nuestros resultados sugieren que el género *Leptonycteris* se originó hace aproximadamente 13.91 millones de años (grupo corona), mientras que las especies *Leptonycteris curasoae* y *L. yerbabuenae* divergieron hace 13.3 millones de años. No se identificó estructura genética definida para la especie, pero sí se detectó una expansión poblacional reciente (hace ~130,000 – 160,000 años). Ello corresponde con modelos de distribución potencial proyectados hacia el pasado y las zonas termodinámicamente más estables, comparándolas con la distribución presente y pasada del género *Agave*. Concluimos así que el centro de origen del género *Leptonycteris* y de la especie *L. yerbabuenae* se localiza en el centro-sur de México, asociado a las zonas de mayor diversidad de especies de *Agave*, aunado a la presencia de otras fuentes de alimento para estos murciélagos nectarívoros.

Abstract

The lesser long-nosed bat, *Leptonycteris yerbabuenae*, is a bat that consumes mainly floral resources (nectar-pollen) to cover its energy requirements. This bat fulfills the role of main pollinator of one of the most emblematic and best ecologically represented groups for Mexico, the *Agave* genus. One of the conservation problems this bat faces is the loss of shelters and foraging areas. Additionally, the lack of knowledge in some communities causes confusion and false alerts, which leads people to vandalize the shelters, not only of this species but of many more bats that inhabit Mexico.

One of the most remarkable ecological characteristics of the tequila-bat is the migratory behavior of a part of the female population, which consists of forming maternity caves to the north of its distribution during the warmer seasons (Spring-Summer), while during the cold season (Autumn-Winter) they take refuge in central-southern Mexico. In this work, based on phylogeographic analysis, the geographical distribution of differentiated lineages throughout their area of distribution was determined. Also, based on phylogenetic analyses, the divergence time was calculated and the center of origin of the species was inferred. Finally, a management plan was proposed that helps the conservation of the bat based on the most important ecological relationship that it has established as the main pollinator of the *Agave* genus.

Our results suggest that the genus *Leptonycteris* originated approximately 13.91 million years ago (crown group), while the species *Leptonycteris curasoae* and *L. yerbabuenae* diverged 13.3 million years ago. No definite genetic structure was identified for the species, but a recent population expansion (~130,000 – 160,000 years ago) was detected. This corresponds to models of potential distribution projected towards the past and the most thermodynamically stable zones, comparing them with the present and past distribution of the *Agave* genus. Thus, we conclude that the center of origin of the genus *Leptonycteris* and of the species *L. yerbabuenae* is located in south-central Mexico, associated with the areas with the greatest diversity of *Agave* species, coupled with the presence of other food sources for these species. nectar-feeding bats.

Introducción general

I. Filogeografía y conservación

A partir de la segunda mitad del siglo pasado, se ha registrado una crisis biológica a nivel global dada por la disminución y pérdida de biodiversidad en todo el mundo, debido principalmente a las actividades humanas (Soulé, 1985; Dirzo et al., 2014). En ese sentido, a este periodo geológico se le ha denominado Antropoceno ya que se ha causado un gran impacto a nivel global (Pievani, 2014; Corlett, 2015). Así, la pérdida de la biodiversidad se ha abordado desde varios enfoques y disciplinas que en conjunto se denominan Biología de la Conservación (Soulé, 1985; Allendorf *et al*., 2007). Estos enfoques surgieron como respuesta a la crisis ambiental y se encargan de estudiar las causas y proponen métodos para tratar de enfrentar las consecuencias de la pérdida de especies y en su caso mitigar los daños que esto causa en la naturaleza (Soulé, 1985; Allendorf *et al*., 2007).

Se han desarrollo herramientas teóricas y tecnológicas que nos permiten estudiar tanto los patrones de diversidad a distintos niveles (genes, especies y ecosistemas), así como proponer proyecciones ante distintos escenarios de cambio climático, degradación de vegetación y pérdida de especies (Allendorf *et al*., 2007), incluyendo información satelital hasta la secuenciación de nueva generación. De esta manera, se han podido integrar estudios genéticos y genómicos como disciplina para el desarrollo de la Genética de la Conservación (Primmer, 2009). Los marcadores genéticos moleculares permiten medir parámetros fundamentales en conservación, como el tamaño efectivo de la población, cuellos de botella y flujo de genes específicos del sexo o contribuciones de los fundadores.

Los marcadores moleculares también se pueden utilizar para inferir la relaciones históricas y geográficas entre linajes (Filogeografía). En ese sentido, los datos de provenientes de muestras antiguas pueden proporcionar información adicional sobre la relación de los grupos contemporáneos (Hedrick, 2001). La información genética se puede complementar con información y análisis biogeográficos (Allendorf *et al*., 2007) para discernir patrones de distribución de la diversidad genética entre las especies y las poblaciones. De esta manera, se pueden considerar elementos de las historias evolutivas de las especies a partir de datos recientes y diseñar estrategias de prevención, mitigación y/o rescate.

II. El murciélago magueyero, descripción y biología básica

Los murciélagos (orden Chiroptera) son un grupo de mamíferos muy numeroso, actualmente se cuenta con alrededor de 1,411 especies registradas en todo el mundo (Burgin et al., 2018). Estos mamíferos presentan características particulares que los distinguen a

simple vista del resto de los grupos que componen a la clase Mammalia. Entre estas características resalta su capacidad de vuelo (única entre todos los mamíferos), la estructura de las alas que consisten en una membrana de piel (patagio) apoyada en dedos elongados, la disposición de las patas traseras y sus hábitos nocturnos (Neuweiler, 2000). El orden Chiroptera se divide en dos subórdenes: Yangochiroptera y Yinpterochiroptera. La principal diferencia es la capacidad de ecolocalización, presente únicamente en Yangochiroptera, que está determinada por la forma y estructuras que componen la laringe y tráquea (Kunz, 1982; Neuwieler, 2000; Teeling et al, 2002, 2005).

En cuanto a su distribución, tan solo cinco géneros de Chiroptera (Myotis, Pipistrellus, Eptesicus y Nycticeius de la Familia Vespertilionideae, y Tadarida de la Familia Molosidae) se encuentran en seis regiones zoogeográficas (Paleártica, Neártica, Neotropical, Indomalasia (región Oriental), Etiope (región Etiope) y Australiana (Australia y Nueva Guinea). Algunas regiones comparten alta similitud de géneros, por ejemplo las regiones Neotropical y Australiana comparten una similitud genérica de 7.6%, la región Etiope y la Oriental el 47% y la Neártica y Paleártica el 40% (Koopman, 1981; Gunell y Simmons, 2005). Los hábitos alimenticios de los murciélagos son muy variados. Se pueden alimentar de insectos, pequeños vertebrados, peces, de otros murciélagos, sangre de algunos mamíferos y aves, además pueden consumir productos vegetales, tales como frutos, néctar y polen (Neuweiler, 2000). Una de las familias que ha llamado la atención por su diversidad de especies, hábitos alimenticios y hábitats que ocupa, es la familia Phyllostomidae (suborden Yangochiroptera), endémica del continente americano, con alrededor de 217 especies conocidas (Teeling et al, 2002, 2005; Burgin et al., 2018).

La familia Phyllostomidae se caracteriza por contar con murciélagos que se han especializado en distintos hábitos alimenticios incluyendo sangre, peces, néctar, insectos, frutos, carne y algunos considerados omnívoros (Kunz, 1982; Neuweiler, 2000; Van de Bussche y Hoofer, 2004). Dentro de esta familia se ha descrito el papel de los murciélagos polinizadores de múltiples plantas, agrupados en la subfamilia Glossophaginae. Uno de los que más ha llamado la atención es el género *Leptonycteris*.

El género *Leptonycteris* cuenta con tres especies: *L. curasoae*, *L. nivalis* y *L. yerbabuenae*. Dos distribuidas en México y sur de Estados Unidos de América (*L. nivalis* y *L. yerbabuenae;* Figura 1.1) y la tercera, *L. curasoae*, que se encuentra únicamente en algunas islas caribeñas (Aruba, Bonaire, Curazao y Margarita) y regiones de Venezuela y Colombia (Cole and Wilson, 2006). El nombre del género, *Leptonycteris*, proviene del griego *leptos*, que significa esbelto, y *nycteris*, que significa murciélago y hace referencia al rostro esbelto de sus miembros (Hensley and Wilkins, 1988). También se les conoce como murciélagos

magueyeros. Las especies con distribución simpátrica en Norteamérica, *L. nivalis* y *L. yerbabuenae* se les denomina comúnmente como murciélago magueyero mayor y murciélago magueyero menor, respectivamente, haciendo alusión a la diferencia del tamaño corporal entre las dos especies. En contraparte, *L. curasoae* se ha denominado comúnmente como el murciélago hocicudo de Curazao.

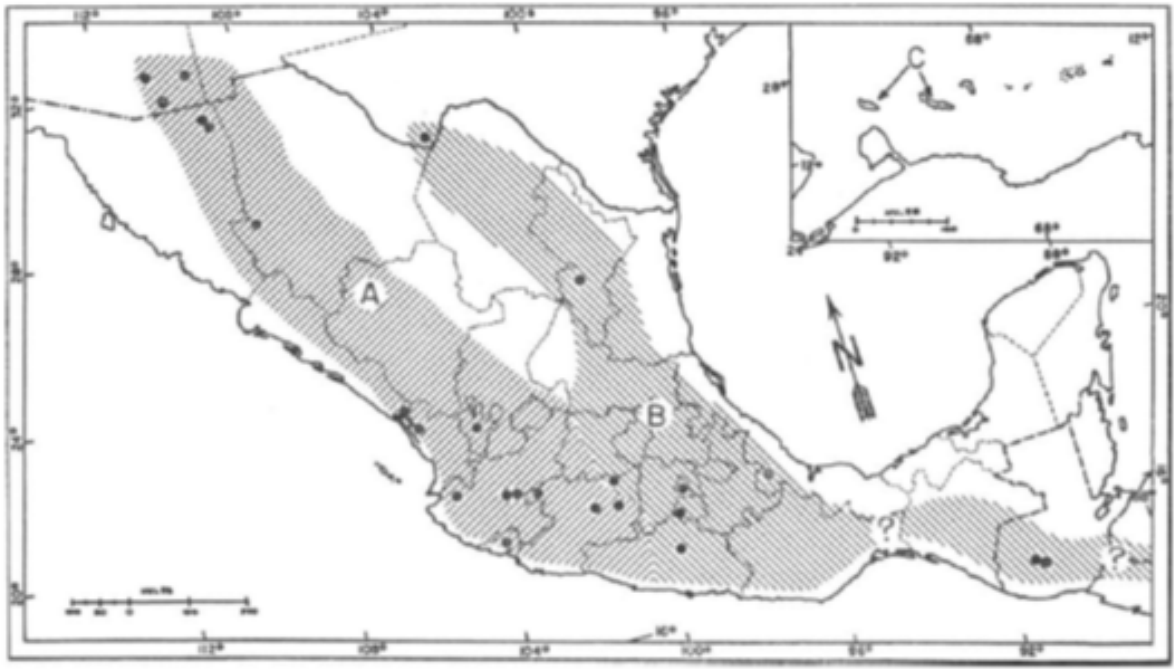

Figura 1.1 Distribución de las especies de las especies de A) *Leptonycteris yerbabuenae* y B) *L. nivalis*. Tomado de Hoffman (1957).

La diagnosis del género se describe por Hoffmeister (1957) como: “Un murciélago de hoja nasal que habita en América (Phyllostomidae) de la subfamilia Glossophaginae en el que la cola está tan acortada que parece ausente pero en realidad consta de tres vértebras caudales; la longitud total de cabeza a cola comprende el rango de 70 a 95 mm; el antebrazo, 46 a 57 mm; el rostro y hocico se aprecian alargados pero menos alargados que en algunas otras especies glosofaginas; la primera falange del tercer dedo mide menos de un tercio de la longitud del metacarpal de la mano; la fórmula dental, $\frac{2}{2}\frac{1}{1}\frac{2}{3}\frac{2}{2}$. Incisivos inferiores presentes; en algunos casos, pueden estar tan desgastados que parecen estar ausentes o pueden perderse por completo”.

Las tres especies del género *Leptonycteris* se alimentan, en mayor o menor medida, de plantas del género *Agave* entre otros grupos como Cactaceae, Bombacaceae y algunas leguminosas (Álvarez y González, 1970; Arita y Santos del Prado, 1999; Arizaga *et al*., 2000; Martino et al., 2002; Ibarra-Cerdeño *et al*., 2005; Molina y Eguiarte, 2003; Moreno-Valdez *et*

al., 2004; Peñalba *et al*., 2003; Rocha *et al*., 2005; Barba, 2012; Trejo et al., 2015), estas evidencias dieron paso a la hipótesis de la "coevolución difusa" entre ambos géneros (Flores-Abreu et al., 2019).

II.1 Murciélago magueyero menor, *Leptonycteris yerbabuenae*

El epíteto específico de *L. yerbabuenae* se refiere al tipo de la localidad, un pequeño pueblo nombrado Yerbabuena, ubicada en el estado de Guerrero, México, de donde proviene el tipo original en una cueva ubicada al sur del poblado, a una elevación poco mayor de 1,800 msnm (Martínez y Villa-R, 1940). *Leptonycteris yerbabuenae* (Martínez y Villa-R, 1940) es una de las 35 especies de la subfamilia Glossophaginae (Cole and Wilson, 2006), para la se tiene información de patrones evolutivos desde enfoques genómicos, filogeográficos y ecológicos (Arteaga et al., 2018; Medellín et al., 2018; Gaona et al., 2019; Trejo et al., 2019; Gutierrez-Guerrero et al., 2020; Menchaca et al., 2020).

Leptonycteris yerbabuenae se puede distinguir de otras especies de la familia por la ausencia de una cola llamativa y la presencia de un pelaje marrón (Arita, 1991). El pelaje de es más corto y más denso en comparación con el pelaje más largo y esponjoso de la especie del mismo género y con la cual comparte algunas zonas de su distribución, *L. nivalis* (Cole and Wilson, 2006). Por su parte, *L. nivalis* es más grande con pelaje grisáceo, uropatagium más estrecho y alas más largas. *L. yerbabuenae* es más pequeño (15-25 g versus 18-30 g), tiene alas más cortas (longitud del antebrazo 51-54 mm versus 56.5-59.5 mm), y un tercer dedo más corto (92-102 mm versus 106-115 mm) que *L. nivalis* (Arita, 1999). La longitud de la cabeza y el cuerpo de *L. yerbabuenae* tiene un promedio de hasta un 10% más corto que el de *L. nivalis* (Hoffmeister, 1957). El cráneo de *L. yerbabuenae* es más pequeño que el de *L. nivalis* (Davis y Carter, 1962). Mientras que el cráneo de *L. curasoae* es generalmente más grande que el de *L. yerbabuenae*. Los incisivos superiores de *L. curasoae* están espaciados uniformemente y son más grandes que los de *L. yerbabuenae* (Cole and Wilson, 2006).

II.2 Evolución

El origen del género *Leptonycteris* no es claro. El registro fósil cuenta con algunos representantes de cada una de las tres especies que lo componen, aunque, de depósitos geológicos recientes (Pleistoceno). En el caso de *L. curasoae*, se ha encontrado un subfósil en la "Isla de Toas" en costas de Venezuela (Rincón, 2001), sin embargo, debido a su estado de "conservación" no es óptimo para obtener mayor dato de antigüedad. En el caso de *L. nivalis*, se cuenta con el registro material depositado en una cueva en el sur de Tamaulipas, México, de una mandíbula sin dientes (Dalquest and Roth, 1970). Para la especie *L. yerbabuenae*, se

cuenta con un ejemplar en depósitos en el estado de San Luis Potosí, México (Arroyo-Cabrales, 1992; Arroyo-Cabrales and Polaco, 2003).

Debido a que se cuenta con pocos ejemplares fósiles y poco informativos. Se han empleado herramientas moleculares para inferir el origen y evolución de este grupo. Algunos autores han propuesto fechas de origen y diversificación para la familia Phyllostomidea brindando información que nos permiten establecer fechas de origen para el género *Leptonycteris*. Una de las primeras hipótesis filogenéticas propuestas estimó una fecha de divergencia entre *Leptonycteris* y su género hermano *Glossophaga* de alrededor de 14 millones de años y una fecha de divergencia entre las especies de *L. curasoae* y *L. yerbabuenae* alrededor de 0.5 millones de años (Datzmann, 2010). Posteriormente, se reportó una edad de 10.4 millones de años entre el género *Leptonycteris* y *Glossophaga* (Rojas et al., 2011; Rojas et al., 2012). Más recientemente, se propuso una relación coevolutiva entre el género *Agave* y el género *Leptonycteris*, estableciendo una fecha de divergencia entre los grupos *Leptonycteris* y *Glossopha* de 10.3-16.6 millones de años y una fecha de divergencia entre *L. nivalis* y *L. yerbabuenae* de 6.63 millones de años (Flores-Abreu et al., 2019). Por otra parte, con el auge de las nuevas tecnologías aplicadas a la secuenciación masiva y análisis genómicos, se ha desarrollado una nueva propuesta para inferir los tiempos de divergencia para las especies del género concluyendo que la separación entre las especies *L. nivalis* y *L. yerbabuenae* ocurrió hace 9 millones de años aproximadamente (Gutierrez-Guerrero et al., 2020).

Por otra parte, se han publicado algunos trabajos filogeográficos de la especie *Leptonycteris yerbabuenae* (antes *L. curasoae*) en poblaciones mexicanas (Ramírez, 2011; Morales-Garza *et al*., 2007; Wilkinson y Fleming *et al*., 1996). Estos trabajos han explorado si las colonias principalmente de cuevas en Sonora, Baja California y Jalisco, corresponden a una población o a poblaciones (migrantes) durante el verano y con flujo genético bajo.

Wilkinson y Fleming (1996) analizaron un fragmento de la región control mitocondrial, propusieron dos rutas migratorias, una por la costa del Pacífico y la otra por las faldas del este de la Sierra Madre Occidental (Wilkinson y Fleming, 1996). Por otra parte, Morales-Garza et al., (2007) mencionan que existen dos poblaciones distintas de la especie *L. yerbabuenae* a lo largo de su distribución, una en el centro-sur de México y otra en la costa del Pacífico hacia el norte, en los estados de Baja California y Sonora. Ramírez (2011) realizó un muestreo amplio de varias localidades en Arizona y México (costa del pacífico) con el cual concluyó que *L. yerbabuenae* forma una población panmíctica. Sin embargo, falta un análisis detallado con los datos de las poblaciones del centro y sur del país, lo cual nos permitiría establecer si hay estructura genética en la distribución de *L. yerbabuenae*. En años recientes, se publicaron dos

trabajos filogeográficos, el primero con un enfoque local en la península de Baja California en el cual mencionan nula estructura genética y una probable expansión demográfica reciente (Arteaga et al., 2018) y el otro basado en cuatro localidades mexicanas en la península de Baja California, Sonora, Colima y Chiapas en donde determinaron nula estructura genética y una expansión reciente (Menchaca et al., 2020).

II.2.1 Ecología y distribución

La distribución del murciélago magueyero menor comprende principalmente el territorio mexicano en donde se encuentran las colonias más grandes. Sin embargo, se ha reportado su presencia desde Honduras y Nicaragua (Tapia et al., 2020), hasta el suroeste de los Estados Unidos. *Leptoncyteris yerbabuenae* vive principalmente en hábitats áridos y semiáridos, se encuentra asociado a vegetación de tipo matorral xerófilo, pastizales, bosque espinoso, bosque tropical caducifolio y subcaducifolio y bosque de pino (Arita y Santos del Prado, 1999; Cole y Wilson, 2006).

Leptonycteris yerbabuenae exhibe un comportamiento migratorio conocido como filopatría, en el cual las hembras realizan un movimiento desde el centro sur de México hasta el sur de los Estados Unidos de América (Arizona) por la costa oeste (Frick et al., 2018). Por lo que se ha establecido que la migración de este murciélago sigue un “corredor de néctar”, en el cual, la floración de cactus ofrece las recompensas durante la temporada de migración durante la primavera y durante el otoño los agaves aportan el recurso en el sur de la distribución (Fleming *et al*., 1993). En primavera-verano, *L. yerbabuenae* forma colonias "de maternidad" en el suroeste de Estados Unidos (Arizona) y en Sonora, México. Sin embargo, se han identificado colonias que se mantienen en un lugar de residencia todo el año y cuevas de maternidad que se forman durante el otoño (Rojas et al., 1996; Rojas et al., 1999; Riechers et al., 2003).

En la reserva de Chamela se han reportado dos periodos reproductivos, de diciembre a marzo y de julio a septiembre. Esta información ha contribuido para describir la migración: Ceballos *et al*. (1997) sugieren que los murciélagos pueden aparearse y viajar hacia el norte de su distribución en donde tienen a sus crías, mientras que Stoner *et al*. (2003) plantean que éstos pueden permanecer en la misma localidad para alumbrar. Por otra parte, en el centro y sur del país hay colonias residentes que aumentan sus números demográficos durante el invierno y durante la época de apareamiento (Rojas-Martínez et al., 1999; Galindo et al., 2004). Este murciélago se considera uno de los principales polinizadores de cactáceas columnares y agaves entre otros grupos como bombacoides y algunas leguminosas. Por esta razón, *L. yerbabuenae* ha sido ampliamente estudiado en cuanto a su papel como polinizador

y/o dispersor de semillas de especies clave de los grupos Agavaceae y Cactaceae (Álvarez y González, 1970; Arita y Santos del Prado, 1999; Arizaga *et al*., 2000; Ibarra-Cerdeño *et al*., 2005; Molina y Eguiarte, 2003; Moreno-Valdez *et al*., 2003; Peñalba *et al*., 2003; Rocha *et al*., 2005).

Las dos especies de murciélagos magueyeros norteamericanos, *L. yerbabuenae* y *L. nivalis* son simpátricas en el centro de México. Por lo que Arita (1991) sugirió que existe una diferencia de nichos entre ellas que les permite coexistir, inclusive si se considera que sus requerimientos ecológicos, y particularmente la alimentación, son similares. Así, *L. yerbabuenae* está presente en altitudes menores a 1,800 msnm mientras que *L. nivalis* se encuentra entre los 1,000 y 2, 200 msnm. Además, *L. nivalis* se presenta en zonas donde la temperatura media es de 20°C, a diferencia de *L. yerbabuenae* que se encuentra en zonas con una temperatura media de 25°C. También, se reconocieron diferencias en la distribución de ambas especies de acuerdo al tipo de vegetación, por una parte *L. yerbabuenae* se asocia a vegetación de bosques secos, como bosque tropical caducifolio y bosque espinoso mientras que *L. nivalis* se asocia más comúnmente a las zonas de transición entre los bosques caducifolios y bosques de pino-encino (Arita, 1991).

II.3 Problemática

El murciélago magueyero menor es una especie que ha generado interés en años recientes. Como se mencionó anteriormente, se han desarrollado trabajos enfocados en varios aspectos de su biología, desde aspectos ecológicos (González-Terrazas et al., 2016; Bogan et al., 2017; Medellín et al., 2018; Gaona et al., 2019; Zamora-Mejías et al., 2020), morfológicos (von Busse et al., 2012), fisiológicos (Gaona et al., 2016; Gaona et al., 2020; Walter et al., 2020), comportamiento (Martínez-Coronel et al, 2014), genética de poblaciones (Ramírez, 2011), filogeográficos (Morales-Garza et al., 2007; Arteaga et al., 2018; Menchaca et al., 2020) y hasta la reciente secuenciación de su genoma completo (Gutiérrez-Guerrero et al., 2020).

Si bien es cierto que *L. yerbabuenae* depende de varios grupos de plantas para su alimentación y que estas son abundantes y tienen distribuciones amplias, existe el riesgo de que la perturbación por parte de la especie humana restrinja cada vez más estas fuentes de alimentación, al igual que otros murciélagos (Allen-Wardell et al., 1998; Medellín, 2003; Frick et al., 2018). Por otra parte, las acciones que realizan algunas personas al intentar exterminar a los murciélagos en sus refugios pueden causar graves daños en las poblaciones (Arita, 1993; Arita and Santos del Prado, 1999). El comportamiento migratorio que presenta esta especie es un factor determinante para su reproducción, por lo que es necesario mantener el

estado de conservación de los hábitats que conforman la ruta que siguen las hembras durante el movimiento migratorio (Frick et al., 2018; Burke et al., 2019).

La falta de información entre la sociedad acerca de la importancia y relevancia de los murciélagos magueyeros y la satanización del grupo Chiroptera por ser considerados transmisores de enfermedades, promueven el vandalismo y la destrucción de las cuevas y del hábitat de forrajeo. La gente no es capaz de reconocer y diferenciar entre las especies hematófagas y el resto de los murciélagos y asumen que todos son un problema para sus actividades agropecuarias o para la salud humana misma. La vulnerabilidad de la selva baja o bosque tropical caducifolio, amenazada debido al cambio de uso de suelo, a la deforestación y la fragmentación del hábitat (Ceballos y García, 1995), puede ser otro factor de riesgo para la especie de *L. yerbabuenae* considerando que sus fuentes de alimentación y refugios se encuentran asociados principalmente a estos ecosistemas.

III. Justificación y relevancia del proyecto

El presente trabajo se pretende explorar algunos aspectos de la historia natural de *L. yerbabuenae* basados en herramientas filogeográficas, determinando de qué manera la migración influye en la distribución de haplotipos paternos y maternos. Debido a la clara relación ecológica que se ha mencionado anteriormente entre los murciélagos y distintas especies de agaves y cactus. Estudiar estos aspectos se considera importante para aplicar los conocimientos desarrollados en las políticas y estrategias de conservación de zonas prioritarias para estos organismos, así como la conservación y buen aprovechamiento de las especies económicamente importantes como son las especies tequileras, mezcaleras y pulqueras del género *Agave*. Consideramos importante conocer la distribución de la diversidad genética de la especie a lo largo de su distribución. Esto nos puede ayudar a identificar áreas prioritarias ante el avance de las actividades humanas, la degradación de los ecosistemas y ante posibles escenarios de cambio climático como el calentamiento global. También, es necesario fortalecer las estrategias de mitigación y conservación de zonas, paisajes y corredores que permitan que la especie mantenga niveles demográficos y conectividad genética entre las poblaciones y acceso a las zonas de forrajeo, cuevas de refugio, apareamiento y maternidad para que la especie continúe jugando el rol que actualmente desempeña como polinizador primordial de distintas especies de plantas.

Los resultados que se generen en la presente tesis sirven de modelos para otras especies de murciélagos migratorios, nectarívoros e incluso en otros grupos como aves que llevan a cabo roles ecológicos similares como polinizadores y que, por consiguiente, enfrentan

condiciones similares de desventaja ante las actividades antropogénicas y su dependencia de zonas de conservación.

V. Presentación

Esta tesis presenta una serie de métodos y análisis con un enfoque evolutivo y ecológico para describir parte de la historia natural de la especie *Leptonycteris yerbabuenae*. Basados en inferencias desarrolladas, principalmente, bajo esquemas de modelos probabilísticos se pretende contribuir al conocimiento del murciélago magueyero menor y aplicar dicho conocimiento a planes y programas enfocados a la conservación de este grupo de mamíferos. Espero que este trabajo pueda ser considerado en un futuro próximo en la toma de decisiones acerca de la conservación de la especie, así como las zonas prioritarias en las que su papel ecológico es fundamental para la polinización de especies vegetales, silvestres y cultivadas, y la dispersión de semillas de algunas especies de cactáceas (Rojas-Martínez et al., 2015; Bustamante et al., 2016; García-Ruíz et al., 2018).

En el capítulo 1 se presenta el artículo principal de la tesis titulado: “Discordance in maternal and paternal genetic makers in lesser long-nosed bat, *Leptonycteris yerbabuenae*, a migratory bat: Recent expansion to the North and male philopatry”, en el cual se realizó un trabajo filogeográfico para describir la diversidad haplotípica de los linajes paterno y materno. Además, con la ayuda de estos marcadores se calculó la estructura genética poblacional para determinar si se trata de una metapoblación o si la especie se encuentra distribuida en subgrupos o subpoblaciones en proceso de aislamiento. Por último, con modelos de distribución potencial construidos a partir de información geográfica y capas climáticas se proyectaron los sitios más estables y favorables para la especie. Finalmente, se desarrolló un análisis para describir la demografía histórica de la especie para determinar si había cambios a lo largo de su historia reciente.

El capítulo 2 consiste en la reconstrucción filogenética de la familia Phyllostomidae. En este capítulo, se realizaron análisis con la finalidad de inferir tiempos de divergencia para los géneros y las especies que componen la familia, así como la evolución del hábito alimenticio a través de la nectarivoría y las áreas de posible origen para los grupos. Además, se calcularon las tasas de diversificación para la familia. Los análisis realizados en este capítulo nos permiten ubicar algunos eventos ecológicos y evolutivos relacionados directamente con el género *Leptonycteris* ya que se cuenta con las tres especies que lo componen, y en particular, con la especie objeto de la presente tesis, *Leptonycteris yerbabuenae*. Los tiempos calculados para este trabajo corresponden con propuestas filogenéticas anteriores (Rojas et al, 2016; Flores-Abreu et al, 2019). Además, es posible identificar algunos eventos geológicos,

climáticos y ecológicos de los cuales se tiene registro para los grupos relacionados, particularmente los agaves (Jiménez-Barron et al., 2020).

En el capítulo 3, se aborda la relación ecológica y evolutiva que mantienen las especies del género *Leptonycteris* que habitan en el territorio mexicano con el grupo *Agave* a través de herramientas biogeográficas. Se cuenta con evidencia de que varias especies de la familia Phyllostomidae han desarrollado una relación ecológica y evolutiva con varias especies de agave (Flores-Abreu et al., 2019). En particular, el murciélago magueyero menor es protagonista de esta relación debido a sus números demográficos (Medellín et al., 2018), distribución) y los movimientos migratorios por parte de las hembras (Burke et al., 2019). Hasta la fecha se ha demostrado el papel como polinizador efectivo en las poblaciones de algunos agaves con los cuales comparte zonas de distribución (Álvarez and González-Quintero 1970; Easterla 1972; Howell, 1979; Howell and Hart 1980; Howell and Roth 1981; Arizaga *et al.* 2000; Silva-Montellano and Eguiarte 2003; Molina-Freaner and Eguiarte 2003; Scott, 2004; Rocha *et al*. 2005; Sánchez and Medellín 2007; Trejo et al. 2015).

Finalmente, el capítulo 4 refleja una propuesta de conservación que bien podría ser incorporada en un futuro como parte del manejo de especies de agave cultivadas, aprovechando el papel cultural y la importancia económica de este grupo en México (Trejo et al., 2016), ayudando por otro lado a la conservación de la especie *L. yerbabuena*, así como su especie hermana *L. nivalis* y afines como *Choeronycteris mexicana* y especies mexicanas del género *Glossophaga*. También, se favorecería a aves e insectos locales que pueden obtener recurso de las mismas plantas. La idea de esta propuesta gira en torno de la conservación de un porcentaje mínimo de inflorescencias de plantas que pertenezcan a cultivos destinados a la producción de tequila y mezcal, y más adelante podrían adherirse bacanora y pulque.

V. Objetivo

General

Describir la historia evolutiva y estructura genética poblacional de la especie de murciélago nectarívoro *Leptonycteris yerbabuenae* con base en los linajes paterno y materno, aportando datos que puedan generar interés para mejorar los planes y programas de conservación de este murciélago.

Particulares

- Describir la diversidad genética de la especie *L. yerbabuenae* basados en marcadores mitocondriales y asociados a cromosoma Y.
- Estimar la estructura genética de las poblaciones de *L. yerbabuenae.*
- Describir la demografía histórica de la especie con base en marcadores moleculares asociados a los linajes paterno y materno.
- Determinar los tiempos de divergencia del género y datar de origen de la especie.
- Generar modelos de distribución potencial en el presente y proyectarlos hacia el pasado.
- Proponer prácticas de conservación con base en la relación de interacción ecológica-evolutiva de la especie del murciélago magueyero menor y el grupo *Agave*.

Capítulo 1.

PeerJ

Discordance in maternal and paternal genetic markers in lesser long-nosed bat *Leptonycteris yerbabuenae*, a migratory bat: recent expansion to the North and male phylopatry

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ABSTRACT

Leptonycteris yerbabuenae, the lesser long-nosed bat is an abundant migratory nectar-feeding bat found in most of Mexico, and in some areas of northern Central America and small sections of southwestern USA. We analyzed the distribution of the maternal and paternal lineages of this species with phylogeographic methods based on two mitochondrial markers, *Cyt-b* and *D-loop*, and a marker located in the Y chromosome, *DBY*. We obtained tissue samples from 220 individuals from 23 localities. Levels of genetic diversity (haplotype diversity, H_d) were high (*Cyt-b* = 0.757; *D-loop* = 0.8082; *DBY* = 0.9137). No clear patterns of population genetic structure were found for mitochondrial markers, while male genetic differentiation suggested the presence of two lineages: one from Mexican Pacific coast states and another from central-southern Mexico; in accordance to strong male philopatry and higher female migration. We used genealogical reconstructions based on Bayesian tools to calculate divergence times, and to test coalescent models to explain changes in *L. yerbabuenae* historical demography. Our results show that recent demographic changes were consistent with global climatic changes (~130,000 kyr ago for *Cyt-b* and ~160,000 kyr for *D-loop*) and divergence times dated from molecular genealogies exhibited older divergence times, *Cyt-b* (4.03 mya), *D-loop* (10.26 mya) and *DBY* (12.23 mya). Accordingly, the female lineage underwent demographic expansion associated to Pleistocene climate change, whereas the male lineage remained constant.

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INTRODUCTION

Climate oscillations have global and local effects on distribution, demographic and genetic diversity patterns (*Bloom et al., 2013*; *Ramírez-Barahona & Eguiarte, 2013*; *Castellanos-Morales et al., 2016*; *Scheinvar et al., 2017*; *Hipp et al., 2018*). In particular, Mexico experienced several geological and climatic changes in the recent past (Pleistocene), which have promoted speciation, extinction and diversification of its flora (*Ramírez-Barahona & Eguiarte, 2013*; *Scheinvar et al., 2017*; *Hipp et al., 2018*) and fauna (*Gutiérrez-García & Vázquez-Domínguez, 2013*; *Trejo-Salazar, 2013*; *Castellanos-Morales et al., 2016*). These phylogeographic and biogeographic analyses have shown that genetic patterns of Mexican taxa have been mediated by ecological, geological and climate changes. Aspects of the natural history of species, such as annual migration, are seldom considered in the analysis of species' ecological and evolutionary history because of its complexity, but potentially can be very important. For instance, migration can affect genetic structure, especially in cases were each sex exhibits different migration patterns during the reproductive season (*Russell, Medellín & McCracken, 2005*).

The lesser long-nosed bat, *Leptonycteris yerbabuenae*, (Glossophaginae) is considered the most widespread nectar-feeding bat in Mexico, pollinating at least 64 plant species, including many *Agave* species, columnar cacti and Bombacoideae trees (*Rojas-Martínez et al., 1999*; *Fleming, Geiselman & Kress, 2009*; *Trejo-Salazar, Scheinvar & Eguiarte, 2015*; *Bustamante et al., 2016*; *Jiménez-Barrón et al., 2020*). Distribution of *L. yerbabuenae* comprises mainly arid and semiarid areas (*Cole & Wilson, 2006*) from Nicaragua to Arizona, including most of Mexico, except for the Yucatan peninsula and north of Baja California, there are no records of its presence in the coast of the Gulf of Mexico and in the northern area of the Chihuahuan desert (*Cole & Wilson, 2006*; *Tapia, Namendy & Martínez-Fonseca, 2020*). It is listed as Near Threatened with decreasing population trends (*IUCN Red List of Threatened Species, 2017*), but was delisted in Mexico since the populations have recovered (*Medellín et al., 2018*).

The lesser-long nosed bat is a migratory species (*Cole & Wilson, 2006*). The migration involves only female groups that travel north from central and southern Mexico during spring-summer to give birth (Arizona and Sonoran Desert), and they travel back south in autumn (*Wilkinson & Fleming, 1996*; *Ceballos et al., 1997*; *Rojas-Martínez et al., 1999*; *Medellín et al., 2018*). This migration is associated with the blooming period of cactus during summer at the northern area (*Fleming, Nuñez & Sternberg, 1993*; *Molina-Freaner & Eguiarte, 2003*; *Burke et al., 2019*) and of agaves during the journey (*Burke et al., 2019*). In addition, all the males and some females remain in permanent roost caves in the center-south of its distribution area year-round (*Rojas-Martínez et al., 1999*; *Riechers, Martínez-Coronel & Vidal, 2003*; *Stoner et al., 2003*; *Galindo et al., 2004*). This raises questions regarding the proportion of the female population that migrates, the magnitude of the sex ratio bias in permanent locations at given times due to female migration, and the identity and steadiness of migrant females. Also, we could expect to find differences in the population genetic structure of locations where the species is present throughout the year.

Previous genetic studies based on mitochondrial, microsatellites and Random Amplified Polymorphic DNA markers (RAPDs) have concluded that there are different genetic groups along the species distribution (*Wilkinson & Fleming, 1996*; *Morales-Garza et al., 2007*). *Wilkinson & Fleming (1996)* proposed a coastal and an inland group, in accordance with migration routes, supported by *Rojas-Martínez et al. (1999)* and *Menchaca et al. (2020)*. However, these studies did not analyze resident groups from permanent roosting caves. *Morales-Garza et al. (2007)* found that the species is composed by at least a "center-south" and a "west-north" group; but their samples only covered six sites (Baja California, Sonora, Hidalgo, Morelos, Puebla and Oaxaca); while *Arteaga et al. (2018)* reported a demographic expansion in Baja California. These studies provide evidence for the existence of at least two genetic groups, and here we analyze if these genetic groups could relate to differences in migration behavior between males and females in *L. yerbabuenae*.

In addition, the distribution of *L. yerbabuena* encompasses several biogeographic regions (Fig. 1), that have influenced the distribution of genetic variation in some bat species. For example, the Isthmus of Tehuantepec is a genetic barrier for *Pteronotus davyi* (*Guevara-Chumacero et al., 2010*), *P. personatus* (*Zárate-Martínez et al., 2018*) and *Natalus mexicanus* (*López-Wilchis et al., 2021*), while *Artibeus jamaicensis* show the presence of two lineages: Gulf of Mexico and Pacific Ocean (*Ruiz, Vargas-Miranda & Zúñiga, 2013*). The region around the Balsas river is also an important barrier for species such as *Sturnira parvidens* (*Hernández-Canchola & León-Paniagua, 2017*). In these studies, geographical barriers together with Pleistocene climate changes promoted lineage divergence followed by range expansion (*Guevara-Chumacero et al., 2010*; *Ruiz, Vargas-Miranda & Zúñiga, 2013*; *Hernández-Canchola & León-Paniagua, 2017*; *Zárate-Martínez et al., 2018*; *López-Wilchis et al., 2021*).

In the present study, we analyzed paternally and maternally inherited molecular markers to uncover the effect of sex-bias in migration patterns on the distribution of genetic diversity in *L. yerbabuenae*. We conducted a phylogeographic analysis with a sampling covering the species distribution in Mexico, and test whether climatic changes have influenced its demographic dynamics. We hypothesize that past global climate change had a differential effect in the genetic diversity, phylogeographic patterns and demographic history of female and male lineages of *L. yerbabuenae*, and migratory movements could be the result of a geographic expansion related to changes that represent better climatic and ecological conditions for this species in the present.

MATERIALS & METHODS

Sampling

Samples were taken from 23 localities in roosting caves and mist nest on field feeding-sites along *L. yerbabuenae* distribution from 2014 to 2016 (Fig. 2), with sampling permit Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT) SGPA/DGVS/07161/15 (Supplemental File S1) following the Animal Care and Use protocols of the American Society of Mammalogists (*Sikes, 2016*). Tissue samples were taken with a three mm^2 biopsy wing punch in an area of the wing with no blood capillaries or nerve terminals. Wing

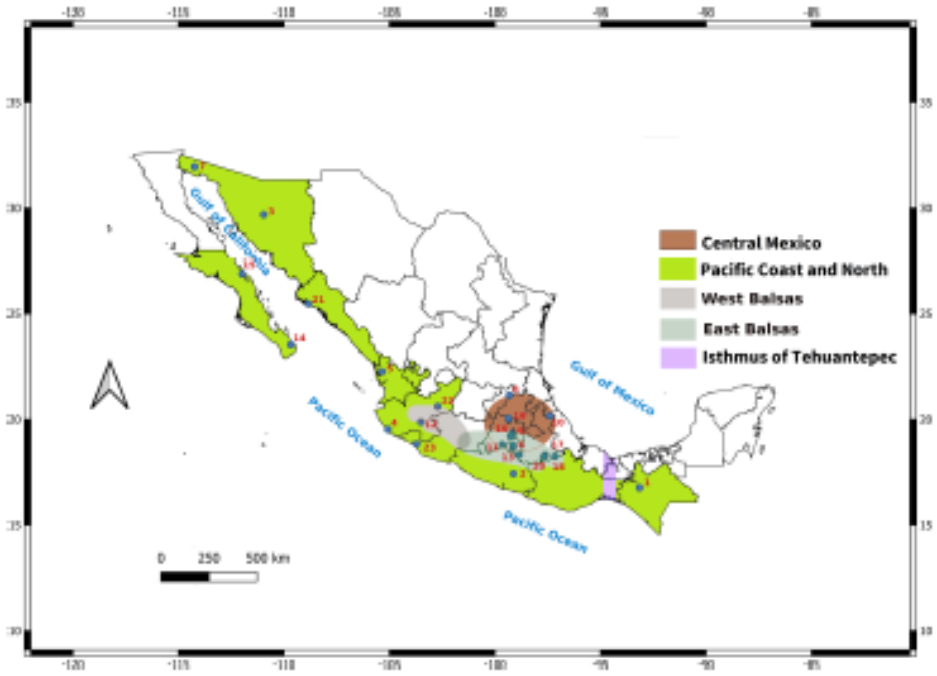

Figure 1 Map depicting sampled localities showing the Balsas River Basin and Isthmus of Tehuantepec. The Balsas River divides the Central Mexico-East Balsas and West Balsas-Pacific Coast groups proposed by *Morales-Garza et al. (2007)*.

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biopsies were fixed in 90% ethanol at environmental temperature and then stored at −20 °C until DNA extraction. Additionally, we used tissue samples from wing punches collected from 2001 to 2003 (*Morales-Garza et al., 2007*) and tissue samples from wing punches from the tissue collection of the Laboratorio de Ecología y Conservación de Vertebrados Terrestres (LECVT), Instituto de Ecología, UNAM (Table 1).

DNA extraction and amplification

Total genomic DNA was extracted following Paboö's modified protocol (*Gasca Pineda, 2015*). Tissue was digested for 12 h at 40 °C in Paboö lysis solution (100 mM NaCl, 100mM Tris HCl and 2mM EDTA, pH8.0) with 20 mg/ml proteinase K, 2% SDS and 0.04M DTT, followed by a phenol: chloroform protocol for DNA isolation (*Gasca Pineda, 2015*). The quality and amount of extracted DNA was visualized in a 1% agarose gel. We performed gel electrophoresis at 90 V for 30 min; gel was stained with Midori green advance solution and visualized in UV light. We sequenced two mitochondrial DNA regions, cytochrome b (*Cyt-b*) and control region (*D-loop*), which are maternally inherited and variable in most mammal species. We also sequenced the region of Dead box associated to the Y chromosome gene (*DBY*) which is located in the Y chromosome and is paternally inherited, this marker has been used before for phylogeographic studies in phyllostomid bats (*Clare et al., 2011*).

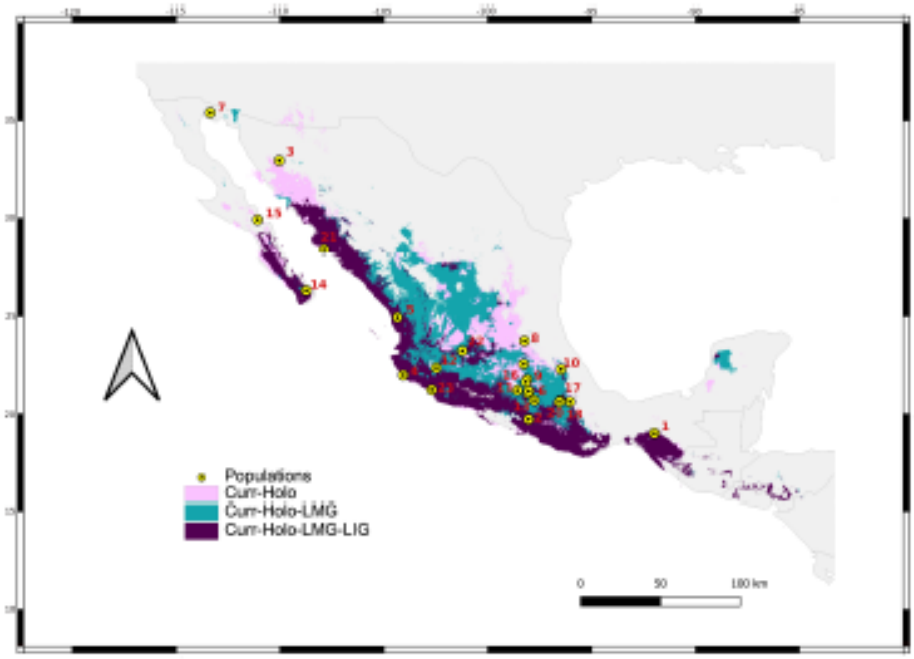

Figure 2 **Sum of maps.** Map depicting species distribution models past projections highlighting areas of environmental stability into the current-Holocene (pink), current-Holocene-Last Glacial Maximum (blue), and current-Holocene-Last Glacial Maximum-Last Interglacial (purple). Yellow points show sampled localities.

Full-size DOI: 10.7717/peerj.12168/fig-2

We obtained 1121 bp of *Cyt-b* with primers L14125 5′TGAAAAAYCATCGTTGT 3′and H15915 5′TCTTCATTTYWGGTTTACAAGAC 3′(*Steppan et al., 1999*). For the *D-loop* region , we amplified 828 bp with primers L15933 5′-CTCTGGTCTTGTAAACCAAAAATG-3′and H637 5′-AGGACCAAACCTTTGTGTTTATG-3′(*Oshida et al., 2001*). PCR for mitochondrial markers were performed in a final total reaction volume of 15 µl, and contained 2 µl of DNA, 2 U of Taq polymerase (GoTaq Flexi DNA Polymerasa, Promega, USA), 0.4 µM of each primer (10 µM), 1x Taq buffer, 2.5 µM of $MgCl_2$ (25 µM), 0.2 µM of dNTPs (10 µM) and 7.325µl of H_2O. The PCR profile for *Cyt-b* was: 5 min of initial denaturation at 95 °C, followed by 35 cycles of 30 s at 96 °C, 1 min at 53 °C, 2 min at 72 °C, and a final extension of 7 min at 72 °C; and for *D-loop*: 3 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 45 s at 54 °C, 2 min of 72 °C, and a final extension of 10 min at 72 °C in an ABI Veriti 96-Well Thermal Cycler (Model: 9902; Thermo Fisher Scientific Inc.).

We amplified a 450 bp fragment from the Dead box associated to the Y chromosome gene (*DBY*) with primers 5′-CCGTTACTTCCATTTTCAAAA-3′and 5′-GCTAAAACCAACGAGATTGGT-3′(*Lim, 2007*; *Lim et al., 2008*); the reaction mixture of 15µl total volume contained 2µl of genomic DNA, 2 µM of each primer (10 µM), 200 µM dNTPs (10 µM), 1.5 mM $MgCl_2$ (25 µM), 2.5 U of Taq DNA polymerase (Promega) and

Table 1 Number of male and female samples for *Leptonycteris yerbabuenae* sequenced for *Cytb*, *D-loop* and *DBY* regions (only males) for each Locality and State. Locality ID key is shown Next to locality.

		Cyt-b			*D-loop*			*DBY*	Geographic coordinates	
Locality		Male	Female	Total	Male	Female	Total	*n*	Lat	Long
1 Los Laguitos, Chiapas	Chi	10	1	**11**	1	10	**11**	1	−93.15098	16.76512
2 Juxtlahuaca, Guerrero	Jux	3	21	**24**	3	22	**25**	9	−99.12634	17.42676
3 La Mariana, Sonora	Son	6	12	**18**	2	14	**16**	1	−110.9587	29.6864
4 Chamela, Jalisco	Chame	11	4	**15**	1	0	**1**	19	−105.0738	19.52677
5 Las Lumbres, Nayarit*	Nay	0	1	**1**	0	0	**0**	8	−105.3465	22.25478
6 Ticuman, Morelos*	Tic	0	10	**10**	0	7	**7**	0	−99.14083	18.77555
7 El Pinacate, Sonora	Pin	0	20	**20**	0	18	**18**	0	−114.2341	31.94816
8 Xoxafi, Hidalgo*	Xox	1	0	**1**	0	1	**1**	2	−99.319	21.137
9 Las Vegas, Puebla*	vegas	0	5	**5**	0	0	**0**	0	−97.40263	20.16594
10 Tzinacanostoc, Puebla*	Tzni	0	1	**1**	0	0	**0**	0	−98.85219	18.32304
11 Tonatico, Estado de México	Ton	2	2	**4**	0	0	**0**	2	−99.65836	18.80343
12 Atotonilco, Jalisco	Ato	4	3	**7**	4	2	**6**	10	−102.7132	20.61148
13 Tetecalita, Morelos (Salitre)	Sal	16	10	**26**	12	8	**20**	21	−99.15740	18.65918
14 Baja California (Las Cuevas)	BC1	2	7	**9**	1	3	**4**	6	−109.6773	23.532
15 Baja California (Mulege)	BC2	8	1	**9**	0	0	**0**	6	−111.9868	26.8785
16 Ciudad de México	DF	2	9	**11**	9	0	**9**	9	−99.19241	19.32163
17 San Juan Raya, Oaxaca	SJR	23	9	**32**	21	6	**27**	16	−97.62269	18.31159
18 San Sebastian Frontera, Oax	SSF	4	1	**5**	3	0	**3**	0	−97.65934	18.24915
19 Tula, Hidalgo	Tul	1	2	**3**	3	0	**3**	1	−99.35589	20.04357
20 Coxcatlán, Oaxaca	Cox	0	1	**1**	0	1	**1**	0	−97.16104	18.25364
21 Navachiste, Sinaloa*	Sin	0	0	**0**	0	0	**0**	1	−108.8382	25.46586
22 Tuxtepec, Jalisco*	Tux	0	0	**0**	0	0	**0**	11	−103.5061	19.8661
23 Callejones, Colima*	Col	0	0	**0**	0	0	**0**	7	−103.7177	18.82904
				213			**152**	**130**		

Notes.
*Samples from LECTV historical tissue collection.

7.7µl of H_2O. Amplification was carried out as follows: 10 min of an initial denaturation at 94 °C, 36 cycles of 45 s at 94 °C, 30 s at 54 °C, 2:30 min at 72 °C, and a final extension of 5 min at 72 °C in an ABI Veriti 96-Well Thermal Cycler (Model: 9902; Thermo Fisher Scientific Inc.). We sequenced each genetic region with forward and reverse primers at Macrogen USA's Maryland headquarters (http://www.macrogenusa.com).

Differences in the final sample number among markers resulted from PCR artifacts, *Cyt-b* was successfully amplified, while for *D-loop* sequences were low quality for several individuals and were discarded from analyses. We amplified a total of 213 individuals for *Cyt-b*, 152 for *D-loop* and 130 for *DBY* (Table 1; Supplemental File S2). All sequences are available in NCBI GenBank (accession number: *Dloop*: MT790834–MT790986; *Cytb*: MT859334–MT859403; *DBY*: MT913638–MT913767).

Data analysis

Genetic diversity

We assessed the quality of DNA sequences and assembled forward and reverse sequences with Consed 29.0 using the default settings (*Ewing et al., 1998*; *Gordon, 2004*). We aligned sequences with CLUSTAL X (*Thompson et al., 1998*), and checked the alignment by hand. Missing data or undetermined bases were excluded from analyses, and we eliminated sequences with more than 50% of missing data.

For each marker, we estimated the number of segregating sites (S), number of haplotypes (h), haplotype diversity (H_d) and nucleotide diversity (π) for each sampled locality using DNAsp 5.10.01 (*Librado & Rozas, 2009*) and Arlequin 3.5.1.2 (*Excoffier & Lischer, 2010*).

Population genetic structure

To visualize the genealogical relationship among haplotypes, we obtained a haplotype network for each molecular marker. Haplotype networks were constructed with the median-joining algorithm (*Bandelt, Forster & Röhl, 1999*) implemented in the program PopART 1.7 (*Leigh & Bryant, 2015a*; *Leigh & Bryant, 2015b*).

To assess genetic differentiation among populations, we calculated pairwise F_{ST} values using the Slatkin method (*Slatkin & Hudson, 1991*) implemented in Arlequin 3.5.1.2 (*Excoffier & Lischer, 2010*). We also conducted a Bayesian analysis of population structure, BAPS 6 (*Corander, Sirén & Arjas, 2008*). BAPS 6 uses maximum likelihood and MCMC under the non-reversible Metropolis-Hasting method to assign individuals to different genetic groups, where the number of groups is given under the assumption of admixia (*Corander, Sirén & Arjas, 2008*). Moreover, BAPS 6 can incorporate geographical coordinates and perform Voronoi's tessellation to explicitly test for spatial genetic structure (*Corander, Sirén & Arjas, 2008*). To test whether we could recover a Pacific-South and Central Mexico groups, following *Morales-Garza et al. (2007)*, we conducted the BAPS 6 analysis without including the geographical information and using a different fixed value of K = 2, 3, 4 and 5 and 10 repetitions for each value. To evaluate the partitioning of genetic variation among sampled localities, we performed an analysis of molecular variance (AMOVA) with poppr (*Kamvar, Tabima & Grünwald, 2014*) in R 1.4.1106 (*R Core Team, 2013*). All samples were treated as a single group to determine the amount of variation partitioned among and within the localities. To assess the significance of the two geographic groups previously analyzed with BAPS 6, we conducted an additional AMOVA. We obtained the level of significance for both tests with 10,000 permutations.

Historical analyses

Divergence times

For divergence time estimations, we used the haplotype identities for each gene (*Cyt-b* ($n = 76$ haplotype sequences), *D-loop* ($n = 43$) and *DBY* ($n = 70$). We conducted separate analyses for each gene, because each region shows a different mutation rate; thus, allowing to make inferences at different time scales and for each sex. We determined the substitution model with best fit to our data with jModelTest 2 (*Posada, 2008*) based on the Akaike Information Criterion (AIC; *Akaike, 1974*). *Cyt-b* and *D-loop* followed a GTR+G+I model with γ-distributed rate heterogeneity, while *DBY* followed a GTR+G substitutions model.

For each region, we obtained an ultrametric tree and estimated divergence times under a relaxed uncorrelated lognormal clock model with BEAST 1.10.4 (*Suchard et al., 2018*), which allows rates to vary among branches. We downloaded from GenBank sequences for each region to be used as outgroups; nevertheless, we had to use different species as outgroups for each analysis in accordance with the available data. For *Cyt-b* we considered *Glossophaga commisarissi* (GenBank accession number: AF382886; *Hoffmann & Baker, 2001*) and *Choeronycteris mexicana* (*Flores-Abreu et al., 2019*) as outgroups. For *D-loop*, outgroups were *Hylonycteris underwoodii* (GenBank accession number: MF804191.1; *Cruz-Salazar et al., 2018*) and *Glossophaga longirostris* (GenBank accession number: AF510544.1; *Newton, Nassar & Fleming, 2003*). Outgroups for the *DBY* genealogy were *Glossophaga soricina*, *Uroderma bilobatum* and *Platyrrhinus helleri* (GenBank accession numbers: JF458413.1, JF458602.1 and JF458470.1, respectively; *Clare et al., 2011*). Genealogies were calibrated using the same four dates. Two calibration points came from the fossil record: glossophaginae 22.8 million years ago (mya) (1.5 Standard Deviation, SD) (*Teeling, 2005*); Choeonycterinii 13 mya (1.0 SD) (*Czaplewski et al., 2003*); Calibration points for *Glossophaga* + *Leptonycteris* clade15 mya (1.0 SD), and *Leptonycteris* 12 mya (1.0 SD) were derived from detailed Bayesian analysis previously reported (*Flores-Abreu et al., 2019*). Priors for BEAST 1.10.4 were set with the default values, running for 500 million generations sampling every 1000 generations, and a 10% burn in. We used Tracer 1.7.1 (*Rambaut et al., 2018*) to evaluate convergence and stationarity of 10,000 trees. The maximum credibility tree was obtained with TreeAnnotator 1.10.4 (*Suchard et al., 2018*) and visualized with FigTree 1.4 (*Rambaut & Drummond, 2007*).

Historical demography analysis

To estimate the demographic dynamics of *L. yerbabuenae* through time we constructed Bayesian skyline plots with BEAST 1.10.4 (*Suchard et al., 2018*). We calculated coalescence times for each locus separately considering all individuals (*Cyt-b* $n = 213$, *D-loop* $n = 156$ and *DBY* $n = 130$) using GTR+G+I in a Piecewise-linear nucleotide substitution model (*Suchard et al., 2018*).

Genealogies and model parameters for each lineage were sampled every 50,000 iterations for 5×10^8 generations under a relaxed lognormal molecular clock with uniformly distributed priors and a pre-burn in of 1000. Demographic plots for each analysis were visualized with Tracer 1.7.1 (*Rambaut et al., 2018*). To scale the time of the Bayesian coalescence of the Skyline (evolutionary time between real time), we used the last divergence time of the branches in the calibrated tree. We also calculated Tajima's *D* (*Tajima, 1989*) and Fu's *Fs* (*Fu, 1997*) with a coalescent algorithm in DNAsp 5.10.01 (*Librado & Rozas, 2009*), as an independent test for demographic inferences and to provide further support to skyline plot results.

Past distribution models

We obtained coordinates for the presence of *L. yerbabuenae* from scientific collections (Colección Nacional de Mamíferos (Instituto de Biología, UNAM), Museo de Zoología "Alfonso L. Herrera" (Facultad de Ciencias, UNAM), Colección de Mamíferos UAM-I (Universidad Autónoma Metropolitana), Colección Mastozoológica ENCB (Instituto

Politécnico Nacional)) and GBIF (*GBIF.org, 2014*) databases and own collections (Supplemental File S3). To avoid spatial autocorrelation and bias in the distribution model estimation, we eliminated duplicate records by pixel and records separated by less than 1 km. An additional validation was carried out using information on habitat, distribution, and taxonomic status.

We used 19 bioclimatic variables (WorldClim, http://www.worldclim.org) to perform a variance inflation factor analysis to eliminate correlated bioclimatic variables, leaving a set of uncorrelated informative variables to be used in environmental niche modeling, and to obtain Mahalanobis distances. The later was used along with occurrence data to identify outlier records that could be a source of error. These points were removed from further analyses. All analyses were performed with R 1.4.1106 (*R Core Team, 2013*).

Climatic Niche Models were constructed with MaxEnt 3.3 (*Phillips & Dudík, 2008*), using the *ad-hoc* selection of variables to reduce overprojection (*Hijmans et al., 2005*). We executed MaxEnt 3.3 using the following settings: 20% random test, 30 bootstrap replicates, 1000 maximum iterations, convergence threshold of 0.00001, with extrapolation and clamping turned off. For the purposes of this study, we derived the distributional model for *L. yerbabuenae* from the average model. We evaluated all the distributional models using the area under the receiver operating characteristic curve (AUC) scores, where values above 0.85 are considered useful (*Elith et al., 2006*).

Climatic niche models (CNM) for *L. yerbabuenae* were obtained with MaxEnt 3.3 (*Phillips, Dudík & Schapire, 2004*; *Phillips, Anderson & Schapire, 2006*; *Phillips & Dudík, 2008*) for four periods: Current, Holocene (Hol ~6,000 years ago), Last Glacial Maximum (LGM ~21,000 years ago) and Last Interglacial (LIG ~120,000–140,000 years ago). We loaded the corresponding past layers of Atmospheric-Ocean General Climate Model (AOGCM), obtained from WorldClim 1.4 dataset (*Hijmans et al., 2005*). The data of these layers were based on the AOGCM of the Community Climate System Model (CCSM; *Collins et al., 2006*). A binary map was created using the percentile value of training sample points as a threshold, assuming that 10% of records used for model generation are susceptible to error. Finally, we built a sum of maps that reflects the most stable climatic zones through these four time periods.

RESULTS

Genetic diversity

We analyzed a total of 250 individuals from 23 sites across Mexico (females = 120, males = 130) (Table 1; Fig. 1). We obtained a total of 1,128 bp for *Cyt-b* ($n = 213$; 76 haplotypes); 828 bp for *D-loop* ($n = 152$; 43 haplotypes) and 450 bp for *DBY* ($n = 130$; 70 haplotypes). Genetic diversity (H_d) values were lower for maternally inherited mitochondrial regions (*Cyt-b* = 0.757; *D-loop* = 0.8082) than for paternally inherited *DBY* (0.91; Table 2), a similar pattern was observed for nucleotide diversity (Table 2).

Genetic structure

The haplotype networks (Fig. 3) exhibited differences among them. Mitochondrial *Cyt-b* and *D-loop* networks showed a main group with most haplotypes from all localities

Table 2 Values of genetic diversity for mitochondrial DNA *Cyt-b* and *D-loop* and for the Y-chromosome *DBY* gene showing total number of amplified individuals (n), number of segregating sites (S), haplotype number (h), haplotype diversity (Hd), and nucleotide diversity (π).

	Sample size (n)	Segregating sites (S)	Number of haplotypes (h)	Haplotype diversity (H_d)	Nucleotide diversity (π)
Cyt-b	213	182	74	0.757	0.03087
D-loop	152	327	43	0.8082	0.04289
DBY	130	178	70	0.9137	0.04781

connected, and smaller groups consisting mainly of members from the Peninsula of Baja California-West of Balsas localities (Figs. 3A–3B). The *DBY* haplotype network consisted of three-star groups with geographic congruence (West of Balsas, Central Mexico and Isthmus of Tehuantepec) connected by several mutational steps (Fig. 3C).

Pairwise F_{ST} showed low genetic differentiation between localities for the mitochondrial markers (Tables S1–S2) but higher genetic differentiation for *DBY* (Table S3). For *Cyt-b* and *D-loop*, the results suggest that locations from the West of Balsas region are moderately differentiated from East of Balsas, with values below $F_{ST} = 0.4$. In contrast, the paternal lineage (*DBY* marker) exhibited a well-defined genetic structure between the Isthmus of Tehuantepec-East of Balsas and West of Balsas (Pacific Coast) with values ranging from 0.6 to 0.98.

Bayesian population analysis (BAPS) for *Cyt-b* (log likelihood = −10512.121) and *D-loop* (log likelihood = −10990.658) detected two genetic clusters ($K = 2$; Fig. S1), consistent with East of Balsas *vs.* West of Balsas (or Pacific Coast) groups. In the case of the *DBY* gen, BAPS detected three different genetic groups, congruent with the geographic distribution of sampled localities ($K = 3$; log likelihood = −6397.346; S2. Fig. S1), corresponding with West of Balsas, Central Mexico, and Isthmus of Tehuantepec zones (Fig. 1).

AMOVA analyses revealed that variance was better explained without considering a hierarchical grouping for both mtDNA regions, while for *DBY* clustering provided a slightly better explanation of genetic variance (Table 3). In all cases, most of the genetic variation was found within-localities (55.2% for *Cyt-b*, 75.7% for *D-loop* and 66.6% for *DBY*) (Table 3).

Historical analyses

Divergence times

The Bayesian genealogy for *Cyt-b* (Fig. 4) and *D-loop* (Fig. 5) show that *L. yerbabuenae* is a monophyletic clade consisting of several lineages. The chronogram of the most probable tree constructed with mitochondrial *Cyt-b* (Fig. 4) indicated that *L. yerbabuenae* originated 4.03 mya (95% HDP, 2.27–8.63 mya). In contrast, for mitochondrial *D-loop* (Fig. 5), we obtained a deeper date of 10.26 mya (95% HDP, 8.73–11.82 mya). The chronogram based on chromosome Y *DBY* gene (Fig. 6) suggests a similar (but not overlapping, according to the 95% HDPs) date for the origin of *L. yerbabuenae*, 12.23 mya (95% HDP, 9.99–13.78 mya).

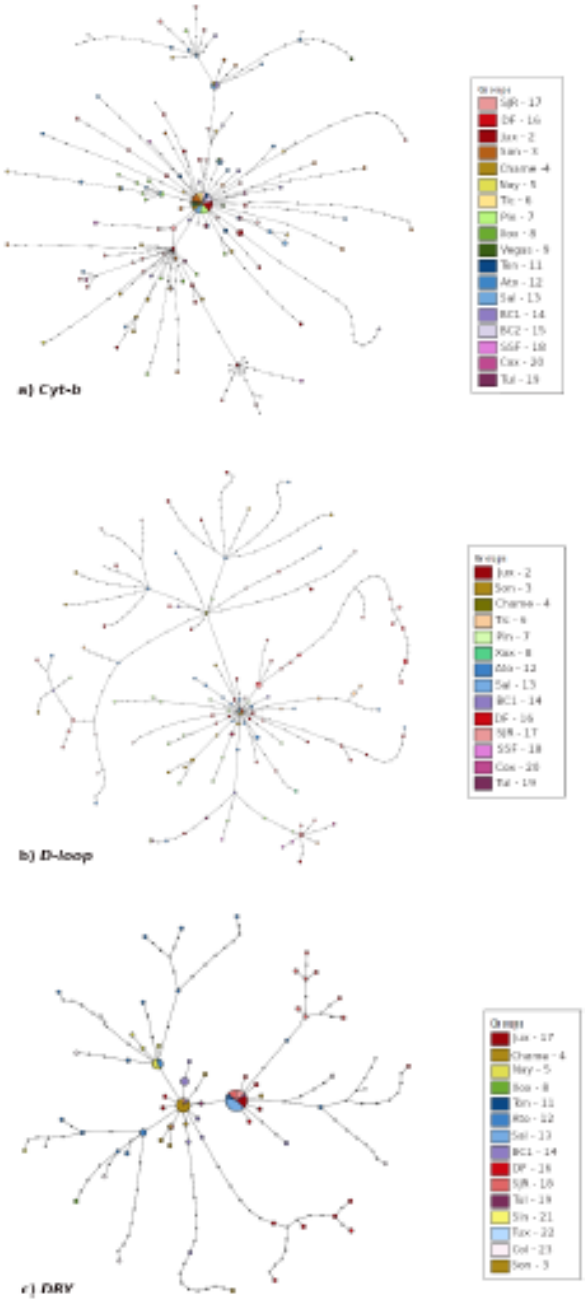

Figure 3 **Median joining haplotype network built based on (A) *Cyt-b*, (B) *D-loop*, and (C) *DBY* markers for *Leptonycteris yerbabuenae*.**

Full-size DOI: 10.7717/peerj.12168/fig-3

Table 3 Results from analysis of molecular variance (AMOVA) for samples from *Leptonycteris yerbabuenae* considering: (A) All populations as a single cluster and (B) two geographic regions of the Balsas River Basin as proposed by Morales-Garza et al.,

Analysis	Source of variation	df	percentage of variation	*F*-statistic
Cyt-b				
A) All populations	Among populations	21	44.74	
	Within populations	191	55.26	$F_{ST} = 0.447$
	Total	212		
B) Clusters				
	Among Cluster	21	21.32	
	Within clusters	13	16.25	
	Within samples	178	50.33	$F_{CT} = 0.334$
				$F_{SC} = 0.244$
	Total	212		$F_{ST} = 0.497$
D-loop				
A) All populations	Among populations	14	24.25	
	Within populations	137	75.75	$F_{ST} = 0.242$
	Total	151		
B) Clusters				
	Among Cluster	1	5.31	
	Within clusters	2	5.71	
	Within samples	148	88.98	$F_{CT} = 0.11$
				$F_{SC} = 0.06$
	Total	151		$F_{ST} = 0.053$
DBY				
A) All populations	Among populations	20	33.37	
	Within populations	104	66.63	$F_{ST} = 0.334$
	Total	124		
B) Clusters				
	Among Cluster	1	9.96	
	Within clusters	123	90.04	$F_{CT} = 0.344$
				$F_{SC} = 0.321$
	Total	124		$F_{ST} = 0.345$

Regarding the geographical distribution of maternal lineages, for *Cyt-b*, individuals from SJR, DF and SSF are paraphyletic and show long branches denoting a possible bottleneck, and the rest of the individuals form a monophyletic clade with short branches denoting a possible population expansion (Fig. 4). For *D-loop*, there is no clear geographic structure, but we can also see that some groups have longer branches suggesting that different lineages might have undergone different demographic trajectories (Fig. 5). For the paternal lineages, the genealogy for the *DBY* gene shows several groups with a few geographical correlations among haplotypes (Fig. 6). The main group contains haplotypes from States of Pacific coast of Mexico and some members from Oaxaca and Morelos (3.06 mya; West Balsas, Pacific Coast and Northern States, Fig. 6). Regarding individuals from Central Mexico, we can see that most individuals from DF form a monophyletic clade with long branches (3.25 mya;

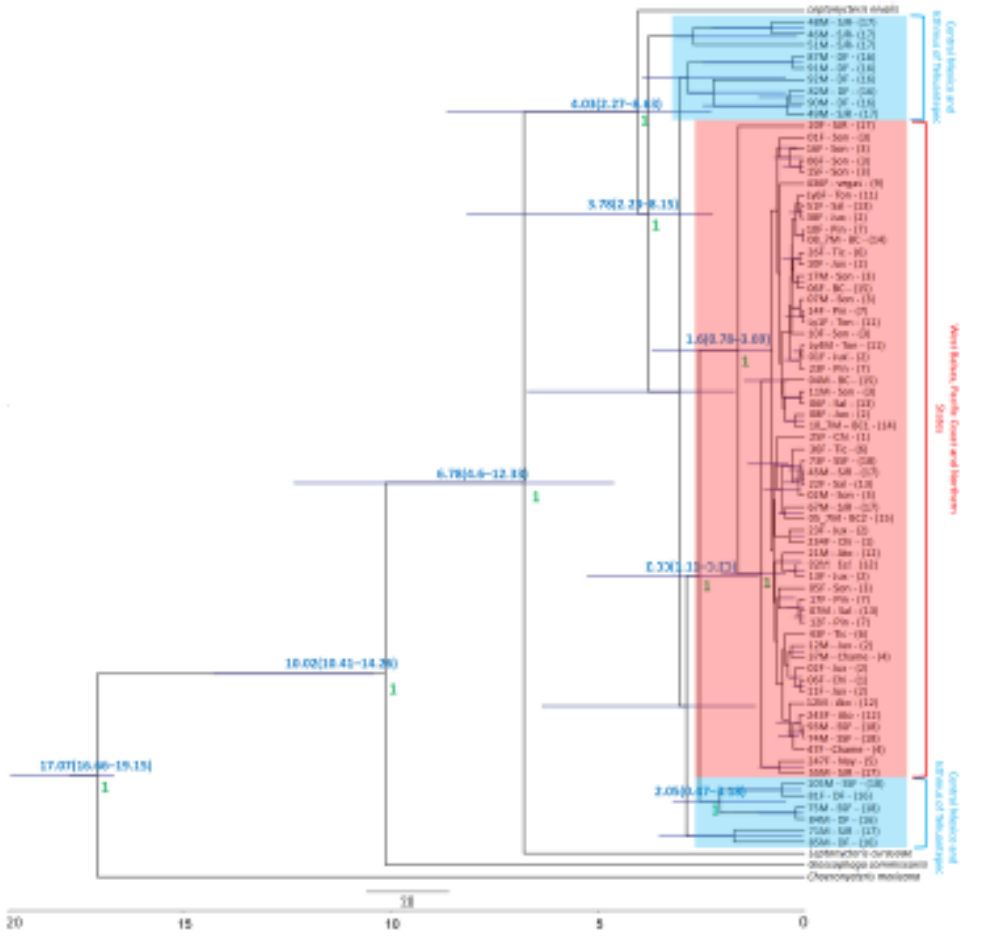

Figure 4 **Gene genealogy and dates of divergence estimated with BEAST based on paternally inherited *Cyt-b* sequences.** Black numbers above nodes depict divergence in million years; green numbers below nodes depict support values (posterior probability). Highlighted in blue color are the individuals from the Central Mexico and Isthmus of Tehuantepec localities, highlighted in red color are the individuals from the localities located in the West Balsas-Pacific Coast and Northern States Region. HDP values are in parentheses above the blue bar.

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Fig. 6), and most individuals from SJR in Oaxaca, form another clade (0.96 mya; Fig. 6). There is a group exclusively composed by members from Jalisco (1.05 mya; Fig. 6).

Historical demography analysis

Bayesian skyline plots (SLP) indicate a late Pleistocene demographic expansion, starting at ~130,000 ya for *Cyt-b* (Fig. 7A) and ~500,000 ya for *D-loop* (Fig. 7B). In contrast, for the *DBY* gene (Fig. 7C), SLP basically exhibits demographic stability, with an older and constant but slight demographic expansion, about 6-7 mya, coincident with its own evolutionary history and divergence times dated in the gene genealogy above.

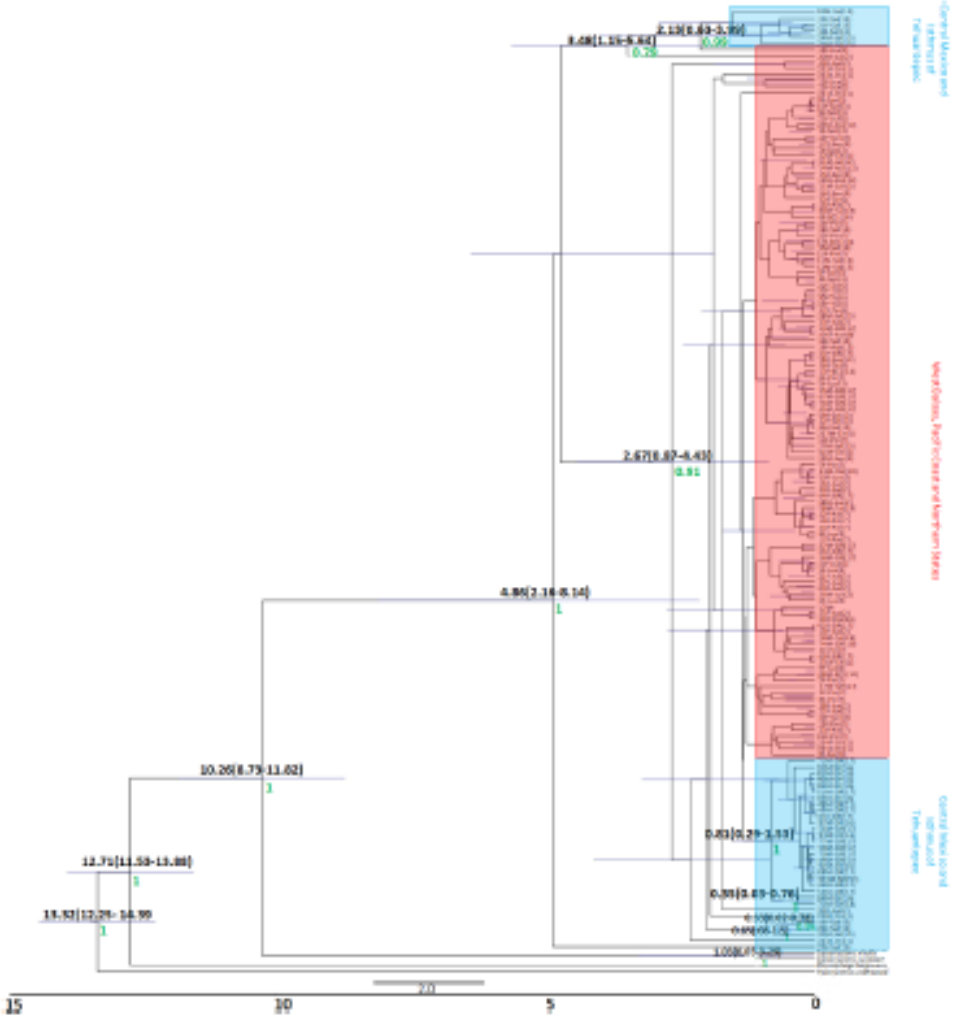

Figure 5 ***D-loop* Gene genealogy.** Gene genealogy and dates of divergence estimated with BEAST based on paternally inherited *D-loop* sequences. Black numbers above nodes depict divergence in million years; green numbers below nodes depict support values (posterior probability). Highlighted in blue color are the individuals from the Central Mexico and Isthmus of Tehuantepec localities, highlighted in red color are the individuals from the localities located in the West Balsas-Pacific Coast and Northern States Region. HDP values are in parentheses above the blue bar.

Full-size DOI: 10.7717/peerj.12168/fig-5

Tajima's *D* was negative and significant for both mitochondrial genes (*Cyt-b* Tajima's D = −1.73, $p = 0.007$; *D-loop* Tajima's $D = -1.6$, $p = 0.023$; Table S4), suggesting a possible historical population expansion, while Tajima's *D* for the *DBY* was positive and non-significant (Tajima's $D = 4.49$, $p = 0.99$; Table S4), suggesting demographic stability at deeper times. Fu's neutrality tests exhibited negative but non-significant values for the mitochondrial regions (*Cyt-b* = −5.86, $p = 0.19$; *D-loop* = −2.05, $p = 0.37799$ and was positive but also not significant for *DBY* Fu's = 0.44, $p = 0.87$).

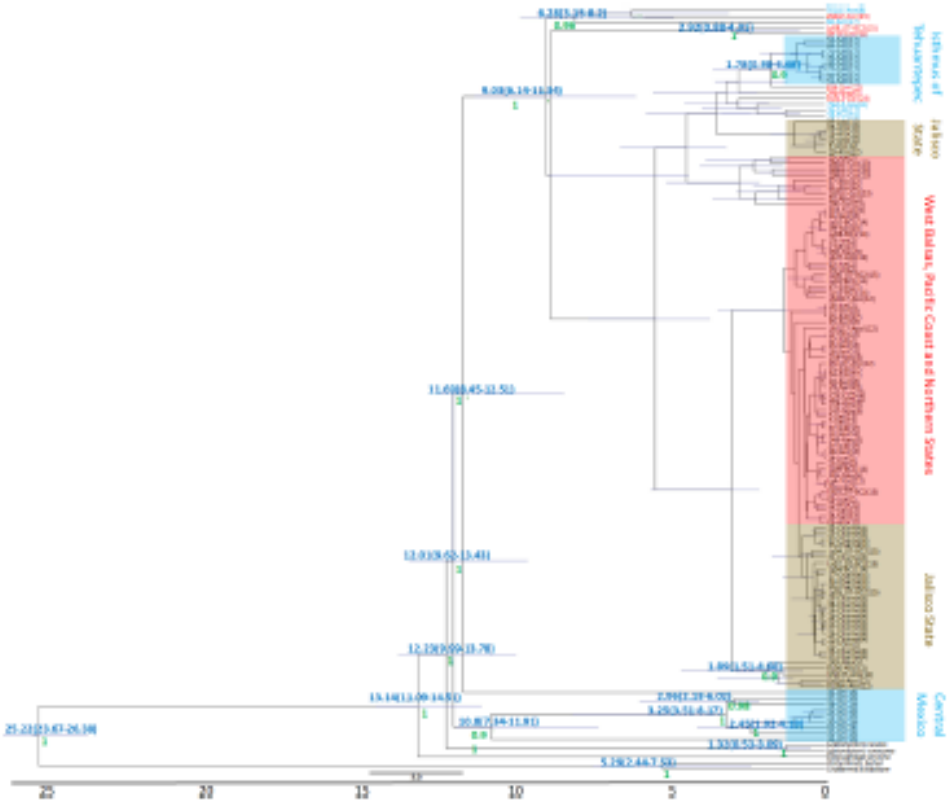

Figure 6 ***DBY* Gene Genealogy.** Gene genealogy and dates of divergence estimated with BEAST based on paternally inherited *DBY* sequences. Blue numbers above nodes depict divergence in million years; green numbers below nodes depict support values (posterior probability). Highlighted in blue color are the individuals from the Central Mexico and Isthmus of Tehuantepec localities, highlighted in red color are the individuals from the localities located in the West Balsas-Pacific Coast and Northern States Region and highlighted in brown individuals from Jalisco State . HDP values are in parentheses above the blue bar.

Full-size DOI: 10.7717/peerj.12168/fig-6

Past distribution models

We retained seven bioclimatic layers (Isothermality, Min Temperature of Coldest Month, Temperature Annual Range, Mean Temperature of Coldest Quarter, Precipitation of Wettest Quarter, Precipitation of Warmest Quarter, Precipitation of Coldest Quarter) to build distribution models. The potential distribution models for *L. yerbabuenae* (Figs. 2; 8) showed stability and good support, for each distribution model the area under the ROC curve (AUC) was >0.8. The projection to the Holocene (Fig. 8B) suggests that the distribution area of species has been stable during this period. Nevertheless, LIG (Fig. 8D) and LGM (Fig. 8C) models do not recover suitable environmental conditions for the presence of the species at higher latitudes, such as Arizona, USA. However, environmental suitability is predicted in West and East Balsas and the Isthmus of Tehuantepec for all periods (Fig. 2).

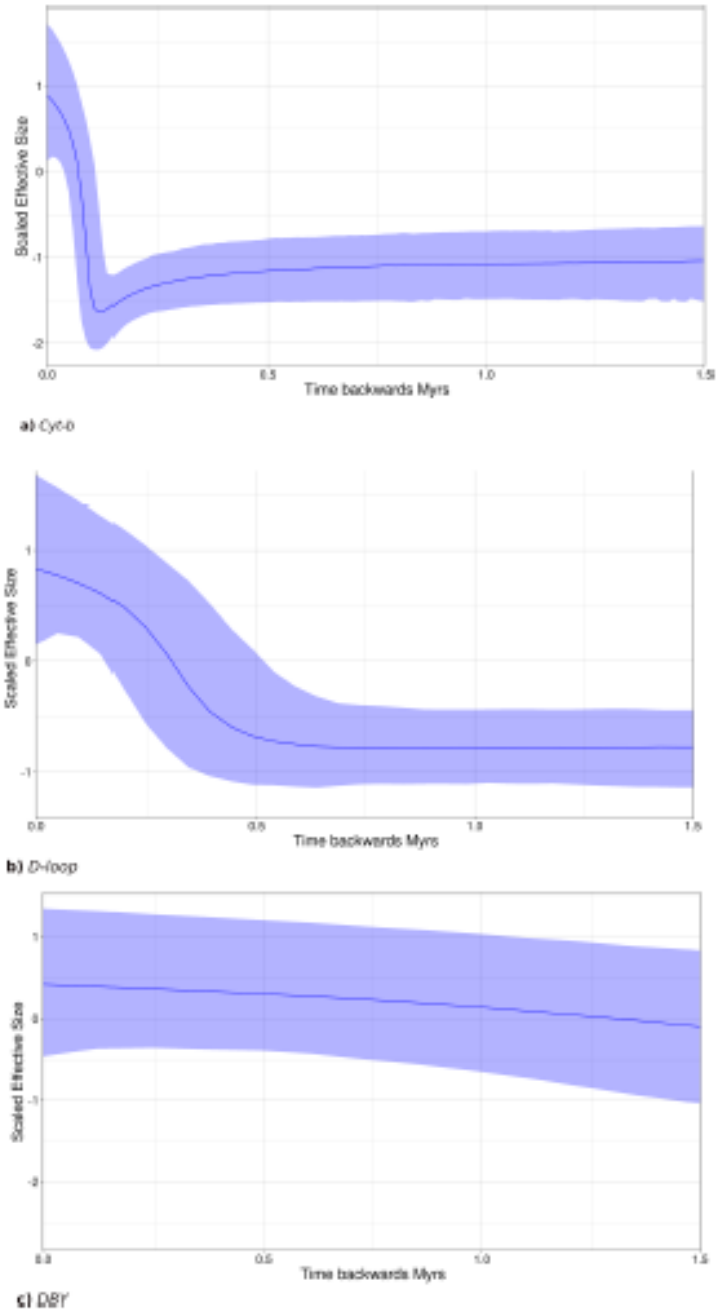

Figure 7 Sky-Line Plots depicting the demographic trajectory of *Leptonycteris yerbabuenae* obtained with BEAST for (A) *Cyt-b*, (B) *D-loop*, and (C) *DBY*. Results show population expansion for maternal lineages starting around the Last Interglacial Period , ~130,000 in (A) and ~500,000 in (B), while the paternal lineage shows demographic stability.

Full-size DOI: 10.7717/peerj.12168/fig-7

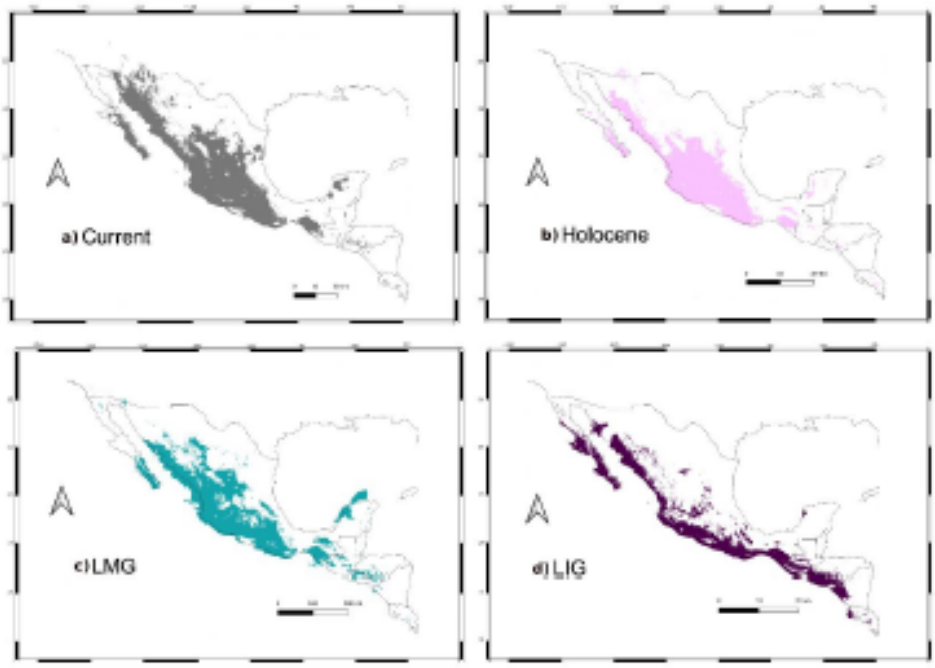

Figure 8 **Past distribution models.** Maps depict (A) Current, (B) Holocene, (C) last maximum glacial and (D) last Interglacial distribution models of *Lepomycteris yerbabuenae*.
Full-size DOI: 10.7717/peerj.12168/fig-8

For the current model, the area with the greatest environmental suitability for the presence of the species is located close to West Balsas (along Sierra Madre Occidental), south of the peninsula of Baja California, the states of Oaxaca and Chiapas on the Isthmus of Tehuantepec zones (coast of the Pacific Ocean states and portions of Central Mexico; Fig. 8A). For the Holocene, *L. yerbabuenae* had a marked contraction specially in Central America, in contrast with the expansion observed in Central Mexico, West Balsas (and Pacific Coast of Mexico) and south of Baja California Peninsula (Fig. 8B). During the LGM, suitable environmental conditions occurred along the Pacific coast including West-Balsas to East-Balsas comprising areas of Central Mexico towards the southern area of the Chihuahuan Desert (Fig. 8C). The distribution of *L. yerbabuenae* apparently contracted during the LIG period but exhibited suitable conditions for the presence of species in West Balsas and the Isthmus of Tehuantepec. Finally, during the transition between the Holocene and Current periods there is an evident expansion towards the north, particularly in Sonora and Arizona (Figs. 8A–8B).

DISCUSSION

This study includes the largest number of samples and localities analyzed so far for *L. yebabuenae* (*Wilkinson & Fleming, 1996*; *Morales-Garza et al., 2007*; *Arteaga et al., 2018*; *Menchaca et al., 2020*). As amplification of the *D-loop* showed difficulties –apparently because of poorer preservation state of the oldest samples or a bias originated by the

oligonucleotide used for its amplification such as mutations in primer binding site –we had sample size differences between molecular markers; thus, some inferences may be interpreted with caution (*Jennions & Møller, 2002*; *Nazareno & Jump, 2012*). In addition, for other migratory species, haplotype diversity is also high, for instance: *Lasiurus borealis*, *L. cinereus* and *Lasionycteris noctivagans* (*Korstian JM & Williams, 2015*; *Sovic, Carstens & Gibbs, 2016*; *Vonhoff & Russell, 2015*). Moreover, species where females move long distances, sex ratio bias is present and should be considered in the analyses. Therefore, we handled parental lineages separately to compare historical demographic patterns and historical distribution (*Prugnolle & De Meeus, 2002*). In contrast, some non-migratory species exhibit low haplotype diversity as *Dasypterus ega* and *D. intermedia* (0.018 and 0.588 respectively; *Chipps et al., 2020*). We found higher levels of genetic diversity, signals of stronger genetic structure and a nearly constant demographic trajectory for paternal lineages, in contrast with maternal lineages that show lower genetic variation, weak genetic structure and signals of demographic expansion.

Genetic diversity

Levels of genetic diversity as measured by haplotype diversity for the three molecular markers (Table 2) were consistent with previous studies of this species (*Wilkinson & Fleming, 1996*; *Morales-Garza et al., 2007*; *Arteaga et al., 2018*; *Menchaca et al., 2020*), and of other American bat species (*i.e., Tadarida brasiliense* (*Russell, Medellin & McCracken, 2005*), *Sturnira parvidens* (*Hernández-Canchola & León-Paniagua, 2017*), and lower than values reported for *Artibeus jamaicensis* (*Ruiz, Vargas-Miranda & Zúñiga, 2013*).

The highest haplotype diversity for both mitochondrial markers was found in the Central Mexico near the Isthmus of Tehuantepec. *DBY* showed the highest genetic diversity in populations from the Pacific Coast (including Baja California Sur = 0.90; Juxtlahuaca, from Guerrero = 0.94 and Arandas, Jalisco = 0.9789) (Table S5). The conflicting patterns of genetic diversity between *DBY* and mitochondrial markers could be related to different possible causes, including their mode of inheritance, patterns of molecular evolution and distinct evolutionary and demographic history (*Tosi, Morales & Melnick, 2000*), and to *L. yerbabuenae*'s female migratory behavior and male philopatry (*Herrera, 1997*; *Rojas-Martínez et al., 1999*); there is a sex bias during spring-summer seasons, females are more abundant at northern latitudes when they live in roosting caves and males leave mating caves at the southern area of their distribution (*Herrera, 1997*; *Rojas-Martínez et al., 1999*; *Medellín et al., 2018*).

Genetic structure

Analysis of genetic differentiation, haplotype network, BAPS and AMOVA for mtDNA showed lower genetic structure for female inherited genetic markers than for the male inherited marker, coherent with reports in other bat species (*i.e., Tadarida brasiliensis* (*Russell, Medellin & McCracken, 2005*), *Miniopterus schreibersii* (*Bilgin et al., 2008b*), *Myotis capaccinii* (*Bilgin et al., 2008a*) *Myotis nattereri* (*Rivers, Butlin & Altringham, 2005*)). In particular, for the *DBY* gene, the analyses indicate strong genetic structure with geographical consistency, comprising three groups: West of Balsas, Central Mexico-Balsas, and Isthmus of Tehuantepec (Fig. 1).

Our results support the previous inferences that *L. yerbabuenae* females move more than males, and that males show philopatric behavior, conducting only local and altitudinal movements (*Cockrum, 1991*; *Herrera, 1997*; *Rojas-Martínez et al., 1999*; *Tellez et al., 2000*; *Medellín et al., 2018*). Migratory females apparently promote gene flow among caves minimizing genetic differentiation, while male philopatry and perennial presence promote genetic differentiation in the *DYB* gene.

Historical analyses

The relationships among haplotypes within *L. yerbabuenae* clade were different in the three gene genealogies; these differences were marked between the paternally (*DBY*) and one of the maternally (*Cyt-b*) inherited molecular markers, although there are some similarities. Genealogical relationship among *L. yerbabuenae* and sister species is not clear, with *D-loop* and *DBY* markers of *L. curasoae* and *L. nivalis* forming a monophyletic group. While in the case of the genealogy based on *Cyt-b*, *L. nivalis* and *L. curasoae* form independent branches, and *L. nivalis* is established as the closest species to *L. yerbabuenae* clade. In addition, we observe some disagreement in haplotype relationships depicted in the maternally inherited *Cyt-b* and *D-Loop* genealogies. In this sense, high homoplasy has been reported for the *D-loop* in several species (*Martes pennanti* in *Finnilä, Lehtonen & Majamaa, 2001*; *Homo sapiens* in *Knaus et al., 2011*).

The major difference occurred with calculated divergence times, for *DBY* marker, divergence times shows 12.01 mya [9.62–13.43 95% HDP] for the origin of *L. yerbabuenae*. These results reflect the evolutionary history for each gene, in particular differences in mutation rates (*Allio et al., 2017*), as well differences in the evolutionary and ecological history of each sex. Some authors have stablished that Y-chromosome genes are less polymorphic than mitochondrial genes and this could be a reason for discordant divergences times (*Boissinot & Boursot, 1997*).

Divergence times of *Leptonycteris yerbabuenae* for *D-loop* (10.26 mya [95% HDP, 8.73–11.82 mya]) and the chromosome Y associated *DBY* marker (12.23 mya [95% HDP, 9.99–13.78 mya]) were older than those obtained for *Cyt-b* (4.03 mya [95% HDP, 2.27—8.63 mya]). Dates of divergence for *DBY* and *D-loop* are consistent with the origin of ecologically related taxa, such as *Agave sensu lato* (4.6–12.3 mya; *Flores-Abreu et al., 2019*; and more recently *Jiménez-Barrón et al., 2020* reported 9 mya for this group). It is interesting to note that date of divergence for *Cyt-b* is consistent with the divergence of *A. lechuguilla* (2.47–6.71 mya; (*Scheinvar et al., 2017*). This is relevant because *Agave* and *Leptonycteris*, are closely associated and they share close evolutionary trajectories (*Rocha, Valera & Eguiarte, 2005*). Times of divergence are consistent with two pulses of acceleration in the diversification rate of *Agave sensu lato*, first 8-6 mya, and second 3—2.5 mya (*Good-Avila et al., 2006*) and with the most recent report with a pulse at 6.18 mya and the second one at 4.91 mya (*Jiménez-Barrón et al., 2020*). Moreover, these dates of divergence also coincide with a temperature decrease for tropical wet climates in Mexico during glacial periods between 5.3 and 1.8 Mya (*Van Devender, 2000*). It is worth mentioning that in all genealogies *L. yerbabuenae* consists of several lineages that may have originated by the isolation of populations during the Pliocene, in particular in the area of Central Mexico.

Nevertheless, an analysis of dates of divergence based on genome wide data will allow a better understanding of the processes leading to lineage divergence within *Leptonycteris yerbabuenae*.

The demographic history of populations also influences the shape of ultrametric trees, where a genetic bottleneck can increase the rate of coalescence of lineages, while a demographic expansion could produce isolated long branches (*Ho & Shapiro, 2011*; *Gattepaille, Jakobsson & Blum, 2013*). Accordingly, during the Pleistocene, the species may have undergone geographic expansion that erased geographic structure. In particular, one shows (*Cyt-b*) signals of demographic expansion, probably associated with Pleistocene climate changes as supported by demographic analyses.

High haplotype diversity and medium-low nucleotide diversity, in addition to a star-like haplotype network, further suggests population expansion (*Slatkin & Hudson, 1991*; *Avise, 2000*). SkyLine Plots for mtDNA suggest that a population expansion began approximately 130,000 and 500,000 years ago, during the Pleistocene; Tajima's *D* results also support demographic expansion for mtDNA. These results are further supported by past distribution models showing an expansion from the LIG through the Holocene.

Pleistocene climate changes influenced the distribution of a variety of living groups. Overall, highland biota was fragmented during warmer interglacial periods (*McDonald, 1993*; *Metcalfe et al., 2000*; *León-Paniagua et al., 2007*; *Ruiz et al., 2010*; *Bryson et al., 2011*; *Gugger et al., 2011*), followed by expansions related to an increase in temperature (*Hundertmark et al., 2002*; *Hofreiter & Stewart, 2009*; *De Bruyn, Hoelzel AR & Hofreiter, 2011*). The transition between the LIG and Holocene distribution models are consistent with warmer conditions in Mexico 15,000–12,000 years ago, and with the colonization of ecologically related groups such as Cactacea and *Agave* towards the north of Mexico (*Metcalfe et al., 2000*). Furthermore, current distribution for *L. yerbabuenae* agrees with the distribution of several species of the genus *Agave* (*Scheinvar et al., 2017*; *Scheinvar, 2018*).

Female migration in *L. yerbabuenae* might have occurred following the changing climatic conditions of the Pleistocene. Our models for LIG, Holocene and current periods suggest an expansion to northern areas along the Pacific Coast towards Sonora. This area is home to numerous maternity colonies of *L. yerbabuenae*, including the important Pinacate roost cave (*Medellín et al., 2018*). The volcanic record indicates that the most recent volcanic activity in the area occurred 12,000 (+/- 4,000) years ago, while the origin of the current ecosystem has been dated 9,000 years ago (*Marshall & Blake, 2009*). These dates for geological and biological events coincide with our estimated expansion model for the Holocene. These models are also supported by *Arteaga et al. (2018)*, who reported a local demographic expansion for *L. yerbabuenae* in northwestern Mexico associated to Pleistocene climate changes.

Demographic analyses among genetic markers are contrasting, suggesting a demographic expansion for the female inherited markers, but not in the male lineages. This recent expansion is further supported by past projection models. Moreover, molecular data and past distribution models suggest that *L. yerbabuenae* female's migration to northern maternal roost has been an ecological and dynamic process originated in the Pacific Coast zone of Mexico and Central-South area (Isthmus of Tehuantepec; Figs. 8B–8D).

This hypothesis is reinforced because the Pinacate area became ideal for the arrival of females only ~8000–16,000 years ago, due in part to more favorable climatic and geologic conditions for the bat species during the climatic transition from LIG-Holocene. Moreover, genetic diversity, Bayesian genealogies for the three markers and the model for more stable thermal conditions from the Pleistocene to Current time, provide information to assume that the site of origin of the species could have been located in the Balsas zone of Mexico and Central-South (Isthmus of Tehuantepec; Fig. 1). According to the basal lineage in the gene genealogy, an ancestor of the living populations of *L. yerbabuenae* existed south of its current distribution. This origin is also supported by the greatest diversity of lineages currently present in the zone of the Isthmus of Tehuantepec. Nevertheless, these biogeographic hypotheses should be tested with other type of molecular markers, such as SNPs, with higher phylogenetic resolution and including the three species of the genus *Leptonycteris*.

CONCLUSIONS

Leptonycteris yerbabuenae is rich in genetic variation, both in the mitochondria and in the DBY gene. The genetic structure suggests that migrant females promote gene flow, maintaining a cohesive species; however, male philopatry promoted genetic differentiation of three different geographic groups –West of Balsas, Central Mexico, and Isthmus of Tehuantepec. Results from demographic analyses are contrasting and suggest demographic expansion for female inherited molecular markers, but not so for male inherited DBY. This expansion is further supported by past projection models.

Information generated in this work can contribute to reinforce conservation and management strategies for *L. yerbabuenae*. Based on highest haplotype diversity sites, it is possible to design corridors in order to keep connectivity among these sites and it is of relevant importance to conservation to allow *L. yerbabuenae* to play its role as main pollinator of genus *Agave* in wild and cultivated species (*Molina-Freaner & Eguiarte, 2003*; *Scott, 2004*; *Rocha et al., 2006*; *Trejo-Salazar et al., 2016*). Thus, it is evident that ecological and evolutionary interactions among lesser long-nosed bat and *Agave* is a key for the maintenance of the major vegetation types in Mexico (dry-forest and xerophytic vegetations). Furthermore, this result can overlap with current conservation programs, as "Batfriendly" which consists of promoting the conservation of agaves grown in strategic ecological and economic areas to support the migratory movements of bats (*Trejo-Salazar et al., 2016*).

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Competing Interests

Luis E. Eguiarte is an Academic Editor for PeerJ.

Author Contributions

- Roberto-Emiliano Trejo-Salazar, Jaime Gasca-Pineda and Luis E. Eguiarte conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Gabriela Castellanos-Morales performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Dulce Carolina Hernández-Rosales performed the experiments, prepared figures and/or tables, and approved the final draft.
- Niza Gámez performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Miguel Rene Morales Garza and Rodrigo Medellin analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

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DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The *D-loop* sequences are available at Genbank: MT790834–MT790986; the *Cytb* sequences are available at Genbank: MT859334 to MT859403 and; the *DBY* sequences are available at Genbank: MT913638 to MT913767.

Data Availability

The following information was supplied regarding data availability:

The aligned FASTA files are available as Supplemental Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.12168#supplemental-information.

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Capítulo 2. Tiempos de divergencia y origen del género *Leptonycteris*

Origin of tequila long-nosed bats: speciation in genus *Leptonycteris* (Chiroptera: Phyllostomidae).

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Author contributions
RT designed the project, collect samples, data base construction, phylogenetics analysis and drafting the manuscript; **JG** participated in the data base construction, computational analysis and drafting manuscript; **AL** contributed with phylogenetic, diversification analysis and drafting manuscript; **SM** contributed to drafting the manuscript; **LL** contributed to the drafting the manuscript; **LE** was the project leader, designed and coordinated the project, helped with the logistics and drafted and corrected the manuscript.

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Abstract

Tequila bats (or Long-nosed bats) *Leptonycteris* are the most important pollinator of genus *Agave* and many other plants species in North America. There are not previous studies about their origin and evolutionary ecology. We consider them as an important genus because of its ecology role as pollinator and its conservancy status and international protection. We proposed an origin in Central Mexico and we thought they have followed a speciation process conducted by global and local geological, ecological and climate conditions.

Results

We generated a new phyllostomids phylogeny which confirms the insectivory ancestor Nectar-feeding bats origin is dated from 28.12 million years ago (MYA) basal group. While Glossophaginae crown group originated about 18.86 MYA. Moreover, the genus *Leptonycteris* (crown group) arose about 13.91 MYA. That supports diversity rate changes concordant with geological and ecological events.

Conclusions

We propose that the species of the genus *Leptonycteris* arose as a result of ecological events associated with the increase in species of the genus *Agave*, as well as the emergence and diversification of groups such as Cactaceae. In addition, the times obtained from the phylogenetic inference allow us to relate global and local climatic events to the separation of the species *L. curasoae*, which is distributed to the south of the continent where it is restricted. Finally, the phylogenetic analyzes allow us to infer that the center of origin of the genus is located in some area of central Mexico.

1. INTRODUCTION

The chiropteran genus *Leptonycteris* comprises three species: *L. curasoae, L. nivalis*, and *L. yerbabueane*. Two of these species (*L. nivalis* and *L. yerbabuenae*) are distributed in North America, mainly in Mexico, where their distribution is sympatric. While *L. curasoae* is restricted to some locations in Venezuela and Colombia and three Caribbean islands off the Venezuelan coast: Aruba, Bonaire, Curaçao and Margarita (Cole and Wilson, 2006). The species of the genus are considered biologically important because they play a very important role as pollinators of a very diverse group of plants in the places they inhabit, among them, they are considered the primary pollinators of the genus *Agave*, which in Mexico has a very important relevance in ecological, economic, and cultural aspects and other groups of plants as Bombacaceae and Leguminosae (Rojas-Martínez et al., 1999; Fleming, et al., 2009). For these reasons, the bats *L. nivalis* and *L. yerbabuenae* are known as tequila bats or long-nosed bat and lesser long-nosed bat, respectively. The three species are in different conservation categories*: L. curasoae* vulnerable (VU-A2c), *L. nivalis* Endangered (EN-A2c) and *L. yerbabuenae* near threatened (Red List IUCN, 2022, see 3.1). Therefore, it is important to know the ecological and evolutionary aspects of the species that we can generate better conservation programs.

These bats have received special attention during recent years because of its metabolic capabilities and migratory behavior (Gutierrez-Guerrero et al., 2020; Pourshoushtari and Ammerman, 2021). The distribution of this genus is remarkable, because, in the case of the North American species, a group of females are migratory during one time of the year (Spring-Summer) in the reproductive stage, each species following a specific route and forming root caves at north of their distributions. While in the Autumn-Winter seasons, another group of females copulate and give birth in the Center-South of its distribution, and they remain in the same latitudes as the males. On the other hand, it is known that *L. curasoae* carries out migratory movements between the islands and the continental areas in which it inhabits (Simal et al., 2015). The speciation process of this genus has not been explained yet, the

phylogenetic reconstructions previously proposed, group the species *L. curasoae* and *L. yerbabuenae* in a clade, while the species *L. nivalis* is located in the basal branch of the genus. It is noteworthy, since the species that is currently geographically isolated is *L. curasoae*.

Evolutionary patterns of diversity and speciation through time have received much attention in recent years (Nee, 2001; Rabosky, 2006; Goldberg et al. 2011; Morlon et al. 2011; Price et al. 2012; Schnitzler et al. 2012; Magallón et al., 2015; Jiménez-Barron et al., 2020). Nowadays, we know that diversification in several living groups has been influenced by a combination of biotic and abiotic factors (Schluter, 2001; Riklefs, 2003; Kozak et al 2005). Different molecular phylogenetic methods have been developed to infer these patterns (Rabosky, 2006; Revell, 2012; Schwallier, 2016) and now it is possible to associate diversification with such factors which can affect evolutionary processes (Rabosky, 2006; Revell, 2012; Schwallier, 2016), such as ecological relationships, climatic conditions, or even geological events. In this sense, some authors have focused on physiological, ecological or behavior characters (Hughes and Eastwood, 2006; Kozak et al. 2006; Rabosky et al., 2007; Kozak et al, 2010; Ricklefs, 2007; Licona-Vera and Ornelas, 2017). Molecular phylogenetic reconstruction are powerful tools for past inference and they can be related to methods which can support us to include some character and recostruct ancestral areas for well know represented groups (Cusimano and Renner, 2014; Wang et al., 2017). Moreover, we have the chance to infer origin centers and calculate clade age, which is a factor that dominates diversity (McPeek and Brown, 2007). Also, diversification rates and patterns of genetic diversity should be important parameters in phylogenetic inference (Rabosky et al., 2014).

One of the best-described group is family Phyllostomidae (Chiroptera) whose members have shown different adaptive radiation phenomena and it is a good example to explain pattern of diversity through time (Simmons, 2005; Monteiro and Nogueira, 2011; Datzmann et al. 2010; Rojas et al., 2011; Rojas et al., 2012; Rojas et al., 2016). Phyllostomidae is the largest endemic Neotropical bat family (grouped in Superfamily Noctilionoidea), and it encompasses

more than 200 species whose most visible characteristic is an elongated leaf-like nose (Wilson and Reeder, 2005).

This family spans from Southern USA to Argentina (Simmons, 2005b). Therefore, phyllostomid bats have members with several different specialist feeding habits such as insectivory, frugivory as well as nectarivory, blood-feeding, carnivory even other bats, and even some of them are omnivory (Wetterer et al. 2000). Feeding habits diversity reflect that phyllostomid bats play different essential roles in ecosystems where they live (Nogueira et al., 2005; Kunz et al. 2011).

Evolutionary history of phyllostomid feeding habits has been described as a process which was originated from an insectivorous ancestor (Ferrarezi and Gimenez 1996; Freeman, 2000; Wetterer et al. 2000; Nogueira et al., 2005; Datzmann et al., 2010; Rojas et al., 2011; Rojas et al., 2012) and the great diversity in feeding habits within Phyllostomidae has resulted from a process of adaptive radiation (Wetterer et al., 2000; Nogueira et al., 2005; Datzmann et al., 2010; Rojas et al., 2011; Rojas et al., 2012). Within Phyllostomidae there are remarkable physiological, morphological, and behavioral differences between members which have developed adaptations for every feeding habit and ecosystem they inhabited (Wetterer et al., 2000; Nogueira et al., 2005; Monteiro and Nogueira, 2011; Datzman et al., 2010; Rojas et al., 2011).

Despite the inferences described before, there is a little information available about the fossils records of phylostomids bats, even it is not clear the origin of the Superfamily Noctilionoidea and it is not possible to compare these results with paleontology material evidence, the oldest phyllostomid fossil is 40 - 35 million years old during Miocene (Gianinni and Velazco, 2020).

A particular group of phyllostomids bats is Glossophaginae that comprises the endemic nectar-feeding bats of American continent. Members of this subgroup pollinate more than 250 genera of plants and there are just about 38 nectar-feeding bats species (Simmons, 2005). Furthermore, Glossophaginae radiation is compatible with the origin of genus *Agave* and other ecological related plants groups (Flores-Abreu et al, 2019). For that reason, estimating the

divergence times of Phyllostomidae bats and reconstruct ancestral characters (feeding habits and areas of distribution) makes possible to answer how evolutionary history processes, geological and environmental factors have influenced the diversification in family Phyllostomidea and specifically, on current ecological and evolutionary interaction between nectarivorous bats of *Leptonycteris* genus with some Cactaceae and *Agave* groups.

We focus our efforts on understanding how genus *Leptonycteris* bats diversified and established a strong ecological relationship with *Agave* and Cactacea. The main goal in our work is inferring on how historical ancestral areas, feeding-habits specialization (i.e., herbivory, carnivory or omnivory) and divergence times have influenced diversification rates in time; and how it impacts speciation and extinction rates on nectar-feeding bats of *Leptonycteris* (long-nosed bats or tequila bats).

2.METHODS

2.1 Sampling sequences and alignment

We analyzed one hundred and fourty-eigth phyllostomid species from 54 phyllostomid genera. Our sampling comprises all extant genera but except *Glyphonycteris*, *Neonycteris*, *Scleronycteris* and *Xeronycteris*. We also included four noctilionid species (*Firupterus horrens, Molossus molossus, Mormoops megalophy,* and *Noctilio leporinus*) as outgroup taxa. Most of sequences were taken from database reported by Rojas et al., (2016) in GenBank Database. We also included 15 new sequences from tissue samples from Flores-Abreu et al. (2019), provided by different museum collections to increase subfamily Glossophaginae species represented, and we contributed with a new sequence for *Leptonycteris curasoae* (OP320926). We used species assignments according to Wilson and Reeder (2005) and IUCN (http://www.iucnredlist.org/). Gene markers selected were genes of nuclear recombination activating gene 2 (*RAG2*), introns of thyrotropin beta chain (*thy*), signal transducer and activator 5A (*stat5a*), autosomal exons of brain-derived neurotrophic factor (*bdnf*), titin 6 (*ttn6*), X-chromosome exon ATPase-7A (atp7a), the 3 untranslated region or phospholipase C beta 4

(*plcb4*) and for the mitochondrial cytochrome b (*cyt-b*), cytochrome oxidase I (*coxI*) genes and the ribosomal RNAs 12S, 16s and tRNAval.

Sequences were aligned using Clustal X (Thompson *et al*. 1997) and checked manually by eye. We concatenated both mitochondrial and nuclear loci, these were used before by several authors to estimate phyllostomid bats phylogenies and divergence times (Baker et al., 2003; Hoffman et al., 2008; Datzmann et al., 2010; Rojas et al., 2011; Rojas et al., 2012).

2.2 Phylogenetic Reconstruction

We used jModelTest 0.1.1 (Posada, 2008) for loci matrix for each gene marker. The best fitting evolutionary model proposed was the general time reversible model of substitution with allowance for gamma distribution of rate variation and proportion of invariant sites (GTR+Γ+I) according to -lnL value, for every gene marker. These loci has been already used by other authors before (Baker *et al*, 2003; Datzmann, 2010, Rojas et al., 2016; Flores et al., 2019). With a database of ten molecular markers, we built a concatenated matrix loci because all of them present the same evolutionary model. Posteriorly, we calculated a likelihood ratio test (LRT) to prove if sequences follow a molecular clock rate (Lewis, 1998). Bayesian analysis was conducted on BEAST 1.8.2 (Drummond et al., 2012), to estimate the molecular phylogeny and divergence times with 500,000,000 of generations and sampling from the chain every 1000 generations. We used software TRACER 1.5 (Rambaut & Drummond, 2007) for a burn-in value of 10% of the samples and results of the Bayesian analyses were resumed with a 50% majority-rule consensus tree with posterior probability values for each node.

2.3 Divergence Times

The phylogenetic reconstruction under Bayesian Inference (BI) and divergence time analyses were performed in BEAST 1.8.2 (Drummond et al., 2012). The maximum clade credibility tree was calculated in TreeAnnotator (Drummond et al., 2012) with 10 million trees being excluded as burn-in from subsequent calculations. The consensus tree was visualized and edited in FigTree 1.4.2 (Rambaut, 2014). Support values were considered high when posterior

probability (PP) was ≥ 0.7 (Pirie, 2015). Divergence time estimates of Phyllostomidae were conducted with an uncorrelated relaxed clock and a lognormal distribution of rates (Gernhard, 2008). Parameters of the MCMC were identical to those used in the phylogenetic analysis. To calibrate the phylogenetic hypothesis, we used four calibration points based on fossil record considering the minimal age of the stratum where the fossil was recorded (Table 1).

Table 1. Calibration points used for calculated divergence times, most of them based on fossil records.

Calibration point	Age (mya)	Reference
Phyllostomidae	27.4 - 27.6	Jones et al., 2005; Flores-Abreu et a., 2019
Mormoopidae	36	Czaplewski and Morgan, 2003
Glossophaginae	22.8	Teeling et al., 2005
Choeronycterini	12-13	Czplewski et al., 2003
Tonatia	16	Czaplewski et al., 2003

2.4 Diversification Rates through Time

We applied data from reconstructed phylogeny of living Phyllostomidae to estimate diversification and transition rates. We analyzed the diversification process by means the speciation/extinction model using the R package BAMM v2.5.0 (Rabosky, 2014). Priors for the BAMM control files were generated using the dated phylogenetic tree input into the function setBAMMpriors in the package BAMMtools 2.5.0 (Rabosky et al., 2014), the control file was set for 10,000,000 generations. The obtained MCMC loglikelihoods were tested against generation number using the coda package (Plummer et al., 2006). All remaining outputs contained in the event data file were analyzed using BAMMtools. The evolutionary rate parameters used were expected number of shifts = 1.0, lamdaIntPrior = 1.0, lambdaShiftPrior = 0.05, and muInitPrior = 1.0. The analysis was conducted with four independent chains for 500,000,000 generations each. We assumed convergence of the chains when the ESS value exceeded 500.

For diversification analyses, we retrieved the configuration of rate shifts with the highest posterior probability through the “getBestShiftConfiguration” function of BAMMtools. These

configurations were depicted as phylorate plots, which represent the analyzed phylogeny with its branches colored to reflect the instantaneous diversification rate. Rates-through-time plots were generated for speciation (λ), extinction (μ), and diversification (r) We used the functions getCladeRates to obtain estimates of the speciation rate (λ) and an extinction rate (μ) for a specific clade.

2.5 Ancestral Reconstruction

2.5.1 Feeding habits. We followed the information about feeding habits from IUCN and we reviewed it bibliographically for each phyllostomids species. Based on that, we determined the most important feeding habits over the rest in the case of facultative phyllostomids bats (Appendix 1). Feeding habits were codified in six different character state, considering their major feed source: 1) Insectivory; 2) Frugivory; 3) Nectarivory; 4) Carnivory; 5) Hematophagous and 6) Omnivore. Finally, a feeding habits database of 125 phyllostomids species and the three outgroup species from different genera was constructed based on those feeding habits.

State character reconstruction was performed with Phytools 3.6, R library (Revell, 2012) based on the Bayesian Phyllostomidae phylogenetic hypothesis, and we tested two model of character reconstruction, “ER” and “ARD” change models for feeding habits with corHHM 1.22 R library (Beaulieu, 2017). Posteriorly, we proved a stochastic mapping with Phytools 3.6 R library (Revell, 2012) with 500 dated trees from the output BEAST phylogentic reconstruction file in order to infer the ancestral character for Phyllostomidae family. We performed a stochastic character mapping (SCM) by using the *make.simmap* function from phytools. We used an MCMC approach to explore the posterior probabilities of all nodes using the rate model “equal rates” (ER). The analysis was run with 100 independent simulations with 1x105 generations each. Using the plotSimmap function, we examined random trees to evaluate the congruency of the pattern’s recovery. Then, we used the describe.simmap function to summarize the result of the stochastic maps.

2.5.2 Ancestral areas

Data on species distributions were compiled indicating the presence or absence of each species in one of the Neotropical geographic areas (Appendix 2). To infer the geographic history of phyllostomid bats and identify important areas for its diversification we perform a BioGeoBEARS analysis to test historical areas reconstruction under different models (Matzke, 2018). Distribution maps of Phyllostomidea species were retrieved from the IUCN data repository (IUCN 2020; accessed from <www.iucnredlist.org>), and were compared with GBIF records. We followed Rojas et al. (2018) and López-Aguirre et al. (2019) ecoregions: NA= North America; SA= South America; CA= Central America; CB= Caribbean and; AN= Andean; to confirm the presence/absence for each species. Taxonomic identities of all sampled species were compared with current taxonomic arrangement of the order according to IUCN data.

We investigated the origin of Phyllostomidae, Glossophaginae and genus *Leptonycteris* using the R (R Core Team, 2013) package 'BioGeoBEARS' (Matzke, 2018). Based on our molecular phylogeny, 'BioGeoBEARS' statistically compares three models of range expansion (using the likelihood ratio test and AIC): DEC (Ree and Smith, 2008), DIVALIKE (Ronquist, 1997) and BAYAREALIKE (Matzke, 2018). Additionally, 'BioGeoBEARS' tests the effect of incorporating founder event speciation (+J) in these models (Matzke, 2018). Parameters integrated in our analysis included 'areas allowed' and 'dispersal multiplier' matrices (Chacón and Renner, 2014), both of which were associated with five time slices periods (Appendix 3).

3. RESULTS

3.1 Phylogenetic relationships of Phyllostomidae

We included 11,726 bp of mitochondrial and nuclear DNA from 152 species and four sister species for our phylogenetic reconstruction. Thus, bayesian analysis using nuclear and mitochondrial genes data set resulted in a well-supported phylogeny of the Phyllostomidae family as a monophyletic clade (Figure 1) and it was widely corroborated in other studies

(Koopman, 1994; Wetterer et al., 2000; Datzmann et al., 2010: Rojas et al., 2016). We based on this phylogeny our ancestral state reconstructions and discussion of the evolution of feeding habits, historic biogeography, and diversity rates. The Bayesian phylogeny of the Phyllostomidae supports the monophyly of the family. It also recovered the traditional subfamilies proposed by Baker et al. (2016) Macrotinae, Desmodontinae, Phyllostominae, Glossophaginae, Lonchophyllinae, Carollinae and Rhinophyllinae.

Additionally, subfamily Glossophaginae, containing exclusively nectarivorous taxa, was also represented as a monophyletic group. Glossophaginae is formed by five groups: Choeronycterini, Phyllonycterini, Brachyphyllini, Anourina and Glossophagini (Fleming et al., 2009; Datzmann et al., 2010). The monophyly and relationships of Glossophaginae tribes are well supported. Inside Glosophagini we recovered genus *Leptonycteris* as a monophyletic group, *L. curasoae* and *L. yerbabuenae* in a clade, and *L. nivalis* as a basal branch. They, also, are represented as sister genus of *Glossophaga* as traditionally has been described (Wetterer, 2000) in accordance with the traditional view (Wetterer et al., 2000; Simmons, 2005; Koopman, 1994; Jones et al., 2002). Monophyly of all subfamilies was verified with most nodes receiving high posterior probability support (pp > 0.5). Basal lineages were confirmed as Rojas *et al*. (2016) which comprises the taxa *Macrotus* and *Micronycteris* (Figure 1).

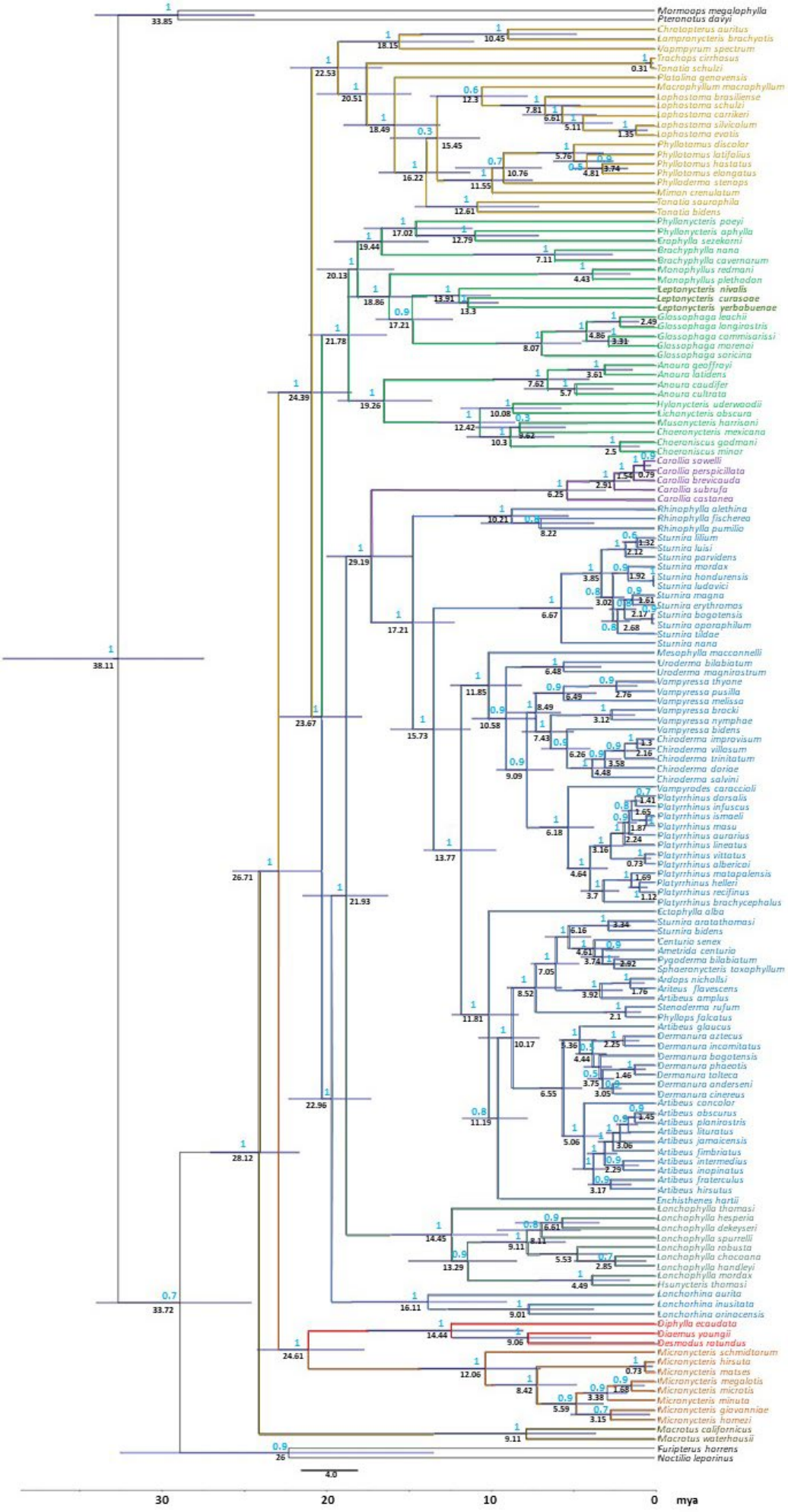

Mormoops megalophylla
Pteronotus davyi
Choeroniscus minor
Carollia sowelli
Carollia perspicillata
Carollia brevicauda
Carollia subrufa
Carollia castanea
Rhinophylla alethina
Rhinophylla fischerae
Rhinophylla pumilio
Sturnira lilium
Sturnira luisi
Sturnira parvidens
Sturnira mordax
Sturnira handurensis
Sturnira ludovici
Sturnira magna
Sturnira erythromos
Sturnira bogotensis
Sturnira oporaphilum
Sturnira tildae
Sturnira nana
Mesophylla macconnelli
Uroderma bilabiatum
Uroderma magnirostrum
Vampyressa thyone
Vampyressa pusilla
Vampyressa melissa
Vampyressa bracki
Vampyressa nymphae
Vampyressa bidens
Chiroderma improvisum
Chiroderma villosum
Chiroderma trinitatum
Chiroderma doriae
Chiroderma salvini
Vampyrodes caraccioli
Platyrrhinus dorsalis
Platyrrhinus infuscus
Platyrrhinus ismaeli
Platyrrhinus masu
Platyrrhinus aurarius
Platyrrhinus lineatus
Platyrrhinus vittatus
Platyrrhinus albericoi
Platyrrhinus matapalensis
Platyrrhinus helleri
Platyrrhinus recifinus
Platyrrhinus brachycephalus
Ectophylla alba
Sturnira aratathomasi
Sturnira bidens
Centurio senex
Ametrida centurio
Pygoderma bilabiatum
Sphaeronycteris toxophyllum
Ardops nichollsi
Ariteus flavescens
Artibeus amplus
Stenoderma rufum
Phyllops falcatus
Artibeus glaucus
Dermanura aztecus
Dermanura incomitatus
Dermanura bogotensis
Dermanura phaeotis
Dermanura tolteca
Dermanura anderseni
Dermanura cinereus
Artibeus concolor
Artibeus obscurus
Artibeus planirostris
Artibeus lituratus
Artibeus jamaicensis
Artibeus fimbriatus
Artibeus intermedius
Artibeus inopinatus
Artibeus fraterculus
Artibeus hirsutus
Enchisthenes hartii
Lonchophylla thomasi
Lonchophylla hesperia
Lonchophylla dekeyseri
Lonchophylla spurrelli
Lonchophylla robusta
Lonchophylla chocoana
Lonchophylla handleyi
Lonchophylla mordax
Hsunycteris thomasi
Lonchorhina aurita
Lonchorhina inusitata
Lonchorhina orinocensis
Diphylla ecaudata
Diaemus youngii
Desmodus rotundus
Micronycteris schmidtorum
Micronycteris hirsuta
Micronycteris matses
Micronycteris megalotis
Micronycteris microtis
Micronycteris minuta
Micronycteris giovanniae
Micronycteris homezi
Macrotus californicus
Macrotus waterhousii
Furipterus horrens
Noctilio leporinus
30
20
10
0
mya

Figure 1. Molecular phylogeny of Phyllostomidae using *Moormops*, *Noctilio*, *Pteronotus* and *Furipterus* as outgroups reconstructed using a Bayesian inference analysis (BEAST software). The numbers next to the nodes indicate the posterior probability (color blue) and divergence date (color black). Color for species names and branch designate traditional subgroups in family.

3.2 Divergence Times

Basal node, which families Moormopidea, Noctilionidae and Phyllostomidea divergence shows an age of 38.1 MYA and Phyllostomidae (basal node) is 28.12 MYA (Figure 1). First, divergence times calculated for Phyllostomidae members were: Phyllostomidae: 27.9 MYA; Glossophaginae: 28.12 MYA.; Choeronycterinae: 12.42 MYA; genus *Leptonycteris:* 17.02 MYA; they differed to previous authors (Flores, 2007; Hoffman et al., 2008). We obtained a crown age for the Phyllostomidae family of 28.12 MYA and for the subfamily Glossophaginae of 18.86 MYA. The origin of genus *Leptonycteris* and *Glossophaga* crown groups were 13.91 and 8.06 MYA respectively. While the divergence between *Leptonycteris*+*Glossophaga* and *Monophyllus* is 18.84 MYA. This are important dates for nectarivorous bats and mainly for bats whose feeding sources depend on *Agave* nectar rewards.

The genus *Macrotus* (mainly insectivorous) shows a date of 9.11 MYA crown age. The subfamily Desmodontinae (crown group), which is made up of the only three hematophagous species, originated 14.44 MYA Phyllostominae (crown group), where we find most of the insectivorous and carnivorous bats, without including all of its members, a date of 22.53 MYA is obtained, originally. Most of fruits-feeding bats (crown group) is estimated to be 21.93 MYA. The genus *Sturnira* is 6.67 MYA. For the Glossophaginae subfamily (basal group) a date of 21.78 MYA. Among the nectar-eating bats we find the clade of the loncophilines with an age of 14.45 MYA, very similar to the age of the Choeronycterini group for which an age of 12.42 MYA was calculated. One of the bats that has been identified with the greatest relationship with plants of the Cactaceae and Agavacea families has been classified within the Choeronycterini (crown group) is *Choeronycteris mexicana*, which is 9.62 MYA. Regarding the clade of the genera *Leptonycteris* and *Glossophaga*, both with representatives in Mexico and associated

with the consumption of nectar and pollination of agaves and cacti, the ancestral node presented an age of 17.21 MYA. The genus *Leptonycteris* has an age of 13.91 MYA while *Glossophaga* is 8.07 MYA.

Diversification rates

We detected two pulses in the diversification rate of the family Phyllostomidae (Figure 2) based on the Bayesian phylogeny we constructed. The first pulse was detected at the Phyllostomidea crown group node (0.1722) when the process of diversification of the family began 28.12 mya. While the second pulse was detected coinciding with Phyllostominae crown group node (the largest group of the family) and it increased (0.296) 15.73 MYA. Topology of phylogenetic reconstruction of Phyllostomidea is congruent with these two pulses, the first one related with the origin of family and the second one related with the Stenodermatinae group (The most numerous group). *Leptoncyteris* arises while diversification rate was between 0.21 and 0.16 sp/million years. In contrast, Stenodermatinae developed all groups whit a diversification rate about 0.29 sp/million years.

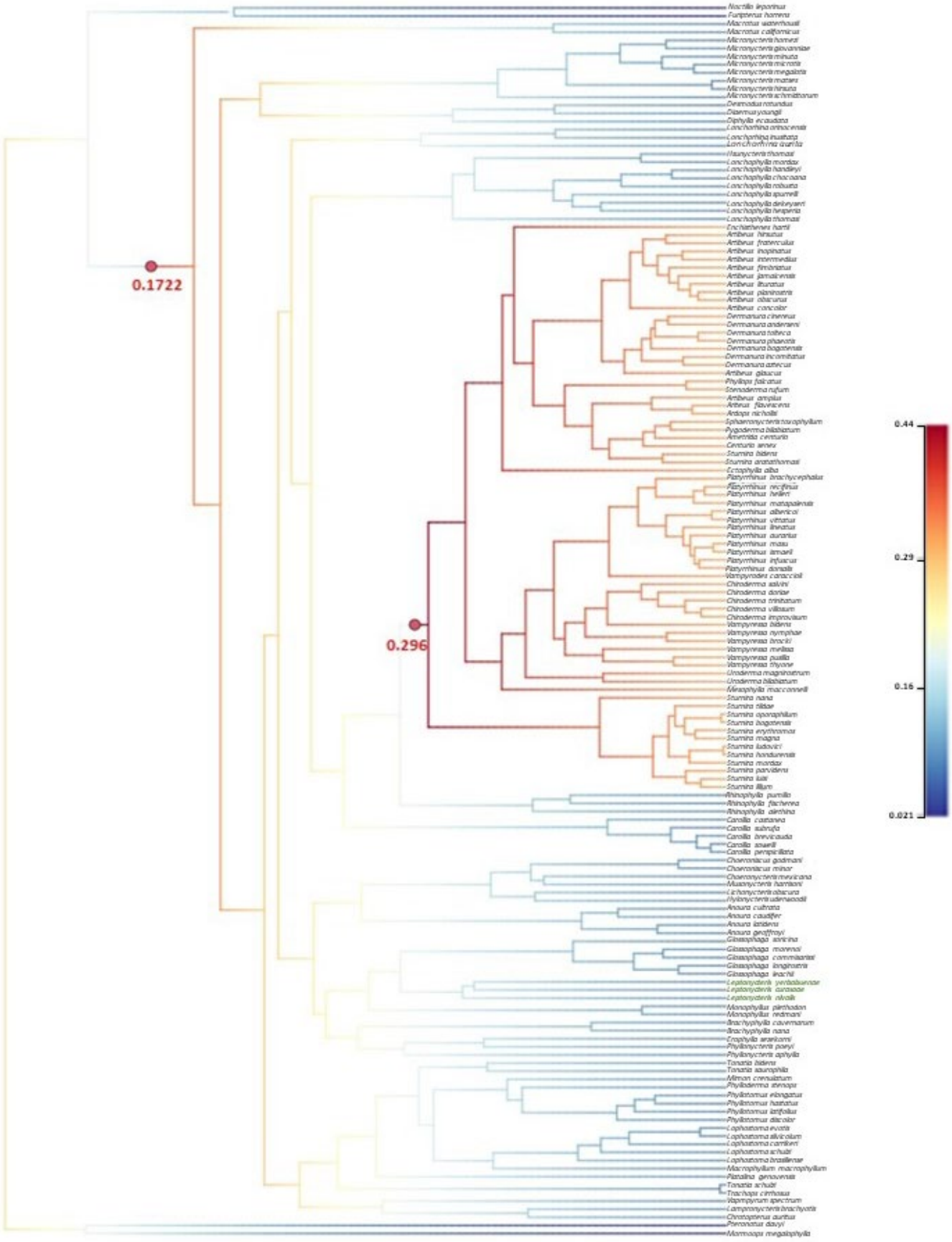


Figure 2. Timing of Phyllostomidae diversification. Chronogram derived from the maximum clade

credibility tree estimated with BEAST. Numbers indicate values of divergence rate, color represent the numeric scale added in right side of the figure.

3.3 Ancestral characters reconstruction

3.3.1 Feeding habits

Our reconstruction of ancestral characters of feeding habits resulted in an insectivore ancestor of the family Phyllostomidae (Figure 3). The outgroups used as sister species of family Phyllostomidea *Pteronotus davyi, Mormoops megalpphylla, and Furipterus horrens* are also insectivorous bats but, in constrat, *Noctilio leporinus* is a piscivore species. However, for the whole Phylogenetic reconstruction the common ancestor is an insectivorous bat.

For the family Phyllostomidea, we observed only one event of fruit-feeding habit origin, in the divergent node of groups Stenodermatinae and Glossophaginae just as the nectarivore origin.

The ancestral reconstruction with phytools software considers the ability of all phyllostomids to consume insects, and in fact in most of the bat members of this group that have been studied in depth, remains of these small arthropods have been found. There are a few representatives of omnivore bats whose species are grouped in the subfamily Phyllostominae, they are five species with a wide range of food sources. And there are an autoapomorphic group with three monospecific genera (Desmodontinae) whose members are exclusive hematophagous, they are the only one's species around de world which can consume blood as feeding habit.

In the case of nectar feeding habit, as mentioned above, emerged in a divergence event between the groups Stenodermatinae and Glossophaginae, but there are some nectar-feeding bats which are able to consume fruit as secondary food source, even *Leptonycteris yerbabuenae* has been recorded feeding of columnar cactus in the center and northern of its distribution (Rojas-Martínez et al., 2012).

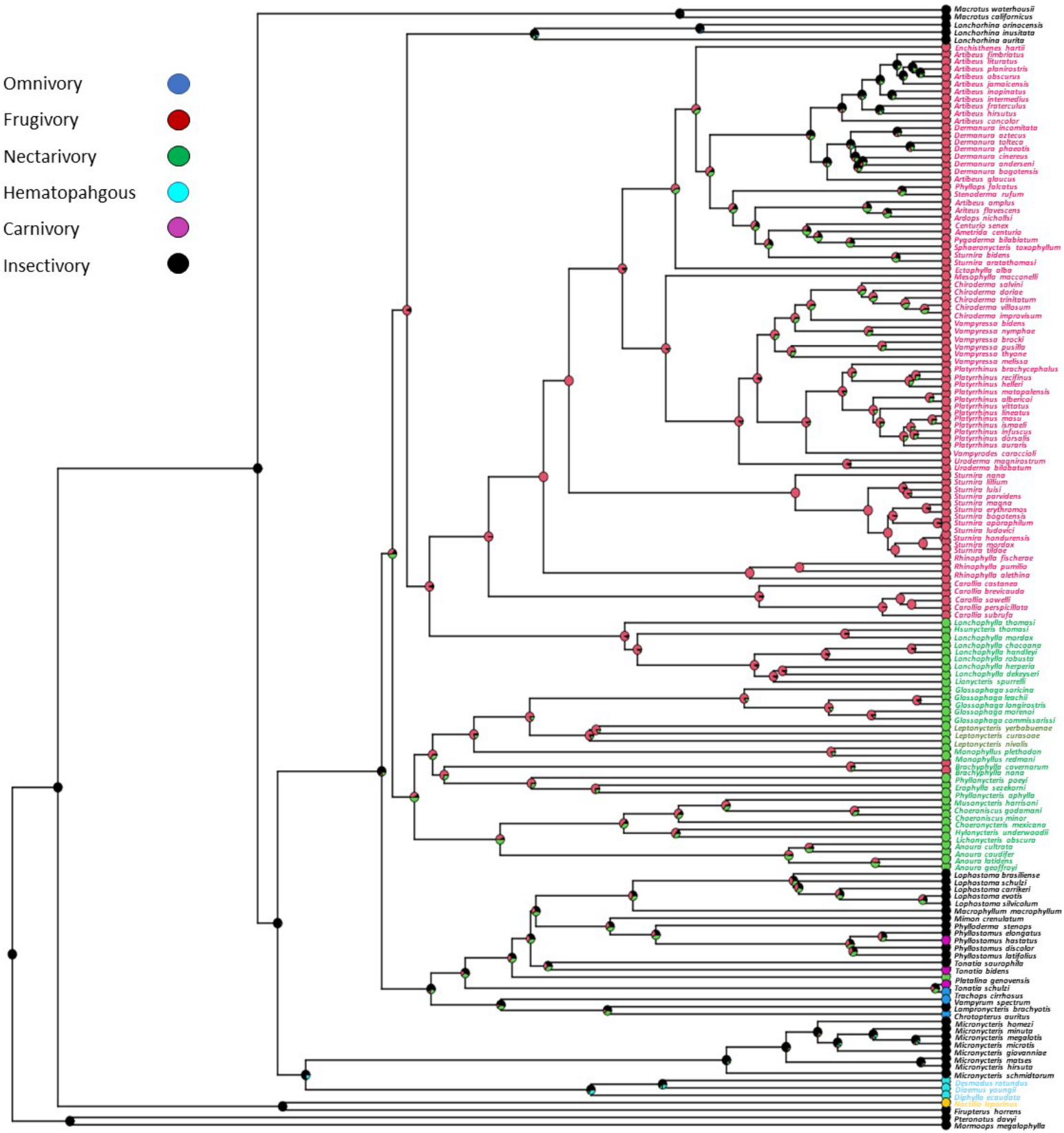


Figure 3. Ancestral reconstruction of feeding habits mapped onto Phyllostomidae chronogram resulting

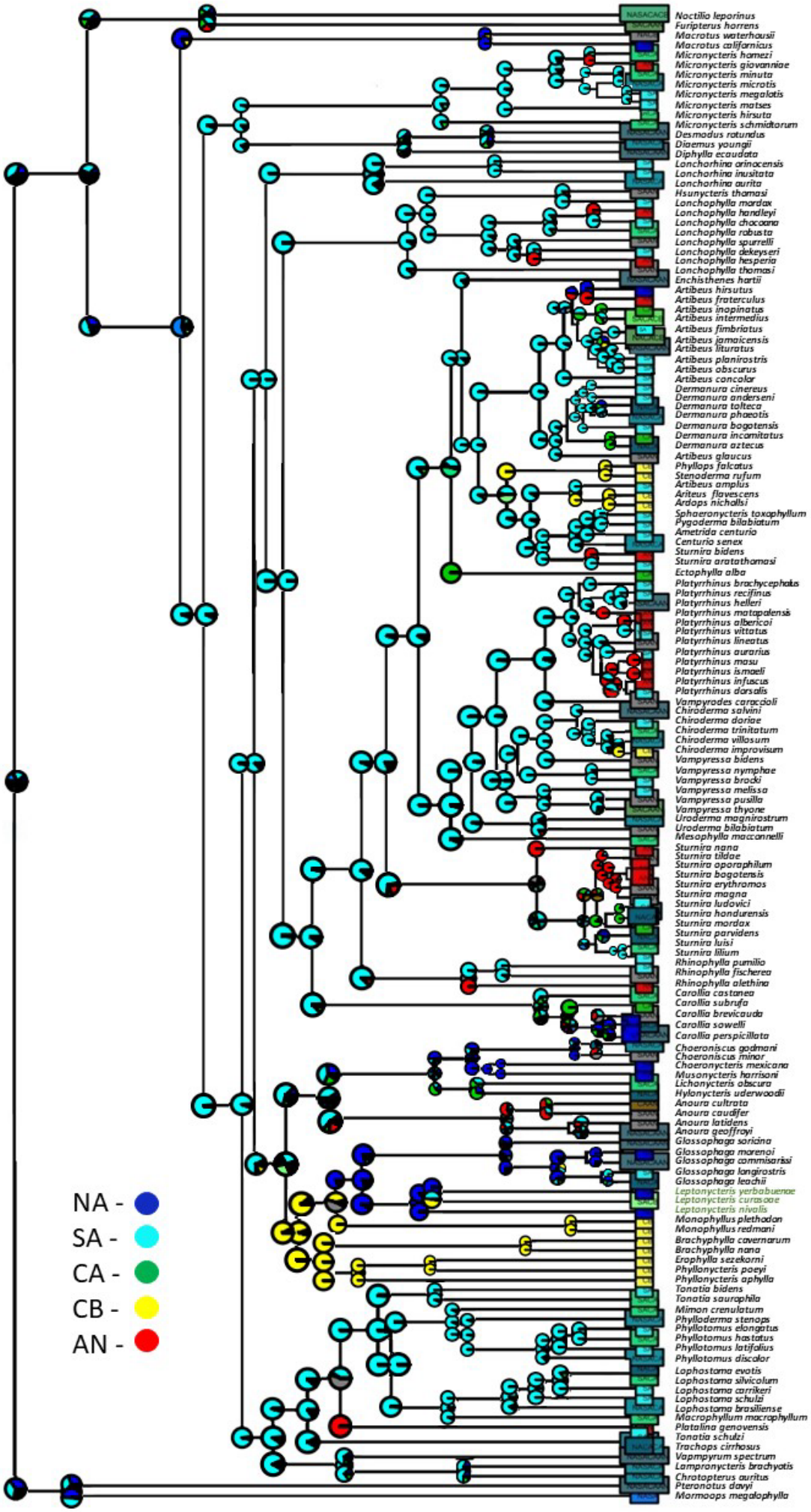
from BEAST analyses. Circles are coded to represent the actual feeding habits. The pie charts show the probability values generated from Phytools for the ancestral feeding habits reconstructed at each node.

3.3.2. Ancestral areas

The dispersal-vicariance analysis (DIVALIKE+J) was the best model selected for the ancestral area reconstruction. We present the results derived from DIVALike+J, LnL = -457.04 (Fig. 4, Table 2). The final mapping on our phylogeny shows that the common ancestor of Phyllostomidae, in our analysis, was present in South America area. In the same way, Glossophaginae crown group arose in South America, but a nectar-feeding bats group arose in NA, including the common ancestor of genus *Leptonycteris*. This model (DIVALike+J) is similar to DEC+J model which assumes founder-event speciation, thus we think that there is some probability for species to colonize adjacent areas.

Table 2. Comparation of BioGeoBEARS results values for six different models with Maximum Likelihood associated values.

	LnL	numparams	d	e	j	AIC	AIC_wt
DEC	-611.5224	2	0.0503716	0.030354	0	1227.04483	2.20E-67
DEC+J	-611.5229	3	0.0503542	0.030384	1.00E-05	1229.04581	8.10E-68
DIVALIKE	-463.7869	2	0.0290587	1.00E-12	0	931.573969	0.00318865
DIVALIKE+J	-457.0424	3	0.0262457	1.00E-12	0.0240896	920.084911	0.99637698
BAYAREALIKE	-498.2564	2	0.0189289	0.065176	0	1000.51285	3.42E-18
BAYAREALIKE+J	-464.7804	3	0.0117561	0.035892	0.034938	935.560841	0.00043438



BioGeoBEARS DIVALIKE+J LnL=-463.79

Figure 4. Ancestral reconstruction of ancestral areas mapped onto Phyllostomidae chronogram resulting from BEAST analyses. Circles are coded to represent the actual biogeographic area of species distribution. The pie charts show the probability values generated from BioGeoBEARS for the ancestral distributions reconstructed at each node.

4. DISCUSSION

Our phylogenetic reconstruction is consistent with proposals made previously, maintaining the main monophyletic groups (Rojas et al., 2016). In our work, the main attention was focused on the Glossophaginae group and particularly on the *Leptonycteris* genus, which maintain the same topology as in previous proposals, even those based on different markers (Datzmann et al., 2010; Rojas et al., 2011; Flores-Abreu et al., 2019). The genealogical relationship between the three species of the genus was maintained in the traditional way (Wetterer et al., 2000), in which *L. curasoae* and *L. yerbabuenae* form a monophyletic group and *L. nivalis* is found as the basal species. Cladogenesis and speciation events underwent accelerations in two different events and temporalities, the first occurred around 28.12 million years ago, associated with the crown node of the Phyllostomidae family. Meanwhile, the second (the highest rate) acceleration in the rate of diversification occurred at 15.72 million years associated with the Stenodermatinae group, the largest group within the phyllostomids (Fig, 2). We expected that the rate of diversification would also increase when nectarivorous species emerged, however, our analysis shows an almost constant rate from emergence and remains so during glossophagine diversification.

The climatic conditions that we can associate to the first acceleration in the diversification rate of the family correspond to a change in global temperature with an increase in temperature during the Oligocene and a subsequent glaciation (Zachos et al., 2001), which gives way to the Miocene and the establishment of most of the current vegetation and ecosystems, when the Miocene begins another variation in global temperature decrease occurs (Roth-Nebelsick, 2004; Blisniuk et al., 2005; Van der Hammen et al. , 2000; Alroy et al., 2000).

During the Miocene (23 - 6 million years ago) a change in the vegetation can be observed in several areas of the continent of about 20 MYA, this change is reflected in the diet of some organism as horses that inhabited North America 20 to 15 MYA and that it was related to a diversification of this group (Wang, 1994). Thus, we can think that this was a very important time for bats that specialized in the consumption of plant sources (either fruits and/or nectar). This time range coincides with the second acceleration in the rate of diversification and during this geological period all the subfamilies and most of their genera arise (Fig. 2).

In the period of the late Miocene and the transition to the Pliocene there is a change in the diet of herbivores and a change in the fauna around the world. Significant faunal changes have been inferred in Pakistan, North America, South America, Europe, and Africa, for instance, in southern North America the change in herbivores diet occurs between 6.8 and 5.5 MYA (Cerling et al., 1997).

In the case of nectar-feeding bats, the diversification conditions could be associated with the increase in floral resources, in terms of the increase in species, as well as the climatic changes that favored this diversity, since there could be a corridor of arid and semi-arid areas connected during the advance of the glacial Pleistocene (Wilkinson and Fleming, 1996; Fleming and Nassar, 2002).

In order to relate more clearly the events of speciation and/or radiation of phyllostomid bats, it is necessary to determine their center of origin. In this sense, there are proposals that, although accepted, have not been able to reach an accurate consensus due to the nature of the fossil record. In that sense, the oldest phyllostomid known fossil record is from La Venta, Colombia (Czaplewski, 2003), indicating that they were found very early in their history in South America (Dávalos, 2006), this information coincides with our inference throughout BioGeoBEARS analysis (Fig. 4). Ancestral area reconstructions show that the ancestor of all Phyllostomidae lived in the SA region in the Eocene. In another study before, they proposed a Neotropical origin for the family (Rojas et al., 2016). The current distribution of phyllostomids in North America has been explained by reversible colonization dynamics from South America to

the Antilles or from South America to Mexico and later colonization to the Antilles. Both hypotheses are not opposed and would help us to explain the dispersal and speciation events in some glossophagine species such as *Glossophaga* and *Leptonycteris* (Dávalos, 2007).

It is very likely that the common ancestor of the genus *Leptoncyteris* was distributed in the NA area (North America) approximately 13-17 million years ago. Based on previous analyzes of the species *L. yerbabuenae* with phylogeographic methods (Trejo et al., 2021) and with climatic data in conjunction with the species *L. nivalis* (Trejo et al., unpublished), we think that the center of origin of the genus is in the Central-Southern area of Mexico, in the same area where there are important diversification centers for the *Agave* and Cactaceae groups.

All three species of the genus *Leptonycteris* had not been included in any of the previously reconstructed molecular marker-based phylogenies. We think that it is very important to be able to carry out the analyzes that would allow us to infer the evolutionary history of the genus. Due to this, the three species were included in this research to be able to calculate more precisely the times of divergence and origin of the three species using phylogenetic tools and evolutionary inferences based on ecological and biogeographic information.

There is some evidence of the characteristics of the Miocene in America, particularly in Mexico. Paleopalynological studies report that there was a high plant diversity with generic flora very similar to the current one since the middle Miocene (Ramírez-Arriaga et al., 2014). It has data on the presence of cloudy mountain forest, pine-oak forest, evergreen sclerophyllous scrub and tropical deciduous forest. Many of these elements have been preserved since the beginning of the Neogene and part of the predominant floristic elements are cacti and members of the *Agave* group, these records belong to the Middle Miocene period (Ramírez-Arriaga et al., 2014). Thus, it makes sense to think that the bats of the genus *Leptonycteris* had different resources in which to specialize, especially considering that these resources were also going through a phase of diversification and expansion in terms of their distribution.

The divergence times within the subfamily Glossophaginae are consistent with two pulses of acceleration in the rate of diversification of the *Agave sensu lato* group, the first 8 MYA in

which the divergence of bats of the genus *Glossopahaga* coincided (8.06 MYA crown group) and the second 3-2.5 MYA (Good-Ávila et al., 2006) and with the most recent reports with a pulse 6.18 and 4.91 MYA (Jiménez-Barron et al., 2020). *Agave sensu lato* has an age of the basal group of 9 million years and the crown group of 6.18 million years. Although the times of diversification of the *Leptonycteris* genus do not directly overlap with the times reported for the *Agave* group, we can observe temporal closeness between the diversification events that involve both groups. The Striata group diversified with a basal age of 4.15 and the crown group with 2.24 MYA. For the *Agave sensu stricto* group, they reported an age of between 3.55-2.68 MYA.

According to the previous data, we could think of a demographic process in which the bats of the *Leptonycteris* genus exploit the resources offered by the members of the *Agave* and thus monopolize this important resource in their distribution areas. Based on the analyzes presented in this thesis, we can begin to explain in a timely manner the circumstances surrounding the species of the genus *Leptonycteris* and, in particular, how these events occurred, mainly the process of divergence between the species *L. curasoae* and *L. yerbabuenae*. Considering that *L. curasoae* is geographically isolated from the other two species (*L. nivalis* and *L. yerbabuenae*).

The speciation event that gave rise to the species *L. curasoae* seems to be the result of a vicariant event in which the long-nosed South American bat was completely isolated when connectivity was broken in Central America, a process that is thought to have taken place during the Pliocene (5.33 – 2.59 mya). This hypothesis is based on the fact that a probable contraction of the montane cloud forest has been reported in the area and expansion of the deciduous tropical forest and arid zones, during the late Pliocene and early Holocene (Rosales-Torres et al., 2017). In addition, all these divergence events coincide with a decrease in the temperature of the humid tropical climates in Mexico during the glacial periods between 5.3 and 1.8 million years ago (Van Devender, 2000).

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Capítulo 3. *Historical, temporal and geographic dynamism of the interaction between Agave* and *Leptonycteris* nectar feeding bats (Enviado a American Journal of Botany)

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Keywords: *Agave*, *Leptonycteris*, ecological interaction, geographic congruence, diffuse coevolution

Abstract

The interaction between ecological and evolutionary processes has been recognized as an important factor shaping the evolutionary history of species. Some authors have proposed different ecological and evolutionary hypotheses concerning the relationships between plants and their pollinators, and a special case is the interaction and suspected coevolution among *Agave* species and their main pollinators, the *Leptonycteris* nectar feeding bats. *Agave* species have in general a pollination syndrome compatible with chiropterophily, including floral shape and size, nocturnal nectar production, as well as nectar quality and sugar concentration. The geographic distribution and flowering time of different *Agave* taxa have been considered as important factors in modeling the distribution patterns of the two Mexican migratory nectar feeding bats. Our goal was to analyze the interaction *Agave* - *Leptonycteris* and its dynamics during the Pleistocene using Ecological Niche Models (ENMs) and three climate scenarios:

Current, Last Glacial Maximum (LGM) and Last InterGlacial (LIG). We modeled the *Agave* - *Leptonycteris* interaction in its spatial and temporal components. We observed the historical presence of *Agave* - *Leptonycteris* interaction corridors, with stability from the LGM to the present. We propose an interaction refuge in the area of Meztitlán/ Tehuacán-Cuicatlán, where *Agave- Leptonycteris* interaction has been probably remained active over the last 2.5 Mya. We found that over the migratory routes of each *Leptonycteris* species analyzed, there is an interaction with different *Agave* species, while during the non-migratory season both bat species consume nectar of almost the same *Agave* taxa, supporting the diffuse coevolution among *Agave* and *Leptonycteris* bats.

Introduction

A fundamental underlying goal of biogeography is to understand the distribution patterns and interactions of the biota, thus enabling the understanding of current ecological processes which influence the distribution of species, allowing to make inferences about the past. Interactions between species are as evolutionarily malleable as the species themselves and have played a central role in the diversification and organization of life, creating complex geographic mosaics of interspecific interactions with the potential to evolve (Thompson, 1999). Moreover, some authors claim that many interaction patterns are maintained over time by phylogenetic relationships and concordance among interacting groups, showing that species can be conservative when it comes to the taxa with which they interact, both spatially and temporally (Balashov, 1984; Thompson et al., 2006; Morgan et al., 2007; Stephens et al., 2009). Despite the central importance of geographic mosaics of interspecific interactions to the diversification of life, much is still unknown about how this mosaic of interactions has changed over time and even more, what can be the possible implications of these changes on evolutionary processes. There is evidence that many of the interaction networks are highly asymmetric (*i.e.,* while many plant species depend strongly on an animal species, the involved animal species depends

weakly on these plants), however, a high degree of dynamism in the interaction can enhance long-term coexistence and facilitate biodiversity maintenance (Bascompte et al., 2006).

Some authors, since Darwin (1959), have proposed different ecological and evolutionary hypotheses concerning the relationship between plants and their pollinators, and for instance, pollination syndrome ideas were developed from those observations (Faegri and van der Pijl, 1979). A special case is the relationship among *Agave* species and some Mexican nectar feeding bats. *Agave* species exhibit morphological features that are congruent with a chiropterophilc pollination syndrome (*i.e.,* floral shape and size, nocturnal nectar production, nectar quality and sugar concentration). Additionally, the broad geographic congruence between *Agave* species and *Leptonycteris* bats, as well as the temporary correspondence between flowering and migration timing (Moreno-Valdez et al., 2004; Molina-Freaner and Eguiarte 2003; Peñalba et al., 2006; Gómez-Ruiz and Lacher, 2016; Burke et al., 2019), have led several authors to suggest that the *Agave-Leptonycteris* interspecific relationship may be an example of diffuse co-evolution (Gentry, 1982; Arita and Humphrey, 1988; Arita and Martínez del Rio, 1990; Rocha et al., 2006; Flores-Abreu et al., 2019), meaning that both groups have evolved to maintain an ecological mutualistic interaction through time, that can be observed in morphological and behavioral features. Under this hypothesis, *Agave* species provide bats with critical resources to carry out their migration, these resources are offered under a chiropterophilic syndrome, and in return the bats ensure cross-pollination, resulting in the maintenance of genetic diversity in *Agave* populations and resilience to environmental stress (Rocha et al., 2006; Trejo et al., 2015).

Indeed, plants from the genus *Agave* are considered as keystone species in the communities of arid and semiarid Mexican areas and are essential for pollinators due to their significant nectar production (Good-Avila et al., 2006; Fleming et al., 2009; Eguiarte et al., 2013). Even when a few *Agave* species are pollinated mainly by daytime visitors (*A. toumeyana, A. parviflora, A. chrysantha and, A. marmorata*), such as birds and insects

(Schaffer and Schaffer, 1977; Slauson, 2000; Ornelas et al., 2002), several researchers have recorded an intense ecological relationship between the genus *Agave* and *Leptonycteris nivalis* and *L. yerbabuenae,* even in *A. chrysantha* that is known to be a daytime pollinated plant (Álvarez and González-Quintero, 1970; Easterla, 1972; Howell, 1979; Howell and Hart, 1980; Howell and Roth, 1981; Arizaga et al., 2000; Molina-Freaner and Eguiarte, 2003; Scott, 2004; Rocha et al., 2005; Sánchez and Medellín, 2007; Trejo et al., 2015; Flores-Torres and Galindo-Escamilla, 2017; Flores-Abreu et al., 2019; Martínez, 2019). While there are several studies that address the *Agave*-bat interaction, these only analyze some *Agave* species (no more than 10; Rocha et al., 2006), so there can be unknown different dynamics of *Agave*-pollinator interactions. Furthermore, none of these studies have made inferences about the stability of this interaction during the Pleistocene, limiting the possibility of drawing conclusions from an evolutionary perspective, such as if there are divergent characters in pollination syndromes or if main pollinators are the same for every species in the genus.

Both *Leptonycteris* bat species from Mexico are migratory, and their distribution covers most of the country territory, as well as some locations in the border with the United States of America (Arita and Santos Del Prado, 1999). *Leptonycteris yerbabuenae* has a wide distribution in Mexico, with a stronger presence in tropical climates than *L. nivalis,* that has a more restricted distribution to Central Mexico and the Mexican portion of the Chihuahuan desert (Easterla, 1988; Hensley and Wilkins, 1988; Arita, 1991; Cole and Wilson, 2006).

However, *Leptonycteris* migratory dynamics are complex (Fig. 1). In the case of *L. yerbabuenae,* it is known that just only a proportion of females migrate (mainly pregnant and nursery females) during spring to give birth and feed offspring during summer (Ceballos et al., 1997; Rojas-Martínez et al., 1999; Stoner et al., 2003; Cole and Wilson, 2006; Medellín et al., 2018), while males stay around their birth caves and just exhibit altitudinal movements (Herrera, 1997). When autumn begins, females and their offspring fly away to winter shelters (Fig. 1; Cole and Wilson, 2006). In addition, there are resident colonies all through the year (Herrera, 1997; Stoner et al., 2003; Riechers et al., 2003; Galindo et al., 2004), with matings

occurring during the autumn and winter months (Galindo et al., 2004).

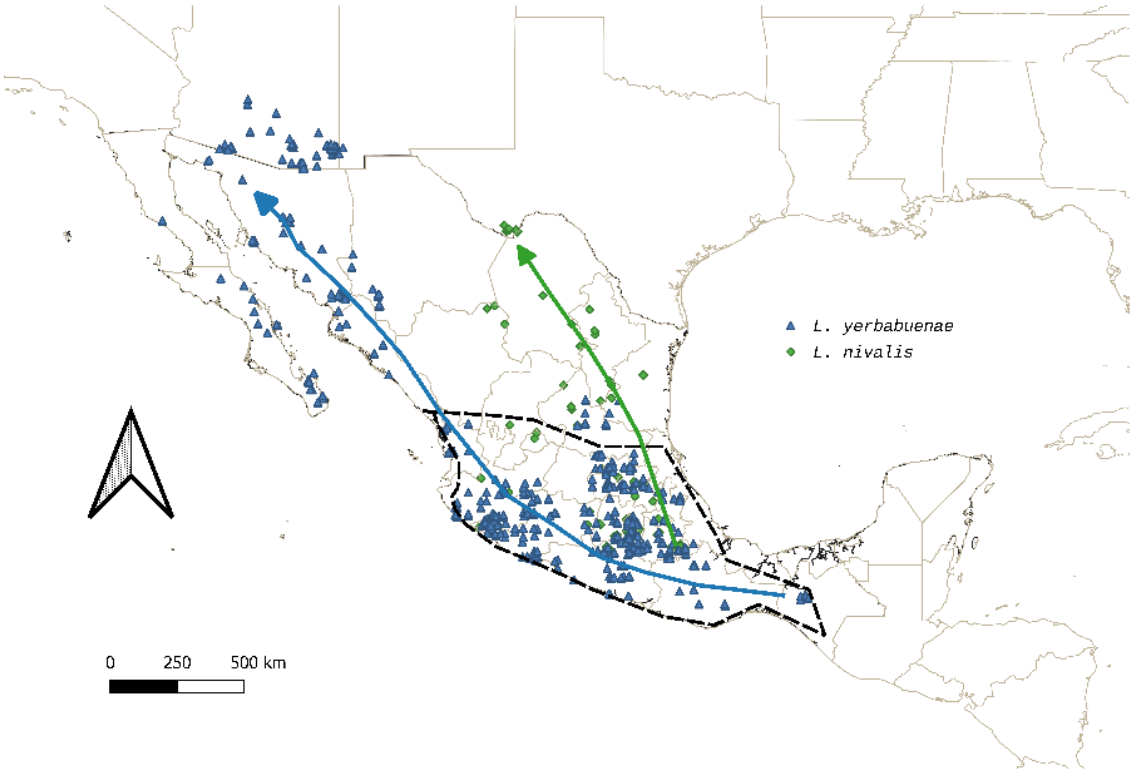

Figure 1. *Leptonycteris* distribution and migration routes. Blue corresponds to *L. yerbabuenae* spring (migratory) movements and green corresponds to *L. nivalis*. The dotted line polygon represents the areas of presence of individuals round-year.

Leptonycteris nivalis performs a migration in the same seasons of the year that *L. yerbabuenae* (Moreno-Valdez et al., 2000), however its migration has been less studied, and thus it is still not clear if movements are made by both sexes or just females, but resident colonies and two different reproductive seasons have been observed at the south of their distribution, similar to *L. yerababuenae* (Ramos-Frías, 2013).

In this study, we proposed a historic biogreographic approach to solve the question ¿How has the ecological interaction among *Leptonycteris* and *Agave* evolved through time? We expected that the ecological, as well as the evolutionary relationship among Mexican *Leptonycteris* species bats and the genus *Agave* would be reflected by overlapping their potential distributions in both current and past time periods. We supported this hypothesis based on evidence of potential *Agave* pollination by *Leptonycteris* bats and of female bats

migration associated with the flowering of several *Agave* species in the migration route. Furthermore, we expected that the 94 *Agave* species used in this study would coincide at least in one time period with the temporal prescence of the *Leptonycteris* species and that *Agave* blooming time in some species would be coincident with the bat migration seasons.

To answer this question and to explore the above hypothesis, we analyzed the interaction between *Agave-Leptonycteris* species at present, and explored the interaction in the past (*i.e.*, in the Last Glacial Maximum (LGM) and Last Interglacial (LIG), with the aim of understanding the ecological and evolutionary history of this interaction. These current and past models can help to solve questions such as for how long the migration of female nectar-feeding bats has occurred. Also, our analyses can help to determine priority conservation areas, where the best and more stable environmental conditions occur for the development and maintenance of the mutualistic interaction.

Methods

DataBases

Agave data: For each *Agave* species, names were homogenized following taxonomy criteria from Gentry (1988) (see details in Scheinvar, 2018). Enrique Scheinvar provided all models of *Agave* taxa distribution; the corresponding methodological details are described in Scheinvar et al., (2016), Scheinvar (2018) and Scheinvar et al., (submitted, "Evolutionary implications of ecological patterns and range shifts during past climate change in *Agave*"). *Agave* data sets were constructed from different presence records databases (GBIF, https://www.gbif.org/; REMIB, http://www.conabio.gob.mx/remib; UNIBIO, http://www.unibio.unam.mx/) as well as personal observations (Scheinvar, 2018). Number of historic records for each species goes in an extensive range from 3 to 705. However, the majority of species are represented by less than 30 historic records. In order to restrict environmental adequacy values and eliminate those that lack interpretive value, we employed the 10th percentile value of training sample points as

a threshold. This value assumes that 10% of records used for model generation are susceptible to error. All values below this threshold were assigned to 0.

To obtain the observed richness map of *Agave* (excluding those with records of daytime effective pollinators; Table 1), we used the modeled distribution of each taxa, and all values over zero were assigned to one; then we constructed algebra maps. We obtained three modeled richness maps as the sum of all individual binomial presence-absence models at Current, LGM an LIG scenarios. All map processing was done in R using the raster library (Hijmans and van Etten, 2012).

Leptonycteris data. We constructed databases with the presence records of *L. yerbabuenae* (n = 499) and *L. nivalis* (n= 137) from museum specimen data, as well as our own observations. We use data from the following collections: National Collection of Mammals Institute of Biology, UNAM (CNMA); Mammal Collection of the Zoology Museum “Alfonso L. Herrera” Science Faculty, UNAM; Mammal Collection of UAM University and Mammal Collection of National School of Biological Sciences of IPN.

The information contained in the database corresponds to the geographical coordinates and date of collection, measurements of the last phalanx from the right wing and record of the presence / absence of hair in the uropatagium and sex, to exclude historical wrong identification confusion among both *Leptonycteris* species (Arita, 1991). According to the month of collection, the records were classified into two categories: reproductive season (from October to February) and birth/breeding season (from March to September). For each species, duplicate records were eliminated, as well as records separated by less than 1km, in order to avoid spatial autocorrelation and bias in the distribution model estimation. Furthermore, we employed the 10th percentile value of training sample points as a threshold, as done for *Agave* models.

Ecological niche models (ENM)

Variable selection

After the initial filter, an additional validation was carried out using information on habitat, distribution and taxonomic status. Once a database with all valid records was constructed, we used 19 bioclimatic variables (from the WorldClim 1.4 data set (Hijmans et al., 2005; http://www.worldclim.org) and six topographical layers from the HYDRO1k Elevation Derivative Database (available at: http://lta.cr.usgs.gov/HYDRO1K) to initially perform a variance inflation factor analysis, in order to select the variables to be used in SDMs, and the calculation of Mahalanobis distances matrix. Correlated bioclimatic variables were eliminated, leaving a unique informative variable (Oren 2003; Yang, 2020). The later was used to identify outlier records that could be a potential source of error, which were removed from further analyses. These analyses were performed using R (http://www.r-project.org).

The predicted distribution was projected into the two time periods in the past: the last interglacial (LIG) (*c.* 120,000–140,000 ka; Otto-Bliesner et al., 2006) and the Last Glacial Maximum (LGM) (*c.* 21,000 ka), based on the general circulation model CCSM (Collins et al., 2006). For North American desert species, the MIROC and CCSM general circulation models have previously resulted in similar responses (Wilson and Pitts, 2012), but CCSM has already been widely used in the reconstruction of past distributions in this region, with congruent results in independent analyses (Martínez-Meyer and Peterson, 2006; Peterson and Nyári, 2007; Gámez et al., 2014; Castellanos-Morales et al., 2016; Scheinvar et al., 2017); for this reason we only used the CCSM model. All current and past layers were resampled to the same projection and to a resolution of 30 arc-seconds (*c.* 1 km) with the raster R package.

For each species, a subset of the less correlated layers was selected (commonality > 0.7 for the most representative, and Spearman's rank correlation coefficient < 0.85 for the less correlated) from the first principal components, which represented at least 80% of total variation (Gámez et al., 2014; Castellanos-Morales et al., 2016). All analyses were performed

with the R package (http://www.r-project.org).

Modeling procedures

In the case of bat species and their associated migratory status, ENMs were derived from three databases: i) total records for the species, ii) records of the species for the non-migrant season, iii) records of the species for the birth/migration season (Figures 1). We generated ENMs for current and past climate conditions with MaxEnt 3.3.3 k (Phillips et al., 2004, 2006; Phillips and Dudík, 2008) using the different databases of occurrence points and the *ad hoc* selection of 11 bioclimatic variables described above to reduce overprojection. We executed MaxEnt using the following settings: 20% random test, 30 replicates, replicate bootstrap type, 1000 maximum iterations, convergence threshold of 0.00001, with extrapolation and clamping turned off. ENMs were built following contemporary published suggestion (Scheinvar et al., 2016; Scheinvar, 2018). However, there are current methods to support models built based on small samples size databases (Morales et al., 2017; Merow et al., 2013).

We derived the distributional model for the species from the average model. We evaluated all the distributional models using the area under the receiver operating characteristic curve (AUC) scores, where values above 0.5 are better than random predictions and those above 0.85 are considered useful (Elith et al., 2006).

Biotic interactions

In order to evaluate the interactions between each *Agave* species and each bat species, Spearman's correlation analyses were carried out (Table 1 and 2). We compared the change in the correlations of each of the 94 *Agave* species and two species of nectarivorous bats and their temporal correspondence throughout their distribution models (Gàbor et al., 2020). This analysis ranges from 0-1 to 1 and based on these values we described intensity of current and historical overlapping distribution and thus, to infer biotic interaction (the R platform 3.6.1 was used for the statistical processing).

We used the distribution maps from the previously obtained niche models of each species of bat (i.e., for *L. yerbabuenae and L. nivalis*), and their corresponding geographical projections to the past (LIG and LGM) to generate a polygon that encompassed the joint geographical distributions for each species and for all the analyzed times (R libraries: raster: Hijmans 2019; rgeos: Bivand and Rundel 2019; maptools: Lewin-Koh et al., 2019; rgdal: Bivand et al., 2019).

The geographical polygons were used as a delimitation area to generate points with a radial distance of 0.2 degrees, which later were used as a reference for the generation of a hexagonal polygon net. For this process we used dplyr (Wickham et al., 2019), tidyr (Wickham and Henry, 2019), sp (Pebesma et al., 2005; Roger et al, 2013), raster (Hijmans, 2019), rgeos: (Bivand and Rundel, 2019), viridis (Garnier, 2018), gridExtra (Auguie, 2017), and rasterVis: (Perpinan-Lamigueiro and Hijmans, 2019).

We used the hexagonal net as a sampling template for each of the relative occurrence rate maps generated by MaxEnt, that range from 0 to 1 (Current, LGM and LIG). For the sampling of these data, we employed the extract function of the raster package (Hijmans, 2019) with the specification that generates an average of the values (by pixel) that would fall within each of the cells of our rack (each hexagon of the network has an apothem of 22.264 km).

The procedure resulted in nine data frames per species of bat (three databases for Current, three for LGM and three for LIG), with the average values per hexagon of 95 taxa, 94 *Agave* and the corresponding bat model. For each Dataframe, a Spearman correlation analysis was performed using psych R library (Revelle, 2019), featured with a Holm-Bonferroni adjustment for p values.

Additionally, in order to analyze the association pattern between the richness of *Agave* vs. the relative occurrence rate of each of the bat species, a correlation analysis was

performed. For this, the values of each *Agave* model were transformed to binary values, where all values below the reference threshold were reassigned with 0 and the rest to 1. We obtained three modeled richness maps, as the sum of all individual presence-absence models at Current, LGM an LIG times. Once the species richness maps were obtained, a Spearman correlation analysis was performed, following the same methodology that for the species-level models, with the difference that in this case the polygon sampling net was constituted by the sum of areas of the richness of *Agave* maps.

Results

Agave distribution patterns

We used a total of 1950 historical records of 54 *Agave* species (Table 1), unfortunately some species were represented by small sample sizes (Scheinvar, 2018), this were going from 3 to 705 records. The current richness map of *Agave* (Figure 2a) shows a distribution from southwestern USA (southern sections of California, Arizona, New Mexico and Texas), over almost all Mexico and to areas of Central America (Pacific slope areas in Guatemala, El Salvador, Honduras and Nicaragua). The most diverse area was the Tehuacán- Cuicatlán valley, with 34 species, as previously reported (García- Mendoza, 2011; Delgado-Lemus et al., 2014) followed by the Central mountains of Oaxaca (i.e., the mezcal region); the Mezquital valley in Hidalgo state and the southern portion of the Sonoran Desert.

The LGM richness pattern (Figure 2b) was very similar to the current richness map, given that the Tehuacán-Cuicatlán valley (with more than 15 predicted *Agave* species at this time), the mountains of Central Oaxaca and the Mezquital valley (with more than 13 *Agave* species) continue with the highest diversity of species, followed by the Sonoran Desert (with more than 10 species). In contrast, the LIG richness pattern of *Agave* (Figure 2c) was different from the Current and LGM. The distribution range of almost all species currently over the Chihuahuan desert, had a reduced distribution to a few pixels; Tehuacán- Cuicatlán valley,

Central Mountains of Oaxaca and the Mezquital valley, and appeared as refugia zones for some species; while those species distributed over the Sonoran Desert, had expanded distribution areas.

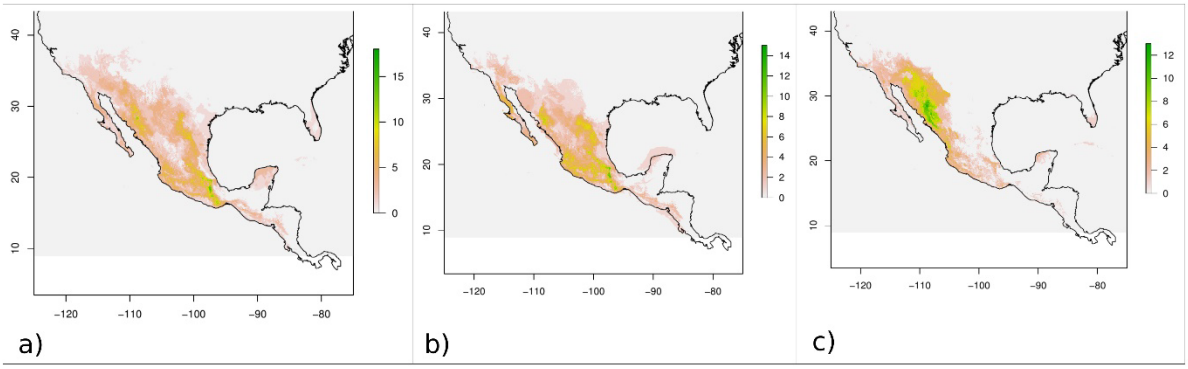

Figure 2. Distribution model of Agave *taxa* richness. 1.a Current, 1.b LGM and 1-C LIG.

Leptonycteris nivalis distribution patterns and correlations with *Agave*. We constructed a database with 137 historical records for describing patterns of distribution of *L. nivalis*. Current time model corresponded with previous studies (Moreno-Valdéz et al., 2000). Furthermore, Current time and LMG models exhibited clear differences from LIG model where distribution was restricted to the center-south of the country.

For the Current time, we detected a significant correlation between the climatic suitability of the species with the climatic suitability of 29 *Agave* members, 21 of these in the area occupied by the species during the reproductive season (15 of these *Agave* species are restricted to this geographical area); and 14 significant correlations with the area occupied during the breeding season, the same ones that were informative for the behavior of the species as a whole, without differentiating the season of the year (Table 1). This suggests that during the breeding season and along the migration route, migrant females can feed of different resources (such as saguaro and other Cactaceae; Medellín et al., 2019) than males or the resident populations.

For the LGM, the interaction with the *Agave* taxa remained similar to the current time in 53% of the species patterns described, being the south of the Chihuahuan desert and central

portion of Mexico, with 15 *Agave* species, the region with the most environmental stable conditions for *L. nivalis* presence, which increased the probability of developing and establishing an abiotic interaction. While for the LIG, the potential distribution geographic models with the most stable conditions showed a lower congruence among *L. nivalis* and *Agave* species, just 3 or 5 cases exhibited geographic congruence, being the most relevant *A. hookeri, A angustifolia* and *A. lechuguilla*. Remarkably, geographic congruence among *L. nivalis* and those three agave species remained stable during the three periods (Current, LGM, LIG); over a small region located in Central-Eastern of Mexico, which today is the southernmost distribution of *L. nivalis* (Table 1, Figure 3).

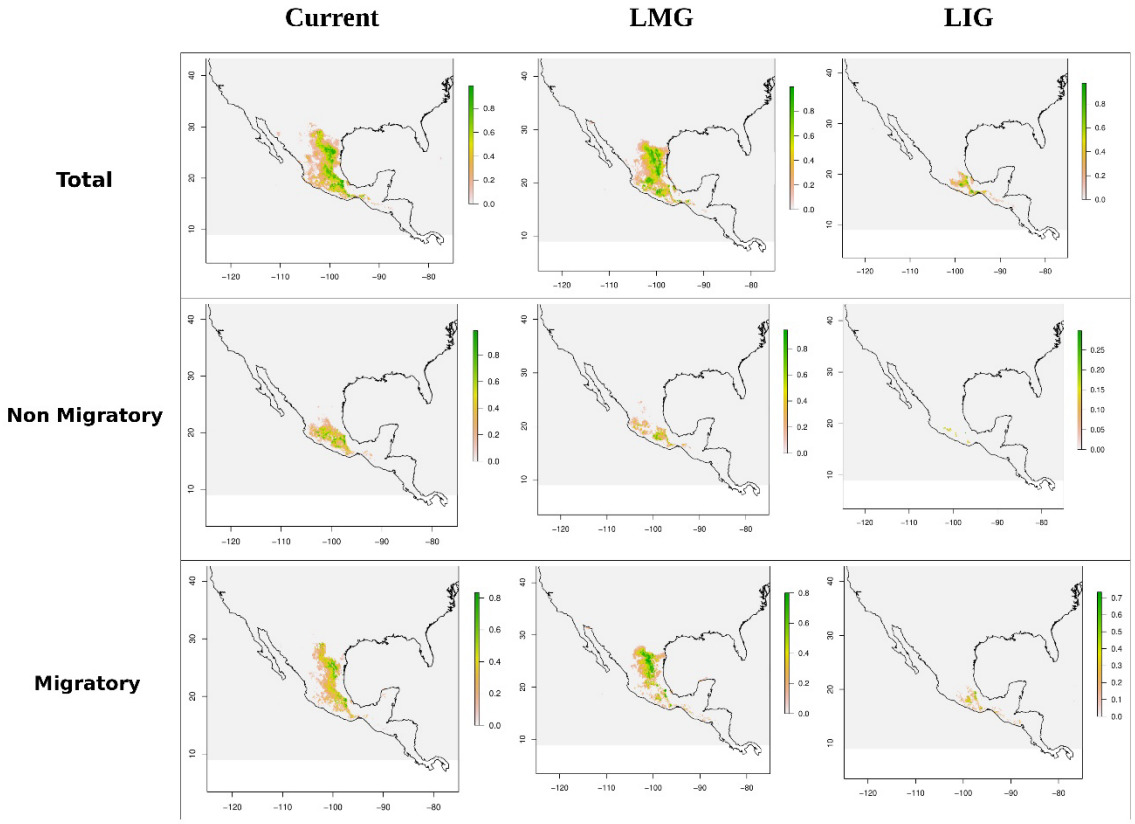

Figure 3. Distribution model of *L. nivalis*, expressed in relative occurrence rate values.

Leptonycteris yerbabuenae distribution patterns and correlations with *Agave*. We used 499 historical records to construct the database for *L. yerbabuenae* ENMs. Current time model

corresponded with previous studies (Fleming et al., 1993; Fleming et al, 1988; Gómez-Ruiz et al., 2016; Burke et al., 2019). Past projections model showed differences from Current time model. However, Current time and LMG models preserved the similar climatic suitability sites, and LIG exhibited more limited climatic suitability areas located on the pacific coast and Mar de Cortés.

For the Current time, we detected a significant correlation between the climatic suitability of the species with the climatic suitability of 33 *Agave* members; of these, 18 also showed a significant correlation with *L. nivalis,* and they are restricted to Central Mexico region, the rest of the species (15) (Table 1, Figure 4), show a wide geographical congruence with the migration route carried out by the females of *L. yerbabuenae* (Figure 1). This suggests that during the birth/migration season and along the migration route, migrant females of both bat species, *L. nivalis* and *L. yerbabuenae*, feed on *Agave* species available on their sites (north and nectar corridor), while the males of both bat species possibly exploit common pollen and nectar resources accessible in the around-year resident sites (Matínez, 2019). There are also 22 Cactaceae members reported as food source in Central Mexico, that are consumed by *L. yerbabuenae* males, while migrant females move to the north (Rojas-Martínez et al., 2012; Martínez, 2019).

For the LGM, the interaction with the *Agave* species remained similar to the current time in 42% of cases, being the area of Central Mexico, with 13 *Agave* species (Table 1), the one that showed the most stable interactions. While for the LIG the geographic congruence between *L. yerbabuenae* and *Agave* taxa was very scarce (3 or 5 cases), being the most constant during the three periods (Current, LGM and LIG) in a small region in Central Pacific coast of Mexico, in the lowlands of Jalisco, Colima and Michoacán (Figures 2, 3 and 4) involving *A. angustifolia*, *A. maximiliana maximiliana* and *A. pedunculifera* (Table 1).

Table 1. Correlation values between the relative occurrence rate values of each *Agave* taxa and *Leptonycteris* species, corresponding to the three climatic scenarios: Current, LMG and LIG. Only shown the cases with "p" value < .001 and "r" value > 0.4

	Current					LMG					LIG				
	Non-Migratory		Migratory		Total	Non-Migratory		Migratory		Total	Non-Migratory		Migratory		Total
Agave species	***L. nivalis***	*L. yerbabuenae*	***L. nivalis***	*L. yerbabuenae*	***L. nivalis***	***L nivalis***	*L. yerbabuenae*	***L. nivalis***	*L. yerbabuenae*	***L. nivalis***	***L. nivalis***	*L. yerbabuenae*	***L. nivalis***	*L. yerbabuenae*	***L. nivalis***	*L. yerbabuenae*
*A. albomarginata***		0.4168	**0.4049**		**0.4049**				0.6287					0.6314		
*A. angustiarum***	**0.5512**	0.5784		0.4617		**0.6564**	0.4178									
*A. angustifolia***	**0.6645**	0.4365		0.6269		**0.6463**			0.6077					0.5124		
*A. applanata***	**0.4278**	0.4348	**0.4032**		**0.4033**									0.4475		
*A. atrovirens_atrovirens***	**0.5686**	0.5637				**0.4711**	0.4310									
*A. attenuata***	**0.4101**	0.4124		0.4617			0.4290									
A. aurea					0.4288				0.6489					0.6187		
*A. bradleana***			**0.5494**		**0.4668**			**0.7564**								
A. bovicornuta				0.4279						**0.6276**						
A. cerulata_cerulata					0.4198				-0.4389					0.4144		
A. cerulata_nelsonii							-0.4062		-0.4189							
A. celsii_celsii			**0.5119**		**0.4723**			**0.4004**		**0.4170**		-0.5500				
*A. chiapensis***	**0.4295**										**0.6623**				**0.4137**	0.4009
A. chrysantha				0.4339										0.4304		0.4558
A. chrysoglossa				0.4641	0.4001				0.5077			0.5594				
A. colimana		0.4129			0.4166											
A. colorata				0.4098	0.4098									0.4398		0.4297
*A. cupreata***	**0.4674**	0.4869		0.4372	0.4236			**0.5804**								
A. delilo_delilo									0.4302			0.4153				
A. deserti_deserti									0.4502			0.5612				
A. deserti_pringlei									0.4116							
*A. difformis***		0.4195	**0.5030**		**0.4636**			**0.4457**								
*A. filifera***	**0.5130**	0.6294	**0.5012**		**0.5131**		0.5032			**0.7495**		0.4063				
A. funkiana			**0.7254**		**0.8641**			**0.7430**	0.4359							
A. gentryi	**0.4454**											0.5502				
A. gracilipes							0.5019		0.4824							
A. guadalajarana							0.4718		0.4288							
A. havardiana								**0.5518**								
*A. hookeri***	**0.5880**				0.4386	**0.5716**	0.4084		-0.5700			-0.5045				
*A. horida***	**0.5045**					**0.5579**										
A. inaequidens_inaequidens	**0.5901**														**0.4467**	
A. karwinskii											**0.6807**		**0.4092**			
*A. kerchoveana***	**0.5078**	0.4456				**0.4334**									**0.4035**	
A. lechuguilla			**0.7563**		**0.6753**			**0.9580**		**0.6581**						
A. macroculmis*			**0.6030**		**0.5144**			**0.4553**		**0.4266**						
*A. majoensis***	**0.6450**	0.6063	**0.4332**		**0.4487**	**0.4945**	0.4294					0.4017				
A. maximiliana_katherinae									0.4157			0.4396				
A. maximiliana_maximiliana				0.4449	0.4097				0.5296			0.4094				0.4178
A. mapisaga*	**0.4107**					**0.4004**								0.4099		
A. marmorata				0.4115	0.4017		0.4254		0.6217			0.6307				
A. ornithobroma					0.4095											
A. parryi_parryi				0.4396					-0.4824			-0.4687		0.4413		
A. parviflora_flexiflora					0.4030											0.4232
A. parviflora_parviflora									-0.4292			-0.4002				
A. pelora																
A. peacockii	**0.4117**										**0.4247**	0.4247	**0.4309**	0.4309	**0.5094**	0.5094
*A. pedunculifera**		0.4356		0.5458	0.5067							0.4332				
*A. polyacantha_polyacantha**	**0.6397**	0.6438		0.5597	0.5600	**0.7307**			0.4116			0.4272				
*A. potatorum***	**0.4811**	0.4437				**0.5033**										
A. rhodacantha				0.5726	0.5648				0.4055			0.4144				
A. schidigera		0.4293		0.4245	0.4089		0.4181									
A. seemaniana							-0.4138									
A. shrevei				0.4118												
A. striata_falcata			**0.6156**		**0.4583**			**0.8376**		**0.5847**						
A. striata_striata			**0.7178**		**0.6429**			**0.5738**		**0.4762**						
*A. stricta***	**0.4900**	0.4726				**0.4193**	0.4753		0.4073			0.4853				
*A. xylacantha***	**0.4389**	0.4726	**0.4752**		**0.6006**											

* *Agave* species distribution which coincide with both *Leptonycteris* at least in one time period and blooming during both migratory and non-migratory bat seasons.

** *Agave* species distribution which coincide with both *Leptonycteris* species at least in one time period.

Bold letters correspond to *Agave* species with blooming temporal coincidence with migratory season of *L. nivalis*.

Leptonycteris patterns: Our results support a sympatric historic zone between *L. nivalis* and *L. yerbabuenae* during the three analyzed time periods, in Central Mexico. However, the most favorable climatic conditions for *L. yerbabuenae* occurred in the West of Mexico (Figure 4), while *L. nivalis* was restricted to a smaller area located at Center-South (Tehuacán-Cuicatlán Valley; Figure 3). For every season model (non-migratory and birth/migratory) we found clear differences between species. *L. yerbabuenae* distribution is wider than *L. nivalis*, especially during the migrant season (Figure 1), and the suitable conditions for *L. nivalis* were more restricted in all scenarios, such that during the LIG the birth/breeding season, they are almost absent (Figure 3).

Historical biotic interactions *Agave* taxa/ *Leptonycteris*

For the analyzed periods (Current, LGM, LIG) in 58 *Agave* taxa we detected a significant correlation between the climatic suitability of the *Agave* species and the climatic suitability of at least one *Leptonycteris* species. Furthermore, the same 58 *Agave* species coincide, at least, with one *Leptonycteris* bat species temporarily during the blooming period and migratory seasons. Of these, 19 species were detected as informative for both bats (Table 1), being important to highlight that all these *Agave* species are distributed in the Central Mexico region and many of them are informative for at least two periods, Current and LGM (Figure 2a and 2b). The same pattern was observed when we visually compared the geographical congruence between the *Agave* species richness and the climatic suitability for *L. nivalis* or *L. yerbabuenae* (Figures 3 and 4).

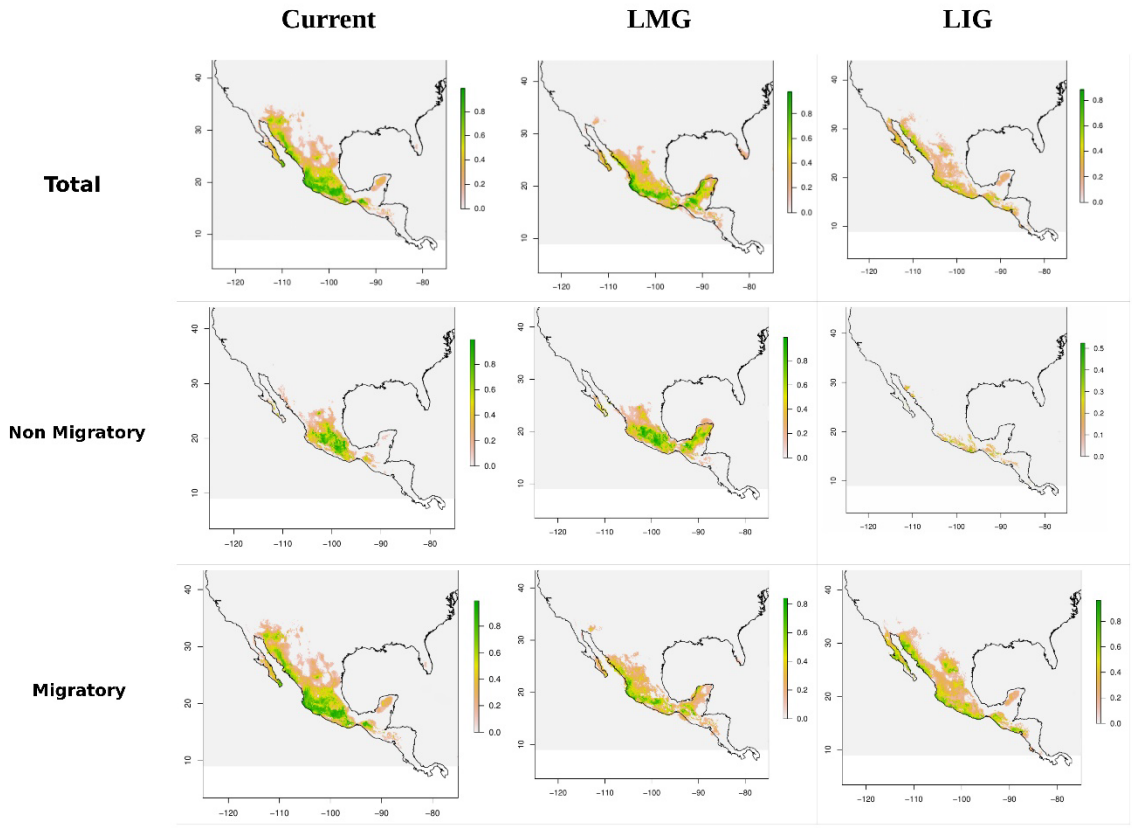

Figure 4. Distribution model of *L. yerbabuenae*, expressed in relative occurrence rate values.

When the possible interaction between the *Leptonycteris* species and the *Agave* group was analyzed in terms of the migratory pattern or past climate changes, it becomes evident that the interaction is dynamic. In both *Leptonycteris* species the geographical distribution of the non-migratory season is a nested subset of the geographical extension of the birth/migration season (Figure 1); and given that the migratory movement of individuals is associated with the females in these species (Medellín et al., 2018), the geographical extension of the birth/migration season is an expression of the space occupied by non-migratory individuals, non-migrant populations or male of migratory populations. This is of relevance because, although the correlation of bat populations during the birth/migration season is not congruent with the flowering season of the *Agave* species, the vast majority of these *Agave* species are an important source of nectar for non-migrant individuals -principally

males- in the Central region of Mexico, while females carry out the breeding season at the Northern part of their distribution.

Geographic Congruence between *Agave* richness and *Leptonycteris* climate suitability

Leptonycteris nivalis. For the Current climatic scenario, we detected a significant correlation between the climatic suitability of the total distribution of *L. nivalis* (including migrant and non-migrant females) and the richness pattern of *Agave* taxa (Figure 2, Table 2). For the LGM, the correlation value with *Agave* richness was significant, being the south of the Chihuahuan desert and Central portion of Mexico, the region with the most stable conditions for interactions (Figure 2, Table 2). While for the LIG time, the correlation value between climate suitability of *L. nivalis* and *Agave* richness was not significant (Table 2).

Table 2. Correlation values between the relative occurrence ratevalues of *Leptonycteris nivalis and Agave richness*, corresponding to the three climatic scenarios: Current, LGM and LIG. The values expressed in red are those with "p" value < .001 and "r" value > 0.4

	Non Migratory	Migratory	Total
Current	**0.4453531737*** **	**0.4090159895*** **	**0.4580725529*** **
LMG	**0.561924466*** **	**0.5618258063*** **	**0.5774861286*** **
LIG	0.218219472	-0.08179878539***	0.08790515572

Leptonycteris yerbabuenae. For the Current climatic scenario, we detected a significant correlation between the climatic suitability of the total distribution of *L. yerbabuenae* (including migrant and non-migrant females) and the richness pattern of *Agave* taxa, considering the center-south of distribution as the site where they inhabit around-year. For the LGM, the correlation with *Agave* richness was significant, being Central Mexico the region with the highest geographical congruence (Figure 4, Table 3), and remained similar and significant over a small region located in Central-Eastern of Mexico, that which today is the southernmost tip

distribution of *L. nivalis* (Figure 2, Table 3). While for the LIG time, the correlation value between climate suitability of *L. yerbabuenae* and *Agave* richness was not significant (Table 3).

Table 3. Correlation values between the relative occurrence rate values of *Leptonycteris yerbabuenae* and *Agave richness*, corresponding to the three climatic scenarios: Current, LGM and LIG. The values expressed in red are those with "p" value < .001 and "r" value > 0.4

	Non Migratory	Migratory	Total
Current	**0.5310633311*** **	**0.5581382832*** **	**0.5570338726*** **
LMG	**0.5217341021*** **	**0.5076377666*** **	**0.4817524615*** **
LIG	0.0831861682	0.320334034	0.347384598

Discussion

While biotic interactions are key elements of ecological and evolutionary processes, it is complicated to obtain evidence to evaluate their relevance (Blois, 2013; Wisz et al., 2013; Pollock et al., 2014). Explaining the dynamics of biotic interactions through time and climate change is an essential task to understand the diversification history of taxa (Wisz et al., 2013; Gavin et al., 2014; Pollock et al., 2014). We constructed three co-presence matrices between *Agave* and both Mexican *Leptonycteris* bats in order to explore the possible dynamics of their biotic interactions based on geographic congruence, incorporating as elements of change the migratory movement of female bats and the past climate changes. From our results, it was possible to make some inferences about the srenght of the biotic interaction between *Agave* taxa and *Leptonycteris* species, and how can this model the evolutionary history of the interaction.

We observed sites with high probability values for the presence of *Agave* and *Leptonycteris* and we inferred this geographic congruence as a probability for the development

and establishment of the interaction and coevolutionary process (Wisz et al., 2013; Pollock et al., 2014). More than 90% of the species of *Agave* analyzed have a flowering time coupled with several months of the birth/migratory season of both *Leptonycteris* species (Table 1); it is during this season that *Agave* - bats biotic interaction is more intense.

We detected 58 *Agave* taxa as potential biotic interactors with at least one mexican *Leptonycteris* species in at least one of three analyzed time periods (Current–LGM-LIG). This is the first study to include a large number of *Agave* taxa to analyze the dynamism of the interactions along migratory movements and during the climate changes of the Pleistocene (i.e., 7 agave taxa in Burke et al., 2019; 9 agave taxa in Gómez-Ruiz and Lacher, 2017; 2019; 94 taxa in this study).

Inferences of biotic interactions were derived from observations of co-presence (Table 1) and relative occurrence rate values from ENMs of the *Agave* taxa and two *Leptonycteris* species. When each *Agave* taxon had a small nested distribution within a bat distribution, the geographic correlation value was not significant, however these is not evidence that the interaction is not happening. Many studies have reported the primary role that nectar-feeding bats play in the pollination of *Agave* (Álvarez and González-Quintero, 1970; Easterla, 1972; Howell, 1979; Howell and Hart, 1980; Howell and Roth, 1981; Arizaga et al., 2000; Molina-Freaner and Eguiarte, 2003; Scott, 2004; Rocha et al., 2005; Sánchez and Medellín, 2007; Trejo et al., 2015; Flores-Torres and Galindo-Escamilla, 2017). For this reason, we believe that as a product of the strict methodology and considering the cases of disparity in the area sizes, the interaction events may have been underestimated in our study. But even with this underestimation bias, it is clear from our results that the biotic interaction between *Agave* - *Leptonycteris* has had adequate conditions to establish a strong interaction, along most of the Mexican territory.

In both *Leptonycteris* species from Mexico, the geographical distribution of the non-migratory bat females during autumm-winter seasons is a nested subset of the geographical

extension of the birth/breeding season, and given that migration in these species is carried out only by females and their young progeny (Medellín et al., 2018), the geographical area of the non-migratory season is an expression of the space occupied by non-migratory individuals (non-migrant populations or male of migratory populations) during birth/migratory season. Some authors have proposed that *L. nivalis* female bat migration is a response to food availability during the migration season and species of *Agave* play a main role as a resource (Moreno-Valdez et al., 2000), also, climate favourable conditions impact migration. In parallel, *L. yerbabuenae* female migration occurs when there is a decrease in food availability and a reproductive event coincides temporarily (spring-summer seasons), but resident males and females throughout the year count with food sources without moving latitudinally (Rojas-Martínez et al., 1999). Moreover, *L. yerbabuenae* exhibit clear differences among migrant - northern members and resident year-round individuals, being that resident year-round members mainly consume C3 plant species (Flemming et al., 1995). Thus, *Agave* play a key role as food resource an energy during long migration movements, from center-south to north and viceversa, this probably occurring in order for females to avoid competence for resources and ensure feeding their offspring. This allowed us to distinguish two geographical zones for the *Agave* - *Leptonycteris* interaction: North of Mexico, occupied by migrant females during birth/feeding (*L. nivalis* through the Mexican Altiplano and north slopes of Mexican Gulf, and *L. yerbabuenae* through the Sonoran Desert and Baja California), and Central Mexico, occupied by non-migrant populations (female and male individuals) and by male individuals of migrant populations, which just perform altitudinal movements close to the original site roots (Herrera-Montalvo, 1997).

Of the total *Agave* taxa detected as potential interactors with *Leptonycteris* (58 *Agave* taxa), 24 are predominantly distributed in the Central Mexico and were relevant for the areas occupied by non-migrant individuals; and 19 of these taxa were detected as interaction elements for both bat species. These 19 *Agave* taxa provide nectar resources to the non-

migratory bat individuals during the birth/breeding season (Rojas-Martínez et al., 1996; Sánchez and Medellín, 2007; López, 2010; Trejo et al., 2015; Martínez, 2019). The remaining 38 *Agave* taxa showed significant correlation values with only one bat species, and they occurred over the areas occupied by bats during the birth/migration season. When we analyze the distribution of *Agave* taxa that were informative for only one bat species, it was observed that these are mainly distributed over the migratory route of the bat in question, that is, the differentiation around the interaction with *Agave* is carried out by the migrant individuals of each bat species.

When we consider the agave-bat interaction over the past climate changes, we observed that the Current environmental scenario suggests a stronger interaction, with 40 cases, many of them also detected for the LGM. In contrast, in the vast majority of these cases, the geographic congruence was not significant for the LIG scenario between *Agave* and bat species and it was observed only over small portions of Central Mexico: for *L. nivalis* with six *Agave* taxa (over Meztitlán, Tehuacán-Cuicatlán and the Oaxaca coast areas) and for *L. yerbabuenae* with seven agave taxa (at the lowlands of the slope of the Pacific in Central Mexico). However, we observed two different bat-agave geographical patterns: in the Eastern portion of Mexico, *L. nivalis* and *Agave* taxa had similar geographical responses, both retracting to the south and significantly reducing their distribution areas; while to the west, *L. yerbabuenae* and *Agave* have different dynamics, given that a large group of *Agave* taxa found favorable climatic conditions for survival over the Sonoran desert, showing a wide distribution, while the distribution of *L. yerbabuenae* contracted and became restricted to the lowlands of the Pacific Coast.

Our results support the existence of an “interaction corridor” between the Mexican *Leptonycteris* species and *Agave* species, previously mentioned by Fleming et al. (1993); Gómez-Ruiz and Lacher (2016, 2019) and Burke et al. (2019), determining that the nature of the interaction is historical. Thus, we suggest that this interaction remained strong during the

low temperatures of the glacial Pleistocene period, particularly for the LGM.

Differences in the plant species from which these two bat species obtain nectar have been reported. The majority of reports in *L. nivalis* involve *Agave* taxa (Sánchez and Medellín, 2007*;* Gómez-Ruiz and Lacher, 2017; Burke et al., 2019; Martínez, 2019), while for *L. yerbabuenae* more than 70 species, some belonging to very distant genera have been reported (see reviews in Valiente-Banuet et al., 1996; Rojas-Martínez et al, 1999; Burke et al., 2019). Furthermore, *L. nivalis* is distributed in a smaller geographical region, with more restricted climatic conditions (Hensley and Wilkins, 1988; Cole and Wilson, 2006; Arita, 1991). The largest populations for *L. nivalis*, including resident bat colonies have been reported in two of the most diverse agave areas, Tehuacán-Cuicatlán and Meztitlán. We can also add that during the LIG, Tehuacán-Cuicatlán and Meztitlán apparently were refuges for many species of *Agave* (see Scheinvar et al., (2016); Scheinvar (2018); Scheinvar et al., (submitted, "Evolutionary implications of ecological patterns and range shifts during past climate change in *Agave*"), so during the LIG, the *Agave* taxa and *L. nivalis* could have kept an intense interaction. Thus we suggest that Tehuacán-Cuicatlán and Meztitlán regions were historical refuge zones for both *Agave* and *L. nivalis* and perhaps even represent a coevolution hot spot for the interaction between them, following the concept proposed by Gomulkiewicz et al. (2000).

On the other hand, the historical relationship between *Agave* and *Leptonycteris* species found in this study seems to support a diffuse coevolutive hypothesis, as suggested by Rocha et al. (2005, 2006) and Abreu-Flores et al. (2019) on which the interrelation between environmental conditions and biotic interactions (i.e., pollination) can act as an evolutionary diversification motor, modeling floral displays, phenology or acting as selective pressures over inflorescence sizes of *Agave* species (Rocha et al., 2006; Blois et al., 2013; Fortelius et al., 2014).

In conclusion, we managed to model the *Agave* - *Leptonycteris* coincidence in their spatial and temporal components. We inferred the historical presence of two *Agave* - *Leptonycteris* interaction corridors, with stability from the LGM to the Current. We also suggest an interaction refuge in the area of Meztitlán/ Tehuacán-Cuicatlán where possibly *Agave*-*Leptonycteris* interaction may have remained active for maybe the last 2.5 Mya.

Additionally, we propose that in the areas related to the migratory bat movements, each bat species interacts with different *Agave* taxa, while in the areas occupied by non-migrant individuals, both bat species consume nectar of almost the same *Agave* taxa (Rojas-Martínez et al., 1996; Sánchez and Medellín, 2007; López, 2010; Trejo et al., 2015; Martínez, 2019).

Author contributions

RT designed the project, and contributed with data base construction and drafting the manuscript; **NG** contributed to design the project, data base construction, ecological analysis and drafting the manuscript; **EE** contributed with ecological analysis; **ES** participated in the data base construction and with ecological analysis; **AM** contributed to drafting the manuscript; **RM** contributed to the drafting the manuscript; **EA** participated in drafting the manuscript; **LEE** was the project leader, designed and coordinated the project, helped with the logistics and drafted and corrected the manuscript.

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Capítulo 4. Save Our Bats, Save Our Tequila: Industry and Science Join Forces to Help Bats and Agaves

Save Our Bats, Save Our Tequila: Industry and Science Join Forces to Help Bats and *Agaves*

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ABSTRACT: The genus *Agave* is one of the most diverse and rich groups of plants of Mexico. Mexican people have developed several technologies to extract products from *Agave*, and for many years they have consumed five different alcoholic beverages derived from *Agave*: Tequila, Mezcal, Bacanora, Raicilla, and Pulque. Additionally, *Agave* has coevolved with nectar-feeding bats, and in several cases, bats play the main role as functional pollinators in this ecological relationship. But with growth in the demand of agave derived products, management practices have reduced dependence on bat pollination, using instead clonal shoots to replant fields and harvesting plants before flowering, thereby negatively affecting both bats (by decreasing food availability) and agaves (by lowering their genetic diversity). We explore the possibility that bat-friendly practices may be incorporated into the production system. We compiled data about the pollination biology of *Agave* to infer how many bats could use the available resources, if Mezcal and Tequila producers allowed 5–10% of agave crop inflorescences to flower based on a linear projection using *Agave angustifolia* (a sister group of *A. tequilana*). If only 5% of the plants in one hectare were allowed to flower (approximately 222 individuals), then, depending on nectar concentration and total volume, a minimum of 89 individual bats could feed every night during flowering period. This means that allowing 5% of the current total population of *A. tequilana* reproductive agaves to flower could feed a total of 2,336,250 nectar feeding bats per month.

Index terms: Agave, bat-friendly, conservation, tequila production

INTRODUCTION

Agave in Mexico

Mexico is a megadiverse country due to its great diversity of many groups of organisms (Ceballos et al. 1998; Frías-Alvarez et al. 2010; Martínez-Meyer et al. 2014). Between 4–8% of the world's flora lives in Mexico, and 51% of the Mexican flora is endemic (Villaseñor 2004; Villaseñor et al. 2005; Sosa and De Nova 2012).

The genus *Agave* is endemic to the American continent; Mexico contains the largest number of agave species and it is the largest genus in the subfamily Agavoidea (Eguiarte et al. 2013). *Agave* is the third largest genus of the Mexican flora, including approximately150 species (García-Mendoza 2002). About 75% of all species of *Agave* are found in Mexico, 69% are endemic (García-Mendoza 2002; Villaseñor et al. 2005). Agaves grow mainly in extreme climates that are dry and hot (García-Mendoza 2002) and are often dominant elements in tropical dry forest, scrub, and xerophytic environments, which occupy more than 50% of Mexico's territory (Rzedowski 1993).

Partly influenced by the complexity of ecosystems in Mexico, agaves (commonly called *maguey*) have diversified into many species and colonized most of the country (Figure 1). They are adapted to arid environments due to their physiological and ecological traits, such as CAM metabolism (Eguiarte et al. 2013). Agaves are perennial and with exception of one small group of species they are semelparous, accumulating huge reserves of carbohydrates that the plant invests in a single flowering event resulting in the development of a vertical flowering stalk with up to thousands of flowers, after which the plant dies (Gentry 1982). They can be distinguished from other plants by their rosette-like growth form, and by their elongated, tapered leaves that have a terminal thorn. The most visible characteristic is the large flowering stalk (also called scape) that they produce at 5–20 years of age, depending on the species. This is the signal that indicates the beginning of the reproductive event and the final life stage of the plant. Flowering stalks can be spicate (without branches) or paniculate (a branched inflorescence, each branch including several flowers) (Gentry 1982).

Mexico is the center of origin of *Agave* (Good-Avila et al. 2006) and it is not surprising that since pre-Columbian times, people in Mexico have developed different technologies to obtain and use several agave products, such as food, fibers, and alcoholic beverages including Mezcal, Tequila, Bacanora, Raicilla, and Pulque (Callen 1965; Kasiak 2012; Radding 2012), which led to the development of a culture around agaves and their products. Interest in *Agave* as an alternative source for biofuel has increased in some regions (Valenzuela 2011; Escamilla-Treviño 2012;

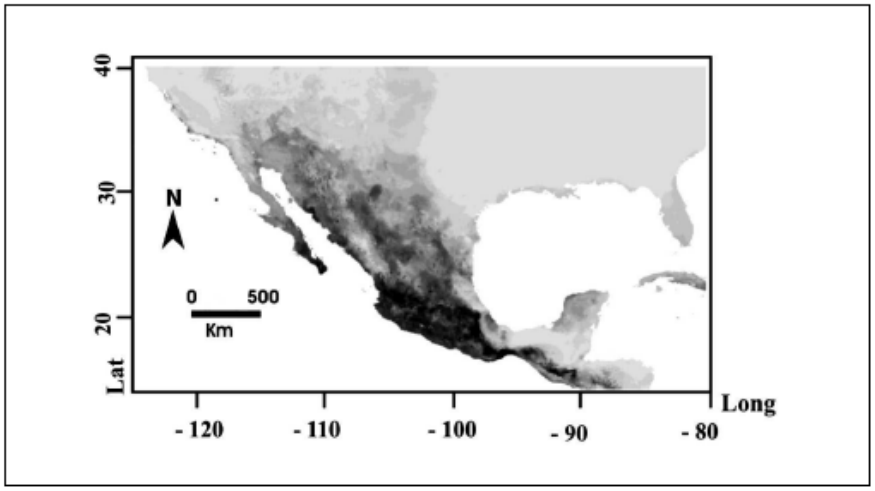

Figure 1. Potential distribution of different species of *Agave* in Mexico according to CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad) and GBIF (Global Biodiversity Information Facility) databases. Darker color represents higher species richness.

Stewart 2015). Today agaves are used for many purposes (Radding 2012; Stewart 2015). However, production of spirits is, by far, the most economically important.

Cultural Landscape of *Agave* Landraces

Agaves have been part of the culture, identity, and tradition of the Mexican people for a very long time (Radding 2012) because of their wide distribution, natural characteristics, and the ancestral human relationship. The use of agave products was documented 9000 years ago in northern Mexico (Callen 1965).

The Mexican states most closely linked with the agave cultural landscape and biological diversity are Oaxaca, with more than 50 agave species; Durango, Puebla, Sonora, and Jalisco with more than 40 species; and Coahuila, San Luis Potosí, Nuevo León, Zacatecas, State of Mexico, and Tamaulipas are inhabited by 25 species of agaves (García-Mendoza 2002). There are more species that have not been described or are still very poorly known and there are several areas of high endemism (Tambutti 2002).

In 1977 and 1995 respectively, Tequila and Mezcal obtained the Appellation of Origin signed before the World Intellectual Property Organization in Geneva, Switzerland. Currently, the two industries are monitored by Mexican technical legislations called NOM (Norma Oficial Mexicana): NOM-006-SCFI-2012 and NOM-079-SCFI-1994 for Tequila and Mezcal, respectively. But these rules also protect the heritage promoting traditional and sustainable use (Diario Oficial de la Federación 2012).

In July 2006, the regions of Magdalena, Amatitán, El Arenal, Tequila, and Teuchitlan within Jalisco, Mexico, were included in the list of World Heritage Sites by UNESCO based on different criteria, but mainly because of the aesthetics of the culture landscape and the traditional distillery practices developed by ancient people. The management plan, prepared by Instituto Nacional de Antropología e Historia (INAH-Mexico), the Government of Jalisco, and World Heritage Mexico, contains the proposal that the Tequila landscape should be part of the list of World Heritage, recognizing that sustainable use should be also a part of that heritage (WIPO 2006).

The production of both agave-distilled beverages, Tequila and Mezcal, has increased in recent years (National Chamber of Tequila Industry 2015); Distilled Spirits Council of the United States (2015) and the production of spirits corresponds to the region where species are distributed. Additionally, different Mexican state governments have requested inclusion in the appellation given that some agave species occur within their boundaries and they use them to produce spirits. These states conform the Tequila and the Mezcal route, however each locality must demonstrate ancestral tradition for these beverages in order to be included in it (Diario Oficial de la Federación 2012).

Tequila Production

From January to December 2014 alone, the National Chamber of the Tequila Industry reported a production of 242 million liters at 40% (alcohol volume) in order to cover the demand of 1615 brands, of which a total of 172.5 million of liters were exported. This production required 788,200 tons of *Agave tequilana* Weber var. azul. On the domestic market, profits reached 13,900 million pesos (National Chamber of the Tequila Industry 2015). In August 2015, this industry represented 70,000 jobs and exports with a profit of 1.2 billion US dollars (National Chamber of the Tequila Industry <https://www.crt.org.mx/EstadisticasCRTweb/> 2015). The export to the United States of America was the most substantial in the period from January to December 2014, and an equivalent to 742 million dollars was exported, where Tequila represented 6.3% of sales spirits in that country (according to the National Chamber of the Tequila Industry 2015). This represents about 117,347,772 liters, considering the total production estimated by the Distill Spirits Council of the United States (2015). Due to high demand, tequila production is a very important economic activity in Mexico. Given their dependency on bats for pollination, developing bat-friendly and ecologically sustainable production techniques is key to keep and expand opportunities for this and other industries, such as Mezcal.

For several decades, there has been a growing tendency for people to acquire products whose production is environmentally friendly, socially responsible, sustainable, and/or organic (Kilbourne and Beckmann 1998; Diamantopoulos et al. 2003). This tendency is paralleled by marketing strategies (Zimmer et al. 1994), primarily in industrialized countries, where consumers

seek government-endorsed labels related with environmentally friendly practices (D'Souza et al. 2007).

Natural *Agave* Landscapes and Bats

Several researchers have recorded and described the relationship between agaves and the nectarivorous bats *Leptonycteris yerbabuenae* (Martínez & Villa-R), *L. nivalis* (Saussure), and *Choeronycteris mexicana* (Tschudi) (Álvarez and González-Quintero 1970; Easterla 1972; Howell 1979; Howell and Hart 1980; Howell and Roth 1981; Arizaga et al. 2000; Molina-Freaner and Eguiarte 2003; Silva-Montellano and Eguiarte 2003; Scott 2004; Rocha et al. 2005; Sánchez and Medellin 2007; Trejo et al. 2015). These three bat species are migratory and their distribution covers most of the Mexican territory (Figure 2), some localities in United States, and small areas in Central America (Arita and Santos Del Prado 1999; Figure 2). All three species are under protection in two categories (Threatened and Endangered) by Mexican, US, or international regulations (Diario Oficial de la Federación 2012; US Fish and Wildlife Service 1995, 2006; IUCN 2016). Although it is unknown whether food is a limiting resource, the tequila landscape was virtually sterile to the bats, with no available nectar for many decades (Zizumbo et al. 2013). Recently, *L. yerbabuenae* populations had recovered from an important decline and for this reason will be delisted in Mexico (Paz 2013) and the official revised list, in press, will reflect this change (C. Alvarez, SEMARNAT, pers. comm.).

Although bat pollination of agaves has been recorded in wild species, and nectar-feeding bats have been noted as the main pollinator, the pollination ecology in both wild and cultivated species of agaves is still scarcely studied. Particularly, the presence of nectar-feeding bats in the flowers of Mezcal agave has been reported for some species, including *A. americana* (Linneo) (Knudsen and Tollsten 1995), *A. angustifolia* (Linneo) (Molina-Freaner and Eguiarte 2003), *A. inaequidens* (Koch) (Sánchez and Medellin 2007), and *A. potatorum* (Zuccarini) (Estrella-Ruiz 2008). Only in a few cases has the role of visitor been described in detail (Table 1). Several publications include reports of bats visiting flowers of additional agave species and the presence of agave pollen on the body or in the feces of bats (Rocha et al. 2006; Eguiarte et al. 2013). The agave anthers dehisce at night and shed pollen before the stigma is receptive (Schaffer and Schaffer 1977; Gentry 1982; Slauson 2000; Rocha et al. 2005). Pollen is able to germinate for only a few hours after dehiscence (Shivanna et al. 1991), meaning that effective pollination primarily occurs at night.

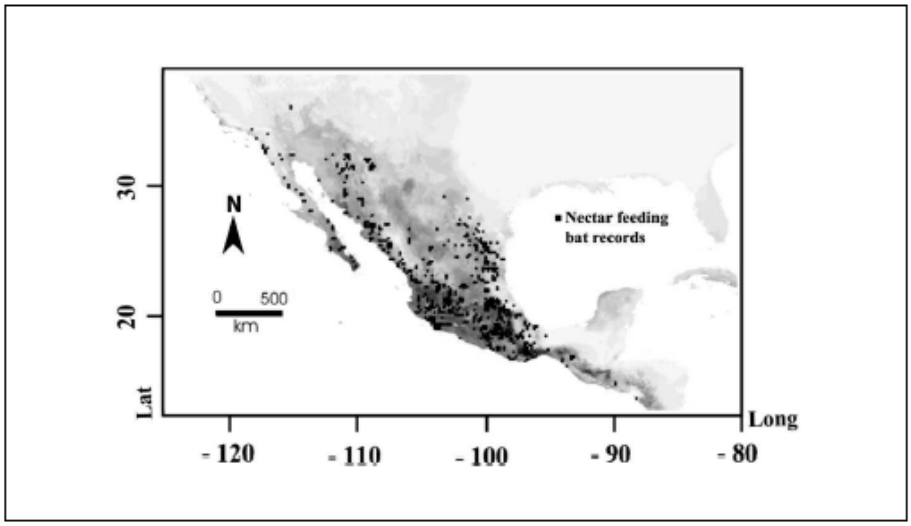

Figure 2. Overlapping distribution of known occurrence of agave-visiting nectar-feeding bats (*Leptonycteris nivalis, L. yerbabuenae* and *Choeronycteris mexicana*) and *Agave* potential distributions in Mexico. Gray scale represents richness of *Agave*.

Dry and semiarid zones cover about 50% of the Mexican territory, where 20% of the total Mexican flora is found (approximately 6000 species), and there is a high incidence of endemism, about 60% (García-Mendoza 2002). This information emphasizes the importance of the conservation of bats and the plants they pollinate in Mexico, which provide benefits not only to natural ecosystems, but also to crops where the bats can help promote genetic diversity. *Leptonycteris yerbabuenae*, *L. nivalis,* and *C. mexicana* are good examples because they feed mainly on plants that dominate the Mexican arid landscapes and these nectar-feeding bats are the principal pollinators of many agave species (Álvarez and González-Quintero 1970; Easterla 1972; Howell 1979; Howell and Hart 1980; Howell and Roth 1981; Arizaga et al. 2000; Molina-Freaner and Eguiarte 2003; Silva-Montellano and Eguiarte 2003; Scott 2004; Rocha et al. 2005; Sánchez and Medellin 2007; Trejo et al. 2015). Ecological relationships between these bats and agaves has been described based on the presence of bats in areas with high abundance and diversity of *Agave* (i.e., Barranca de Metztitlán and Tehuacán Valley) during their flowering period (Rojas-Martínez and Valiente-Banuet 1996; Rojas-Martínez et al. 1999; Moreno-Valdez et al. 2000; Moreno-Valdez et al. 2004; Rocha et al. 2005). Additionally, the migration of the two Mexican *Leptonycteris* species coincides with the flowering period of some agaves in the north of their distribution (Molina-Freaner and Eguiarte 2003; Moreno-Valdez et al. 2004; Peñalba et al. 2006).

Pollination ecology of plants of the genus *Agave* is scarcely studied; there are only a few records of nectar production for some species (Table 1). In addition, consumption of nectar by bats is poorly studied; a few authors have calculated total food intake in nectar-feeding bats (Ayala-Berdon et al. 2008; Ayala-Berdon et al. 2009; Ayala-Berdon and Schondube 2011; Ayala-Berdon et al. 2011a; Ayala-Berdon et al. 2013). Early estimates suggested that an average bat weighing 11.5 g can consume around 15 ml of nectar per night (Helversen and Reyer 1984). A more recent study reports that a Mexican long-nosed bat (*L. nivalis*) consumes a

Table 1. Sugar concentration and total volume of nectar production in different paniculate *Agave* species. Modified from Rocha et al. 2006.

Species	Concentration (%)	Total volume (µl)	Bat visits recorded
A. angustifolia	18–26	180	Yes
A. chrysantha	14–18	470	No
A. macroacantha	--	110	Yes
A. marmorata	9–37	576.61	No
A. palmerii	14–19	713	No
A. salmiana	12	102	Yes
A. subsimplex	22–25	40	Yes

wide range from 18,500–119,000 mg of nectar in one night, depending on sugar concentration (Ayala-Berdon et al. 2013). Additionally, the lesser long-nosed bat (*L. yerbabuenae*) and Pallas´s long tongued bat (*Glossophaga soricina* Pallas) can change their total consumption in response to sugar concentration and energy requirements in different seasons (Ayala-Berdon et al. 2008; Ayala-Berdon et al. 2009; Ayala-Berdon et al. 2011; Ayala-Berdon and Schondube 2011; Ayala-Berdon et al. 2013).

Agave inflorescences are capable of producing thousands of flowers (Sutherland 1987), but flowering is progressive so that flowers display pollen by spatiotemporal clusters within the inflorescence. Nectar production and concentration is variable in every species (Table 1). For instance, each flower of *A. marmorata* (Roezl) produces about 570 µl of nectar in one night (Ornelas et al. 2002), while *A. angustifolia*, a species closely related to *A. tequilana*, produces about 180µl in one night (Molina-Freaner and Eguiarte 2003). Agave species also differ in flowering timing. For instance, *A. angustifolia* begins flowering in January and ends in May, and highest abundance of inflorescences is documented to occur in March (Molina-Freaner and Eguiarte 2003). Sugar concentration in nectar in *Agave* spp. ranges from 18–26% (Rocha et al. 2006). In general, nectar-feeding bats consume nectar from 5–29% sugar concentration (Tschapka and Dressler 2002).

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 order to digest that meal, then they visit flowers again. In this way they can visit up to 1000 flowers per night (Tschapka and Dressler 2002).

In this paper the mutualistic relationship between bats and agaves is explored, along with the ensuing interaction with the tequila industry, and how the three elements (bats, agaves, and industry) can benefit from fostering the interaction.

METHODS

Maps of the richness distribution of *Agave* and nectar-feeding bats were built using Rstudio v0.98.501 based on GBIF and CONABIO database information. We used data from five agave species (Table 1) to explore how much resource they contribute to the bat diets. There is no information about flowering production of *A. tequilana*, so we proposed *A. angustifolia* as a model to project data in an *A. tequilana* crop, due to their close phylogenetic relationship (Eguiarte et al. 2013). Nectar consumption by bats was compiled from the literature (see above; Table 1).

Currently, commercial producers of *A. tequilana* var. blue cultivate contiguous individual plants every 1.5 m (Zizumbo-Villareal et al. 2013); therefore, one hectare can contain approximately 4444 individuals. Unfortunately, as mentioned above, there is no data about pollination biology of *A. tequilana,* so we used data from closely related species to represent possible scenarios. It is also true that these fields are empty of food resources for bats, since the industry maximizes taking the accumulated sugars in agave heads to produce tequila by harvesting them before they produce any flowering stalks. Each new planting season, farmers replant their fields with the clonal shoots produced by a small selection of the plants, thus resulting in further genetic diversity loss.

We developed a linear model with Rstudio v0.98.501 to predict how many bats can feed from different agave species. We considered the value of nectar volume and concentration from *A. angustifolia* to represent the potential contribution of food available for nectar-feeding bats (Table 2). Nevertheless, these measures are only a crude approximation, as volumes and concentration of sugar in nectar vary widely among different *Agave* species (Tables 1 and 2). The only available data on the number of active agave flowers per night is an average of 100 flowers (Rocha et al. 2005).

In order to perform these analyses, volume units expressed in microliters were transformed to equivalent weight using the formula presented by Dafni (1992):

Milligrams of sugar in volume of nectar = (% sugar reading of the refratometer)/100 × volume in µ1 × Density of sucrose at the obs. concentration
Density of sucrose = 1.59 mg/µl (Dafni 1992).

RESULTS

Available data (Table 2) indicate that one *L. nivalis* bat, which is the largest of the nectar-feeding bats in Mexico, can consume the nectar produced by 2.48 in-

Table 2. Number of inflorescences required to feed one *Leptonycteris nivalis* bat (18,500 mg of food), considering 100 active flowers per night per inflorescence.

Species	Total production of sucrose per flower per night (mg)	Number of necessary inflorescences to feed one *L. nivalis* bat	References
A. angustifolia	74.41	2.48	Molina-Freaner and Eguiarte 2003
A. chrysantha	134.51	1.37	Rocha et al. 2006
A. marmorata	339.22	0.54	Ornelas et al. 2002
A. palmerii	215.39	0.85	Rocha et al. 2006
A. salmiana	19.46	9.5	Rocha et al. 2006
A. subsimplex	15.9	11.63	Rcoha et al. 2006

florescences of *A. angustifolia* (Table 2). Approximately 89 *Leptonycteris* (or other nectar-feeding bats species) could feed each night during the six month flowering period (Molina-Freaner and Eguiarte 2003) in 1 hectare of agaves for tequila production (with approximately 4444 plants) if only 5% of inflorescences (222 inflorescences) are allowed to flower with a sugar concentration of 26% and 180µl of total nectar production (as in *A. angustifolia*) in 100 active flowers per night per inflorescence. If the percentage of inflorescences from cultivated plants increased up to 10% (444 inflorescences), then the number of individual bats fed would be a minimum of 178. A case that illustrates the amount of resources offered to flower visitors is the Mezcal agave species *A. marmorata*, which is capable of producing 576 µl of nectar per flower at a sugar concentration of 37% (Ornelas et al. 2002). Thus, the offer of floral rewards to bats by this Mezcal species is much larger than that of other species. Following the linear projection, there is a two to one relationship among them (Table 2).

Bat-Friendly Tequila Concept

As estimated above, if 5% of the agaves in one hectare intended for the tequila industry are allowed to flower, they can provide food for 89 bats. According to the National Chamber of Tequila Industry (August 2015), there are about 105,000 ha of *A. tequilana* Weber var blue cultivated in appellation of origin territory (Cámara Nacional de la Indistria del Tequila 2015). Thus, the amount of potential nectar-feeding bats feeding on these fields could be as high as 9,345,000 during the four-month flowering period (Gentry 1982), or 2,336,250 bats each month. The greater the number of agaves that are allowed to flower, the greater the number of pollinators that can be sustained.

In addition, given that cultivated blue agaves have extremely low genetic diversity (Eguiarte et al. 2013), fertilization of flowers of cultivated agaves and some wild individuals of *Agave tequilana*, often found in the barrancas and other landscapes close to many of these fields, will likely help recover genetic variation.

DISCUSSION

There are about 750 species of plants pollinated by bats in the world (Kunz et al. 2011) and around 360 species inhabit tropical America. Most records of bat-pollinated families are Cactaceae, Bignoniaceae, Bombacoidea (a clade of the Malvaceae) and in the genus *Agave* (Arizmendi et al. 2002). It has been suggested that the loss or decrease in nectar-feeding bat populations can cause a negative impact on some cultivated plants as well as wild plant communities (Allen-Wardell et al. 1998).

Farmers do not allow flowering of several species of cultivated agaves so pollination dynamics in many species is disrupted, but this has not been studied in detail. The relationship between these cultivated agaves and bats is unknown. In order to ensure the future of agaves, nectar-feeding bats, Mezcal, Tequila, and other related products, data on the floral biology of all cultivated and sustainably used *Agave* species, and their relationship with bats, are urgently needed. Direct measurements of nectar production by flowers, number of flowers, pollen production and fertilization, and cross-breeding experiments of *A. tequilana* flowers should also be part of this research agenda.

Some authors have reported that *A. tequilana* and *A. americana* produced just a few viable seeds when they were tested in self-pollination experiments (Escobar-Guzmán et al. 2008). *A. angustifolia* is self-incompatible or has very strong inbreeding depression, thus, floral visitors are essential for fecundity since they move pollen between individuals (Rocha et al. 2006). In the same sense, the role of pollinators in outcrossing is crucial to increase genetic diversity of commercial varieties of *A. tequilana* Weber var. blue.

An increasing percentage of plantations of *A. tequilana* Weber var. blue are being severely affected by the bacterium *Erwinia caratovora* (Jones) and the fungus *Fusarium oxysporum* (Schlechtendal) (Small and Catling 2002). The documented low genetic diversity in blue agave may cause greater vulnerability to attack by these and other diseases and bacterial infections (Eguiarte et al. 2013). And again, the role of bats as pollinators may help solve this serious issue.

Because the nectar-feeding bat species that

pollinate agaves move across the Mexico-United States international border (including *L. nivalis*, *L. yerbabuenae,* and *C. mexicana*), conservation of these species is particularly important and there should be a bi-national strategy and effort to protect the three species. Their movements are critical for the survival and reproductive success of the plants they feed on. The protection of roosts and migratory routes that they follow are also of utmost importance and their foraging resources should be a priority conservation objective. Nectarivorous bats forage in the areas of both continuous habitats and fragments, thus migratory bats help to maintain genetic connectivity between the different fragments and plant populations visited. It is, therefore, timely that only in April of 2015, the need to protect bats was recognized as a priority by the federal governments of Canada, Mexico, and the United States (US Fish and Wildlife Service 2015).

The concept of environmentally friendly products is gaining traction in the general population. It opens a brand new door for collaboration among producers, bottlers, importers, conservationists, and consumers. Given that Tequila is a two billion USD a year industry, and that the economy of 40,000 families is linked to blue agaves (and thus, indirectly to bats), it is in the best interest of all stakeholders (from small producers to industrial giants such as Sauza, Cuervo, and Patron) and from government to the individual consumer, to protect the future of Tequila and Mezcal agave crops under sustainable practices, which incorporate protection to pollinators and genetic sources as key safeguards. This collaboration is already promoting bat friendly products using species such as *A. cupreata* (Trel. & A. Berger), a species endemic to the states of Guerrero and Michoacán that reproduces only sexually through bat-mediated pollination. Producers of *A. cupreata*-based mezcal long ago adopted the practice of allowing about 10% of the plants (they leave the largest plants for this process) to flower so that they obtain the seeds to plant the next generation of agaves (E. Vieyra, pers. comm.). Because of these conditions, *A. cupreata*-based mezcal is, by definition, bat friendly.

In addition, *A. cupreata*-based mezcal produced in central Michoacán is one of the very few alcoholic beverages in the world that does not come from monocultures, but from thinned-out forests in which agaves are interspersed (R. Medellin, pers. obs.). The Mezcal Regulatory Council, and other organizations including the Tequila Interchange Project and the National Autonomous University of Mexico, are cooperating to ensure that these practices do not only remain in place, but become mainstream in the agave spirits industry.

Only consistent cooperation among all the sectors involved, from conservation professionals and land owners to the final consumer, will secure the future of these practices and, thus, of the very old (about 6–9 million years; Trejo 2013) and tightly linked mutualistic interaction between bats and agaves, as well as high quality agave-derived spirits.

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Roberto-Emiliano Trejo-Salazar studied biology and conducted field work on the pollination ecology of agaves as an undergraduate. More recently he obtained a Master Degree from Universidad Nacional Autónoma de México with a titled thesis, "Divergence Times in Phyllostomidae: Origin of nectarivory." Currently, he is a PhD student working in the phylogeography of Leptonycteris in Laboratorio de Ecología y Conservación de Vertebrados Terrestres, Instituto de Ecología, UNAM.

Luis Eguiarte Fruns is Senior Professor at the Institute of Ecology UNAM. He has conducted research about ecology pollination and genetic population of many agaves' species. He also has developed projects with other important crop plants from Mexico, including maize (Zea mays) and pumpkins (the genus Cucurbita). Nowadays he is conducting a genome project of Leptonycteris yerbabuenae.

David Suro-Piñera is the President and Co-Founder of the Tequila Interchange Project (TIP), a 501(c)3 nonprofit organization that advocates the preservation of sustainable, traditional, and quality practices in the industries of agave distilled spirits. The work of the organization that he presides facilitates the translation of academic work, and sponsors research projects such as the Bat Friendly Program. TIP considers the Bat Friendly Project to be fundamental in establishing sustainable practices for the fragile ecosystem of bats and agave.

Rodrigo Medellín is Senior Professor at the Institute of Ecology UNAM, and has conducted research for the conservation of bats and other mammals for over 30 years. His research seeks to inform and orient policy and decision-making processes in conservation. Partly, his work led to delisting of Leptonycteris yerbabuenae in Mexico. Rodrigo is Co-Chair of the Bat Specialist Group of IUCN.

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Discusión

En este trabajo se presenta el mayor esfuerzo de muestreo, hasta el momento, en relación al rango de distribución de la especie *L. yerbabuenae* para un análisis de su genética de poblaciones o filogeografía. Gracias a este esfuerzo podemos comparar la diversidad haplotípica de *L. yerbabuenae* con trabajos anteriores de la misma especie y con otras especies. En ese sentido, la diversidad haplotípica del murciélago magueyero menor es muy similar a la reportada por otros autores anteriormente (Morales-Garza et al, 2007; Arteaga et al., 2018; Menchaca et al., 2020) y similar a otras especies de murciélagos, sobre todo en especies que presentan comportamientos migratorios como: *Lasiurus borealis*, *L. cinereus* and *Lasionycteris noctivagans* (Korstian et al., 2015; Sovic et al., 2016). Además, anteriormente no se habían realizado análisis enfocados en ambos linajes parentales, particularmente en el linaje paterno, mostrando los niveles de diversidad haplotípicos congruentes con el linaje materno (Tabla 5.1). En contraste, en algunas especies no migratorias, la diversidad haplotípica es baja, por ejemplo: *Dasypterus ega* and *D. intermedia* (0.018 y 0.588 respectivamente; Chipps et al., 2020).

Una de las principales preocupaciones al realizar e interpretar los análisis presentados en la tesis, era el de los sesgos en la proporción de sexos en las cuevas o refugios que ocupa el murciélago magueyero menor. En el caso de encontrarnos con estos sesgos, las razones que nos explicarían esos resultados estarían relacionados con el comportamiento filopátrico por parte de las hembras que migran y en cuyo caso, podríamos tener errores de interpretación en cuanto a los valores de diferenciación entre los linajes y localidades. Por lo tanto, planteamos dos hipótesis de trabajo, una dirigida a encontrar diferencias significativas debido a la migración de las hembras y otra que la especie se comporte como una metapoblación genéticamente hablando, en donde las hembras pueden volar hacia las cuevas de maternidad durante las estaciones más calientes del año y al regresar desviarían su ruta original y se agruparían en nuevas colonias distintas a las que ocupaban durante el inicio del ciclo de migración. En ese sentido, contamos con sesgos en el número de secuencias analizadas por localidad y sexo, lo cual nos dificulta la comparación estadística entre ambos sexos y sitios de colecta (Tabla 5.1). A pesar de esto, no se encuentran diferencias significativas en cuanto a la diversidad haplotípica para ninguno de los marcadores. Para resolver estos sesgos, será más conveniente realizar un muestreo sistemático que nos permita representar cada localidad en ambas etapas del ciclo migratorio tanto para machos como para hembras, lo cual nos permitiría describir con mayor precisión la estructura de la población, así como explicar de manera más detallada quién está migrando y hacia dónde

regresan estas hembras migrantes. Así como las localidades con mayor diversidad genética y que conexión existe entre ellas.

Tabla 5.1. Diversidad haplotípica (Hd) y nucleotídica (π) para cada marcador, localidad y sexo.

	Cytb				D-loop				DBY	
Locality	Hd		Π		Hd		π		Hd	π
	Male	Female	Male	Female	Male	Female	Male	Female		
Los Laguitos, Chiapas	1	0.5758	---	0.0031	1	0.9111	0	0.0342	1	0
Juxtlahuaca, Guerrero	1	0.7571	0.0024	0.0025	1	0.8615	0.0617	0.0333	0.9444	0.0188
La Mariana, Sonora	1	0.7727	0.0031	0.0058	1	0.3956	0.0232	0.0098	---	---
Chamela, Jalisco	0.8727	1	0.0021	0.0065	1	---	0	---	0.6725	0.0171
Las Lumbres, Nayarit	---	1	---	0	---	---	---	---	0.8182	0.0082
Ticuman, Morelos		0.6667		0.0023	---	0.7143	---	0.0131	---	---
El Pinacate, Sonora	---	0.5842	---	0.0014	---	0.5686	---	0.0125	---	---
Xoxafi, Hidalgo	---	---	---	---	---	0	---	0	1	0.6734
Las Vegas, Puebla	---	---	---	---	---	---	---	---	---	---
Tzinacanostoc, Puebla	---	0.8	---	0.006	---	---	---	---	---	---
Tonatico, Estado de México	1	1	0.0009	0.0009	---	---	---	---	1	0.0022
Atotonilco, Jalisco	0.8333	0.6667	0.0049	0.0046	0	1	0	0.0217	0.9789	0.1401
Tetecalita, Morelos (salitre)	0.875	0.7556	0.0027	0.0025	0.9848	1	0.0908	0.0834	0.875	0.0081
Baja California (las cuevas)	1	0.4	0.0121	0.0007	1	0.6667	0	0.0107	0.9091	0.2345
Baja california (mulege)	0.786	0	0.0041	0	---	---	---	---	---	---
Ciudad de México	1	1	0.0561	0	0.8611	---	0.0413	---	1	0.0937
San Juan Raya	0.83	0.5833	0.0653	0.0338	0.781	0.9333	0.0301	0.0246	1	0.0328
San Sebastian Frontera	1	1	0.0879	0	1	---	0.0526	---	---	---
Tula, Hidalgo	1	0	---	0	0	---	0	---	1	0
Coxcatlán, Oaxaca	---	1	---	0	---	1	---	0	---	---
Navachiste, Sinaloa	---	---	---	---	---	---	---	---	1	0
Tuxtepec, Jalisco	---	---	---	---	---	---	---	---	1	0
Callejones, Colima	---	---	---	---	---	---	---	---	1	0.3983
Total	0.9238	0.6437	0.04808	0.0087	0.8757	0.8325	0.061	0.0354	0.9137	0.04781

Originalmente, esperábamos encontrar diferencias en la distribución de los linajes causadas por el comportamiento migratorio de parte de las hembras. Esto debido a la conectividad en la migración, los sesgos por proporción de sexos en las cuevas y localidades, además de los procesos históricos, incluyendo extinciones locales y colonizaciones (Moussy, et al., 2012). Sin embargo, al igual que en trabajos anteriores no ha sido sencillo detectar de manera precisa dicha estructura y menos aún se ha determinado el nivel de flujo genético entre las localidades y la tarea es más compleja cuando se considera la alta movilidad de estos organismos ya que en una sola noche pueden volar alrededor de 80 km (Medellín et al.,

2018). Además, no se conoce mucho acerca de los movimientos de los machos y no se tiene claro de donde provienen las hembras que forman las cuevas de maternidad al norte de su distribución. Por esas razones, en este trabajo se decidió utilizar un marcador que nos permitiera seguir el linaje paterno (*DBY*, asociado al cromosoma Y). El análisis de este marcador nos permite describir otro aspecto de la distribución de los linajes en la especie, siendo una región asociada al cromosoma sexual masculino no presenta recombinación, con lo cual podemos inferir de manera indirecta el comportamiento perenne de los machos y comparar los análisis de distribución de ambos linajes en las localidades incluidas en el muestreo.

Los valores de Fst entre las localidades del murciélago magueyero menor son bajos y para el caso de algunos análisis es casi nula, similar a los cálculos de estructura genética poblacional de otras especies de quirópteros que muestran un comportamiento migratorio como: *Nyctalus noctula*, *Tadarida brasiliensis* y *Pteropus spp*. (Svoboda et al. 1985, McCracken et al. 1994, Sinclair et al. 1996, Webb y Tidemann 1996, McCracken y Gassel 1997, Petit y Mayer 1999, 2000, Petit et al. 2001, Russell et al. 2005). Cabe destacar que estas especies no han sido analizadas a través de sus linajes parentales.

El estudio de la diversidad haplotípica nos permitió realizar análisis demográficos desde un punto de vista histórico, mismos que al compararlos con trabajos publicados anteriormente reflejan un patrón similar, una expansión demográfica reciente aunque con diferencias marcadas en la magnitud temporal, es decir, Arteaga et al. (2018), calcularon una expansión demográfica hace aproximadamente 34,737 años atrás y 47, 808 años atrás, mientras que en esta tesis se estableció una expansión que comenzó alrededor de 130,000 a 150,000 años atrás, durante el Pleistoceno. Además, la expansión ha sido apoyada con pruebas de neutralidad en los tres trabajos publicados en los últimos cinco años, lo cual nos da una mayor certeza de la información presentada en una expansión demográfica para el mtDNA. También, se cuenta con proyecciones basadas en modelos de distribución potencial hacia el pasado, y corresponden con una expansión geográfica de las condiciones óptimas climáticas para la especie durante el Último Interglacial y el Holoceno (Trejo-Salazar et al., 2021).

La expansión demográfica se pudo ver favorecida por los cambios climáticos registrados para el Pleistoceno, los cuales influenciaron la distribución de una gran variedad de grupos actuales. Sobre todo, la biota en zonas altas fue fragmentada durante los periodos interglaciales más cálidos (McDonald, 1993; Metcalfe et al., 2000; León-Paniagua et al., 2007; Ruiz et al., 2010; Bryson et al., 2011; Gugger et al., 2011), seguidos de expansiones relacionadas con un incremento en la temperatura (Hundertmark et al., 2002; Hofreiter and

Stewart, 2009; de Bruyn et al., 2011). La transición entre los modelos del último interglacial y el Holoceno son consistentes con las condiciones más cálidas en México hace 15,000-12,000 años atrás, y con la colonización de grupos ecológicamente relacionados con el murciélago magueyero, como Cactaceae y el género *Agave* principalmente hacia el norte de México y sur de los Estados Unidos (Metcalfe et al., 2000). En la actualidad, la distribución actual del murciélago magueyero menor corresponde con la distribución de muchas especies del género *Agave* (Scheinvar et al., 2017; Scheinvar, 2018).

Considerando lo anterior, la migración de las hembras de *L. yerbabuenae* puede haber comenzado al seguir los cambios en las condiciones climáticas del Pleistoceno. Los modelos que representan el Último Interglacial, Holoceno y el periodo actual, sugieren una expansión hacia el norte de México, siguiendo la Costa del Pacífico hasta llegar a Sonora y Arizona. Estas zonas albergan numerosas colonias de maternidad, incluyendo la cueva de maternidad más concurrida por la especie, la cueva del Pinacate (Medellin et al., 2018). El registro geológico para esta zona indica que hubo actividad volcánica reciente, aproximadamente hace 12,000 (+/- 4,000) años en el pasado, mientras que el origen del ecosistema actual ha sido fechado con una edad aproximada de 9,000 años (Marshall and Blake, 2009). En esa región del país, también se tiene evidencia de una expansión demográfica cactáceas asociadas a cambios climáticos durante el Pleistoceno-Holoceno (Clark-Tapia et al., 2003; Fehlberg y Ranker, 2009; Sosa et al., 2009; Cornejo-Romero et al., 2017). Estas fechas reportadas para los eventos biológicos y geológicos coincide con la estimación de la expansión geográfica en la proyección del modelo de distribución potencial para el Holoceno.

Por otra parte, los análisis demográficos basados en los marcadores genéticos son contrastantes, sugiriendo una expansión demográfica para los marcadores heredados vía materna, pero no para los linajes parentales. Esta expansión reciente es apoyada por las proyecciones de los modelos de distribución potencial hacia el pasado. Además, basados en estos análisis y los modelos de distribución potencial, también podemos inferir que la migración de las hembras hacia el norte ha sido un proceso ecológico dinámico que se pudo originar en la zona de la costa del Pacífico y el centro sur (cerca del Istmo de Tehuantepec). Esta hipótesis se refuerza porque en el desierto sonorense, específicamente en el Pinacate, las condiciones climáticas y geológicas ideales para el arribo de los murciélagos magueyeros se establecieron alrededor de 8,000 a 16,000 años atrás (durante la transición del Último Interglacial y el Holoceno).

La diversidad genética, las genealogías bayesianas y los modelos de las condiciones térmicas más estables desde el Pleistoceno hasta la actualidad, proveen información para asumir que el sitio de origen de la especie de murciélago magueyero menor se localiza

alrededor de la región del Balsas y la zona Centro-Sur del México. De acuerdo con los linajes basales de las genealogías propuestas en el capítulo 1, el ancestro de las poblaciones actuales de *Leptonycteris yerbabuenae* se encontraría al sur de la distribución actual. Esta hipótesis de origen de la especie al sur de su distribución actual está apoyada por la gran diversidad de linajes actuales que se pueden detectar al sur de la distribución, alrededor de la zona de la reserva Tehuacán-Cuicatlán.

Aunque aún no es posible determinar con plena certeza el centro de origen de la especie, se han propuesto distintas hipótesis evolutivas de la familia Phyllostomidea (Datzmann et al., 2010; Rojas et al., 2011; Rojas et al., 2012; Rojas et al., 2016; Abreu-Flores et al., 2019). Sin embargo, en ninguna de las filogenias reconstruidas con anterioridad, se habían incluido las tres especies del género *Leptonycteris*. Debido a esto, en este trabajo se incluyeron las tres especies para poder calcular de manera más precisa los tiempos de divergencia dentro del género y el origen de este a través de herramientas filogenéticas e inferencias evolutivas basadas en información ecológica y biogeográfica.

Las genealogías presentadas en el capítulo 1 agrupan al género *Leptonycteris* de manera monofilética con la especie *L. nivalis* como rama basal del género, y son congruentes con propuestas evolutivas presentadas con anterioridad. Sin embargo, resalta la diferencia en los tiempos de divergencia calculados para cada una de las tres genealogías construidas con los linajes parentales. Estas diferencias se pueden explicar por las diferencias en las tasas de mutación, los puntos de calibración y las especies externas utilizadas para poder realizar las reconstrucciones. Uno de los marcadores mitocondriales, *D-loop* (linaje materno) y el marcador asociado al cromosoma Y, *DBY* (linaje paterno) fueron congruentes 10.26 m. a. y 12.23 m. a. respectivamente. En contraste, para la genealogía basada en *Cyt-b*, la fecha de divergencia para el nodo corona de la especie se calculó en 4.03 m. a.

Cómo se mencionó anteriormente, las genealogías presentadas en el capítulo 1 no nos permitieron establecer con claridad los tiempos de divergencia entre las tres especies del género y tampoco establecer con certeza la fecha de origen de este. Por lo tanto, se construyó una hipótesis filogenética considerando a miembros de toda la familia Phyllostomidae. Con esta propuesta, podemos comparar las fechas presentadas en el capítulo 1. Por una parte, la monofilia del género se mantuvo en el mismo orden que las genealogías con linajes parentales. Mientras que las fechas de divergencia para el género *Leptonycteris* y las tres especies que se agrupan en él son coincidentes con el marcador mitocondrial *D-loop* y el linaje paterno, *DBY*. La fecha de divergencia calculada en la filogenia de la familia Phyllostomidae, para el grupo basal del género *Leptonycteris* se calculó en 17.21 m.a. y el

grupo corona en 13.91 m.a, mientras que la fecha para la especie *L. yerbabuenae* fue de 13.3 millones de años.

Con base en los cálculos de los tiempos de divergencia podemos relacionar diversos eventos evolutivos dentro de la familia Phyllostomidea y particularmente dentro del género *Leptonycteris* con eventos climáticos, geológicos, ecológicos y evolutivos de otros grupos para explicar la historia evolutiva de las especies de interés para este trabajo. Así, los tiempos de divergencia calculados con herramientas bayesianas y los eventos demográficos pueden estar relacionados con eventos climáticos a escala global que afectaron el territorio de distribución actual, no solo para el murciélago magueyero menor, sino para las tres especies del género. Durante el Mioceno (23-5 m.a.) se registraron cambios de temperatura que provocaron cambios en la vegetación y diversos eventos de diversificación en varios grupos de organismos. La primera coincidencia con los murciélagos nectarívoros en América se encuentra a principios de este periodo, ya que la fecha de divergencia para la subfamilia Glossophaginae fue de 23.67 m.a. grupo basal y 21.78 grupo corona. También, a mediados y finales del Mioceno es cuando coinciden las fechas de origen y diversificación para las tres especies de *Leptonycteris* mencionadas anteriormente (13.91 mya; Fig. 5.1).

Se cuenta con algunas evidencias de las características del Mioceno en América, particularmente en México, estudios paleopalinológicos reportan que había una alta diversidad vegetal con flora genérica muy parecida a la actual desde el Mioceno medio (Ramírez-Arriaga et al., 2014), se cuenta con datos de la presencia de bosque mesófilo de montaña, bosque de pino-encino, matorral esclerófilo perennifolio y bosque tropical caducifolio. Muchos de estos elementos se han conservado desde comienzos del Neogeno y parte de los elementos florísticos predominantes son las cactáceas y miembros del grupo *Agave*, estos últimos con registros desde el Mioceno-medio (Ramírez-Arriaga et al., 2014). Por lo que tiene sentido pensar que los murciélagos contaban con diferentes recursos en los cuales especializarse, sobretodo considerando que estos recursos también estaban pasando por una fase de diversificación y expansión en cuanto a su distribución.

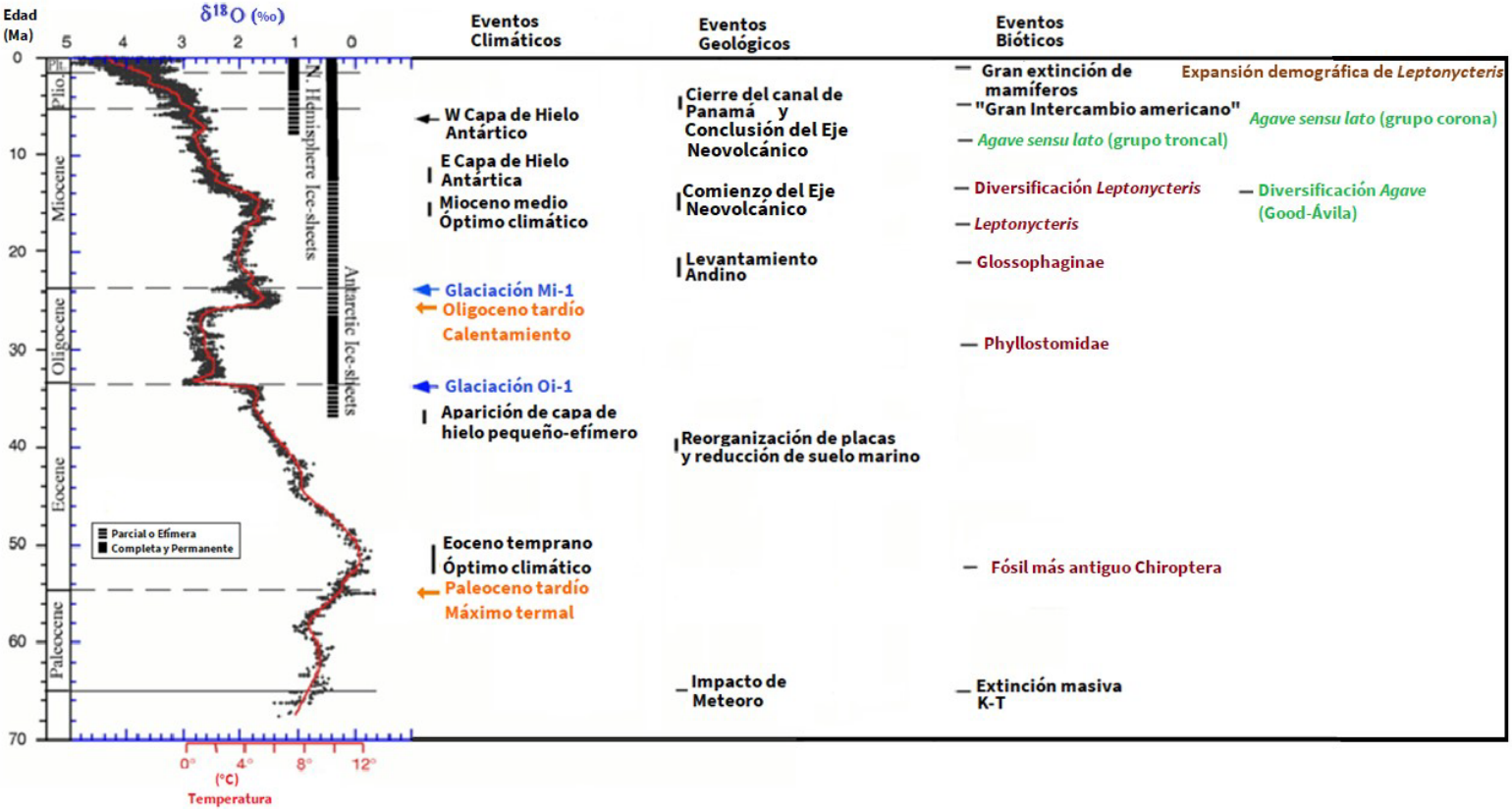

Figura 5.1. Diagrama temporal de calentamiento global con diversos eventos climáticos, téctonicos y biológicos en el territorio de distribución actual del género *Leptonycteris*. Modificado de Zachos *et al*., (2001).

Los tiempos de divergencia dentro de la subfamilia Glossophaginae son consistentes con dos pulsos de aceleración en la tasa de diversificación del grupo *Agave sensu lato*, la primera hace 8 millones de años atrás que coincide con la divergencia de los quirópteros del género *Glossopahaga* (8.06 m.a. grupo corona) y la segunda hace 3-2.5 millones de años (Good-Ávila et al., 2006) y con los más recientes reportes con un pulso hace 6.18 y 4.91 millones de años atrás (Jiménez-Barron et al., 2020). *Agave sensu lato* tiene una edad del grupo basal de 9 millones de años y el grupo corona de 6.18 millones de años. Si bien, los tiempos de diversificación del género *Leptonycteris* no se sobreponen de manera directa con los tiempos reportados para el grupo *Agave*, si podemos observar cercanía temporal entre los eventos de diversificación que envuelven a ambos grupos. El grupo *Striata* diversificó con una edad basal de 4.15 y el grupo corona con 2.24 m.a. Para el grupo *Agave sensu stricto* se calculó una edad de entre 3.55-2.68 m.a.

De acuerdo con los datos anteriores, podríamos pensar en un proceso demográfico en el que los murciélagos del género *Leptonycteris* explotara los recursos ofrecidos por los miembros del género *Agave* y de esta manera acaparar este importante recurso en sus áreas de distribución. Con base en los análisis presentados en esta tesis, podemos comenzar a

explicar de manera puntual las circunstancias que rodeaban a las especies del género *Leptonycteris* y en particular como ocurrieron dichos eventos, principalmente el proceso de divergencia entre la especie *L. curasoae* y *L. yerbabuenae*, ya que *L. curasoae* se encuentra aislada geográficamente respecto de las otras dos especies (*L. nivalis* y *L. yerbabuenae*).

El evento de especiación que dio origen a la especie *L. curasoae* parece ser el resultado de un evento parapátrico en el que el murciélago magueyero de Sudamérica se aisló completamente al romperse la conectividad en Centroamérica, proceso del que se piensa, tuvo lugar durante el Plioceno (5.33 – 2.59 mya). Esta hipótesis se basa en que se ha reportado una probable contracción del bosque mesófilo de montaña en la zona y expansión del bosque tropical caducifolio y zonas áridas, durante el Plioceno tardío y Holoceno temprano (Rosales-Torres et al., 2017). Además, todos estos eventos de divergencia coinciden con un decremento en la temperatura de los climas húmedos tropicales en México durante los periodos glaciales entre 5.3 y 1.8 millones de años atrás (Van Devender, 2000).

Aunado a los tiempos de divergencias, se cuenta con los cambios en las tasas de diversificación. El análisis realizado con base en análisis filogéneticos muestra dos cambios (aceleraciones). El primero se detectó hace 24 millones años aproximadamente, coincidiendo con el origen de la familia Phyllostomidae, lo que nos habla de un proceso de diversificación acelerado (radiación adaptativa). El segundo cambio en la tasa de diversificación se ubica en el grupo corona de la subfamilia Stenodermatinae, en donde se encuentran los murciélagos primordialmente frugívoros y el cual cuenta con el mayor número de especies de toda la familia. Nuevamente, los puntos de aceleración de las tasas de divergencia coinciden con los datos mencionados previamente, la mayoría ocurren durante el Mioceno cuando hay un cambio climático y de vegetación en las zonas de distribución de los murciélagos magueyeros.

En la historia más reciente de la especie *L. yerbabuenae*, contamos con la expansión demográfica detectada a través de métodos coalescentes y la información en la que se basan los modelos de distribución potencial de las especies. Es decir, conocemos la distribución actual pero aún no hemos determinado con certeza las causas históricas para la separación entre las dos especies mexicanas y el aislamiento de la especie caribeña *L. curasoae*. Por esta razón, a través de inferencias filogenéticas, se obtuvo información biogeográfica, ecológica y evolutiva del género. Los modelos de distribución potencial fueron construidos basados en registros históricos en colecciones científicas para las especies mexicanas (para el caso de *L. nivalis* y *L. yerbabuenae*) y los registros de la base de datos GBIF de las tres especies. Con base en esta información, pretendemos describir el centro de origen del género y de ser posible las especies. En complemento, se proponen hipótesis de especiación dentro

del género basados en la información ecológica y evolutiva que se generó a partir de los diferentes análisis.

En cuanto a la distribución histórica de las tres especies, tenemos algunas restricciones que no nos habían permitido definir con claridad los espacios que pudieron ser aptos para que estas se desarrollaran. Una de estas limitantes es el escaso registro fósil, para el grupo *Leptonycteris* no se cuenta más que con un fósil (Arroyo et al., 1992), el cual es muy reciente en comparación con los tiempos de divergencia, estos restos se encontraron en depósitos pleistocénicos, más específicamente del Pleistoceno tardío en una cueva ubicada en el norte de México, lo cual nos genera más dudas, ya que según los análisis desarrollados, durante el pleistoceno podríamos describir procesos demográficos históricos, y por otro lado, el fósil pertenece a la especie *L. nivalis*, lo cual nos indica que el proceso de especiación ya había originado a las especies actuales del género.

Determinar las causas de la separación de las especies es muy complejo, ya que las especies con mayor cercanía filogenética son *L. curasoae* y *L. yerbabuenae*, de manera histórica estas dos especies se han mantenido como un grupo monofilético y *L. nivalis* como la especie basal en el género (Wetterer et al., 2000). En la filogenia presentada en el capítulo 3 de esta tesis, se muestra la misma topología para el caso del género *Leptonycteris*. Para explicar las causas que originaron la divergencia de estas tres especies, se deben considerar circunstancias históricas en cuanto a la ecología de las especies. Sin embargo, es complicado encontrar diferencias en las características ecológicas entre los tres murciélagos magueyeros ya que en México se les encuentra en los mismos climas y vegetación. Además, se pueden alimentar de los mismos grupos de plantas, e incluso de las mismas especies. De la misma forma, *L. curasoae* consume néctar y frutos de los mismos grupos Cactacea y *Agave* principalmente, Bombacáceas y leguminosas y también habita en climas y tipos de vegetación similares (selva seca tropical, matorral espinoso y bosques de cardones) aunque su distribución se encuentra restringida a otra zona del continente. Por lo tanto, el papel ecológico de las tres especies es el mismo en cada zona en que se distribuyen, mostrando un paralelismo en cuanto a sus hábitos alimenticios, comportamientos migratorios y rol ecológico.

Con base en la reconstrucción filogenética de la familia Phyllostomidae y el análisis de reconstrucción de áreas ancestrales, el género *Leptonycteris* tuvo su origen en Norteamérica, lo cual coincide con los datos y evidencias aportadas con los análisis filogeográfico y biogeográficos, en donde se representan a través de una genealogía que la mayor diversidad se encuentra en el centro-sur de México, mientras que a través de los modelos y proyecciones de distribución hacia el pasado, esta misma zona ha sido la más estable climáticamente hablando para mantener a los murciélagos de las especies *L. nivalis* y *L. yerbabuenae*.

Además, en la misma zona se encuentran sobrepuestas las zonas de mayor diversidad del género *Agave* y corresponde con zonas de presencia de zonas representativas de los paisajes mexicanos (Selvas secas y matorrales). Por lo que pensamos, que el centro de origen para el género se encuentra en la zona central de México, muy probablemente cerca de las zonas que conocemos con mayor diversidad de agaves y cactus en México.

Al igual que los demás murciélagos nectarívoros, los murciélagos magueyeros cuentan con un ancestro que potencialmente tendría la capacidad de consumir frutos e insectos además del néctar. Esto es importante porque actualmente sabemos que los murciélagos de la especie *L. yerbabuenae* consumen frutos principalmente de cactus columnares (Rojas-Martínez et al., 2012; Rojas-Martínez et al., 2015; Aliperti et al., 2017) y aunque no es su dieta principal, pueden explotar este recurso también. De la misma forma, se han detectado restos de insectos en las heces fecales y en los análisis de materia en los estómagos de varios individuos de la especie (Sperr et al., 2011). La capacidad de consumo de insectos en las especies nectarívoras no parece una cuestión fuera de lo común, considerando la posibilidad de encontrar estos artrópodos en las flores o bien, en la búsqueda de fuentes de proteína cuando no se cuenta con polen y néctar disponible para cubrir la necesidad alimenticia.

El papel del murciélago magueyero menor como polinizador eficiente y primario en varias especies de *Agave* no está en duda, sin embargo, no se han realizado observaciones directas en la gran mayoría de las especies de *Agave*, pero si se han registrado las características florales y en su mayoría están asociadas a una polinización quiropterofílica (Eguiarte et al., 2021). A pesar de que en México se encuentran 12 especies de murciélagos nectarívoros, solo dos especies son numerosas demográficamente hablando, los murciélagos magueyeros (género *Leptonycteris*). De estas dos especies de murciélagos magueyeros, el murciélago magueyero menor (lesser long-nosed bat, *L. yerbabuenae*) cuenta con un número mayor de colonias, área de distribución y números demográficos en comparación con su especie hermana *L. nivalis* (Rusell and Wilson, 2006), lo que representa una mayor capacidad y por tanto, un papel más relevante como polinizador de plantas silvestres en la mayoría del territorio mexicano.

En términos ecológicos, es evidente la estrecha relación que existe entre el género *Leptonycteris* y el grupo *Agave*. Los periodos de floración de varias especies de *Agave*, corresponde con los periodos de migración de las hembras de las dos especies de murciélagos nectarívoros, lo cual ya había sido mencionado por algunos autores (Gentry, 1982, pp. 417–481; Fleming et al. 1993; and Wilkinson and Fleming 1996), incluso se han publicado esfuerzos parecidos con herramientas basadas en sistemas de información Geográficos (SIG; Gómez-Ruiz and Lacher, 2016). Originalmente, la hipótesis fue propuesta

por Gentry, 1982 y la llamó “corredor de néctar”. Siguiendo esta idea, los análisis presentados en este trabajo modelan la distribución de las especies en el pasado y sugieren como posibles refugios pleistocénicos para las dos especies de murciélagos: 1) a lo largo de la costa del pacífico, concretamente alrededor de Jalisco y Colima y; 2) La zona Centro-Sur de México, específicamente en los estados de Puebla y Oaxaca. Ambas zonas corresponden con las zonas de mayor diversidad de agaves en México, sugiriendo que estas dos pueden ser “hotspots” de coevlución entre estos dos grupos.

Por último, retomando los análisis de demografía histórica basados en coalescencia, se detectó que *L. yerbabuenae* comenzó una expansión demográfica reciente, aproximadamente hace 140 a 150 mil años. Esta edad corresponde con los estimados reportados para la especie *Agave lechugilla* (Scheinvar, 2018) apoyando así una historia de procesos demográficos y evolutivos al menos entre estas especies. Es probable que si pudiéramos realizar este tipo de ejercicios con más especies del género *Agave* se podría determinar cuántas de ellas mantienen una relación tan estrecha históricamente hablando con el género *Leptonycteris*, al menos para las de más amplia distribución se podrían definir claramente estos patrones. El ejercicio realizado en esta tesis, en el cual se simularon las condiciones climáticas más estables durante cuatro temporalidades de la era Cuaternario, se debe reproducir para simular las condiciones que ha atravesado el murciélago *L. curasoae* y los probables escenarios en los que se ha desarrollado su relación ecológica con las especies de *Agave* presentes en su zona de distribución actual. Sabemos que en Venezuela se distribuyen al menos tres especies de agaves: *A. americana*, *A. buldinghiana* y *A. cocui*, las cuales son nativas de Venezuela (Rodríguez et al., 2021).

Conclusiones

Diversidad genética

El murciélago magueyero menor, se encuentra genéticamente “saludable”. De acuerdo con los estimados, los tres marcadores analizados mostraron alta diversidad genética ($H<0.8$). No se encontraron diferencias significativas entre los sexos en las localidades del murciélago *L. yerbabuenae*, a pesar de que hay sesgos en algunos muestreos debido al comportamiento migratorio de algunas hembras.

Historia Evolutiva del murciélago magueyero menor

El murciélago *L. yerbabuenae* surgió alrededor de 13 millones de años en el pasado. Ha pasado por una expansión demográfica hace 130,000 a 150,000 años atrás. Esta

expansión coincide con algunos eventos climáticos y se relacionan de manera estrecha con una expansión en la distribución del género *Agave* a lo largo de su distribución. Se considera que la especie surgió en la zona del centro-sur de México (Puebla-Oaxaca) y a partir de ahí ha expandido su distribución, aprovechando las condiciones climáticas y ecológicas antes mencionadas.

Coevolución con grupo *Agave*

La coevolución entre los agaves y las especies del género *Leptonycteris*, en especial con *L. yerbabuenae* es un hecho, aunque no hay una coevolución lineal y directa entre ambos grupos. En el presente trabajo se aportan evidencias ecológicas y evolutivas para apoyar esta idea. Encontramos una edad de 13.91 millones de años para el género *Leptonycteris* y 21.78 millones de años para el grupo Glossophaginae.

El análisis basado en modelos de distribución temporales nos indica una estrecha dinámica espacio-temporal entre ambos grupos, sobreponiéndose sus distribuciones en cada uno de los tiempos analizados (Último interglacial, Máximo glacial, Holoceno y Actual). Este análisis respalda la evidencia ecológica reportada por trabajos anteriores con especies de agaves observados, así como trabajos en donde se construyen modelos de distribución actuales y pasados para las especies del género *Agave* por separado (Sheinvar et al, 2019).

Reforzar los planes de manejo para la especie manteniendo corredores que permitan el consumo de sus principales fuentes de alimentación.

Se debe considerar que, en el escenario actual, lo más conveniente es generar planes y programas de manejo y conservación de corredores que permitan la conectividad entre las localidades, las zonas de forrajeo y las rutas migratorias, así como con las cuevas de maternidad, permitiendo así que se conserve la diversidad genética en los niveles adecuados. En ese sentido, se propuso el programa Bat-Friendly en la producción tequilera y mezcalera para que se apoyara las rutas migratorias de las hembras de murciélagos del género *Leptonycteris*, así como las zonas de su presencia en todo el año, principalmente en el centro-sur del país.

Perspectivas

A lo largo de este trabajo, se han citado diversas fuentes en donde se reportan trabajos evolutivos, genómicos y ecológicos enfocados en las especies del género *Leptonycteris* y particularmente en *L. yerbabuenae*. Sin embargo, aún quedan muchas preguntas por responder para entender la naturaleza de esta especie y contrastarla con otros murciélagos y

otros mamíferos para describir las dinámicas ecológico-evolutivas, y aplicar todo este conocimiento a los programas de manejo y recuperación cuando esta sea necesario.

Por el momento, se pueden seguir varias líneas de investigación. Entre estas, considero importante trabajar con nuevas tecnologías y análisis, como la secuenciación masiva para lograr mayor resolución con análisis de genómica poblacional, filogeografía, genética del paisaje o bien la búsqueda de "genes candidatos" a través del muestreo de Single Nucleotide Polymorphims (SNP's). Este tipo de marcadores y la tecnología de secuenciación actual nos abren una gama mayor de posibilidades en donde podemos inferir escenarios futuros bajo cambio climático y calentamiento global, degradación y fragmentación de hábitats o decrecimiento de las poblaciones de estos organismos.

La biología de los quirópteros en general, y de los murciélagos magueyeros en particular, les permite el consumo de azúcares en alta concentración, así como frutos. Además, el gasto energético que realizan al volar grandes distancias los convierte en especies de interés humano para comprender sus rutas metabólicas y realizar prospección con un enfoque biomédico, por ejemplo, para entender padecimientos como la diabetes (Gutiérrez-Guerrero et al., 2020). La investigación con nuevas tecnologías de secuenciación masiva nos permite la exploración en aspectos de preocupación actual como el sistema inmune y todas aquellas enfermedades que, en algún momento, se pueden volver emergentes (como ha ocurrido en años recientes).

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Apéndices

A.1

Oikos=18

Roberto Trejo, Luis E. Eguiarte y Rodrigo A. Medellín

¿Quién no disfruta una copa de un buen tequila? ¡Eso ni se discute! Pero lo que poca gente sabe es que le debemos el tequila, la bacanora y el mezcal al trabajo paciente y nocturno de un murciélago: el murciélago magueyero, en latín *Leptonycteris*, que durante millones de años ha ido seleccionando cuidadosamente los mejores agaves para que produzcan más y mejor néctar, del que luego él se alimenta. Esta selección ha llevado a la evolución de una gran diversidad de especies de agaves, plantas únicas en el mundo, que posteriormente los mexicanos hemos aprendido a utilizar, entre otras cosas, para producir mezcales (ver *Agave, mezcal tradicional, cultura y diversidad*) y tequilas. Desafortunadamente, en la actualidad las poblaciones de los diferentes murciélagos nectarívoros se encuentran amenazadas, y debemos hacer lo posible para protegerlas; así aseguramos, a largo plazo, el bienestar de los agaves comerciales. Para entender esta historia, debemos conocer un poco sobre la biología y más específicamente sobre la ecología de los murciélagos, es decir sus relaciones con otras especies y con las condiciones ambientales que los rodean.

Los misteriosos murciélagos

De todos los organismos que habitan la Tierra, indudablemente los murciélagos representan uno de los grupos más misteriosos, diversos, interesantes y, ya que uno los conoce más, fascinantes. Los murciélagos son los únicos mamíferos voladores de nuestro planeta, y lo han habitado durante más de 50 millones de años. A partir de su origen se han diversificado en sus formas corporales y hábitos alimenticios, y han colonizado casi todos los ecosistemas de la Tierra. Actualmente los científicos reconocen más de 1,300 especies de estos mamíferos.

Durante el día podemos encontrarlos en cuevas o refugios similares; en cavidades y hoyos en los árboles, e incluso debajo de las hojas o bajo la corteza de los árboles, en bajo-puentes y en casas y construcciones abandonadas o poco utilizadas, incluyendo pirámides e iglesias. Durante la noche, los murciélagos salen de sus refugios y se alimentan. La mayoría de las especies comen insectos, pero hay otras que se alimentan principal o únicamente de néctar y polen, de frutas, o de semillas; otros son carnívoros y comen ranas, peces y pequeños mamíferos. Por último, debemos mencionar un pequeño grupo de sólo tres especies, que se alimentan de sangre de mamíferos o de aves; éstos son los verdaderos vampiros.

Por desgracia, los murciélagos son muy vulnerables a las actividades humanas, ya que es fácil destruir sus refugios y son muy frágiles ante la toxicidad de los insecticidas usados en cultivos; en otros casos son agredidos directamente por la gente, ya que les temen y acaban con ellos al confundirlos con los vampiros. Además, en años recientes se ha prestado mucha atención a los murciélagos porque se cree erróneamente que son vectores de muchas enfermedades letales como el Ébola en África (los vectores son, según la Organización Mundial de la Salud, "organismos vivos que pueden transmitir enfermedades infecciosas entre personas, o de animales a personas"). Merlin D. Tuttle, en su artículo *Give Bats a Break*, demuestra que los artículos científicos que los acusan de ser vectores de Ébola, MERS, Henipa y otros virus contienen serias especulaciones y exageraciones infundadas.

Por otra parte, las actividades humanas afectan a todos los ecosistemas (por ejemplo: desiertos, selvas, pastizales, bosques, humedales y cuevas), y en muchos casos lo hacen de

manera negativa: los contaminan y destruyen, con lo cual contribuyen al calentamiento global y a la disminución o pérdida de especies y, a mayor escala, a la pérdida de los servicios que brindan los ecosistemas naturales que se sostienen gracias a las interacciones entre los organismos (animales, microorganismos, hongos y plantas) y su ambiente. Entre las diversas actividades humanas que alteran los ecosistemas, destaca el cambio del uso de suelo de zonas naturales para uso agrícola y ganadero y para la construcción de infraestructura urbana.

Se considera que un ecosistema es saludable cuando se mantiene funcionando y cuando, desde el punto de vista humano, puede ser valorado por los servicios que provee, conocidos como servicios ecosistémicos (ver *Es posible vivir con diferentes tipos de transporte. Pero no podemos vivir sin comer*). Entre los servicios ecosistémicos que puede proporcionar un ecosistema boscoso, por ejemplo, están la purificación del agua y aire, la estabilización de suelos, la mitigación de enfermedades e inundaciones y la regulación del clima. En este sentido, los murciélagos son especialmente importantes en muchos ecosistemas, porque contribuyen a regular las poblaciones de insectos (lo cual es particularmente relevante cuando se trata de insectos nocivos) y por el servicio de polinización y dispersión de semillas que llevan a cabo.

La polinización es uno de los servicios ecosistémicos más importantes que brindan los murciélagos de zonas tropicales y subtropicales. Estos murciélagos polinívoros (o nectarívoros) son polinizadores notables, ya que pueden visitar flores en distintas localidades y transportar el polen a grandes distancias —hasta unos 90 kilómetros— polinizando de manera muy eficiente a las plantas, entre ellas los magueyes y plantas que sirven para producir todo tipo de mezcales (cualquier derivado fermentado de agave) y pulque. Además, los murciélagos polinizan a la mayoría de las cactáceas columnares de México, y a árboles mexicanos tan característicos y representativos como las ceibas, los pochotes (el árbol sagrado de muchos pueblos mesoamericanos) y los cazahuates (varias especies de *Ipomoea*), entre otros.

Agaves para beber

En el continente americano viven 38 especies de murciélagos nectarívoros, y 12 de estas especies las podemos encontrar en México. Una de las más importantes es el *Leptonycteris yerbabuenae*, un murciélago nectarívoro conocido como murciélago magueyero. Éste es un interesante animal que forma colonias que pueden albergar hasta 200,000 mil individuos en un mismo refugio. Es muy eficiente al visitar las flores y polinizarlas gracias a sus adaptaciones morfológicas y fisiológicas, y en nuestro país casi siempre se le encuentra asociado a las floraciones de los agaves y cactáceas columnares.

México cuenta con la mayor diversidad de especies del género *Agave* en el mundo; de 200 especies, el 75% son nativas del país y el 69% son endémicas (sólo se encuentran en México, no crecen de manera natural en ningún otro lado del mundo). La diversidad de los agaves corresponde con lo que se conoce como una radiación adaptativa, generada por su co-evolución —un tipo de evolución en la que dos especies van cambiando de forma paralela por selección natural recíproca— con el murciélago magueyero (ver *The Evolution of Bat Pollination: a Phylogenetic Perspective*). En el estudio *Pollination Biology and Adaptive Radiation Of* Agavaceae*, With Special Emphasis On The Genus* Agave demostramos que los dos grupos surgieron y evolucionaron al mismo tiempo y que, con el paso del tiempo, los murciélagos fueron seleccionando activamente las plantas que producían más néctar, más flores e inflorescencias más altas. Los agaves gastan tanta energía en reproducirse que por eso se mueren después de producir sus flores y semillas; ¡de agotamiento sexual! Toda esta energía se almacena en las cabezas o piñas de los agaves justo antes de la floración, y estos recursos, en especial los azúcares de la savia, son los que usamos como materia prima para producir las bebidas emblemáticas de México en el mundo: el tequila, el mezcal y el pulque (para saber más acerca de estos procesos, ver los artículos *Agave, mezcal tradicional, cultura y diversidad* y *De dioses a* hipsters*: el resurgimiento del pulque, una moda de antigua tradición*).

Indudablemente, una de las especies más importantes del género *Agave* es el famoso *Agave tequilana* Weber var. *azul*, a partir del cual se produce el tequila. Para llevar a cabo el proceso de destilación tanto del tequila como del mezcal, las plantas se cosechan antes de que se produzca la floración. Esta práctica busca aprovechar al máximo los azúcares que el agave ha acumulado durante varios años, pero impide que los murciélagos puedan consumir el néctar y que transporten el polen de una planta a otra. Para replantar sus campos, los productores usan los hijuelos, plantas clonales que crecen en la base de plantas adultas, pero que son copias genéticas exactas de la planta madre. El hecho de que se impida la polinización y se usen hijuelos para repoblar los campos de agaves tequileros y mezcaleros ha provocado que los cultivos sean cada vez más vulnerables al ataque de enfermedades, ya que ahora las poblaciones de *A. tequilana* Weber var. *azul* tienen poca variación genética —son básicamente un gran clon—. Esto las hace susceptibles a los ataques de insectos y a sufrir infecciones como las causadas por la bacteria *Erwinia* y el hongo *Fusarium* de la enfermedad "Tristeza y Muerte"

del agave.

Según el Consejo Regulador del Tequila, para el año 2015, se produjeron poco más de 228 millones de litros de esta bebida. Para producir tal cantidad se requirió cortar casi 789 mil toneladas de cabezas de *A. tequilana* Weber var. *azul*. Estas exportaciones produjeron una ganancia de unos 1.1 miles de millones de dólares, y en el mercado nacional 13,900 millones de pesos. Y, como ya mencionamos, el tequila no es el único producto derivado de los agaves; a partir de otras especies o variedades se obtiene pulque, mezcal y bacanora, entre otros. Sumado a esto, cada vez se produce, exporta y consume más mezcal en México y en todo el mundo. Por si fuera poco, hay algunas localidades de México en las que, además de usarlos para producir bebidas, los agaves se explotan para producir fibras y sus hojas se usan para cocinar barbacoa y mixiotes, entre otros usos culinarios.

Bebidas amigables con los murciélagos

Los murciélagos son tan importantes para los agaves, que la enorme riqueza que estas plantas generan se la debemos a estos animalitos que han sido y siguen siendo una gran fuerza de selección evolutiva. Así que, ¿acaso no podríamos destinar una fracción minúscula de los recursos generados por esta industria para el estudio y conservación de los polinizadores de los agaves? Para lograr esto y por el bien del capital natural de México y de las industrias que explotan estas plantas, se necesita urgentemente dedicar esfuerzos a la conservación de los agaves silvestres, muchos de los cuales aún se utilizan para producir mezcal. Una propuesta modesta es que los productores dejen que florezca un pequeño porcentaje de las plantas que cultivan, para que éstas proporcionen néctar a las poblaciones de murciélagos magueyeros. De esa manera se podrá mantener la variación genética de las poblaciones de agaves, y éstos producirán suficientes semillas para que las poblaciones futuras logren regenerarse de forma natural. Para subsistir, cada murciélago necesita visitar cientos de flores cada noche, y las colonias del murciélago magueyero pueden estar compuestas por muchos miles de individuos, así que es inimaginable la cantidad de flores que una colonia de estos murciélagos nectarívoros puede visitar y, potencialmente, polinizar. Todos estos murciélagos necesitan muchísimas flores para alimentarse cada noche.

La propuesta de nuestro programa de tequilas y mezcales *Bat Friendly*MR es que, de cada 20 agaves que se utilizan, ya sea que provengan de poblaciones silvestres o de cultivos, se deje una inflorescencia sin cortar para que produzca sus flores y néctar de manera natural. Además, sugerimos que el 0.1% de las ganancias netas derivadas de estas bebidas se invierta en el estudio de los agaves y sus polinizadores y en la conservación de las poblaciones silvestres de ambos grupos: magueyes y murciélagos. Hoy el proyecto *Bat Friendly*MR cuenta con la participación de varios productores de Jalisco y de Michoacán, que han asumido la responsabilidad y el compromiso de cuidar a los murciélagos para así proteger a los agaves, y también de embotelladores y bartenders de Filadelfia, San Antonio, Washington, D. C., Nueva York, y muchos otros sitios que, enterados de la importancia de los murciélagos para la industria, promueven esta información entre sus clientes.

Esta modesta inversión en plantas y recursos económicos va a evitar, a la larga, que la industria se vea afectada por los problemas que algunos productores ya empiezan a tener a causa de la baja variación genética de las plantas productoras de tequila. Ésta no es la primera vez que surge un problema de esta naturaleza en un monocultivo propagado a partir de clones y asociado a una planta productora de bebidas alcohólicas; algo semejante sucedió en Europa a finales del siglo XIX con las vides y la filoxera; si bien esta enfermedad importada de América atacó tanto a vides silvestres como cultivadas, la baja variación genética de los monocultivos contribuyó a la propagación de la epidemia, que acabó con una enorme cantidad de plantas. La filoxera prácticamente acabó con el cultivo de la uva en Europa, con un costo humano y económico brutal. De igual manera sucedió durante la gran hambruna de Irlanda, entre 1845 y 1849, cuando millones de personas murieron, debido a la pérdida de cultivos de papa cuya variación genética era muy baja en esos tiempos.

Así que cuando pidan un mezcal o un tequila, pregunten por las marcas que portan el distintivo *Bat Friendly*MR. Los murciélagos, los agaves, y su propio paladar, se los agradecerá.

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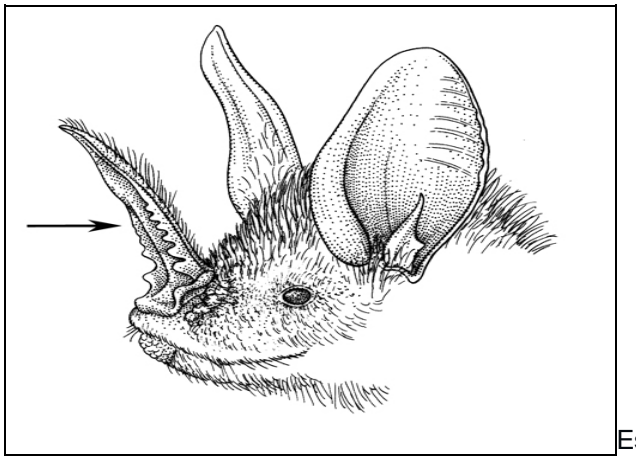
A.2

HISTORIA DE LA EVOLUCIÓN DE LOS MURCIÉLAGOS DE HOJA NASAL

Hits: 8437

Roberto E. Trejo Salazar. 2013. Tiempos de Divergencia de la Familia Phyllostomidae (Chiroptera): Origen de la Nectarivoría. Instituto de Ecología, UNAM. Tesis de Maestría, Programa de Ciencias Biológicas, UNAM. Director de tesis Dr. Luis E. Eguiarte. Esta tesis recibió el reconocimiento **Bernardo Villa** ***en 2014.***

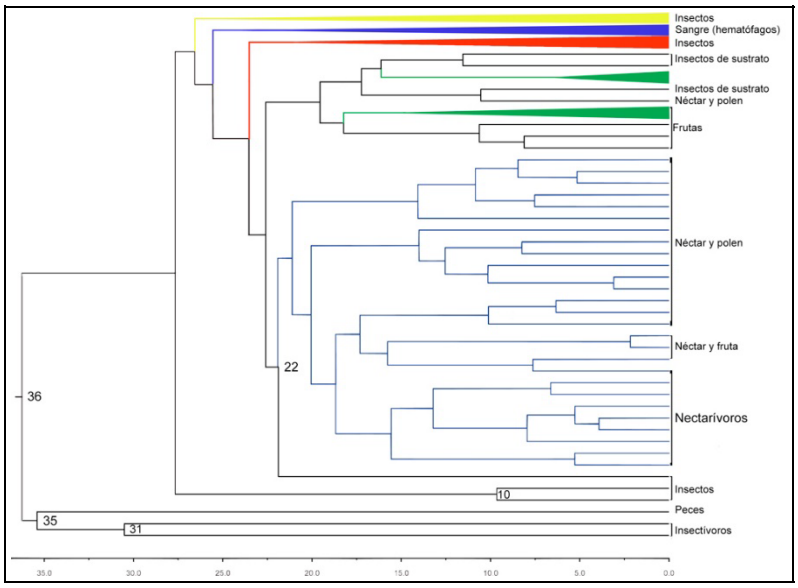


Estudiar los patrones evolutivos a partir de la ecología de los organismos no es un enfoque nuevo. Este tipo de estudios se ha desarrollado desde los trabajos clásicos de naturalistas como Charles Darwin y Alfred R. Wallace del siglo XIX. Sin embargo, fue hasta 1958 que León Croizat acertadamente señaló que "la Tierra y la biota evolucionan juntas". Para comprender la relación entre ecología y evolución, considero que uno de los mejores modelos son los murciélagos, particularmente los miembros de la familia Phyllostomidae. Este grupo de quirópteros tienen nariz en forma de hoja y sólo se encuentran en América (Figura 1).

La familia Phyllostomidae, además de poseer ecolocalización para ubicarse en el espacio y rastrear el alimento, es también una de las más diversas del orden Chiroptera, con poco más de 170 especies, sólo debajo de la familia Vespertilionidae y Pteropodidae. Los murciélagos de la familia Verspertilionidae viven en todo el mundo y los segundos, los zorros voladores –Pteropodidade–, sólo se encuentran en la región tropical y subtropical del viejo mundo. En la familia Phyllostomidae podemos observar todos los tipos de alimentación de los murciélagos. Así, se conocen especies que sólo consumen sangre (hematófagas), frutas (frugívoras) y algunas son carnívoras, hay otras que pueden combinar su alimentación (omnívoras), incluso hay reportes de especies que comen hojas y por supuesto, también existen las que consumen polen y néctar. Gracias a su gran diversidad de formas alimenticias, los miembros de la familia Phyllostomidae juegan distintos papeles ecológicos, algunos muy importantes en las comunidades donde habitan. Los insectívoros ayudan al control de plagas; los frugívoros ayudan a la dispersión de semillas y por supuesto aquellos que llevan a cabo la polinización de una gran variedad de plantas son los nectarívoros o polinívoros.

Los murciélagos considerados estrictamente nectarívoros que habitan en México se han agrupado en la subfamilia Glossophaginae, pero también se conocen especies de las subfamilias Brachyphyllinae y Phyllonycterinae que contribuyen de manera eficiente a la polinización de varias especies de plantas en Centroamérica y el Caribe, todos ellos son miembros de la familia Phyllostomidae. Los glosofaginos mexicanos en su mayoría, habitan en zonas secas y tropicales, con temperaturas relativamente altas y precipitación baja. Pueden vivir en bosques caducifolios y subcaducifolios, en bosques de arbustos espinosos y zonas semidesérticas y desérticas. Entre los glosofaginos mexicanos tenemos dos especies de *Leptonycteris* (*L. yerbabuenae*, *L. nivalis*), a *Choeronycteris mexicana* y a varias especies del género *Glossophaga* que en general son más de zonas tropicales. Estos murciélagos son los principales polinizadores de plantas como agaves, cactáceas columnares, y árboles como los pochotes (varias especies del género *Ceiba*) y los cazahuates (varias especies de *Ipomoea*. Para saber más vea De calorías y murciélagos en el *Blog* La Huella del Jaguar). La pérdida o disminución de las poblaciones de murciélagos nectarívoros puede ocasionar un impacto negativo para estas especies vegetales, muchas de las cuales son especies clave o dominantes ecológicamente, y otras pueden tener valor económico, como los agaves que se usan para producir bebidas como los mezcales y el tequila.

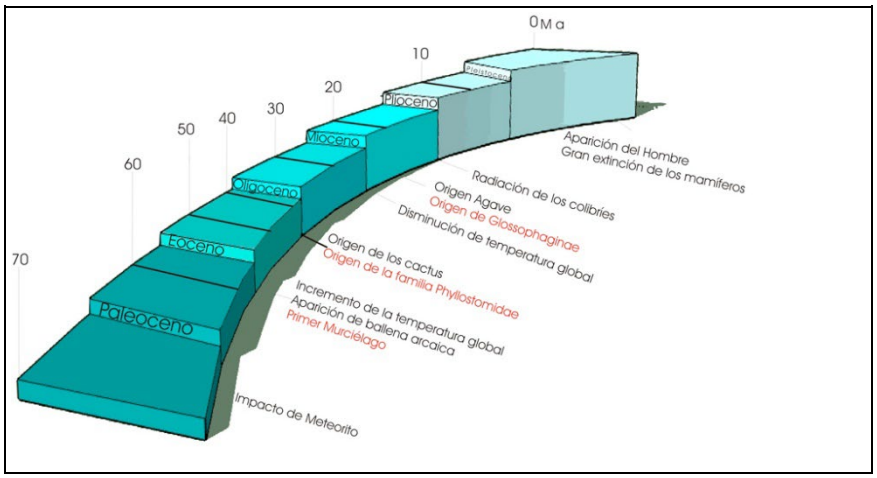
El objetivo de mi tesis fue analizar a detalle la filogenia o genealogía de las especies y entender cómo ha evolucionado la familia Phyllostomidae en el tiempo. Esto se puede estudiar a partir de secuencias de ADN del gen citocromo-b (Cyt-b) que está en la mitocondria y del gen RAG2 que se encuentra en el núcleo de la célula. En particular, buscaba contribuir a entender cómo evolucionó la nectarivoría, es decir el gusto por el polen y néctar de las flores. Analicé 120 especies, 22 pertenecen a la subfamilia Glossophaginae.



Encontré que los filostómidos se agruparon en su mayoría en las subfamilias y tribus que reconocen los especialistas en murciélagos (Figura 2), las cuales además coinciden con la naturaleza de sus dietas. Es decir, los murciélagos hematófagos (Desmodontinae) forman un grupo exclusivo (monofilético), al igual que los frugívoros (Stenodermatinae) y nectarívoros (Glossophaginae); los únicos murciélagos que no forman un grupo monofilético que mantenga a todos sus miembros juntos, fue el de los consumidores de insectos (subfamilia Phyllostominae). El grupo de los murciélagos consumidores de néctar surgen, al igual que el resto de los miembros de la familia, a partir de un ancestro que consumía insectos y es un grupo hermano de los murciélagos que consumen frutos.

A partir de la reconstrucción filogenética y los cálculos de edades, pudimos establecer que el origen de la familia Phyllostomidae ocurrió hace aproximadamente 28 millones de años (m.a.); dentro de ésta, el grupo de los murciélagos especializados en consumo de frutos, Sternodermatinae, se originó hace unos 15 m.a. La familia Glossophaginae, los necatrívoros, aparecieron hace aproximadamente 23 m.a. y dentro de esta subfamilia encontramos a la tribu Choeronycterini con una edad de casi 13 m.a. y las especies del género Leptonycteris, tal vez el grupo más estudiado por su tarea como polinizador, que cuenta con una edad de poco más de 7 m.a (Figura 1).

Las edades que se mencionan anteriormente coinciden con algunos eventos ecológicos, climáticos y geológicos ocurridos a nivel global, lo cual hace muy interesante el análisis de ésta familia y particularmente del grupo de los consumidores de néctar. La mayoría de las subfamilias de murciélagos filóstomidos aparecieron durante el Mioceno, incluyendo a los nectarívoros. Lo mismo ocurrió con los colibríes, quienes muestran su mayor radiación en el Nuevo Mundo durante la misma época, hace unos 17 m.a.



La tasa de diversificación es un índice que se representa como el número de especies que surgieron por cada millón de años, y este cálculo se puede realizar únicamente para grupos monofiléticos como los que se obtuvieron en este trabajo. Por lo tanto, fue posible utilizar dicha tasa para conocer la velocidad con la que han aparecido las especies de los diferentes grupos que conforman a la familia. Así fue que para el conjunto de Phyllostomidae, determiné que la tasa de diversificación promedio fue de 0.19 especies por millón de años. Para los grupos monofiléticos dentro de la familia, obtuve diferentes resultados. Por ejemplo, la subfamilia de los vampiros tiene la tasa de diversificación más baja de sólo 0.052 especies por millón de años. La tasa más alta fue la de los frugívoros, Stenodermatinae, 0.284 especies por cada millón de años, mientras que los nectarívoros resultaron intermedios, con una tasa de 0.175 especies por millón de años. Estos datos son interesantes al compararlos con las tasas de diversificación de otros grupos, como por ejemplo el de las plantas con flores. Así lo realizaron Susana Magallón y Amanda Castillo en el 2009, en su trabajo sobre diversificación de las angiospermas en el tiempo. Las autoras mencionan que aumentó el número de especies de las plantas con flores hace 34 m.a., en el periodo entre el Eoceno y Oligoceno, fecha que se encuentra muy cercana al momento en el que se separa la familia Phyllostomidae del ancestro común que compartía con las familias Mormoopidae y Noctilionidae. Además, algunos grupos de plantas también coinciden en tiempos de divergencia con especies de murciélagos nectarívoros. Por ejemplo, el origen de los cactus coincide con el origen de la familia Phyllostomidae (Figura 3); más recientemente, cuando se originan los agaves también se originan los murciélagos nectarívoros hace más de 20 millones de años (Figura 3). Actualmente los murciélagos nectarívoros se alimentan del néctar que producen estas plantas. Estos patrones evolutivos muestran claramente que el botánico franco-italiano León Croizat tenía razón cuando propuso, hace más de 50 años, que "la Tierra y la biota evolucionan juntas".

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