Therya

Volumen 17 Número 1 Enero 2026

AMMAC

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La portada

El zorro cangrejero (*Cerdocyon thous*) se distribuye desde el norte de Colombia y Venezuela, a través de gran parte de Brasil, el este de Bolivia, Paraguay y Uruguay, hasta el norte de Argentina. Este individuo fue fotografiado en el municipio de Salamina, en el departamento de Caldas, Colombia. Fotografía de Jeremy Domínguez.

Issue cover

The crab-eating fox (*Cerdocyon thous*) ranges from northern Colombia and Venezuela through much of Brazil, eastern Bolivia, Paraguay, Uruguay, and into northern Argentina. This individual was photographed in the municipality of Salamina, in the Department of Caldas, Colombia. Photograph by Jeremy Domínguez.

Nuestro logo "Ozomatli"

El nombre de "Ozomatli" proviene del náhuatl, se refiere al símbolo astrológico del mono en el calendario azteca, así como al dios de la danza y del fuego. Se relaciona con la alegría, la danza, el canto, las habilidades. El signo decimoprimero en la cosmogonía mexica, "Ozomatli", es una representación pictórica del mono araña (*Ateles geoffroyi*). La especie de primate de más amplia distribución en México". Es habitante de los bosques, sobre todo de los que están por donde sale el sol en Anáhuac. Tiene el dorso pequeño, es barrigudo y su cola, que a veces se enrosca, es larga. Sus manos y sus pies parecen de hombre; también sus uñas. Los Ozomatin gritan y silban y hacen viajes a la gente, arrojan piedras y palos. Su cara es casi como la de una persona, pero tienen mucho pelo."

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Contenido

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Therya

El objetivo y la intención de THERYA es ser una revista científica para la publicación de artículos sobre los mamíferos. Estudios de investigación original, editoriales y artículos de revisión son bienvenidos.

THERYA, 2026, Vol. 17(1):1-2

DOI: 10.12933/therya.2026.6254 ISSN 2007-3364

Editorial

El monitoreo de mamíferos

Desde tiempos ancestrales los mamíferos han sido objeto de fascinación para el ser humano. Ya en épocas pleistocénicas, los primeros habitantes del Continente Americano utilizaban tácticas de sigilo y aproximación para lograr una cacería exitosa. Estos eventos de caza quedaron plasmados en numerosas pinturas rupestres encontradas en cuevas, en donde la fauna de mamíferos era abundante y diversa. Hoy en día los mastozoólogos conservamos esa curiosidad innata por examinar y rastrear a los mamíferos; sin embargo, nuestro objetivo no es la cacería de subsistencia, sino contribuir con nuevos datos a la ciencia mediante el uso de diversas técnicas y herramientas. Debido a su naturaleza, los mamíferos suelen ser animales esquivos y de hábitos nocturnos, por lo que su estudio en campo presenta desafíos importantes. Esta dificultad ha llevado a los científicos a ingeniárselas desarrollando nuevos métodos y herramientas que permitan monitorear a estos animales de manera más eficaz.

El monitoreo de mamíferos ha sido posible gracias a la variedad de instrumentos utilizados para localizar en campo a las diferentes especies de este grupo de vertebrados. Existen métodos tradicionales indirectos, mediante la identificación de sus rastros (huellas y heces); métodos basados en ecolocalizadores para mamíferos voladores como murciélagos; y métodos directos que implican la captura de individuos vivos, como las trampas Sherman para pequeños mamíferos, principalmente roedores, trampas Tomahawk para mamíferos medianos, trampas con cepos para mamíferos grandes, redes de niebla, redes de arpa y el uso de la radiotelemetría, entre muchos otros. Estas técnicas han aportado información valiosa y han permitido describir nuevas especies, determinar abundancias, establecer índices de diversidad, delimitar áreas de distribución y realizar colectas científicas, entre otros usos. Sin embargo, el diseño de algunas de estas metodologías ha sido considerado demasiado invasivo para los individuos capturados, ya que en muchos casos implicaba el sacrificio del animal.

Actualmente los avances científicos y tecnológicos han

Figura.1. Zorrillo atrapado en trampa Tomahawk para monitoreo de especies en vida silvestre (Foto Alberto González).

Figura 2. Colocación de redes de niebla para la captura de murciélagos en bajo puente (Foto César Guzmán)

permitido incluir nuevas herramientas en el estudio de los mamíferos, revolucionando en cierta forma la manera de colectar información. Gracias a pequeñas muestras ambientales, colectadas de forma indirecta y de manera no invasiva, es posible tener una buena aproximación de las especies que habitan una zona en particular. Una de estas nuevas técnicas es el ADN ambiental (eDNA), que permite detectar especies a partir de pequeñas partículas celulares depositadas en el medio ambiente y capturadas mediante bombas de vacío y membranas de filtración. Esta novedosa técnica ha permitido tener aproximaciones muy certeras sobre el monitoreo de los mamíferos en particular y de otros organismos en general.

Otra herramienta que ha resultado muy efectiva en el monitoreo de mamíferos son las cámaras trampa, las cuales son colocadas en lugares precisos o de forma sistemática, con separaciones de entre 300 y 1000 m, dependiendo del objetivo del estudio. Estas cámaras permitien documentar, mediante registros fotográficos y video tomados durante largos períodos, el paso de los animales por un sitio determinado, además de poder determinar sus patrones de actividad. Esta técnica ha permitido registrar la presencia de grandes mamíferos cuya detección antes resultaba casi imposible debido su comportamiento elusivo, e incluso ha contribuido al redescubrimiento de especies que se creían extintas en vida silvestre. Es un método ampliamente utilizado para estudiar comunidades de mamíferos, sus interacciones, uso del hábitat, variaciones temporales y su presencia en diversos ecosistemas y regiones.

Los mastozoólogos han llegado a un punto en el que deben combinar las metodologías tradicionales de monitoreo con las nuevas herramientas, lo que permite un enfoque más integral, y al mismo tiempo, más respetuoso con el medio ambiente y su fauna. Esto se debe a que muchas de las herramientas recientes no son invasivas y resultan eficientes y objetivas, permitiendo responder preguntas muy concretas sobre los mamíferos de una región determinada. No obstante, aún existen obstáculos que limitan la accesibilidad de estas nuevas herramientas tecnológicas para todos los científicos, un ejemplo es el potencial de análisis de una gran cantidad de datos que se generan a través de rutas bioinformáticas, o bien los costos asociados para su implementación. Este nuevo enfoque está abriendo nuevas posibilidades sin precedentes para el estudio de los mamíferos, de una manera más ética y con mayor capacidad para responder un amplio número de preguntas de ámbito ecológico, genético, conductual y de otras áreas del conocimiento.

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THERYA, 2026, Vol. 17(1):3-16 DOI: 10.12933/therya.2026.6211 ISSN 2007-3364

Diet diversity of four herbivores in Coahuila, Mexico

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Understanding the annual and seasonal composition of herbivore diets is essential for effective population management and habitat conservation. This study aimed to evaluate the annual and seasonal dietary composition and diversity of four wild herbivores in a desert scrubland of Coahuila, Mexico, during the dry season (October 2018 and February 2019) and the wet season (May and August 2019). The research was conducted at the Rancho San Juan Wildlife Management Unit (UMA) using captive populations of desert bighorn sheep (*Ovis canadensis mexicana*), aoudad (*Ammotragus lervia*), white-tailed deer (*Odocoileus virginianus texanus*), and mule deer (*O. hemionus*). A total of 280 fecal group samples per species (140 per season) were collected and analyzed using microhistological techniques. Dietary diversity was estimated using Hill numbers by season. Differences in dietary composition were assessed with the Kruskal–Wallis test and principal component analysis. Aoudad exhibited the highest dietary diversity (64 species), followed by desert bighorn sheep (50), white-tailed deer (49), and mule deer (43). Shrub species predominated in all diets. No significant differences were detected between seasons, although grouping patterns were observed in plant occurrence frequencies. Principal component analysis indicated that 55% of the consumed plant species constituted the common dietary base of the four herbivores. White-tailed deer and mule deer, as browsing ruminants, showed greater selectivity for shrubs, while mule deer stood out for including lechuguilla (*Agave lechuguilla*), a dominant species in the rosetophyllous desert scrub. These findings underscore the importance of incorporating dietary diversity into wildlife management strategies and highlight the need for long-term studies to better understand patterns of plant resource use in arid ecosystems.

Key words: *Acacia rigidula,* agaves, management, microphyllous desert scrub, rosetophyllous desert scrub, *Opuntia engelmannii.*

Comprender la composición anual y estacional de las dietas de los herbívoros es esencial para una gestión efectiva de las poblaciones y la conservación del hábitat. El objetivo de este estudio fue evaluar la composición y diversidad dietaria anual y estacional de cuatro herbívoros silvestres en un matorral desértico de Coahuila, México, durante la estación seca (octubre de 2018 y febrero de 2019) y la estación húmeda (mayo y agosto de 2019). La investigación se llevó a cabo en la Unidad de Manejo para la Conservación de la Vida Silvestre (UMA) Rancho San Juan, utilizando poblaciones en cautiverio de borrego cimarrón del desierto (*Ovis canadensis mexicana*), arruí (*Ammotragus lervia*), venado cola blanca (*Odocoileus virginianus texanus*) y venado bura (*O. hemionus*). Se recolectaron y analizaron un total de 280 muestras de grupos fecales por especie (140 por estación) mediante técnicas microhistológicas. La diversidad dietaria se estimó por estación utilizando los números de Hill. Las diferencias en la composición de la dieta se evaluaron mediante la prueba de Kruskal–Wallis y un análisis de componentes principales. El arruí presentó la mayor diversidad dietaria (64 especies), seguido por el borrego cimarrón del desierto (50), el venado cola blanca (49) y el venado bura (43). Las especies arbustivas predominaron en todas las dietas. No se detectaron diferencias significativas entre estaciones, aunque se observaron patrones de agrupamiento en las frecuencias de ocurrencia de las plantas. El análisis de componentes principales indicó que el 55 % de las especies vegetales consumidas constituyeron la base alimentaria común de los cuatro herbívoros. El venado cola blanca y el venado bura, como rumiantes ramoneadores, mostraron una mayor selectividad por los arbustos, mientras que el venado bura destacó por incluir la lechuguilla (*Agave lechuguilla*), una especie dominante en el matorral desértico rosetófilo. Estos hallazgos subrayan la importancia de incorporar la diversidad dietaria en las estrategias de manejo de vida silvestre y resaltan la necesidad de realizar estudios a largo plazo para comprender mejor los patrones de uso de los recursos vegetales en los ecosistemas áridos.

Palabras clave: *Acacia rigidula,* agaves, manejo, matorral desértico micrófilo, matorral desértico rosetófilo, *Opuntia engelmannii.*

Coahuila, Mexico, is home to native species of large herbivores, which play a central role in the nutrient dynamics of ecosystems by participating in plant phenology through herbivory, contributing to soil compaction and nutrient supply (Gastelum-Mendoza *et al.* 2019), and constituting a source of food for natural predators (Rosas-Rosas *et al.* 2003). The white-tailed deer (*Odocoileus virginianus texanus*) is one of the 14 subspecies recorded in Mexico, distributed throughout the country, except for the Baja California peninsula (Mandujano *et al.* 2010; De la Rosa-Reyna *et al.* 2012). This species is mainly associated with the desert shrublands of northeastern Mexico and the southern United States of America; it represents an alternative for the development of the rural economy and livestock production through sustainable hunting (Valdez *et al.* 2006; Lozano-Cavazos *et al.* 2020).

Similarly, the mule deer (*Odocoileus hemionus eremicus*), whose distribution area in Mexico mainly includes the Sonoran Desert, is a species adapted to arid environments in northern Mexico, thriving in desert shrublands and mountain ranges (Weber and Gonzalez 2003). This subspecies plays an important ecological role as a browser, influencing the structure and composition of vegetation (Krausman *et al.* 1999). In addition, its hunting value has supported its inclusion in intensive management programs in Coahuila (Velázquez *et al.* 2010), and its adaptation to desert shrublands in northeast Mexico is considered viable due to the similarity in habitat conditions (Olivas-Sánchez *et al.* 2018b).

Additionally, some mountain ranges in Coahuila were part of the natural distribution of bighorn sheep (*Ovis canadensis*) until the mid-nineteenth century, when their populations were extirpated from northeastern Mexico by poaching and disease transmission from domestic livestock (O'Farrill *et al.* 2019). In this regard, the Mexican Official Standard NOM-059-SEMARNAT-2010 has listed *O. canadensis* as Special Protection (Pr; DOF 2019), and reintroduction programs have been promoted in regions such as the Sierra Maderas del Carmen and private land in the state of Coahuila (Espinosa and Contreras-Balderas 2010). However, the presence and rapid expansion of Barbary sheep (*Ammotragus lervia*), an exotic bovine native to North Africa, represents a threat to wildlife diversity in northern Mexico, as it competes directly for food and space with the species mentioned above, in addition to being a carrier and vector of parasites and diseases (Ben Mimoun and Nouira 2013; 2015; Gastelum-Mendoza *et al.* 2023).

Managing these herbivores requires knowledge about the plant species that are consumed as food (Gastelum-Mendoza *et al.* 2019), as it provides key information on herbivory pressure, which can adversely affect the dispersal and diversity of plant species, in addition to being useful for estimating carrying capacity (Serna-Lagunes *et al.* 2024), assessing the nutritional status of populations, and establishing priority areas for conservation (Saucedo-Uuh *et al.* 2024). In this regard, several studies in Mexico and the United States indicate that the white-tailed deer is a selective browser, feeding preferentially on twigs of shrubs and some herbaceous plants (Fulbright and Ortega-Santos 2007; Lozano-Cavazos *et al.* 2020). In contrast, mule deer show a greater capacity to adapt to changes in habitat conditions, modifying their diet according to seasonal forage availability (Olivas-Sánchez *et al.* 2018b). On the other hand, studies on bighorn sheep and Barbary sheep have documented that both species have opportunistic feeding habits (Ben Mimoun and Nouira 2015; Gastelum-Mendoza *et al.* 2021). In addition, habitat factors, such as topography and escape vegetation cover, are key drivers of their distribution and population development. Information available on the diet of exotic species of herbivores in Mexico is currently scarce (Olguín-Hernández *et al.* 2017).

Therefore, the objective of this study was to identify and compare the composition and diversity of the seasonal diet of bighorn sheep, Barbary sheep, white-tailed deer, and mule deer in north Coahuila. The results obtained are relevant to identifying potential areas for the reintroduction and management of these species in desert scrub ecosystems in northeastern Mexico.

Materials and methods

Description of the study area. The study was carried out at the *Rancho San Juan* Unit for Wildlife Conservation, Management, and Sustainable Use (*Unidad para la Conservación, Manejo y Aprovechamiento Sustentable de la Vida Silvestre*; UMA, in Spanish) (26°49'31.11" N, 101°01'57.77" W), located in the municipality of Monclova, state of Coahuila de Zaragoza, Mexico (Figure 1). Rancho San Juan includes four areas dedicated to intensive wildlife management. The first, with an area of 450 ha, is home to 70 bighorn sheep from Tiburón Island, Sonora. The second area, comprising 1020 ha, is dedicated to the management of 550 Texas white-tailed deer; the third, with an extension of 200 ha, is intended for the conservation of 20 mule deer from the state of Sonora. Additionally, Sierra Las Hormigas, a mountainous area of 1200 ha, is used to manage approximately 120 Barbary sheep in confinement. These four populations are isolated from each other. In addition, in the intensive management units for white-tailed deer, mule deer, and bighorn sheep, alfalfa food supplementation is carried out during the driest months of the year (July and August) to mitigate the effects of natural forage shortages.

In the areas of intensive management of the two deer species, microphyllous desert shrubland predominates, characterized by shrubs of the genus *Acacia* and cacti of the genus *Opuntia*, as well as extensive areas of open grasslands. In these areas, 46 species of plants have been reported, some of which have high forage value, such as *Acacia berlandieri* and *A. rigidula*, as well as others that provide thermal protection for cervids, such as *Cenchrus ciliaris* and *Yucca filifera* (Gastelum-Mendoza *et al.* 2020). On the other hand, the management areas of the two species of bovids show the dominance of rosetophyllous desert shrublands,

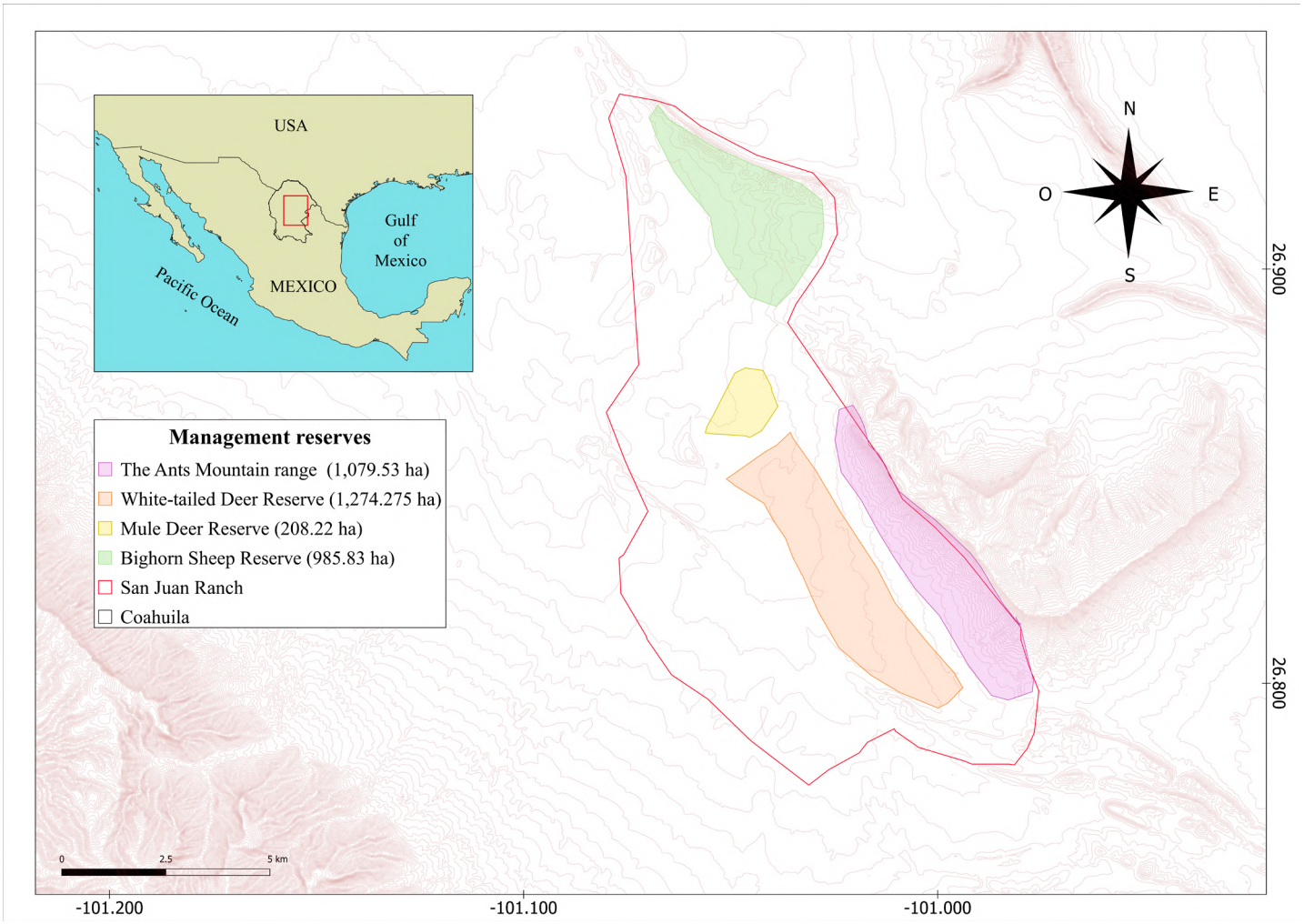

Figure 1. Location and delimitation of reserves for the management of wild herbivores in Rancho San Juan UMA, Coahuila, Mexico.

with low shrubs and abundant succulent species such as *lechuguilla* (*Agave lechuguilla*), *guapilla* (*Hechtia glomerata*), and *candelilla* (*Euphorbia antisyphilitica*; Miranda and Hernández 1963; Gastelum-Mendoza *et al.* 2019).

The local climate is semi-arid (BS), with a mean annual temperature of 21 °C, which can exceed 40 °C in summer and drop below 0 °C in winter. Annual precipitation ranges between 200 mm and 900 mm (García 1988).

Analysis of the composition and diversity of diets. The plant species that make up the annual and seasonal diet of bighorn sheep, Barbary sheep, white-tailed deer, and mule deer were identified using the microhistological technique. This methodology allows the identification and quantification of plant epidermal structures in fresh feces through microscopic analysis (Peña and Habib 1980). To this end, fresh feces from the four herbivore species studied were collected during the dry season (October 2018 and February 2019) and the wet season (May and August 2019). The samples were placed in paper bags, labeled, and transferred to the Wildlife Laboratory of the Faculty of Forestry Sciences of the Autonomous University of Nuevo León, Nuevo León, Mexico. These samples were dried in a 120 VAC, 60 Hz stainless steel oven at 75 °C for 48 hours. Once dried, the samples were sorted by time of year and ground in a Wiley mill using a No. 10 sieve (1.70 mm mesh opening). A composite sample was obtained from each seasonal group, clarified with sodium hypochlorite, and fixed on slides following the protocol described by Peña and Habib (1980). A total of 80 slides were mounted (10 per season for each species), and 800 microscope fields (10 per slide) were analyzed using an OMAX M82ES 40X–2000X® microscope with a 10X objective and a 10X ocular lens.

To identify and quantify plant cell fragments in fecal samples, a reference catalog was prepared consisting of photomicrographs of characteristic epidermal structures — trichomes, stomata, silica cells, and crystals, among others — corresponding to 141 plant species present in the study area. These were classified according to their biological form (shrub, herbaceous, grass, and succulent) and by family and species. The plant samples underwent the same drying, rinsing, and grinding procedures as fecal samples to ensure a comparison of the cell structures.

Numerical analysis. The diet composition of each herbivore species was determined by the frequency of each plant species in fecal samples, following the methodology of Fracker and Brischle (1944). The diversity of the diet for the four herbivore species was compared by estimating the true diversity profile based on Hill numbers (Hill 1973). This

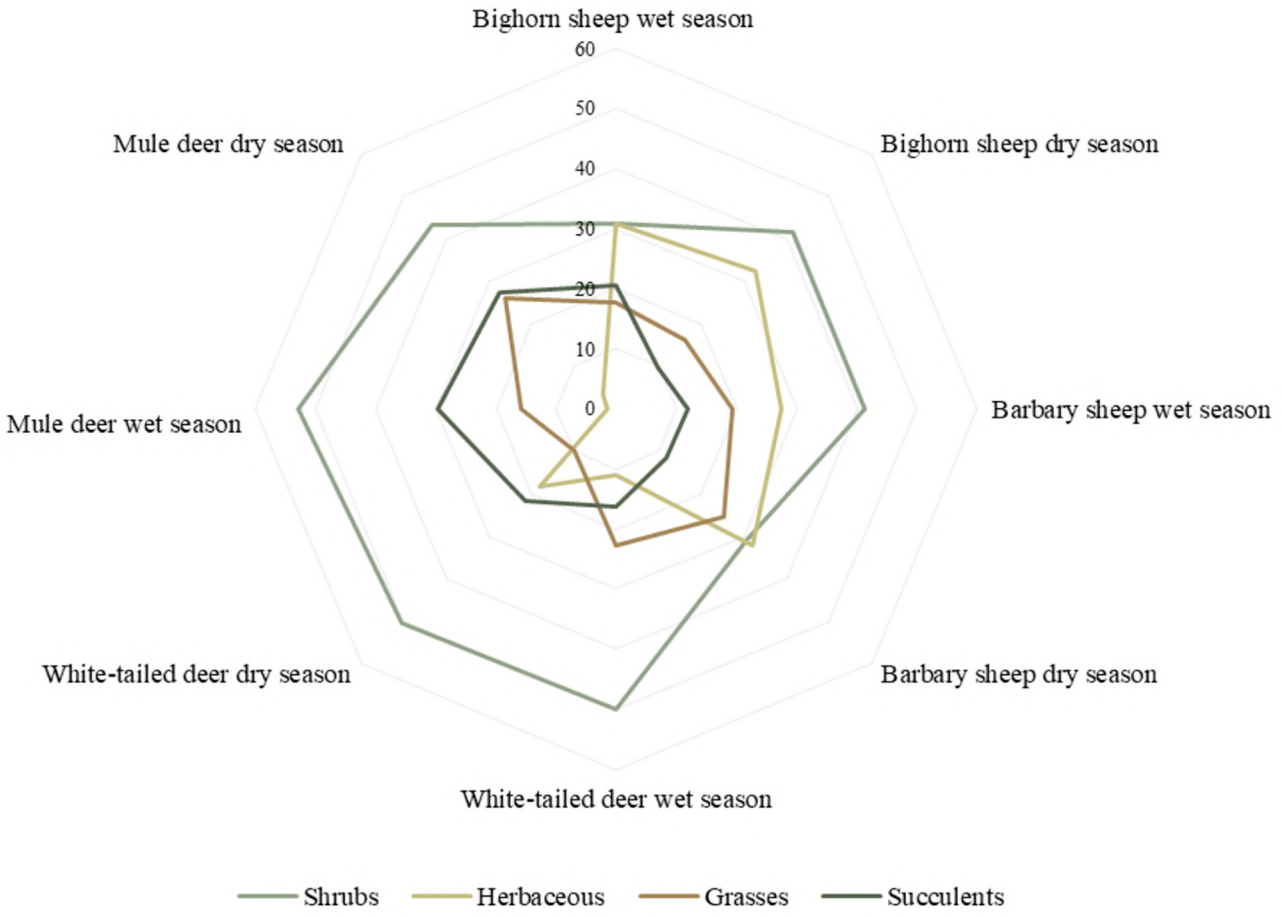

Figure 2. Variation in diet composition according to season, biological form of the forage consumed, and species of herbivores in Rancho San Juan UMA, Coahuila, Mexico (values expressed in relative frequency).

approach allows for the construction of diversity curves based on species richness and the presence/absence of the species recorded in the samplings. This analysis also yields sample coverage and sample size through inter- and extrapolations (along with 95% confidence intervals) calculated from 1000 bootstrap replicates, using the online platform of the iNEXT software (Chao *et al.* 2016). The comparison between species was performed considering the effective number of species for the orders q0 (species richness), q1 (exponential of the Shannon index), and q2 (inverse of the Simpson index) (Chao *et al.* 2014). Differences ($\alpha \leq 0.05$) between dietary diversity profiles were assumed when the confidence intervals did not overlap (Cumming *et al.* 2007).

Food similarity between the four species was evaluated by calculating the Jaccard and Sorensen indices (based on the presence or absence of plant the species consumed), as well as the Horn, Morisita-Horn, and Bray-Curtis indices (based on the relative abundance of the species consumed) (Chao *et al.* 2000; Pan *et al.* 2009) using the SpadeR software available online (Chao *et al.* 2015). In addition, the completeness of the sampling between species was compared using the sample coverage estimator, which indicates the degree of completeness of the dietary inventory. Coverage close to 100 % suggests that the sampling effort and technique were sufficient to adequately characterize the diet (Chao and Jost 2012), supporting valid comparisons between assemblages with a similar completeness level (Magurran and Henderson 2010).

Diets of the herbivore species were compared using an analysis based on frequency density ($\alpha \leq 0.05$). The similarity between diets was assessed using a cluster analysis, with Euclidean distance as a measure of similarity. Additionally,

Table 1. Diversity indicators according to Hill numbers for four species of herbivores living in Rancho San Juan UMA, Coahuila, Mexico.

Indicators	Bighorn sheep	Barbary sheep	White-tailed deer	Mule deer
Sample size	394	409	399	400
q0	50	64	49	43
q1	24	28	27	26
q2	14	17	19	20
Sample coverage	0.97	0.94	0.97	0.97
Percentage of sample coverage	97 %	94 %	97 %	97 %

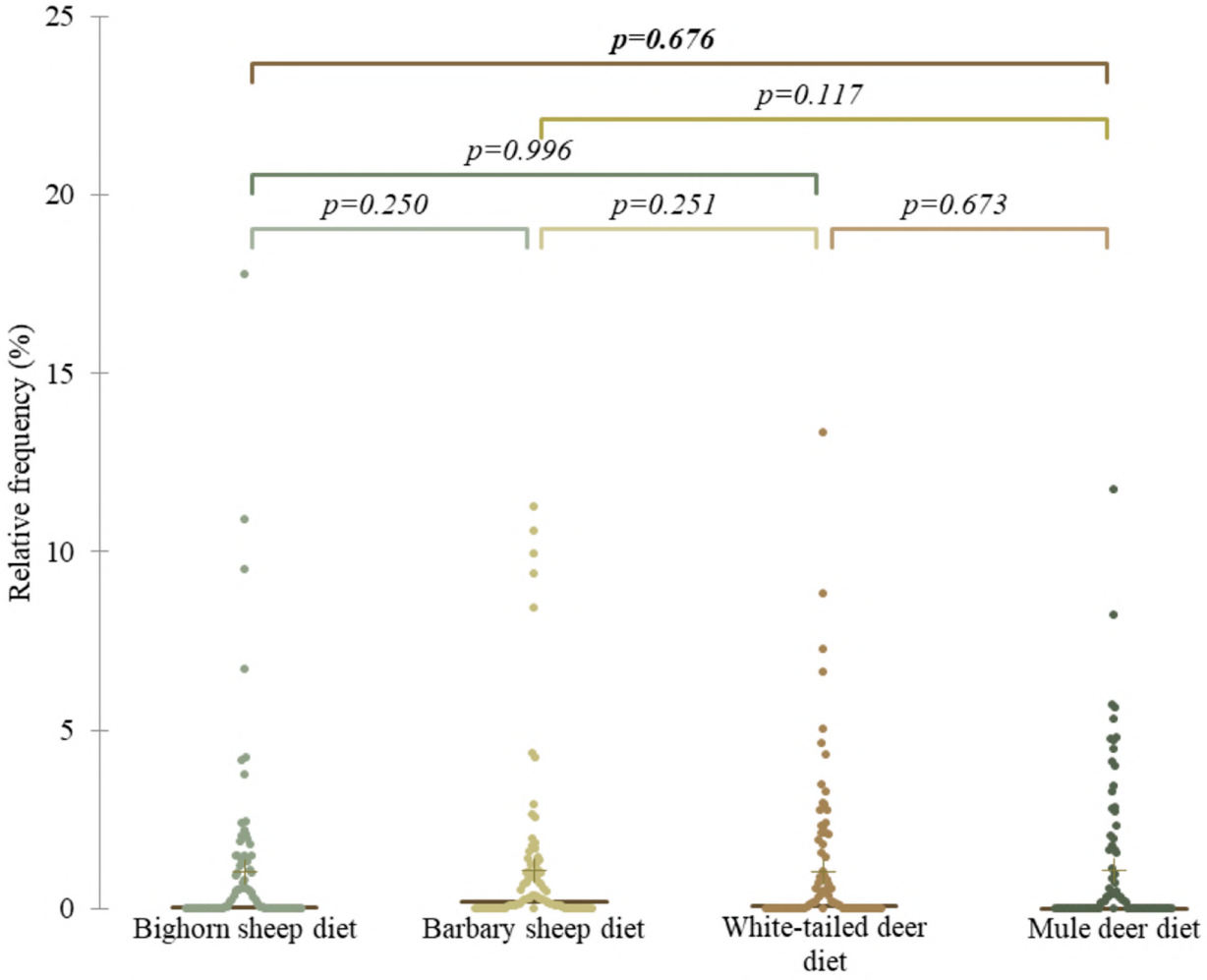

Figure 3. Comparison of the relative frequency of plant species in the diet of four wild herbivores living in Rancho San Juan UMA, Coahuila, Mexico, using the nonparametric Kruskal-Wallis test.

the proportion of consumed plant species shared by the four herbivore species was analyzed by applying the Whittaker index ($\alpha \leq 0.05$).

Likewise, nonparametric statistical tests were applied to evaluate differences in diet composition between species and times of the year. These included the Kruskal-Wallis test ($\alpha \leq 0.05$) and the paired Mann-Whitney test ($\alpha \leq 0.05$). A principal component analysis (PCA) was performed using the frequencies of consumption of plant species as independent variables and the diversity of plants consumed as dependent variables. This analysis allowed for the identification of correlations between variables through principal components, which were represented in a three-dimensional space (factorial planes). The percentages of variance explained by each principal component were calculated. The statistical analyses were performed in Past 3.0 and XLSTAT.

Results

Composition and diversity of diets. A total of 280 fecal samples were collected per species, evenly distributed between the two seasons of the year (140 per season). The sample coverage was greater than 90% for the four species of herbivores, which indicates a representative sample (Table 1). The richness of plant species varied between herbivore species, ranging from 43 to 64. The diet of the bighorn sheep included 50 plant species (q0): 28 shrubs, seven herbs, 11 grasses, and four succulents. Annually, the most consumed species were woody crinklemat (*Tiquilia canescens*; 17.75 %), Torrey's croton (*Croton torreyanus;* 10.89 %), and rabbit cactus (*Opuntia microdasys*; 9.51 %; Table 2). Regarding the seasonal contribution of biological forms in the diet of bighorn sheep, shrub species predominated throughout the year (Figure 2). No differences were found between the consumption of the different biological forms and the time of year (Figure 3). However, a higher consumption of shrubs and herbaceous plants was observed during the dry season (41.54 % and 32.59 %, respectively), while the consumption of grasses and succulents was higher in the wet season (17.74 % and 20.57 %, respectively; Figure 2).

Barbary sheep consumed 64 species (q0), including 34 shrubs, 12 herbs, 15 grasses, and three succulents. *Tiquilia canescens* (11.28 %), *chaparro prieto (Acacia rigidula*; 10.6 %), and desert prickly pear (*Opuntia engelmannii*; 9.96 %),

Table 2. Seasonal composition of the diet of bighorn sheep, Barbary sheep, white-tailed deer, and mule deer living in Rancho San Juan UMA, Coahuila, Mexico, expressed in percentage of the consumption values.

	Ovis canadensis mexicana		*Ammotrgus lervia*		*Odocoileus virginianus texanus*		*Odocoileus hemionus eremicus*	
Species	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**
Abutilon wrightii		4.16	1.03	1.47	1.96	1.64		
Acacia berlandieri		0.98	2.58		0.29		2.88	1.04
Acacia farnesiana	2.35	5.12	0.26	1.14	0.43	0.33		
Acacia rigidula	1.00		9.59	11.60	11.93	5.73	7.24	2.26
Acourtia runcinata			0.25		0.14			
Agave lechuguilla	0.62	0.44					12.65	10.81
Agave sp.							2.54	2.89
Allionia incarnata	1.86	0.53		0.22	0.99	4.85		
Aloysia macrostachya	0.13	2.80	2.96	0.37	1.41	0.44	2.16	1.35
Aloysia wrightii		1.06		0.15				
Ambrosia dumosa	0.66	1.33	0.13	2.01	0.14	4.11		
Aristida adscensionis	0.26	0.65	0.39	0.37	0.57	0.77		
Aristida adscensionis			1.35					0.63
Aristida purpurea	0.78	1.90	0.65	3.04	0.83	2.07		
Astrolepis integrifolia	2.71	0.88	4.87	0.22				
Baccharis glutinosa								0.81
Baccharis texana					1.11	0.65		
Bothriochloa laguroides								0.95
Bothriochloa saccharoides			0.62					
Bouteloua curtipendula			2.25	1.29		0.98	0.74	2.50
Bouteloua eriopoda	0.25	2.68	0.13	0.37				
Bouteloua gracilis			0.13				4.97	3.28
Bouteloua hirsuta	0.13	0.11	0.13	1.98	0.28	1.77		
Bouteloua sp.	0.74	1.41						
Calliandra sp.							3.26	
Casimiroa edulis			0.99					
Castela texana		0.33	0.13	0.44				0.91
Celtis pallida							0.73	0.72
Cenchrus ciliaris	3.60	4.88	2.58	6.12	3.80	2.10	2.93	8.48
Chamaecrista greggii		0.11	0.74					
Chilopsis linearis					0.42			
Cordia parvifolia	0.26	0.19	2.19	1.73				
Croton dioicus			3.07	2.23				
Croton pottsii				0.30				
Croton punctatus		0.10	0.65	1.10		4.66		
Croton sp.				0.15				
Croton torreyanus	9.22	12.57	0.90	4.94	0.99	7.60		
Cynodon dactylon	1.93	1.05						
Cynodon dactylon					0.42	0.34		
Dalea aurea	0.90	0.52	0.78	1.02				
Dalea bicolor	1.15	0.84		0.44	2.42	1.44		
Dalea greggii							1.11	
Diospyros texana					0.29		0.37	
Ephedra pedunculata					0.55	0.12	4.05	
Ephedra trifurca	0.99			0.59				
Erioneuron pulchellum	7.55	0.78	10.55	6.34	12.69	0.56		
Euphorbia antisyphilitica	0.50	1.31		1.10	2.22	4.71	4.33	1.35
Evolvulus alsinoides			5.22	3.22	0.57	0.44		
Eysenhardtia texana	3.47	0.95	0.65	0.77	7.99	6.55		

Ferocactus sp.							1.79	0.41
Flourensia cernua							4.85	4.07
Forestiera angustifolia	0.26	0.11	1.60	0.15	0.83	2.29	2.93	2.69
Gochnatia hypoleuca	0.50	1.06	3.20				0.35	0.32
Guaiacum angustifolium	1.18		1.39		5.15	0.33	4.00	3.99
Gymnosperma glutinosum	0.13			0.15		0.11		
Hechtia glomerata							1.08	0.63
Heteropogon contortus	0.89	1.94	0.52	2.39	3.84	0.34		
Hilaria mutica	0.63			0.30	0.29		4.26	5.35
Hymenoxys odorata			0.13	0.15	0.15	0.22		
Jatropha dioica					0.15		4.35	6.27
Karwinskia humboldtiana		1.09		0.11	0.15	0.12	3.63	1.04
Koeberlinia spinosa								0.50
Krameria erecta	0.13	0.95	0.13	0.15	1.00	0.11		
Lantana camara			0.13					0.32
Larrea tridentata	0.37	0.33	1.39	0.26	0.43	0.68		
Lesquerella fendleri			0.37					
Leucophyllum frutescens	1.85	1.93	2.59	0.15	1.25	3.52	1.82	1.28
Lippia graveolens	0.50			0.15	0.29		2.84	6.62
Medicago sativa	6.52	6.86	6.94	11.79	3.52	3.02		
Mimosa zygophylla	1.40	3.41	0.49	0.55	2.66	2.86		
Opuntia engelmannii	4.45	0.42	10.29	9.63	11.51	15.15	7.95	8.52
Opuntia leptocaulis	2.23	1.66	1.11	0.88	4.86	5.20	2.93	3.59
Opuntia microdasys	12.28	6.75	0.52	1.10		0.23	0.73	
Panicum hallii			0.13	2.68				
Parthenium argentatum			1.03	0.85	0.15		0.37	
Parthenium hysterophorus	0.89	2.08						1.89
Parthenium incanum	0.00		1.97					
Parthenium sp.					0.28	1.15		
Paspalum notatum							2.48	4.36
Phaulothamnus spinescens								0.63
Physaria fendleri				0.55		1.58		
Prosopis glandulosa	0.50	2.10			6.33	2.91	5.42	5.86
Salvia coccinea							0.36	
Setaria leucopila		0.11		0.44				
Sidneya tenuifolia	1.85	2.22						
Solanum elaeagnifolium		0.10		0.15	0.42	0.45		
Solanum nigrum			0.37	0.11				
Telosiphonia macrosiphon								0.81
Tiquilia canescens	18.45	17.05	9.93	12.63	1.15	3.11		
Tridens muticus				0.11				
Viguiera stenoloba			0.13					
Wedelia texana								0.63
Yucca filifera								0.32
Ziziphus obtusifolia					3.43	1.09		

were the most consumed species. In addition, Barbary sheep consumed mostly shrubs throughout the year (Table 2), although with a higher consumption in the wet season (41.2 %). Herbaceous plants and grasses were more common in the diet during the dry season (32.17 % and 25.39 %, respectively). Succulents were equally consumed in both seasons (Figure 2).

The diet of white-tailed deer consisted of 49 plant species (q0), including 27 shrubs, ten herbs, nine grasses, and three succulents. *Opuntia engelmanni* (13.33 %), *A. rigidula* (8.83 %), and Texas kidneywood (*Eysenhardtia texana*; 7.27 %) were the dominant plant species in the diet throughout the year (Table 2). Shrub species represented more than one-half of food consumption during the dry season (50.47 %). Also, herbs (18.16 %) and succulents (21.53 %) were consumed more commonly in the dry

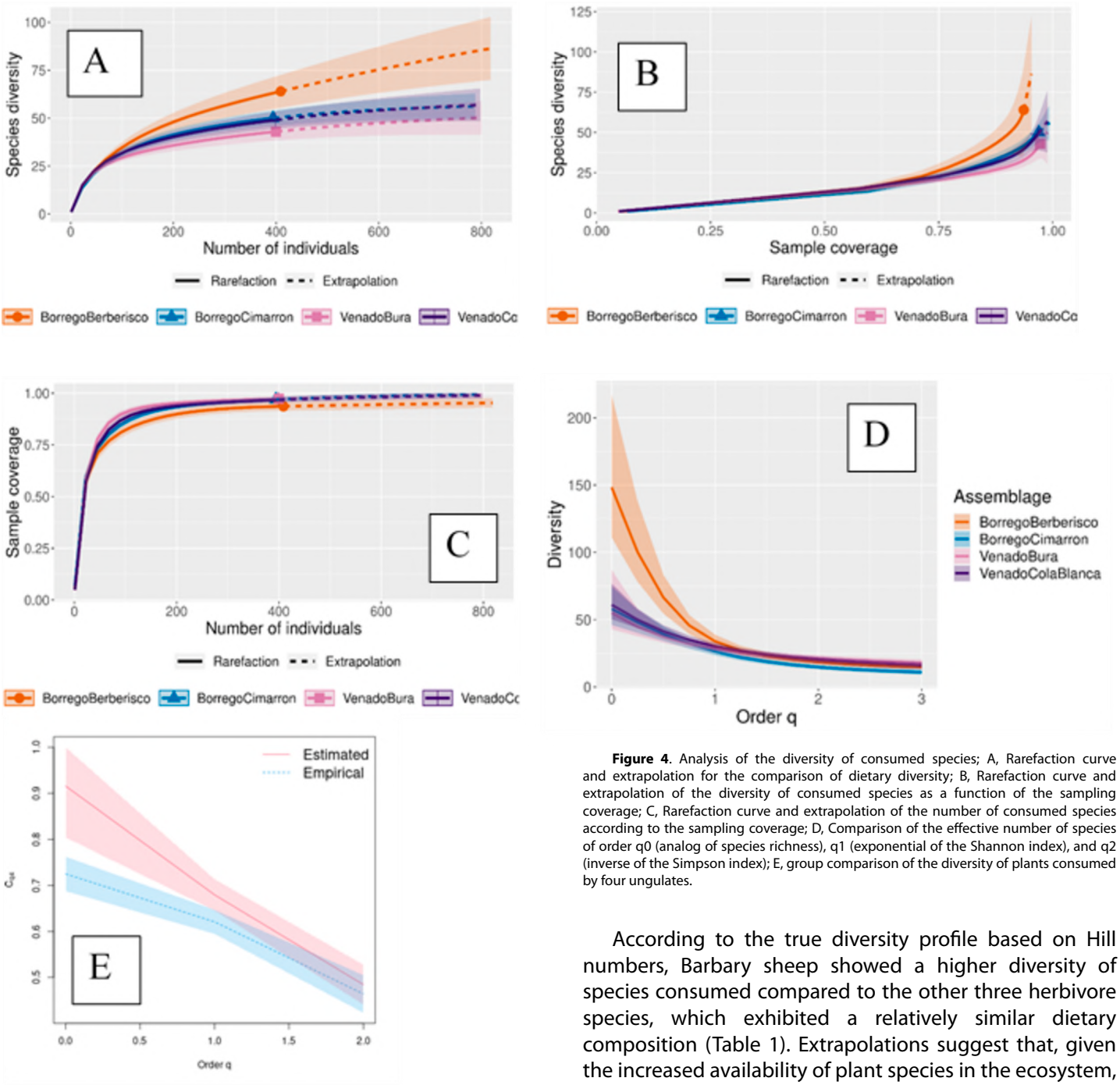

Figure 4. Analysis of the diversity of consumed species; A, Rarefaction curve and extrapolation for the comparison of dietary diversity; B, Rarefaction curve and extrapolation of the diversity of consumed species as a function of the sampling coverage; C, Rarefaction curve and extrapolation of the number of consumed species according to the sampling coverage; D, Comparison of the effective number of species of order q0 (analog of species richness), q1 (exponential of the Shannon index), and q2 (inverse of the Simpson index); E, group comparison of the diversity of plants consumed by four ungulates.

season, while grasses were consumed mainly during the wet season (22.7 %; Figure 2).

The diet of mule deer consisted of 43 plant species (q0), of which 25 were shrubs, four herbs, seven grasses, and seven succulents. The most representative plant species consumed all year round were *Agave lechuguilla* (11.73 %), *O. engelmannii* (8.23 %), and *Cenchrus ciliaris* (5.7 %; Table 2). The consumption of shrub species was predominant throughout the year, with a higher incidence during the wet season (52.83 %). Succulents also contributed significantly at this time of the year, accounting for 30 % of the diet. In contrast, grasses were consumed mainly during the dry season (26 %), while herbs made a low proportion of the diet throughout the year (Figure 2).

According to the true diversity profile based on Hill numbers, Barbary sheep showed a higher diversity of species consumed compared to the other three herbivore species, which exhibited a relatively similar dietary composition (Table 1). Extrapolations suggest that, given the increased availability of plant species in the ecosystem, herbivores would be able to incorporate a diversity of plants proportional to that of the habitat (Figure 4). When comparing the diversity of the diet between the four species, we observed that the observed diversity exceeded the expected one (Figure 4E), with significant differences ($P \leq 0.05$) in the composition of the plants consumed. In particular, Barbary sheep showed the highest species richness in their diet (q0). However, in terms of the number of common species (q1) and the number of dominant species (q2) in the diet, no significant differences were observed between the species analyzed (Figure 4D).

Similarity of diet composition and diversity. According to the results of the Kruskal-Wallis test ($X^2 = 2.48, df = 3, P = 0.68$), no statistically significant differences were observed in the

Table 3. Significance values of the paired Mann-Whitney test ($\alpha \leq 0.05$) according to the diet composition of the herbivorous species and the seasons of the year.

Species – season of the year	Bighorn sheep – wet season	Bighorn sheep – dry season	Barbary sheep – wet season	Barbary sheep – dry season	White-tailed deer – wet season	White-tailed deer – dry season	Mule deer – wet season
Bighorn sheep – dry season	0.63						
Barbary sheep – wet season	0.36	0.61					
Barbary sheep – dry season	0.37	0.63	0.97				
White-tailed deer – wet season	0.73	0.89	0.61	0.55			
White-tailed deer – dry season	0.89	0.82	0.43	0.45	0.87		
Mule deer – wet season	0.6	0.36	0.15	0.14	0.36	0.48	
Mule deer – dry season	0.84	0.47	0.27	0.27	0.62	0.69	0.77

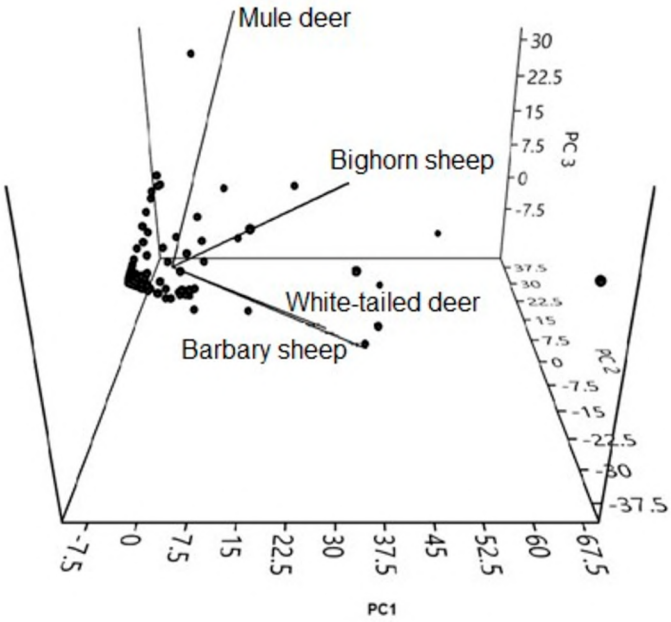

Figure 5. Principal Component Analysis (PCA) of the seasonal composition of the diet of four wild herbivores at Rancho San Juan UMA, Coahuila, Mexico.

annual diet composition between the herbivore species studied (Figure 3). Similarly, no significant differences were found in the consumption of the different biological forms of forage between seasons ($X^2 = 3.04$, $df = 7$, $P = 0.82$). Furthermore, the results of the Mann-Whitney paired test indicated that there are no differences in diet composition between herbivore species or between seasons of the year (Table 3). The cluster analysis indicated two groups based on the similarity of the diets (Figure 5). The first group included the seasonal diet composition of white-tailed deer, bighorn sheep, and Barbary sheep; the second included the diet of mule deer in both periods (Figure 6).

In the PCA (Figure 5), PC1 and PC2 explained 55.20 % and 25.5 % of the variance, respectively; together, the first two components accounted for 80 % of the variance. This variation is explained by the correlation between the diversity of plant species consumed by bighorn sheep and mule deer, which are associated with PC3. This position in the two-dimensional plane highlights the difference with the white-tailed deer and Barbary sheep, which correlate in PC2, suggesting the similarity in the diet of these two species.

The Whittaker comparison analysis (Table 4) indicated the proportion of plant species consumed by the four

Table 4. Whittaker's comparison analysis to determine the plant species consumed by ungulates (proportion of species shared).

	Bighorn sheep	Barbary sheep	White-tailed deer	Mule deer
Bighorn sheep				
Barbary sheep	0.23			
White-tailed deer	0.29	0.29		
Mule deer	0.59	0.61	0.57	

ungulates, with a greater similarity of species consumed between Barbary sheep and mule deer (61 %), mule deer and bighorn sheep (59 %), white-tailed deer and mule deer (57 %), and white-tailed deer, bighorn sheep, and Barbary sheep (29 %).

Discussion

Smaller herbivores select a higher-quality diet due to their relatively high nutritional requirements (Ramírez *et al.* 1997). This preference is related to a distinctive characteristic of small ruminants classified as browsers, such as white-tailed deer and mule deer, which have morphological adaptations in the digestive tract that allow them to be more selective regarding the species and parts of plants they consume (Ramírez-Lozano 2004). In contrast, ruminants classified as grazers, such as bighorn sheep and Barbary sheep, have larger molars and digestive tracts, allowing them to digest a greater diversity of plant species more efficiently, especially grasses with a high fiber content and lower nutritional quality (Guerrero-Cárdenas *et al.* 2018). This explains why shrub browsing was the most important food component for white-tailed deer and mule deer, while the percentages of shrubs in the diet of the two bovid species were lower (Figure 2).

Each of the four herbivore species exhibits evolutionary adaptations that influence their patterns of food use and selection. For example, the habitat requirements of bighorn sheep are strongly conditioned by topography; this species depends on areas with canyons, steep slopes, and vegetation cover that facilitates the detection of predators (Tarango *et al.* 2002). This habitat component is even more relevant than food availability, as bighorn sheep are considered opportunistic foragers that can feed on a wide variety of plants (Gastelum-Mendoza *et al.* 2021; Méndez-Rosas *et al.* 2025). For its part, Barbary sheep, an exotic species in Mexico, shows a high plasticity in habitat

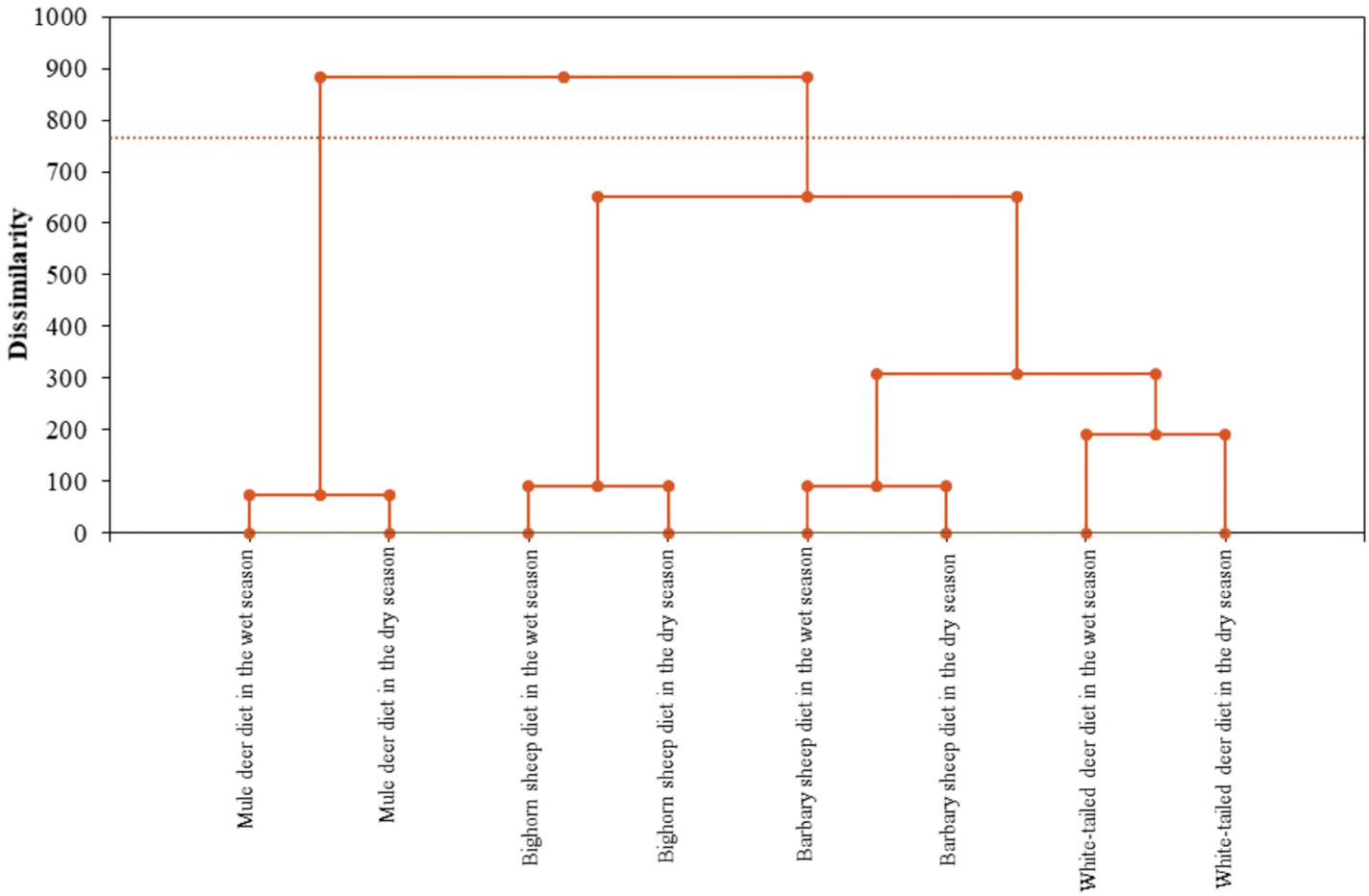

Figure 6. Dendrogram of dissimilarity in the seasonal diet composition of four wild herbivores at the Rancho San Juan UMA, Coahuila, Mexico.

use and food selection (Ben Mimoun and Nouira 2013). In Texas, USA, one of the few studies on this topic reveals that in the presence of white-tailed deer, Barbary sheep avoid browsing shrubs to prevent competition (Ramsey and Andereg 1972). This strategy suggests that, despite the high dietary similarity between white-tailed deer, bighorn sheep, and Barbary sheep (Figure 3), the habitat resources available for these species to compete for are different. In particular, competition could be expressed in the use and selection of escape terrain or water sources. Although common plant species were identified in the diet of the four herbivore species studied, they were not consumed in the same proportions. In particular, *A. rigidula* accounted for a significant percentage of the annual diet of Barbary sheep, but only contributed 0.5 % of the diet of bighorn sheep (Table 2), and few species accounted for high consumption percentages (Figure 3).

From a habitat management perspective, forage species for wild herbivores are classified as declining when their availability decreases in response to herbivory pressure (Fulbright and Ortega-Santos 2007). In the case of bovids, *T. canescens*, an annual herbaceous species, was classified as a declining species because it was the most representative in the annual diet of bighorn and Barbary sheep, with 17.75 % and 11.27 %, respectively. However, specific differences in diet composition were observed between the two species. A high percentage of occurrence of *C. torreyanus* was found in the annual diet of bighorn sheep (10.9 %), but not in the diet of Barbary sheep (2.9 %; $P \leq 0.05$). In contrast, *A. rigidula* was consumed in a higher proportion by Barbary sheep (10.6 %) compared to bighorn sheep (0.5 %; $P \leq 0.05$). As for cervids, both species showed a high consumption of *O. engelmannii* (Table 2). However, *A. lechuguilla*, a dominant succulent plant in the desert shrublands of northeastern Mexico (Alanís-Rodríguez *et al.* 2015), was consumed in a high proportion (11.7 %) only by mule deer. On the other hand, low woollygrass (*Erioneuron pulchellum*) was recorded exclusively in the diet of white-tailed deer (6.6 %).

Studies on forage competition are complex, since this ecological phenomenon occurs when multiple species or individuals simultaneously use a resource whose availability is insufficient to meet the minimum survival and development requirements of the individuals or populations involved (Olguín-Hernández *et al.* 2017). Under this definition, similarity in diet composition is considered a primary indicator of interspecific competition for food. In Mexico, studies on food competition between wild herbivores are scarce. In La Michilía Biosphere Reserve, Durango, studies on the long-term feeding habits of white-tailed deer and mule deer concluded that there is no significant competition in forage use between the two species (Gallina and Ezcurra 1981; Gallina 1993). For their part, Olguín-Hernández et al.

(2017), in a study carried out in Tamaulipas, identified that the most intense food competition between white-tailed deer and exotic species occurred in spring. During this season, a high similarity was observed between the diet of white-tailed deer and sika deer (*Cervus nippon*, 49 %), red deer (*Cervus elaphus*, 54 %), and eland antelope (*Taurotragus oryx*, 47 %). However, no studies have been carried out in Mexico on the competition between bighorn sheep and Barbary sheep. The cluster analysis (Figure 4) suggests that competition for forage use might be more likely among bighorn sheep, Barbary sheep, and white-tailed deer. Likewise, the consumption of herbs and grasses was more common in the diet of bovids, while shrubs and succulents were consumed more frequently by deer.

Shrub species constitute the food base of wild herbivores in arid ecosystems (Guerrero-Cárdenas *et al.* 2018; Bautista De Luna *et al.* 2022). During their development, shrubs that thrive in arid zones allocate nutrient reserves to building new tissues, which results in a relatively high crude protein content compared to some herbaceous and grass species (Mazaika *et al.* 1992; Memmott *et al.* 2011). In desert shrublands in northern Mexico, succulent plants represent an alternative source of water for herbivores during drought periods (Tarango *et al.* 2002; Gastelum-Mendoza *et al.* 2020). Within this group, *O. engelmannii* was recorded in high proportions in the diet of all species studied, except for bighorn sheep, whose consumption frequency was 2 %. This species showed a greater preference for *O. microdasys* (Table 2). In this regard, Gastelum-Mendoza *et al.* (2020) state that *O. engelmannii* is one of the dominant species in the study area, with a mean annual IVI of 77.09 ± 6.05 %. However, *O. microdasys* was one of the least available species in shrublands. Considering that bighorn sheep and Barbary sheep require particular topographic elements for their development and survival (Tarango *et al.* 2002), competition between the two species could be intense when they share the same habitat. In this sense, and due to the limited information available on the simultaneous use of the topographic space by these two species, it is not recommended that they share the same management area.

Studies on the diet of mule deer in northern Mexico and the southern U.S. have reported that it adapts to the consumption of a wide variety of plant species (Olivas-Sánchez *et al.* 2018b). In the Chihuahuan Desert, its diet mainly consists of browsing leaves, the regrowth of shrubs and succulents, and herbs as a key emerging resource during the period after rain. In the Mapimí Biosphere Reserve (state of Durango), *ocotillo* (*Fouquieria splendens*) inflorescences, which emerge in March, are an important nutritional contribution during the dry season. These resources, which are highly digestible (≈ 85 %), are consumed intensively during critical periods (Gallina *et al.* 2017). Likewise, studies carried out in Texas (Trans-Pecos and Panhandle) reveal that the annual diet of mule deer is composed on average of 70 % of shrub browsing, 25 % of grasses, and 5 % of grasses (Anderson 1949). The consumption of herbs increases markedly after summer rains, while it may include wheat and other crops growing in agricultural landscapes in winter (Short 1977). In general, mule deer require diverse shrubs and patches of herbaceous plants to maintain their body condition and promote reproduction.

The results of this study are consistent with Olivas-Sánchez *et al.* (2018a), who found that mule deer consume mainly shrubs and succulents throughout the year, with herb and grass consumption being less frequent (Figure 2). Unlike the white-tailed deer, which is a selective browser (Ramírez-Lozano 2004), the mule deer is considered an opportunistic forager, meaning its diet depends on the local availability of resources (Hanley 1997). This explains why *A. lechuguilla* was found in high percentages only in the mule deer diet (Table 2). Similarly, Geist (1981) points out that this species changes its diet from one based on herbs and grasses to one dominated by shrubs in response to extrinsic factors. The results of the present study suggest that the low availability and nutritional quality of herbaceous plants in the region are insufficient to meet the requirements of mule deer adequately. To better understand the trophic interactions between these four herbivore species, we recommend expanding these results with studies of simultaneous habitat use and analyses of the nutritional profile and availability of the main plant species consumed.

Conclusions

Barbary sheep showed a higher species richness in their diet compared to the three herbivores that share the same habitat. This high richness of species consumed by Barbary sheep may be related to its nature as a generalist herbivore capable of incorporating a wide variety of plants without showing a marked preference for any of them. In this sense, the creation of forage banks could contribute to reducing overgrazing in desert shrubland ecosystems. In general, shrub species formed the basis of the diet of the four species analyzed. Herbaceous plants were consumed in a greater proportion by sheep, while deer preferred succulents. No differences were recorded in the consumption of biological forms between seasons of the year or between herbivore species.

The principal component analysis revealed that the mule deer has the most distinct diet compared to the other herbivores. In contrast, the evidence collected in the present study suggests a low risk of trophic overlap between cervid populations, which could facilitate their management in shared areas without major adverse implications. Finally, the most consumed species can be a useful criterion for identifying new sites for repopulation, by delimiting key foraging areas in the desert shrublands of northeastern Mexico.

Acknowledgments

The authors thank Ing. Gerardo Benavides Pape, owner of UMA Rancho San Juan, for allowing access to his property and providing the necessary conditions for conducting

field sampling. We also express our appreciation to Leonel Reséndiz Dávila, Alejandra Galván Ramírez, César Uribe Sierra, Daniel López Carmona, and Juan Ángel Reyna González from the Faculty of Forestry Sciences at the Universidad Autónoma de Nuevo León for their technical support during the fieldwork.

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Associated editor: Rafael Reyna Hurtado
Submitted: June 6, 2025; Reviewed: September 12, 2025
Accepted: October 2, 2025; Published on line: November 6, 2025

THERYA, 2026, Vol. 17(1):17-30 DOI: 10.12933/therya.2026.6224 ISSN 2007-3364

Potential distribution of medium-sized felines in Morelos: climate suitability and coverage in protected natural areas

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The presence of the six species of felids distributed throughout Mexico has been documented in the state of Morelos. These species face serious threats from habitat loss and fragmentation driven by human activities. Some, particularly medium-sized felines, such as margay, jaguarundi, ocelot, and bobcat, remain poorly studied. Our objective was to identify their potential distribution areas within Morelos, assess the impact of human activities on these areas, and evaluate the role of Protected Natural Areas (PNAs) in conserving their potential habitats. We used the Maximum Entropy algorithm to model the ecological niches of the four species and generate potential distribution maps using bioclimatic variables from WorldClim. We estimated the potential distribution areas for each species and identified zones suitable for the coexistence of all four felines. These models were superimposed on digital maps of human settlements, agricultural fields, and bare soil to quantify anthropogenic impacts and to assess the effectiveness of PNAs in protecting these habitats. Our results indicate that human activities reduce the potential distribution areas of the four species by an average 42%, and only 880.56 km^2 (18%) of the area with primary or secondary vegetation is protected by any PNAs. Although we identified areas with high climate suitability for these species, no research has yet confirmed their presence. We therefore propose targeted monitoring of these areas to gather critical data that can inform conservation strategies for medium-sized felines and their habitats in Morelos.

Keywords: Agriculture, ecological niche model, *Herpailurus yagouaroundi, Leopardus pardalis*, *Leopardus wiedii*, *Lynx rufus*, urbanization.

En el estado de Morelos se ha reportado la presencia de las seis especies de félidos que se distribuyen en México, estas se encuentran gravemente amenazadas por la pérdida y fragmentación de su hábitat provocadas por las actividades humanas. Algunas de estas especies han sido poco estudiadas, particularmente los felinos medianos (tigrillo, jaguarundi, ocelote y gato montés), por lo que nuestro objetivo fue identificar las áreas de distribución potencial dentro de Morelos, evaluar los efectos que tienen los impactos antropogénicos sobre las áreas estimadas y analizar la importancia de las ANP estatales en la protección de las áreas potenciales de distribución de estas especies. Se utilizó el algoritmo de Máxima Entropía para modelar el nicho ecológico de las cuatro especies y poder obtener mapas de distribución potencial considerando las variables bioclimáticas disponibles en WorldClim. Se estimaron las áreas de distribución potencial para cada especie y se identificaron áreas idóneas para la coexistencia de los cuatro felinos. Los modelos fueron superpuestos sobre mapas digitales de asentamientos humanos, áreas agrícolas, suelo desnudo, para cuantificar los efectos que tienen estas actividades sobre las áreas de distribución estimadas y analizar la importancia que tienen las ANP en la protección de estos felinos. Nuestros resultados indican que las actividades humanas reducen en promedio un 42% las áreas de distribución potencial de las cuatro especies y solo 880.56 km^2 (18%) del área con vegetación primaria o secundaria está protegida por algún ANP. Identificamos áreas de alta idoneidad climática para estas especies, sin embargo, no existen trabajos que comprueben su presencia, por lo que proponemos el monitoreo en las zonas con el fin de obtener información relevante que nos pueda ayudar a desarrollar estrategias de conservación para los felinos medianos y su hábitat en Morelos.

Palabras clave: Agricultura, *Herpailurus yagouaroundi, Leopardus pardalis*, *Leopardus wiedii, Lynx rufus*, modelado de nicho ecológico, urbanización.

Habitat loss and fragmentation are among the main factors threatening biodiversity (Crooks and Sanjayan 2006; Ryser *et al.* 2019). The first reduces the habitat area, potentially affecting species richness (Fahrig 2003; Galán-Acedo *et al.* 2023), while the second divides the habitat into increasingly smaller patches, exposing species to external threats. Additionally, the distance between patches complicates the displacement of individuals, influencing gene flow between populations (Fahrig 2003; Holderegger and Di Giulio 2010). The creation of Natural Protected Areas (PNAs) is an essential strategy to counteract the effects of habitat loss and fragmentation and preserve biodiversity (Gray *et al.* 2016). However, this strategy has been insufficient, as the effects of habitat fragmentation are still evident within the PNAs. On the other hand, the distance between PNAs contributes to the isolation of animal populations, mainly due to habitat transformation outside them (Santiago-Ramos and Feria-Toribio 2021; Yuan *et al.* 2024).

Felids are particularly vulnerable to habitat loss and fragmentation (Crooks *et al.* 2011; Zanin *et al.* 2015; Butti *et al.* 2022). Six felids are distributed in Mexico (Ceballos and Oliva 2005): *Leopardus wiedii* (margay), *Herpailurus yagouaroundi* (jaguarundi), *Leopardus pardalis* (ocelot), *Lynx rufus* (bobcat or red lynx), *Puma concolor* (puma), and *Panthera onca* (jaguar). There has been a considerable reduction in the distribution areas of these felids in

recent years, mainly due to the loss and fragmentation of their habitat as a result of anthropogenic activities such as the expansion of crop fields and urban areas, which compromises survival and puts felid populations at risk (Carrillo-Reyes and Rioja- Paradela 2014; Dirzo *et al.* 2014; SEMARNAT 2018; Solari *et al.* 2018). Furthermore, these species are hunted or captured for trade or due to the growing conflicts between wildlife and humans, as wild felids are considered a threat to domestic animals or humans (Inskip and Zimmermann 2009; CITES 2010; Solari *et al.* 2018). Therefore, at the national and international levels, wild felids have been listed in some risk category — Endangered, Threatened, or Near Threatened — although some species, such as the bobcat and the puma, are not yet listed in a risk category (IUCN 2010; SEMARNAT 2010).

Felid conservation is essential for the integrity of ecosystems, as they regulate the population sizes of other species, influencing the dynamics and structure of natural communities, which is why they are considered indicators of habitat quality (Miller *et al.* 2001; Nagy-Reis *et al.* 2017; Tossens *et al.* 2024). In addition, these mammals require extensive areas with little human intervention for their survival, so the area allocated for felid conservation can potentially serve for the protection of other species and in territorial planning through the establishment of protected areas and to support conservation-related decision-making (Ceballos *et al.* 2002; Nuñez *et al.* 2002; Carrillo-Reyes and Rioja- Paradela 2014; Ashrafzadeh *et al.* 2020; Vega and Farías 2021)

The state of Morelos is home to the six felid species present in Mexico (Guerrero *et al.* 2020; Valenzuela *et al.* 2020). Four of these species are medium-sized felines that weigh between 101 g and 10 kg (Ceballos and Oliva 2005; Cervantes and Riveros Lara 2012). Information on the distribution of medium-sized felines in Morelos is limited to presence records in some localities and PNAs (Vargas *et al.* 1992; Valenzuela *et al.* 2013; Aranda *et al.* 2014; Aranda and Valenzuela 2015; Valenzuela *et al.* 2015; Vera-García *et al.* 2023). Although the potential distribution of these felines in Mexico has been modeled (Monroy-Vilchis *et al.* 2019), the models were developed at the biogeographic province level. This implies that areas with a suitable climate in Morelos have not been specifically identified, which is crucial for the conservation of these felines in the state. Therefore, it is necessary to identify potential areas where medium-sized felines can live in the state of Morelos, to support the development of conservation strategies that help prevent and mitigate the risks threatening their populations and habitats.

Our objectives were the following: a) identify the potential distribution areas for medium-sized felines in the state of Morelos using ecological niche models; b) analyze the extent to which the Natural Protected Areas in Morelos protect the potential distribution areas; and c) identify unprotected areas that could facilitate the connectivity of the populations of these species.

Materials and methods

Study area. The state of Morelos is located in central Mexico, between coordinates 18°20′, 19°07′ N and 98°37′, 99°30′ W. It covers an area of 4893 km^2, representing 0.25% of Mexico's territory (INEGI 2021a). Morelos includes five types of climates, ranging from cold subhumid to warm subhumid. The mean annual temperature is 21.5 °C and the mean annual precipitation is 900 mm, with summer rainfall (INEGI 2021a). The state is divided into three ecological regions: a) the northern mountainous region, represented by primary temperate forests, b) the intermontane valley dominated by crops and some disturbed patches of low deciduous forest, and c) the southern mountainous region, characterized by the largest extension of low deciduous forest in the state (Monroy and Colín 1991).

Fourteen PNAs have been established in Morelos (Figure 1) — five federal, seven state, and two municipal — which together comprise an area of 1196.9 km^2 (Table 1). These PNAs seek to protect and conserve biological diversity and natural resources in the state. Some have patches of habitat for different mammals, while others are corridors that maintain structural connectivity for populations of several species (González-Flores and Contreras-MacBeath 2020).

Ecological niche model. Ecological niche models (ENM) were used to identify potential distribution areas of the four medium-sized felines in Morelos. These models are tools for exploring the relationship between presence records and the associated environmental variables to construct potential or actual species distribution models (SDMs) (Guisan and Zimmermann 2000; Phillips *et al.* 2006; Peterson and Soberón 2012). The ENMs and SDMs for each species were generated using the MaxEnt algorithm version 3.4.4 (Philips *et al.* 2006). This is one of the most widely used algorithms for modelling ecological niches due to its high predictive power. In addition, the results allow predicting the availability of suitable areas for each species, generating a geographical representation of this information (Elith *et al.* 2006; Phillips *et al.* 2006; Kumar and Stohlgren 2009; Merow *et al.* 2013).

Species records were obtained from the scientific literature published between 2005 and 2020. A database was constructed from the geographic coordinates of occurrence records of the four medium-sized felines at the national and state levels, supplemented with records from the online database of the Global Biodiversity Information Facility (GBIF). Records of subspecies distributed in Mexico that could be present in Morelos were downloaded. Duplicate records, those without geographic coordinates, and those outside of Mexico were excluded. In total, 568 records of margay, 252 of jaguarundi, 1029 of ocelot, and 1819 of bobcat were obtained countrywide.

An ENM was generated for each feline species using its presence records in Mexico and coverage information on 19 climatic variables obtained from WorldClim that have been previously used for feline ENMs (Martínez-Calderas *et al.* 2015, 2016; Pérez-Irineo *et al.* 2019; Morales-Delgado *et al.*

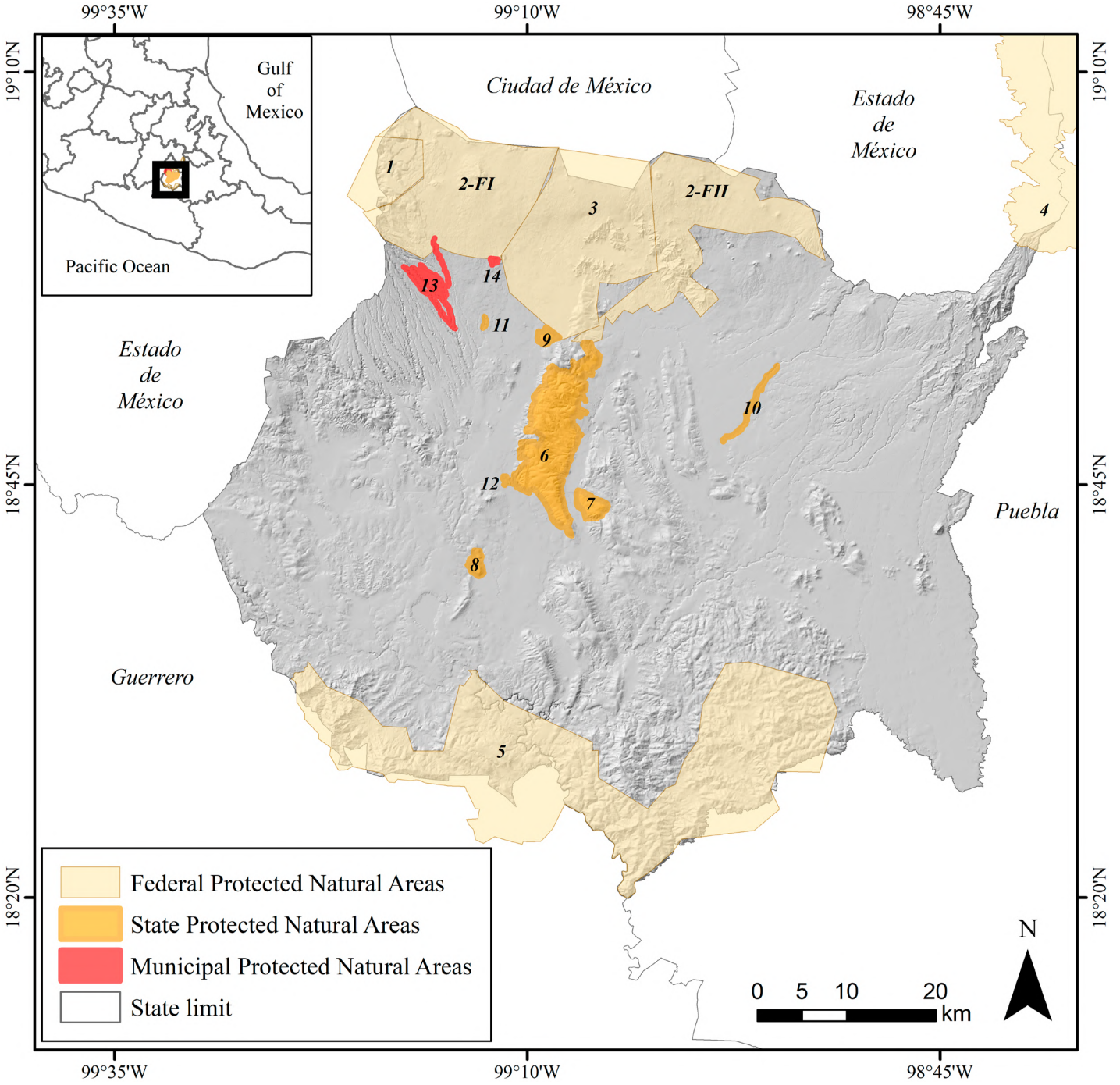

Figure 1. Map of the state of Morelos and its PNAs (acronyms after the name in Spanish). 1. Lagunas de Zempoala National Park (PNLZ), 2. Chichinautzin Biological Corridor Flora and Fauna Protection Area (APFFCBC), 3. El Tepozteco National Park (PNET), 4. Iztaccíhuatl-Popocatepetl National Park (PNIP), 5. Sierra de Huautla Biosphere Reserve (REBIOSH), 6. Sierra de Monte Negro State Reserve (RESMN), 7. Las Estacas State Reserve (RELE), 8. Cerro de la Tortuga State Park (PECT), 9. El Texcal State Park (PEET), 10. Los Sabinos, Santa Rosa, San Cristóbal Ecological Conservation Zone (ZSCESSS), 11. Barranca de Chapultepec Urban State Park (PEUBC), 12. Cueva El Salitre Wildlife Refuge (RVSCS), 13. Barrancas Urbanas de Cuernavaca Protected Natural Zone (ZNPBUC), 14. Bosque Mirador Protected Natural Area (ANPBM).

2021). Climate data were limited to the period 1970–2000 and had a spatial resolution of 1 km. The accessible area (*M*-area) was delimited (Soberón *et al.* 2017) by selecting the global terrestrial ecoregions reported by Olson *et al.* (2001) that coincided with the location of records for each species and with the country area. The resulting areas were used to delimit the set of bioclimatic layers for each species.

Based on records obtained via spatial filtering, the values of the 19 bioclimatic layers were extracted for each species, and a correlation test was performed to remove redundant information (Zurell *et al.* 2020; Passos *et al.* 2024). Layers with a Pearson correlation coefficient greater than 85% were considered correlated variables (Dorman *et al.* 2013; Passos *et al.* 2024). Based on these results, we selected the layers with simpler interpretations and a direct effect on the biology of the four species.

Initially, all records for each species were included in the niche models, with the climate variables selected after the correlation analysis. To avoid overrepresentation of records without affecting model fitness, spatial filtering was

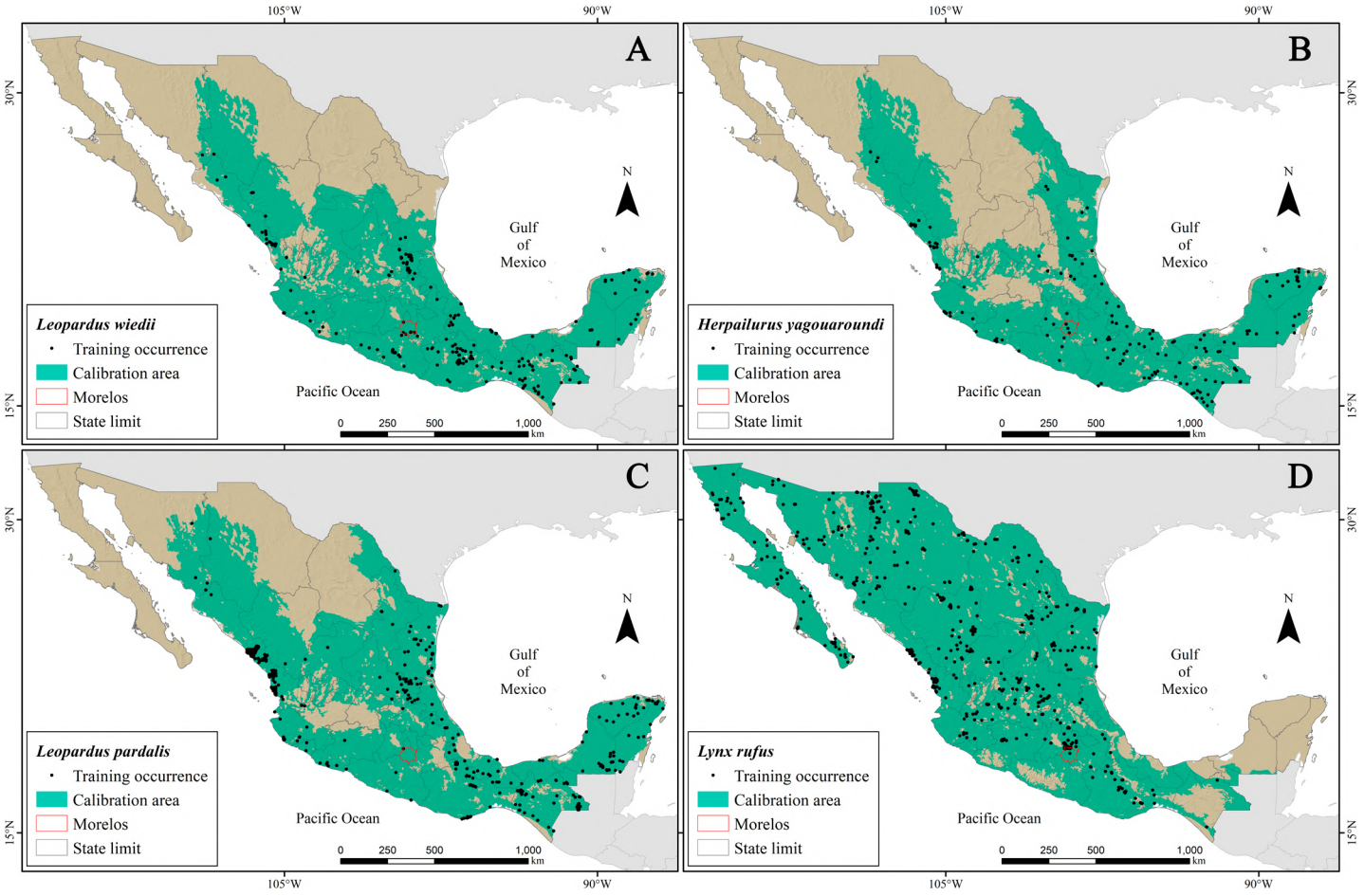

Figure 2. Maps of calibration areas and records of each medium-sized feline species in Mexico.

performed at different distances from presence records. The 1 km distance produced the best response. The 1km spatial filtering produced 302 records for margay, 240 for jaguarundi, 643 for ocelot, and 804 for the bobcat (Figure 2). Of the total records obtained, 70% were randomly selected to calibrate the ENM and 30% to validate the SDM using the partial ROC method available in the *ntbox* package in R (Osorio-Olvera *et al.* 2020).

Since using a large number of bioclimatic layers in models can lead to prediction errors (Peterson and Nakazawa 2008), these were reduced based on three reliable criteria in the MaxEnt output: 1) Jackknife plots, which evaluate the relative importance of environmental variables. For all species, the variables maintained in the models were those with the highest contribution and those that most affected the model (Phillips 2010; Merrow *et al.* 2013; Golden *et al.* 2022). 2) The table of the percentage contribution and importance of the permutation of the variables to each model (Phillips 2010). 3) The final model was the one with the lowest number of climatic variables with an AUC value greater than 0.70.

Species distribution model. The SDM models were created from ENMs and validated with the partial ROC method available in the *ntbox* package in R (Osorio-Olvera *et al.* 2020). The bootstrap technique was used, selecting 50% of the validation records for each of the 500 iterations performed.

The potential distribution of the four felines in the state of Morelos was spatially represented using binary models. The presence-absence cut-off threshold was set at the 10th percentile of the training presence method because there were no data on actual absence (Brito *et al.* 2009) and because it is a good threshold that accurately recovers the distribution of mammals (Escalante *et al.* 2013). This threshold was also selected based on the model with the smallest predicted area, with the lowest omission and commission rates.

Considering that 11 records were obtained for margay, 4 for jaguarundi, 2 for ocelot, and 27 for bobcat in Morelos, the cut-off threshold by species was set to include most of these records; only one record for ocelot was not predicted. After defining the cut-off threshold, binary climate suitability (CS) maps were generated in the calibration area. Subsequently, the CS areas for each species in Morelos were delimited, and a consensus was derived by superimposing these areas to identify the potential distribution range of the four species in the state of Morelos.

Contrast with human activities and Natural Protected Areas. Exclusion zones for the distribution of the four felines were delimited on the generated binary DMs, assigning a suitability value of 0 (zero) to areas that do not correspond to primary vegetation, secondary tree vegetation, and secondary shrub vegetation. According to the land use and vegetation layer series VII (INEGI 2021b),

Table 1. Protected Natural Areas in the state of Morelos. The names of the PNAs are presented along with their category. The numbering corresponds to that in Figure 1. The acronyms used in the text are indicated in parentheses.

Protected Natural Area	Jurisdiction*	Area in Morelos (km^2) *	Vegetation types *	Primary vegetation (km^2) **	Secondary tree vegetation (km^2) **	Secondary shrub vegetation (km^2) **	Medium-sized felines reported in Morelos ***
1. Lagunas de Zempoala National Park (PNLZ)	Federal	30.04	Aquatic, pine-fir forest, alpine grassland	28.67	0	0	Bobcat and ocelot
2. Chichinautzin Biological Corridor Flora and Fauna Protection Area (APFFCBC)	Federal	369.87	Pine, pine-oak, fir, and mountain cloud forest, oak forest, low deciduous forest, and crasicaule shrub	160.92	32.43	45.71	Bobcat and margay
3. El Tepozteco National Park (PNET)	Federal	209.54	Pine, pine-oak, fir, mountain cloud forest, and low deciduous forest	40.61	52.42	51.26	Bobcat
4. Iztaccíhuatl-Popocatépetl National Park (PNIP)	Federal	4.44	Pine-fir forest, alpine moorland and grasslands	3.89	0	0	No report
5. Sierra de Huautla Biosphere Reserve (RBSH)	Federal	488	Low deciduous forest	40.97	41.17	298.98	Bobcat and margay
6. Sierra de Monte Negro State Reserve (RESM)	Estatal	77.25	Low deciduous forest	0	41.68	30.46	Margay
7. Las Estacas State Reserve (RELE)	State	6.52	Low deciduous forest, riparian forest, and aquatic and underwater vegetation	0	0	5.52	No report
8. Cerro de la Tortuga State Park (PECT)	State	3.10	Low deciduous forest	0	0	2.93	No report
9. El Texcal State Park (PEET)	State	2.6	Low deciduous forest	0	2.42	0	No report
10. Los Sabinos Santa Rosa San Cristóbal Zone Subjected to Ecological Conservation (ZSCESSS)	State	1.52	Aquatic and riparian forest	0	0	0	No report
11. Barranca de Chapultepec Urban State Park (PEUBC)	State	0.13	Aquatic and riparian forest	0	0	0	No report
12. Cueva El Salitre Wildlife Refuge (RVSCS)	State	0.0003	Low deciduous forest	0	0	0	No report
13. Barrancas Urbanas de Cuernavaca Protected Natural Zone (ZNPBUC)	Municipal	3.7	Aquatic and riparian forest	0.84	0	0	No report
14. Bosque Mirador Protected Natural Area (ANPBM)	Municipal	0.22	Pine-oak forest	0	0	0.16	No report
Total surface area		**1196.93**		**275.90**	**170.12**	**435.03**	

*Data from González-Flores and Contreras-MacBeath 2020.
**Cover according to the land use and vegetation layer, series VII of INEGI (2021b).
***Records obtained from GBIF, Valenzuela et al. (2013), Aranda and Valenzuela (2015), and Vera-García et al. (2023).

the primary vegetation covers an area of 332.48 km^2; the secondary tree vegetation, 318.12 km^2; and the secondary shrub vegetation, 1184.62 km^2. In the resulting SDMs, we quantified the potential distribution areas for each species. The SDMs of each species were superimposed to estimate the areas of medium-sized feline richness in Morelos. Finally, the federal, state, and municipal PNAs were superimposed, and, according to the CS, the number of medium-sized feline species that each PNA could potentially host and the potential distribution area of each species protected by the PNAs were counted.

Results

The final ENMs were calibrated with 212 records for margay, 168 for jaguarundi, 403 for ocelot, and 563 for bobcat, using a combination of variables associated with the presence records for each species (Table 2). According to the contribution and permutation values and the Jackknife method, the minimum temperature of the coldest month is the most important variable for margay, jaguarundi, and ocelot, while the seasonality of precipitation is the key variable for bobcat. The second most important variable differed among species: annual temperature range for

Table 2. Importance of climatic variables and evaluation of models with the partial ROC method, according to the Jackknife output. An asterisk indicates that the variable produces a better model fit; two asterisks indicate that the absence of the variable reduces the model fit.

Species	Climatic variable	Percentage contribution	Percentage permutation	Mean AUC ratios (partial ROC)	*p*-value for the partial ROC analysis
Margay	Bio6 (minimum temperature of the coldest month) **	53.8	53.6	1.36	<0.00001
	Bio7 (annual temperature range) *	36.4	34.3		
	Bio15 (precipitation seasonality)	6.4	7.3		
	Bio10 (mean temperature of the warmest quarter)	3.4	4.7		
Jaguarundi	Bio6 (minimum temperature of the coldest month) */**	77.9	13.1	1.28	<0.00001
	Bio4 (temperature seasonality)	13.3	23.1		
	Bio10 (mean temperature of the warmest quarter)	5.5	33.4		
	Bio7 (annual temperature range)	3.3	30.4		
Ocelot	Bio6 (minimum temperature of the coldest month) *	74.2	20.2	1.25	<0.00001
	Bio1 (mean annual temperature) **	16.1	47.2		
	Bio7 (annual temperature range)	9.8	32.6		
Bobcat	Bio15 (precipitation seasonality) */**	36.8	25.8	1.12	<0.00001
	Bio10 (mean temperature of the warmest quarter)	26.8	33.8		
	Bio6 (minimum temperature of the coldest month)	14	6		
	Bio19 (precipitation of the coldest quarter)	12.3	14.5		
	Bio3 (isothermality)	10.1	20		

margay, temperature seasonality for jaguarundi, mean annual temperature for ocelot, and mean temperature of the warmest quarter for bobcat (Table 2).

Based on the MaxEnt response curves (Philips *et al.* 2006) and information on environmental variables associated with the records, we described the relationships between the climatic variables and the records of the four species. The minimum temperature of the coldest month was used for all four species, which showed a positive relationship between 0 °C and 22 °C for margay, jaguarundi, and ocelot, but a negative relationship between -6 °C and 20 °C for bobcat. The temperature of the warmest quarter was used in three species, revealing a positive relationship for jaguarundi (between 14 °C and 30 °C) and ocelot (between 12 °C and 30 °C), and a negative relationship for bobcat (between 8 °C and 33 °C). The annual temperature range was relevant for margay, jaguarundi, and ocelot, with a negative relationship between 10 °C and 34 °C. Precipitation seasonality was considered for the bobcat only, with a negative relationship and coefficients of variation ranging from 47 to 140.

The partial ROC evaluation of the SDMs indicates that the predicted potential distribution of the species is greater than expected by chance, with average values of AUC ratios of 1.36 ($p < 0.0001$) for the margay, 1.28 ($p < 0.0001$) for the jaguarundi, 1.25 ($p < 0.0001$) for the ocelot, and 1.22 ($p < 0.0001$) for the bobcat (Table 2). Binary SDMs predict an area with CS of 4327.28 km^2 for margay, 3564.6 km^2 for jaguarundi, 3280.2 km^2 for ocelot, and 2926 km^2 for bobcat. Of these areas, 20% correspond to water bodies, induced grasslands, induced palm forest, bare soil, and devoid of vegetation; 9%, to human settlements; and 33%, to agricultural areas. By reducing these binary model areas, the area with CS decreases to 38% (± 0.74) for each species (Table 3, Figure 3).

Of the total area considered potentially viable, 7% corresponds to primary vegetation, an additional 7% to secondary tree vegetation, and 24% to secondary shrub vegetation. In terms of extension, most of the potential distribution of neotropical felines (ocelot, jaguarundi, and ocelot) is concentrated in the central and southern regions of the state, although the models also consider regions to the north for the ocelot. For bobcat, a large part of its potential distribution is concentrated in the north and center of the state, largely coinciding with that of margay (Figure 3).

Considering the reduction of SDMs associated with the absence of primary or secondary vegetation, the area that

Table 3. Potential distribution area for the four medium-sized felines present in the State of Morelos. Estimates of the area with climate suitability (CS) that coincides with primary and secondary vegetation (tree and shrub) are shown, as well as the area within and outside protected natural areas.

Species	CS area (km^2)	CS area with vegetation cover (km^2)	CS area with vegetation cover within PNAs (km^2)	CS area with vegetation cover outside PNAs (km^2)
Margay	4327.28	1646.21	726.36	919.85
Jaguarundi	3564.56	1352.99	487.07	865.92
Ocelot	3280.16	1298.02	490.78	807.24
Bobcat	2926.04	1125.85	514.50	611.35

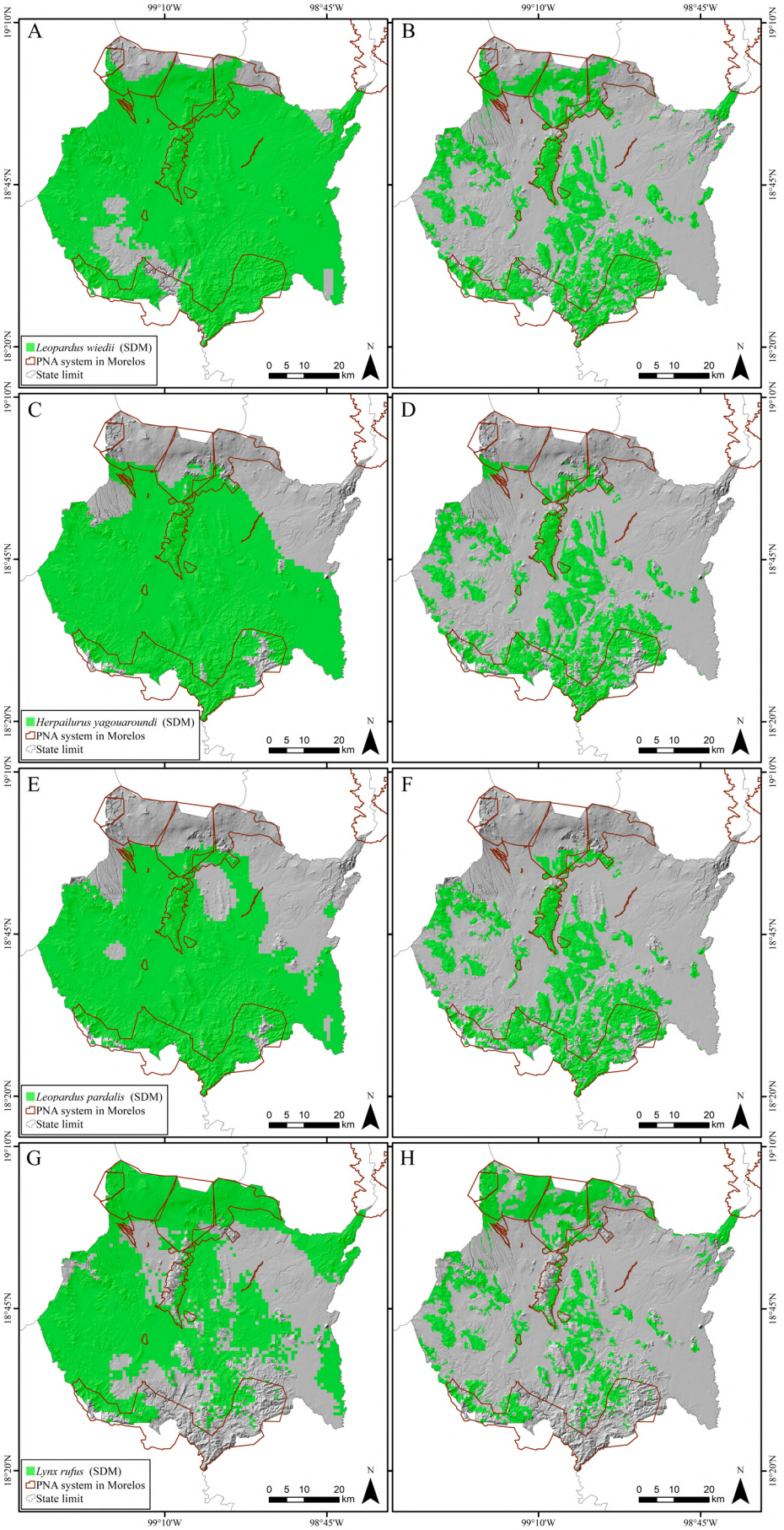

Figure 3. Potential distribution of the four medium-sized felines in the state of Morelos. Sections A, C, E, and G depict the models obtained from MaxEnt. Subparagraphs B, D, F, and H correspond to areas with climate suitability that coincide with primary vegetation, secondary tree vegetation, and secondary shrub vegetation (INEGI 2021b).

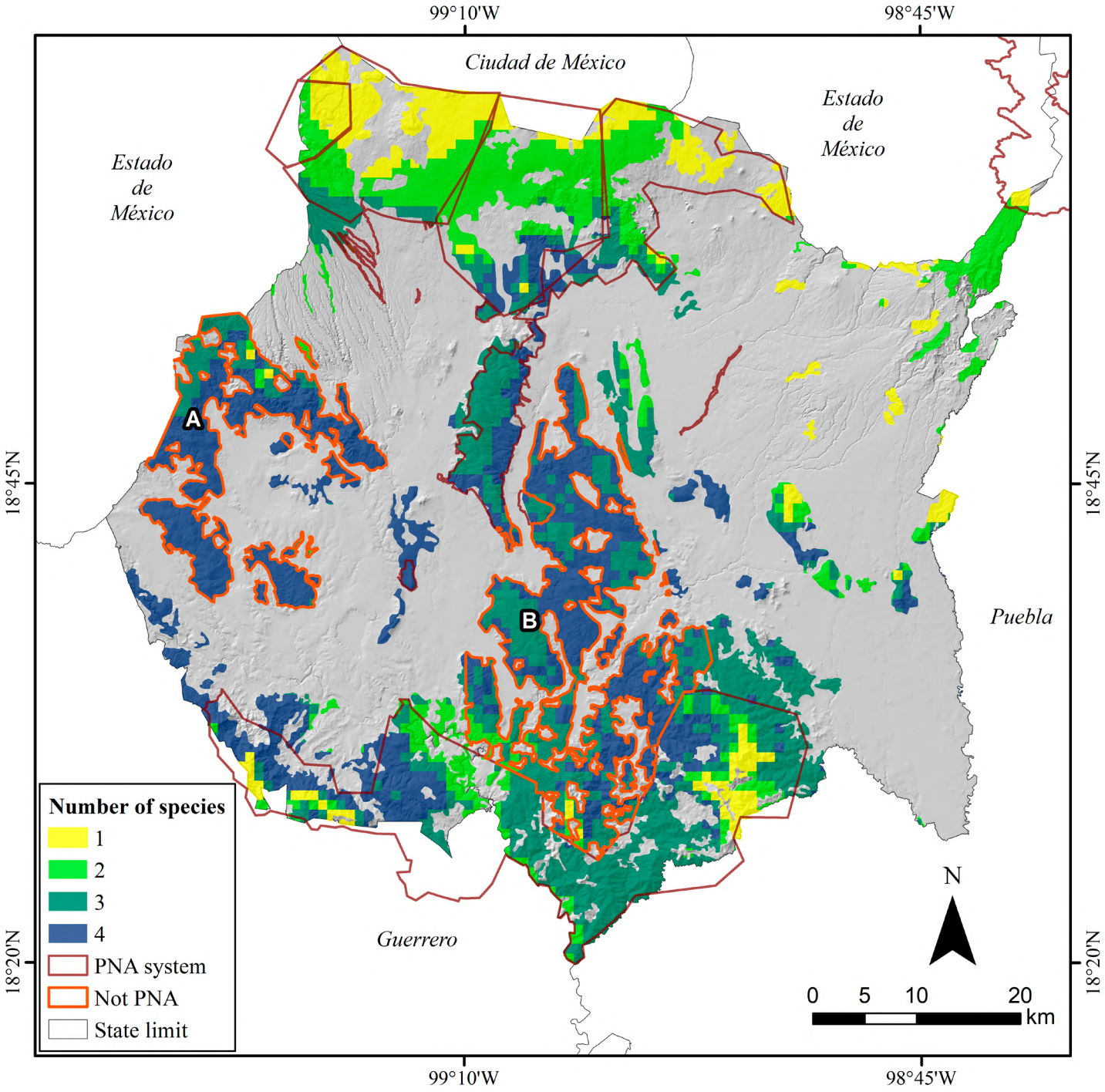

Figure 4. Richness model of the potential distribution of the four feline species in the state of Morelos. Blue shades indicate areas where the four species of medium-sized felines are potentially distributed. A) Region with a continuous area with climate suitability for four species in the west of the state; B) region with a continuous area with climate suitability for four species between RELE and RBSH.

can potentially host one feline species is 189.70 km^2; two species, 360.43 km^2; three species, 625.92 km^2; and the area with CS and vegetation cover in which the four species could potentially be present is 658.69 km^2, with region A (208 km^2) and region B (404 km^2) in Figure 4 being the largest.

By superimposing the PNAs on the richness areas model, the PNAs showing CS and vegetation cover for four species are APFFCBC FII, PNET, RBSH, RESMN, RELE, PEET, PECT, and ZPNBUC; it is worth mentioning that the last four have an area of less than 7 km^2 (Figure 4). The PNAs that show CS and vegetation cover for three species are APFFCBC FI and ANPBM, the latter with an area of only 0.22 km^2, but contiguous to APFFCBC FI (Figure 4). Finally, the PNAs with CS and vegetation cover for two species are PNIP and PNLZ, in which the potential distributions of margay and bobcat overlap (Figure 4).

Discussion

ENMs show climatic segregation, consistent with the Neotropical and Nearctic affinities reported for these felines (Sunquist and Sunquist 2002; Solari *et al.* 2018). The presence records of margay, jaguarundi, and ocelot are associated with

warm and temperate climates, which prevail in the study area and favor a large CS area in Morelos for these species. On the other hand, bobcat presence records are associated with low temperatures, which explains the availability of CS areas in the north of the state.

The SDMs show that, despite differences in the importance of climate variables, their combination predicts potential overlapping distribution ranges for the four species. However, it is worth noting that only 38% of the CS area has native vegetation cover, with 24% being secondary shrub vegetation. Therefore, factors such as habitat patch size and conservation status could limit the use of CS areas, as they are not sufficiently large and conserved to support populations of the four felines (Fahrig 2003; Lawrence *et al.* 2018; Cudney-Valenzuela *et al.* 2021).

When SDMs were superposed, two CS areas for the potential distribution of a single species were predicted, covering 3.9% of the area of Morelos. The first is located in the northern part of the state, corresponding to a large part of the potential distribution of the bobcat; the second corresponds to part of the potential distribution of the margay and is located in the southeast and northeast of the state (Figure 4).

The area where the potential distribution ranges of two feline species overlap is equivalent to 7.4% of the state (Figure 4). The combinations predicted are as follows: bobcat and margay in the north of the state, margay and jaguarundi in the center, and ocelot and jaguarundi in the south of the state. The area potentially inhabited by three feline species covers 12.8% of the state, resulting from the intersection of the CS areas of margay, ocelot, and jaguarundi, mainly in the central and southern regions of the state. Intersections of potential ranges of bobcat with margay and ocelot, and with jaguarundi and ocelot, and with jaguarundi and margay were also found, but in a smaller proportion. Finally, the results showed that the potential area where the four species could be found is equivalent to 13.5% of the state area, mainly in the west (region A, Figure 4) and the center-south of the state (region B, Figure 4).

Although our results show areas in Morelos where all four species could be found, interactions between these felines should also be considered, as these may also limit the presence of a given species. Previous studies indicate that the medium-sized felines studied could compete for similar resources, hampering their coexistence (Hutchinson 1957; Jacksic and Marone 2007). However, they could display spatial or temporal segregation mechanisms that could favor sympatry (Núñez *et al.* 2002; Di Bitetti *et al.* 2010; Bianchi *et al.* 2014; Carrera *et al.* 2018).

The constant spatial and demographic growth of urban areas or the expansion of the agricultural frontier have contributed to the transformation and degradation of natural systems, with a negative impact on biodiversity, limiting resources, and the ability to establish populations for some species (Monroy and Velázquez 2002; Sierra 2012; Newbold *et al.* 2015). This effect is evident in the 42% reduction in CS areas for felines in the state. However, it should be noted that 33% of the potential distribution range of medium-sized felines corresponds to agricultural areas, which are considered the productive base of the primary sector (Monroy and Colín 1991; Escandón *et al.* 2018). Therefore, habitat conservation strategies for medium-sized felines should be designed considering these activities.

Our findings show that the PNA complex in the north of the state (PNLZ, APFFCBC, PNET, and PEET) mainly protects CS areas for bobcat and margay and, to a lesser extent, for ocelot and jaguarundi. RESMN and REBIOSH, located in the center and south of the state, respectively, have climatic conditions and primary, secondary tree, and secondary shrub vegetation that are ideal for the potential distribution of the four species. According to previous reports, APFFCBC, PNET, and REBIOSH have high CS for the distribution of margay (Morales-Delgado *et al.* 2021), which is consistent with our results.

The protected areas are supplemented by smaller PNAs that have CS for at least one species (ZNPBUC, ANPBM, PEET, PECT, and RELE). However, due to their extension, they cannot house or conserve a population of felines, as their area is smaller than the home ranges reported in Mexico for bobcat, ocelot, and jaguarundi (Elizalde-Arellano *et al.* 2012; Caso 2013; Giordano 2016). Nonetheless, some small PNAs are located adjacent to larger PNAs, and others, such as RELE, are part of large areas outside PNAs that are covered by vegetation and have ideal climatic conditions for the establishment of feline populations (region B in Figure 4). In addition, small PNAs could serve as stepping stones to facilitate the movement of individuals between PNAs (Dueñas-López *et al.* 2015; Herrera *et al.* 2017; Luja *et al.* 2017). However, to favor the role of these small PNAs as stepping stones, the design of structural corridors connecting them to larger PNAs or to regions A and B is required (Figure 4).

Although our results suggest that at least 11 of the 14 PNAs in Morelos have a high CS for the four medium-sized felines, records of these species in PNAs are scarce, and their presence has only been reported in five of them. The first record of the ocelot was reported in PNLZ in 2014. The presence of ocelot has also been confirmed in APFFCBC, REBIOSH (Valenzuela *et al.* 2013; Aranda Valenzuela 2015), and recently in RESMN (Vera-García *et al.* 2023). There are several records of bobcat in PNLZ, APFFCBC, and PNET (Monroy and Velázquez 2002; Uriostegui-Velarde *et al.* 2015), and, to a lesser extent, in RBSH (Valenzuela *et al.* 2013). There are no published records of jaguarundi confirming its presence in PNAs of Morelos, although there are anecdotal records that await confirmation.

This work also identified areas outside of the PNAs with primary, secondary tree, and secondary shrub vegetation that have CS for the potential distribution of the four medium-sized felines. One such area is region A (Figure

4), which, according to the richness model, covers 208.01 km^2 and is mainly composed of low deciduous forest (INEGI 2010a; Miranda-González *et al.* 2011). In this region, Álvarez *et al.* (2009) reported the presence of jaguarundi, ocelot, and bobcat in the community of Paredón, municipality of Miacatlán. Another important area is region B (Figure 4), located between RESMN-RELE and REBIOSH, covering 404.25 km^2 of low deciduous forest (INEGI 2010b; INEGI 2010c) with CS for the four felines.

Studies in regions A and B addressing the local fauna are scarce. Therefore, it is recommended to implement systematic monitoring to evaluate the conservation status, potential threats, and the presence of key or indicator species, such as felines. On the other hand, although there are remnants of relatively conserved vegetation, no measures have been put in place to protect and conserve them in these regions. Consequently, evaluations should be conducted to incorporate these areas into the Morelos protected natural areas system.

The results of the present study highlight the importance of designing monitoring programs to confirm the presence of the studied feline species in the different Morelos localities where their presence has not yet been substantiated. In addition, we suggest supplementing the programs with restoration actions, as between 40% and 58% of the final areas predicted by the models correspond to secondary shrub vegetation, which would restrict their viability. On the other hand, the potential distribution of these four felines should be considered in the State Urban Development Program and the Ecological Land-Use Planning Program to prevent further degradation of their habitat. It is therefore necessary to conserve and protect the areas that contribute to the structural connectivity between the PNAs. With this information and with the participation of different sectors of society, comprehensive conservation strategies can be established that guarantee the protection and restoration of the areas potentially inhabited by medium-sized felines in the state of Morelos.

Acknowledgments

We thank Areli Rizo Aguilar, Luis Gerardo Ávila Torresagatón, and Zuri Samuel Vera García for their valuable comments on an earlier version of the manuscript, as well as the associate editor and two anonymous reviewers whose thoughtful comments and suggestions greatly enriched this research.

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Associated editor: Eduardo Mendoza Ramírez
Submitted: August 7, 2025; Reviewed: October 18, 2025
Accepted: October 30, 2025; Published on line: November 24, 2025

THERYA, 2026, Vol. 17(1):31-44 DOI:10.12933/therya.2026.6207 ISSN 2007-3364

Trophic ecology of *Cerdocyon thous* and *Lycalopex gymnocercus* within the Iberá Ecoregion in Argentina

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This study analyzed the diet, trophic niche overlap, and resource selection of two sympatric foxes, *Cerdocyon thous* and *Lycalopex gymnocercus*, in Mburucuyá National Park, a protected area within the Iberá Ecoregion, Argentina. Between December 2014 and November 2015, a total of 293 scat samples were analyzed, with 44% identified as *C. thous* and 56% as *L. gymnocercus*. The analysis revealed 11 plant species and 27 animal taxa that were consumed by both foxes. The results suggest that both species are hypocarnivorous and have overlapping trophic niches throughout the year, although they exhibit seasonal variations in their trophic amplitudes. During the winter months, when fruit availability was low, both species displayed more active foraging behavior. This selective foraging was evidenced by their consumption of specific palm species, which likely represent a critical nutritional source. Although insects and arachnids (weighing between 0.1 and 10 grams) were their most common prey, meso and small mammals constituted approximately 90% of the consumed biomass due to their larger size. Further research should focus on the trophic plasticity of these foxes in other environments and on quantifying the nutritional contributions of different food sources. Comparing these findings from a protected area to those from anthropogenically disturbed environments will be crucial for understanding the species´ conservation needs.

Keywords: Canids, coexistence, food availability, Mburucuyá National Park, resource partitioning, trophic overlap.

Este estudio analizó la dieta, la superposición de nichos tróficos y la selección de recursos de dos zorros simpátricos, *Cerdocyon thous* y *Lycalopex gymnocercus*, en el Parque Nacional Mburucuyá, un área protegida dentro de la Ecorregión Iberá, Argentina. Entre diciembre de 2014 y noviembre de 2015, se analizaron un total de 293 muestras de heces, de las cuales el 44% se identificaron como de *C. thous* y el 56% como de *L. gymnocercus*. El análisis reveló 11 especies de plantas y 27 taxones de animales que fueron consumidos por ambos zorros. Los resultados sugieren que ambas especies son hipocarnívoras y presentan superposición de nichos tróficos a lo largo del año, aunque muestran variaciones estacionales en sus amplitudes tróficas. Durante los meses de invierno, cuando la disponibilidad de fruta era baja, ambas especies mostraron un comportamiento de búsqueda de alimento (forrajeo) más activo. Este forrajeo selectivo se evidenció por su consumo de especies de palmeras específicas, lo que probablemente representa una fuente nutricional crítica. Aunque los insectos y arácnidos (con un peso entre 0,1 y 10 gramos) fueron sus presas más comunes, los meso y pequeños mamíferos constituyeron aproximadamente el 90% de la biomasa consumida debido a su mayor tamaño. Es necesario que la investigación futura se centre en la plasticidad trófica de estos zorros en otros entornos y en cuantificar las contribuciones nutricionales de las diferentes fuentes de alimento. La comparación de estos hallazgos de un área protegida con aquellos de ambientes sujetos a perturbación antropogénica será crucial para comprender las necesidades de conservación de las especies.

Palabras clave: Cánidos, coexistencia, disponibilidad de alimento, Parque Nacional Mburucuyá, partición de recursos, superposición trófica.

The competitive exclusion principle, states that competitors using identical resources cannot coexist (Hardin 1960). Competitive exclusion can manifest either as exploitative competition, where species vie directly for limited resources, or as apparent competition, which is mediated by shared natural enemies (Johnson and Bronstein 2019). To avoid it, sympatric species often differentiate their use of available resources, a phenomenon known as niche differentiation (Kooyers *et al.* 2017). Niche partitioning fundamentally explains how different species within a community divide and use space and resources to reduce interspecific competition, thus allowing for their coexistence (Pianka 1986; Petalas *et al.* 2021; Říha *et al.* 2025). To study these dynamics, ecologists measure niche overlap, which assesses the degree of shared resource use between species, facilitating the analysis of potential competition (Colwell and Futuyma 1971; Hurlbert 1978). From an ecological perspective, coexistence depends on morphological, physiological and/or behavioral divergences. These differences can lead to differential resource utilization or spatial or temporal variation in the use of similar resource (Schoener 1974). Furthermore, at finer spatial scales, variations in resource use by a species have been directly linked to greater niche overlap or partitioning (Anderson *et al.* 2011; Ávila-Nájera *et al.* 2020).

Given their phylogenetic proximity and morphological similarities (Xiaoming *et al.* 2004), canids (Carnivora, Canidae) are an ideal subject for this analysis. In

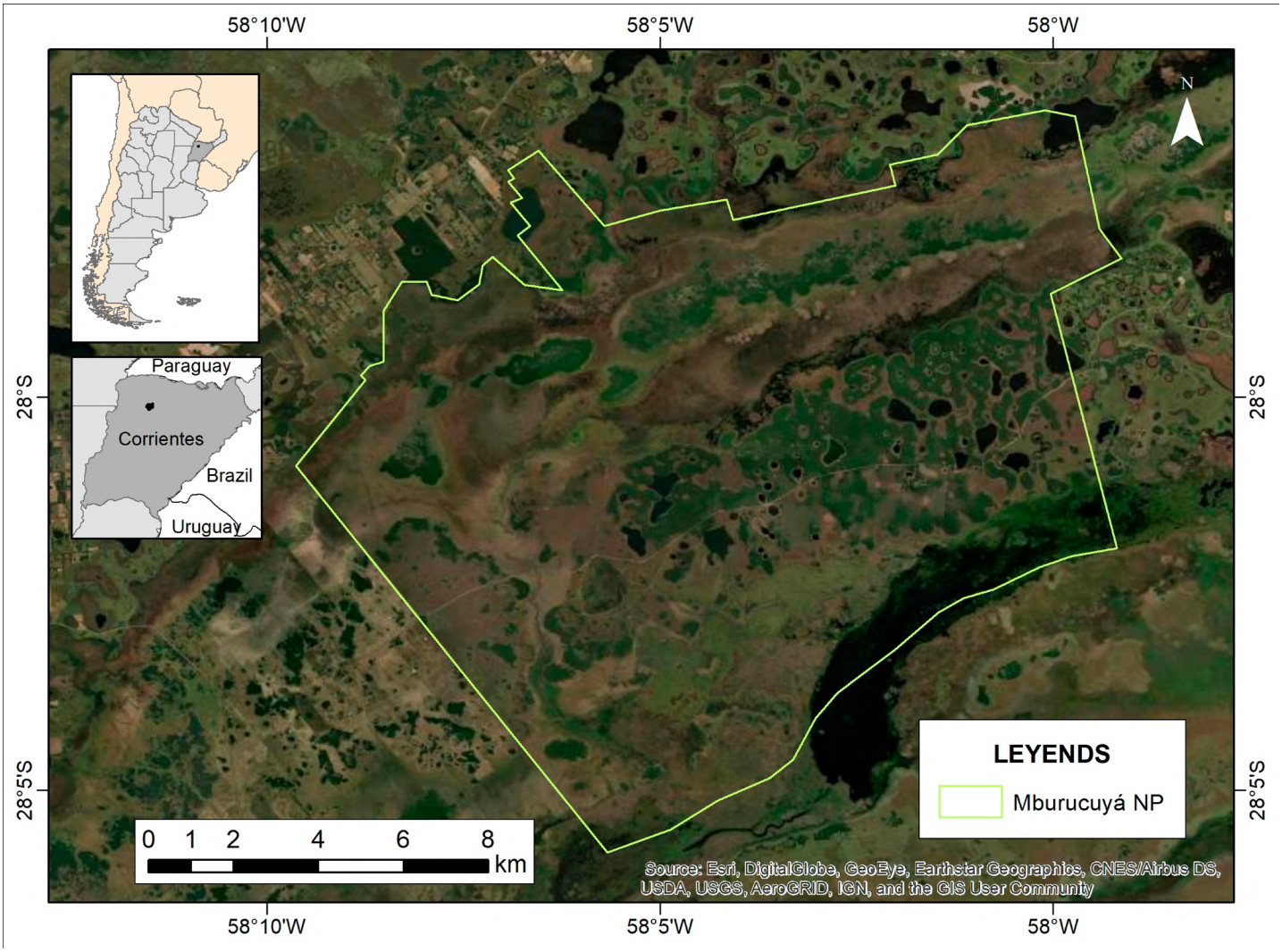

Figure 1. Study Area. Geographical location of Mburucuyá National Park (Corrientes, Argentina).

northeastern Argentina, two sympatric species with similar characteristics coexist: the crab-eating fox *Cerdocyon thous* (Linnaeus, 1766) and the pampas fox *Lycalopex gymnocercus* (G. Fischer, 1814). Both foxes are medium-sized, with *C. thous* weighing 4.5–8.5 kg (head-body length 54–77.5 cm) and *L. gymnocercus* weighing 3–8.2 kg (head-body length 44–72 cm) (Castelló 2018). As opportunistic omnivorous, their diets include a wide range of food items, including fruit, carrion, and prey ranging from such as ungulates, armadillos, capybaras, small mammals, birds, reptiles, amphibians, crustaceans, and insects (Sillero-Zubiri *et al.* 2004; Luengos Vidal *et al.* 2019).

The geographical ranges of these foxes overlap considerably (Sillero-Zubiri *et al.* 2004; Di Bitteti *et al.* 2009). The distribution of *C. thous* extends from northern Colombia and Venezuela to a substantial portion of Brazil, eastern Bolivia, Paraguay, Uruguay, and on to northern Argentina. It is highly resilient, able to utilize a wide variety of environments, including savannas, swamps, mesophileous forests, lowlands within the Amazon rainforest zone, and anthropogenic areas such as plantations, agricultural fields, and/or regenerating developments (Eisenberg and Redford 1999; Courtenay and Maffei 2004). However, its presence in anthropogenic areas, such as plantations and agricultural fields, highlights its tolerance for disturbed habitats, though this resilience often comes at the cost of increased exposure to zoonotic diseases, such as severe scabies (Oliveira *et al.* 2025). In turn, the distribution of *L. gymnocercus* ranges from southern Bolivia and Brazil to Chile, Paraguay, Uruguay, and Argentina, reaching as far south as Tierra del Fuego (Luengos Vidal *et al.* 2019). Due to the potential for competition in their overlapping habitats, studying these species in sympatry offers a valuable opportunity to investigate the mechanisms facilitating their coexistence (Bossi *et al.* 2018).

Several studies have compared the ecological niche of *C. thous* and *L. gymnocercus* in both Brazil and Argentina (Vieira and Port 2007; Di Bitetti *et al.* 2009; Faria-Corrêa *et al.* 2009; Bossi *et al.* 2018; Di Bitteti *et al.* 2022; Bay-Jouliá *et al.* 2024; Romero *et al.* 2025). Focusing on the niche complementarity hypothesis which posits that some niche dimensions are partitioned when there is high overlap in another (Schoener 1974). Vieira and Port (2007) found a high degree of dietary overlap, between these foxes in

Table 1: Occurrence data and percentage obtained from the diet composition of canids, *Cerdocyon thous* and *Lycalopex gymnocercus,* in Mburucuyá National Park, Corrientes, Argentina (2014-2015). References: O, occurrence; OP, occurrence percentage.

	Cerdocyon thous **(scat samples: 129)**			***Lycalopex gymnocercus*** **(scat samples: 164)**		
FLESHY FRUIT PLANTS	**Count**	**O**	**OP**	**Count**	**O**	**OP**
Syagrus romanzoffiana	108	15	7.65	36	11	5.7
Butia yatay	161	34	17.35	239	44	22.8
Bromelia serra	15	7	3.57	14	6	3.11
Ocotea acutifolia	885	28	14.29	370	16	8.29
Eugenia uniflora	38	2	1.02			
Ficus luschnathiana	818	20	10.20	320	9	4.66
Citrus	5	3	1.53	4	3	1.55
Chrysophyllum gonocarpum	22	2	1.02			
Chrysophyllum marginatum	200	1	0.51	975	7	3.63
Psidium guajava	15	2	1.02	3	1	0.52
Solanaceae	35	2	1.02	80	2	1.04
ANIMAL CATEGORIES						
INVERTEBRATES						
Gastropoda						
Pomacea canaliculata				11	3	1.55
Crustacea						
Trichodactylus kensleyi	4	4	2.04	3	3	1.55
Arachnidae						
Ixodidae	3	1	0.51	1	1	0.52
Scorpiones cf. *Bothriurus* sp.	3	3	1.53	1	1	0.52
Araneae				1	1	0.52
Insecta						
Mantidae	1	1	0.51			
Acrididae	96	28	14.29	63	29	15.03
Gryllidae				1	1	0.52
Tettigonidae				2	1	0.52
Lepidoptera	3	3	1.53			
Formicidae				1	1	0.52
Scarabeidae	6	5	2.55	14	9	4.66
Cicindelinae	2	2	1.02			
Carabidae	1	1	0.51			
Unidentified beetle	3	3	1.53	1	1	0.52
Other unidentified insects	61	3	1.53	26	6	3.11
VERTEBRATES						
Unidentified fish	1	1	0.51	1	1	0.52
Unidentified reptiles	2	2	1.02	2	2	1.04
Colubridae				1	1	0.52
Lacertilia	2	2	1.02			
Eggs	1	1	0.51			
Unidentified small birds	1	1	0.51	1	1	0.52
Passeriforme				8	8	4.15
Mammals						
Cavia aperea	1	1	0.51	2	2	1.04
Hydrochoerus hydrochaeris	2	2	1.03	2	2	1.04
Unidentified small mammals	7	7	3.57	7	7	3.63
Unidentified medium mammals	9	9	4.59	13	13	6.74
TOTAL	2511	196	100	2203	193	100

the Aparados da Serra National Park (Southeastern Brazil, 29°10'S, 50°25'W), whilst they partitioned habitat use over time and space: *C. thous* exhibited a more nocturnal activity pattern and was observed more frequently at forest edges, in grasslands, and on roads, whereas *L. gymnocercus* was more prevalent in open areas (Vieira and Port 2007). Likewise comparative diet analysis across three protected areas in northeastern Argentina (Mburucuyá National Park, Portal de San Nicolás, and Rincón de Santa María Natural Reserve) also evidenced high degree of niche overlap among these species, along with considerable dietary breadth (Bay-Jouliá *et al.* 2024).

Morphological convergence, specifically comparable body weights, minimizes intraguild conflict a conclusion consistent with the Donadio and Buskirk (2006) framework, which links similar body size ratios among carnivores to reduced intraguild killing (Di Bitetti *et al.* 2022). This effect is complemented by significant ecological partitioning, evidenced by divergent niches and distinct habitat preferences (Di Bitetti *et al.* 2009). Further evidence comes from Romero *et al.* (2025), who reported mean densities of 0.27 individuals/km^2 for *L. gymnocercus* and 0.50 individuals/km^2 for *C. thous* in Mburucuyá National Park. Their density model revealed that greater plant cover positively influenced *C. thous* but negatively affected *L. gymnocercus*, confirming a differentiated habitat use that sustains local coexistence.

In this study, we assess the diet, resource availability, and the selection of resources by *C. thous* and *L. gymnocercus* to understand the potential partitioning or overlap of their trophic niches. The research was conducted on protected populations in the Iberá Region, within Mburucuyá National Park (Corrientes, Argentina). Given the diversity of habitats and food resources within the park, and building upon empirical observations from previous ecological studies, we formulated three main hypotheses: the dietary composition of both species is similar, which will be evident in shared food items and a high niche overlap index; the consumption of fleshy fruits by both species will adjust to their environmental availability, with the percentage of fruit in their diets increasing when these items are abundant and decreasing when availability is scarce; and finally, the fox species inhabit environments with a greater richness of fruit-bearing plants, indicating that the active foraging behaviour for these resources could be happening.

Materials and methods

Study area. The study was conducted in Mburucuyá National Park (MNP), spanning 17,086 hectares in the central-northwestern part of Corrientes province (27°58'S and 58°08'W), northern Argentina (Figure 1). The park's landscape is characterized by a topography of sandy ridges—relicts of an ancient alluvial megafan of the Paraná River—and slow-draining wetlands, locally known as 'esteros' (Contreras and Contreras 2017). From a phytogeographical perspective, the MNP is in the Iberá

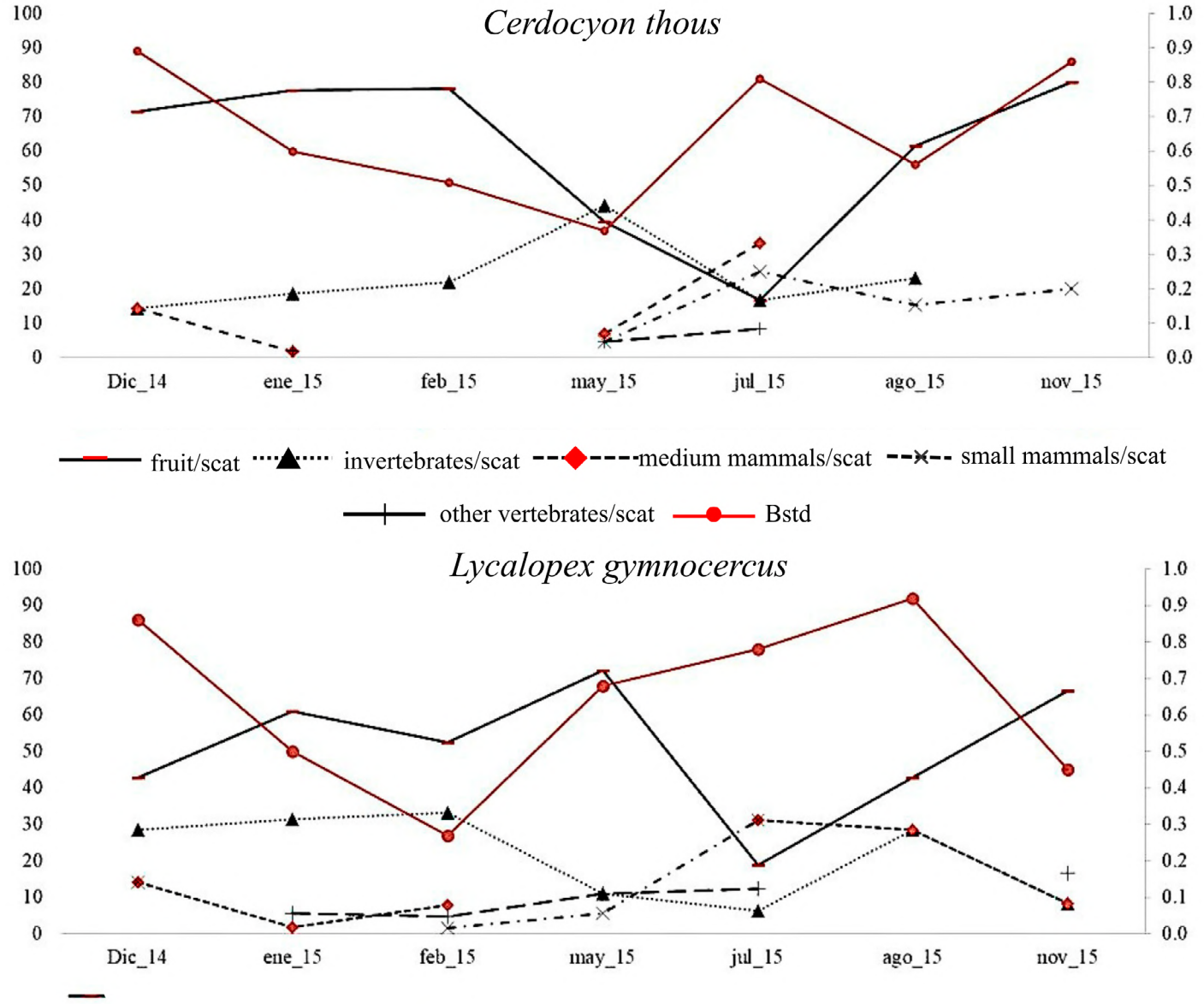

Figure 2: Food items variation (%) in canids *Cerdocyon thous* and *Lycalopex gymnocercus* 2014-2015, measured using the standardized Levins Index, in Mburucuyá National Park (Corrientes, Argentina).

Ecoregion, with a biodiversity that includes plant species from the Eastern Humid Chaco, Paranaense, and Espinal districts (Cabrera 1976; Arbo 2004). Its main habitats consist of tall grasslands, mesophileous forests, and palm groves of *Butia yatay* (Mart.) Becc., where wetlands, including lakes and streams, constitute 64% of the total area, providing crucial ecological functions.

The climate is classified as humid subtropical, with an average annual temperature of 21°C, reaching maximum values above 40°C in summer, but without a defined thermal winter (Contreras *et al.* 2020). Precipitation is rainfall, with an annual average of 1,400 mm, predominantly occurring from spring to autumn (October - May), with peak rainfall in April and May. In contrast, precipitation during winter (June - September) is minimal or absent (Smichowski *et al.* 2022; Smichowski and Contreras 2024).

Field work. Between December 2014 and November 2015, scat samples were collected along twelve transects (1 to 4.5 km in length) located in three MNP habitats: mesophileous forests, grasslands, and *B. yatay* palm groves. Samples were identified in the field by their size, shape, odour, the presence of hair or fruits, location of deposition, and their association with fox tracks (Chame 2003; Pedó *et al.* 2006; Vieira and Port 2007; Varela *et al.* 2008). Scat that could not be attributed with certainty to the species under investigation was discarded, and fragments found within a 0.5 m^2 area were considered a single defecation (Vieira and Port 2007). In the laboratory, samples were assigned to species level by identifying bile acid patterns using thin-layer chromatography (Cazón *et al.* 2009; Casanave *et al.* 2012). Scat analysis was chosen as a reliable, cost-effective, and non-invasive method for

Table 2: Trophic niche overlap. (Pianka Index) and diet composition of canids, *Cerdocyon thous* and *Lycalopex gymnocercus*, in Mburucuyá National Park, Corrientes, Argentina, during winter-summer 2014-2015. References: CT, *C. thous*; LG, *L. gymnocercus*.

	Winter seasonal		Summer seasonal	
	Occurrence percentage (%)			
	CT	LG	CT	LG
Scat samples	49	51	80	113
FLESHY FRUIT PLANTS				
Syagrus romanzoffiana	14.5	19.5	4.7	2.1
Butia yatay	9.7	2.4	22.1	29.7
Bromelia serra	11.3	14.6		
Ocotea acutifolia			22.1	11
Eugenia uniflora			1.6	
Ficus luschnathiana	3.2	2.4	14.2	5.5
Citrus	4.8	7.3		
Chrysophyllum gonocarpum			1.6	
Chrysophyllum marginatum			0.8	4.8
Psidium guajava			1.6	0.7
Solanaceae			1.6	1.4
ANIMAL CATEGORIES				
Invertebrates	35.5	12.2	21.3	31
Fish	1.6			0.7
Reptiles	1.6		1.6	2.1
Birds		9.8	0.8	3.5
Small mammals	11.3	14.6	0.8	2.1
Medium mammals	6.5	17.1	5.5	5.5
PIANKA INDEX	0.75		0.90	

estimating the diet of carnivores, a technique widely used in this type of study (Vieira and Port 2007; Marucco *et al.* 2008; Bay-Jouliá *et al.* 2024)

Fleshy-fruit Availability. The phenology and abundance of 11 species of fleshy-fruited plants—previously detected in the diets of the two fox species under study (Bueno and Motta-Junior 2004; De Almeida Jácomo *et al.* 2004; Pedó *et al.* 2006; Varela *et al.* 2008; Vieira and Port 2007)—were monitored monthly from January to November 2015 at the MNP. For this purpose, 20 sampling sites (100 m x 20 m) were established, distributed equitably and strategically across three vegetation strata: seven in mesophileous forests, seven in grasslands, and six in palm groves, following the methodology proposed by Ganzhorn *et al.* (2011). At each site, the number of individuals per species and their phenological data (flowering, fruit ripening, and percentage of fruit/flower) were recorded. To calculate the biomass of consumed fruits, samples of fruiting plants were obtained to determine the mean mass of their fruits.

Laboratory Analysis. In the laboratory, scat samples were dried in an oven at a temperature of 60°C until they reached a constant weight. Subsequently, samples were disaggregated under water using a 0.5 mm mesh sieve and examined under a stereoscopic binocular microscope (4–40X). Each food item was classified into one of seven main categories: fruits; invertebrates (which included crustaceans, mollusks, arachnids, and insects); fish; amphibians; reptiles; birds; and mammals (small and medium-sized species). The classification of each item was conducted at the most specific taxonomic level possible (species, genus, family, or order), based on the identification of undigested macroscopic structures such as seeds, exoskeletons, hair, bones, and dental remains. The presence of guard hairs in the scats was identified as an important tool in the identification of mammal species, as proposed by Quadros and Monteiro-Filho (2006a, b). The identification of fruits was achieved through a comparison of ingested seeds with the morphological characteristics of seeds from the main plant species in the MNP. The identification of both animal remains and fruits was carried out with the assistance of literature on regional flora and fauna and with the help of specialists in the field (Giraudo *et al.* 2006; Casco *et al.* 2008; Cano *et al.* 2011; Fontana 2017).

Dietary and Statistical Analyses. The diet of *C. thous* and *L. gymnocercus* were analyzed based on three key parameters: occurrence, percentage of occurrence, and consumed biomass. These methods were utilized to ascertain the significance of each food item and to facilitate direct comparisons with other dietary studies on these species and other carnivores (Pia *et al.* 2003; Bueno and Motta-Junior 2004, 2006; Rodrigues *et al.* 2007). Occurrence was defined as the frequency of a particular item relative to the total number of occurrences (Queirolo and Motta-Junior 2007), while percentage of occurrence was the proportion of a given item relative to the total number of items consumed (Pia *et al.* 2003; Bianchi *et al.* 2014).

Furthermore, for the animal items, the numerical frequency percentage (PF) for each item was calculated by determining the ratio between the minimum number of individuals of each category recorded in all scats and the sum of all individuals recorded across all prey categories, multiplied by 100 (Farias and Kittlein 2008). The relative biomass contributed by each animal item was estimated by multiplying its biomass by the PF, and was expressed as the total percentage of consumed biomass (BC) (Farias and Kittlein 2008). In the case of small mammals and birds, the consumption of biomass was calculated using correction factors that had previously been estimated for *Vulpes vulpes* (Linnaeus, 1758) (Ferreras and Fernández-de-Simón 2019). The correction factor is a number that, when multiplied by the total weight of indigestible matter, yields the original weight of the prey ensuring an accurate estimation of the ingested biomass from scat remnants. The biomass of consumed fruits was estimated by multiplying the pulp weight in grams of the found species by the number of records of each item found in the diet (Rodrigues *et al.* 2007), and it was assumed that each fruit was ingested whole. The body mass of animal prey was obtained from extant literature (Canevari and Vaccaro 2007), whereas the biomass of fruit was measured *in situ*.

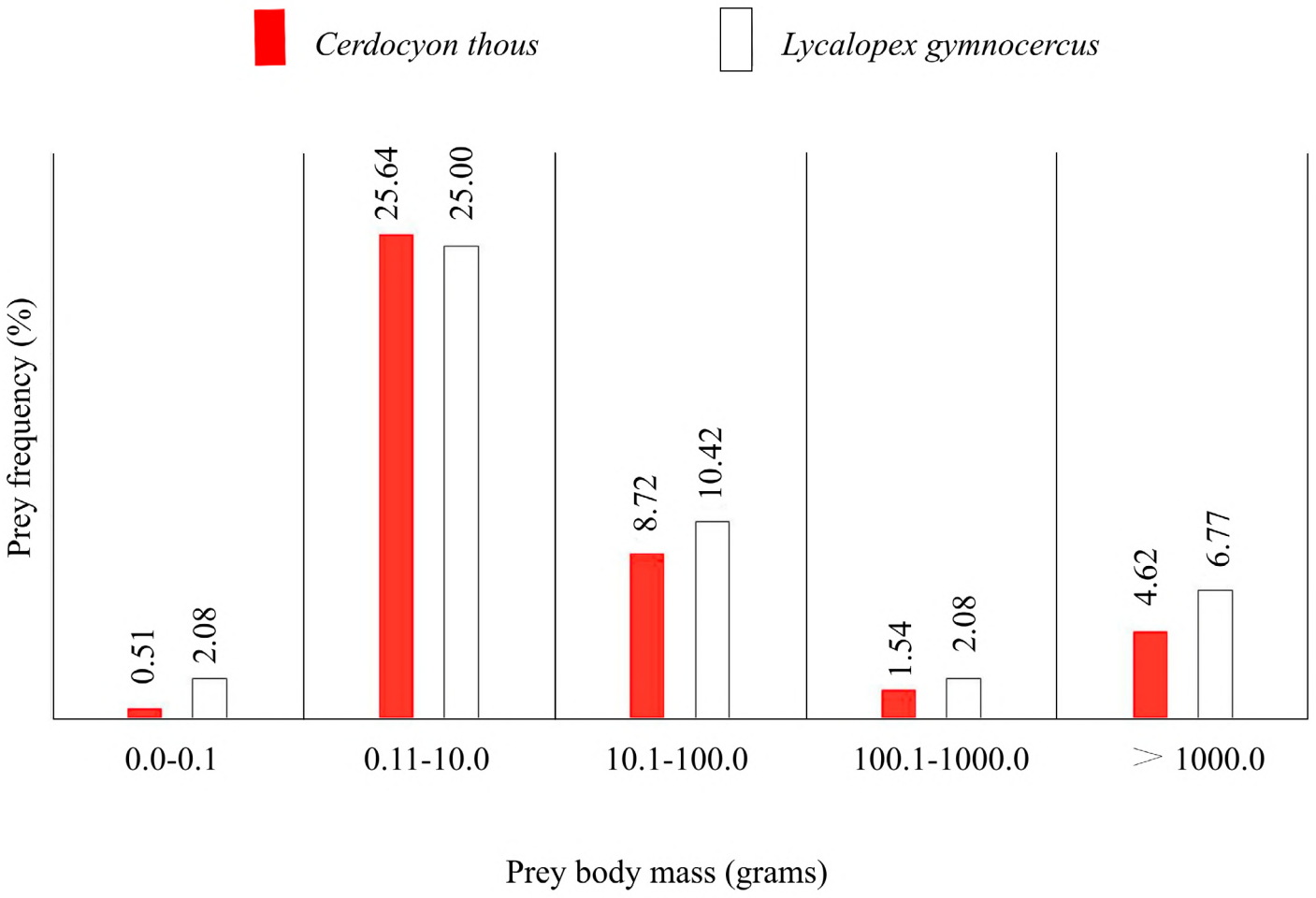

Figure 3. Frequency of consumption (%) of prey of different sizes, in grams, in the diet of canids *Cerdocyon thous* and *Lycalopex gymnocercus* in Mburucuyá National Park, Corrientes, Argentina (2014–2015).

The statistical analysis involved the assessment of dietary similarity. We evaluated diet similarity for each season using Pianka's index: Ojk =∑ *pij pik*/(∑ *pij*2 *pik*2)1/2, where pi is the frequency of occurrence of prey item i in the diet of species j and k (Pianka 1973). Pianka's index (O) varies between 0 (total separation) and 1 (total overlap). This approach facilitates comparisons with other studies (Juarez and Marinho-Filho 2002; De Almeida Jácomo *et al.* 2004; Vieira and Port 2007; Bay-Jouliá *et al.* 2024). The trophic niche breadth was determined using the standardized Levins index (*Bstd*), which is based on the frequency of each food item and ranges from 0 (minimum breadth) to 1 (maximum breadth). In order to establish seasonal variations in the diet of the canids, the percentage of occurrence of plant and animal items found in the scats were compared using a Chi-squared test (Silva and Talamoni 2003). For fruit consumption, a Chi-squared test was utilized to evaluate selectivity (Martínez *et al.* 1993), and the Spearman's correlation coefficient (*rs*) was utilized to ascertain the selection of resources to the seasonal variation in the percentage of occurrence of fruiting species (Silva and Talamoni 2003; Bueno and Motta-Junior 2006). These statistical tests were selected based on similar studies conducted on the diets of canid species from the region, such as *Chrysocyon brachyurus* (Illiger, 1815) in Brazil and *Lycalopex griseus* (Gray, 1837) in Chile (Silva and Talamoni 2003; Bueno and Motta-Junior 2006).

Results

We analyzed a total of 293 scat samples, comprising 129 from *C. thous* and 164 from *L. gymnocercus*. The analysis of these samples revealed a total of 38 food item types, including 11 of plant origin and 27 of animal origin. Animal prey constitutes 41% of the total food intake for *C. thous* and 49% for *L. gymnocercus*, with the remaining percentage composed of fruit. The animal origin categories for both fox species included invertebrates (crustaceans, mollusks, arachnids, and insects) and vertebrates (fish, amphibians, reptiles, birds, and small and medium-sized mammals) (Table 1). Overall, fruits from a total of nine species, one genus, and one family of plants were identified (Table 1).

The dietary overlap between *C. thous* and *L. gymnocercus* was consistently high throughout the year. For instance, the Pianka index showed a high value of 0.90 in summer, which decreased slightly to 0.75 in winter (Table 2). However, the breadth of their respective trophic

Table 3. Animal and vegetal estimated biomass consumed by canids, *Cerdocyon thous* and *Lycalopex gymnocercus*, Mburucuyá National Park, Corrientes, Argentina (2014-2015). References: n, number of records; NFP, Numerical Frequency Percentage; g, mass in gram; EB, Estimated biomass (g); CF, Conversion factors: passeriforme (CF = 45), *Cavia aperea* (CF = 44), Small mammals (CF = 23).

	Cerdocyon thous				*Lycalopex gymnocercus*			
FLESHY FRUIT PLANTS	**n**	**NFP (%)**	**Mass (g)**	**EB**	**n**	**NFP (%)**	**Mass (g)**	**EB**
Syagrus romanzoffiana	108		9.43	1018.4	36		9.4	339.5
Butia yatay	161		6.4	1030.4	239		6.4	1529.6
Bromelia serra	15		5.7	86.1	14		5.7	80.4
Ocotea acutifolia	885		1.2	1044.3	370		1.2	436.6
Eugenia uniflora	38		0.5	18.6				
Ficus luschnathiana	818		1.08	883.4	320		1.1	345.6
Citrus	5		230.7	1153.5	4		230.7	922.8
Chrysophyllum gonocarpum	22		10.2	225.3				
Chrysophyllum marginatum	200		0.1	28	975		0.1	136.5
Psidium guajava	15		23.4	351.3				
Solanaceae	35		7	244	80		7	557.6
Subtotal				6083.3				4348.5
ANIMAL CATEGORIES								
Gasteropoda (*Pomacea canaliculata*)					11	4.6	0.1	0.5
Crustacea (*Trichodactylus kensleyi*)	4	5.3	20	105.3	3	4.6	20	90.9
Ixodidae	3	15.8	0.1	1.6	1	4.6	0.1	0.5
Scorpionidae	3	5.3	0.6	3.2	1	4.6	0.6	2.7
Aranidae					1	4.6	0.6	2.7
Mantidae	1	5.3	0.6	3.2	1	4.6	0.6	2.8
Acrididae	96	5.3	0.7	3.7	63	4.6	0.7	3.2
Gryllidae					1	4.6	0.6	2.7
Tettigonidae					2	4.6	1	4.4
Lepidoptera	3	15.8	0.6	9.5				
Formicidae					1	4.6	0.2	1.
Scarabeidae	6	5.3	1	5.3	14	4.6	1	4.6
Cicindelinae	2	5.3	0.2	1				
Carabidae	1	5.3	1	5.3				
Coleoptera not identified	3	5.3	0.6	3.2				
Insecta not identified	61	5.3	0.6	3.2				
Subtotal				144.2				115.9
Fish not identified	1	5.3	100	526.3	1	4.6	100	454.6
Reptilia not identified	2	5.3	22	115.8	2	4.6	22	100
Culibridae	1	5.3	22	115.8	1	4.6	22	100
Lacertilia	2	5.3	22	115.8				
Egg	1	5.3	10	52.63				
Birds not identified	1	5.3	20	105.3		4.6	20	90.9
Passeriforme					8	4.6	20	4090.9
Cavia aperea	1	5.3	300	69473.7	2	4.55	300	60000
Hydrochoerus hydrochaeris	2	5.3	800.1	4210.5	2	4.55	800	3636.4
Small mammals not identified	7	5.3	20	2421.1	7	4.55	20	2090.9
Medium mammals	9	5.3	2000	10526.3	13	4.55	2000	9090.9
Subtotal				87663.2				79654.6
Total				93890.7				84119

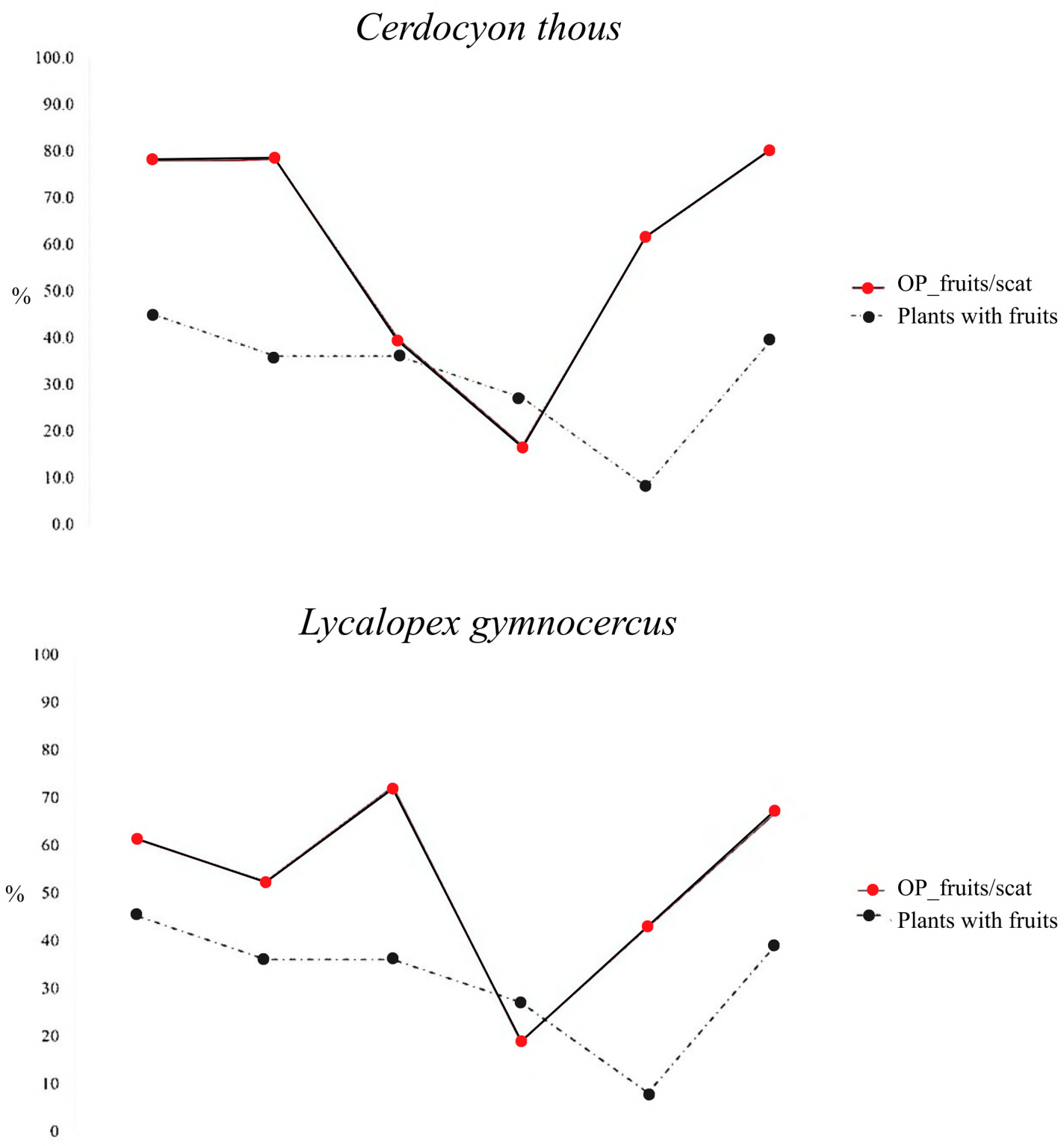

Figure 4. Percentage of plant species in scat samples of canids, *Cerdocyon thous* and *Lycalopex gymnocercus,* and percentage of plants with fruit in Mburucuyá National Park (2015).

niches fluctuated monthly (*C. thous*: *Bstd* = 0.37 to 0.89; *L. gymnocercus*: *Bstd* = 0.3 to 0.9), suggesting differences in food consumption despite the overall dietary overlap (Figure 2). Regarding the diet composition across seasons (Table 2), *C. thous* showed a higher consumption of fruits during the summer, primarily from *B. yatay* (22.1%), *Ocotea acutifolia* (Nees) Mez (22.1%), and *Ficus luschnathiana* (Miq.) Miq. (14.2%). This seasonal variation, which included a higher consumption of invertebrates and small mammals throughout the study, was statistically significant ($\chi 2 = 12.4$, $P = 0.0004$). In contrast, the diet of *L. gymnocercus* did not exhibit significant seasonal fluctuations ($\chi 2 = 1$, $P = 0.32$), maintaining a consistent consumption of *B. yatay* fruits (29.7%) and invertebrates (31%) in summer, and shifting

Table 4: Fleshy fruits consumed (%) monthly in the diet of canids, *Cerdocyon thous* and *Lycalopex gymnocercus*, Mburucuyá National Park, Corrientes, Argentina (2015). References: CT, *C. thous*; LG, *L. gymnocercus*.

	January		February		May		July		August		November	
	CT	LG	CT	LG	CT	LG	CT	LG	CT	LG	CT	LG
Scat samples	37	25	23	40	26	15	12	32	10	8	4	13
Syagrus romanzofianum				1.6		16.7	16.7	18.8	53.8	28.6	20	16.7
Butia yatay	24.1	27.8	40.6	41.3	11.6				7.7	14.3		
Bromelia serra					16.3	33.3						
Ocotea acutifolia	35.2	25.9	25	3.2								
Eugenia uniflora											40	
Ficus luschnathiana	18.5	7.4	3.1	1.6	4.7	5.6						
Citrus					7	16.7						
Chrysophyllum gonocarpum											1.6	
Chrysophyllum marginatum											20	50
Psidium guajava			6.3	1.6								
Solanaceae			3.1	3.2								

slightly to mammals (31.7% of small and medium-sized mammals) and *Syagrus romanzoffiana* (Cham.) Glassman fruits (19.5%) in winter (Table 2). The size of prey consumed by both fox species ranged from 0.10 to 2000g (Figure 3), with no significant differences between prey size categories ($\chi2$ =1.28, df = 4, P = 0.87) for either species. In relation to consumption frequency, invertebrates were the most common food items (27.5% for *C. thous* and 29.5% for *L. gymnocercus*). However, when considering the contribution to the biomass consumed (Table 3), the diets of these foxes were predominantly composed of small mammals, which represented 78% to 81% of the total.

The selection of resources such as fleshy-fruited species was similar for both fox species when comparing the percentage of occurrence in scat samples with fruit availability in the environment (Figure 4). Specifically, between January and February 2015, the percentage of consumed fruits exceeded the proportion of fruiting species by 25% to 30%. In this period, a high consumption of fruits from *B. yatay*, *O. acutifolia*, and *F. luschnathiana* was observed in both species (Table 3). Notably, *C. thous* also consumed fruits from *Psidium guajava* L. and Solanaceae. This pattern shifted in the first months of winter, with a lower consumption of fruits by *C. thous*. Conversely, *L. gymnocercus* showed a significant peak in consumption of fruits from *Bromelia serra* Griseb., *S. romanzoffiana*, *Citrus* L., and *F. luschnathiana* (Table 4).

The selection of resources by *C. thous* and *L. gymnocercus* was evaluated by analysing the correlation between the percentage of fruit occurrence in their diet and its availability in different habitats during the summer and winter seasons. During summer, no significant correlation was observed for *C. thous* (rs = 0.47, P = 0.27). However, a significant correlation was found between the diet of *L. gymnocercus* and fruit availability (rs = 0.64, P = 0.04).

In summer, an association was observed between the consumption of both foxes' species and the availability of fruits from specific plants, including *S. romanzoffiana*, *O. acutifolia*, and *F. luschnathiana* in mesophileous forests, and *B. yatay* in palm groves. In contrast, during the winter season, the effect of fruits availability on their occurrence percentage in the diet of *C. thous* ($\chi2$ = 33.52, P = 0.03) and *L. gymnocercus* ($\chi2$ = 27.24, P = 0.04) was significant. Our dietary analysis identified fruits from *S. romanzoffiana* and *B. yatay*, even though these had not been detected in the field during vegetation surveys, while the highly available.

Eugenia uniflora L., *Chrysophyllum gonocarpum* (Hook. & Arn.) Radlk., *P. guajava*, *Citrus*, and *B. serra* were either minimally represented or completely absent from the diets of *C. thous* and *L. gymnocercus*.

Discussion

This study analyzed the diet, trophic niche overlap, and resource selection of two sympatric foxes, *C. thous* and *L. gymnocercus*, in Mburucuyá National Park (MNP), a protected area within the Iberá Ecoregion, Argentina. The objective was to compare their feeding strategies and assess the potential niche overlap and/or partitioning between these species. The dietary composition of *C. thous* and *L. gymnocercus* in the MNP confirms their classification as hypocarnivorous and omnivorous canids, an ecological trend established by several studies across the Neotropics (Varela *et al.* 2008; Vieira and Port 2007; Rocha *et al.* 2008; Bay-Jouliá *et al.* 2024). However, biomass analysis reveals a crucial trophic dynamic governing coexistence within our study area (MNP): whilst arthropods and insects exhibited the highest frequency of occurrence (the most common, yet lowest energy-yielding outcome), approximately 90% of the total consumed biomass for both species was contributed by small and medium-sized mammals (e.g., *Cavia aperea*, *Hydrochoerus hydrochaeris*). This finding shows that, despite the broad resource base explored, the foraging strategy in the MNP is orientated towards maximizing energy gain from specific animal items.

In the diet of *C. thous*, a higher proportion of fruit was found, which is similar to a study conducted in the Emas National Park (Goiás State, Brazil, 18°19' S, 52°45' W), where vegetable items had a 60% occurrence (De Almeida Jácomo *et al.* 2004). However, when compared with the MNP, the Emas National Park has an area of 132,000 ha, with a grassland predominance of 97% and a small presence of Cerrado shrubs and riparian forests (3%). These differences in vegetation are reflected in the differences in the plant species consumed *by C. thous* in our study area. The diet of *L. gymnocercus* in the MNP exhibited equal proportions between plant and animal food categories throughout the year. While this proportion is highly variable by location, other studies report different trends. Varela *et al.* (2008), for instance, discovered that fruits were predominant over animal items in the wet and dry seasons (frequency of occurrence: 69%) at the Los Colorados and Campo Grande Biological Station (Salta, Argentina, 24°43' S, 63°17' W). In that analysis, *Sarcomphalus mistol* (Griseb.) Hauenschild was the predominant plant food source, followed by arthropods and vertebrates. Similarly, at the Peruvian site of Lambayeque, *Lycalopex sechurae* (Thomas, 1900), a congeneric species, exhibited a highly hypocarnivorous diet with a high occurrence (84.2%) of vegetable items, dominated by *Neltuma* L. (70.5%), a protein and carbohydrate-rich legume (Prokopiuk *et al.* 2000).

These variations in diet composition show that the tendency towards hypocarnivorous or hypercarnivorous diets is influenced by food resource availability. The diet composition of *C. thous* and *L. gymnocercus* varies according to the study site, ranging from strictly hypercarnivorous diets (Farias and Kittlein 2008) to mixed diets and diets that tend towards hypocarnivory, such as the percentages of plant food occurrence above 50% determined in the present study in the MNP and other protected areas of the Iberá Ecoregion (Bay-Jouliá *et al.* 2024) and other sites in Argentina (Varela *et al.* 2008) and Brazil (De Almeida Jácomo *et al.* 2004). Consequently, the availability of trophic resources, climate, and—potentially—social organisation (Eisenberg and Redford 1999; Courtenay and Maffei 2004) are influential factors in the diet of these canids.

Most of the consumed biomass by *C. thous* and *L. gymnocercus* in the MNP consisted of mesomammals and small mammals (90%), with *Cavia aperea* (Erxleben, 1777) contributing the most significant amount (over 70%). However, when the frequency of prey size was analyzed, the most prevalent were those measuring between 0.10 and 10 grams (i.e. insects and arachnids). These results were consistent with the diet of *C. thous* at the Itapetininga Experimental Station in São Paulo (Brazil), which exhibited a high consumption of insects (Acrididae), with some individuals demonstrating hunting and capture behavior towards small prey (Bueno and Motta-Junior 2004). With regard to the consumption of *C. aperea*, evidence has been documented of its consumption by *C. thous* in Brazil (Bueno and Motta-Junior 2004; Pedó *et al.* 2006; Rocha *et al.* 2008), and by *L. gymnocercus* in Argentina (Farias and Kittlein 2008).

In relation to other prey species, two species of snakes *Helicops leopardinus* (Schlegel, 1837) and *Philodryas olfersii latirostris* (Cope, 1862) have been documented as components of the diet of *C. thous* in the MNP and the San Nicolás Portal of the Iberá National Park (Corrientes province) in Argentina (Ruiz-Garcia *et al.* 2020). The present study also makes a novel contribution by documenting, for the first time, the presence of the gastropod *Pomacea canaliculata* (Lamarck, 1828), *Phrynops hilarii* (Duméril and Bibron, 1835) eggs, and the freshwater crustaceans *Trichodactylus kensleyi* (Rodríguez, 1992) in the diet of *L. gymnocercus*, in addition to scorpions of the genus *Bothriurus* sp. (Peters, 1861).

The Pianka index indicated a high dietary overlap between *C. thous* and *L. gymnocercus* in the study area, with results analogous to those observed in Aparados da Serra National Park in Brazil (Vieira and Port 2007). According to these authors, this high overlap could be attributed to the consumption of small mammals throughout the year, a situation similar to the diet obtained for these species in the MNP, where small mammals contributed the highest with the consumed biomass. However, Vieira and Port (2007) observed a lower percentage of fruit occurrences in their diet, a phenomenon attributed, in part, to the prevalence of grasslands and a limited variety of plant species that produce fleshy fruits in the Aparados da Serra National Park (Brazil).

In the diet of *C. thous* and *L. gymnocercus*, the ingestion of fleshy fruits varied seasonally, characterising an opportunistic behaviour that was linked with MNP peak fruiting. A distinctive finding of our research is the clear manifestation of seasonal trophic plasticity in both foxes and its relationship to the selective consumption of palms (*Butia yatay* and *Syagrus romanzoffiana*). The consumption of *S. romanzoffiana* during the winter should not be regarded as a random trophic event, but rather an essential adaptive strategy. We demonstrate that when the general availability of fleshy fruits decreases drastically during the cold season, both species switch to active foraging behaviour specifically targeting this palm. This critical resource allows both canids to maintain a fundamental energetic contribution when other plant resources are scarce. This temporal partitioning of key resources is the mechanism that likely permits coexistence and mitigates intra-guild conflict, as the exploitation of these key resources during periods of scarcity reduces competitive pressure within the shared ecological niche. The capacity to modify their dietary breadth in this way evidences that, whilst trophic overlap is high, seasonal flexibility mediated by local resources maintains the stability of the predator community in the MNP.

The dietary records of these foxes revealed the presence of fruits from *B. yatay*, *O. acutifolia*, and *F. luschnathiana* during the summer months, while in winter, fruits from *S. romanzoffiana*, *B. serra*, *F. luschnathiana*, and *Citrus* were documented. The evidence of the opportunistic behaviour of *L. gymnocercus* was the correlation between the

consumption of fleshy fruits and its availability in summer, a behaviour also observed for the species in the province of Salta (Varela *et al.* 2008). During winter, when fruit availability was scarce, both foxes exhibited active foraging behaviour in search of certain plant species. This has been considered key to the diet of frugivorous mammals in the Paranaense rainforest of Misiones province (Argentina), with *S. romanzoffiana* being a species that fructified more than once and asynchronously (Giombini 2013). This foraging behaviour has also been reported for *Lycalopex vetulus* (Lund, 1842) in the Cerrado of Mato Grosso (Brazil), where it consumed the fruits of *Hancornia speciosa* Gomes during times of scarcity of other plant species, replacing its consumption with fruits of *Solanum lycocarpum* A. St.-Hil. when other species were abundant (Dalponde and De Souza Lima 1999). However, the presence of fruit from certain plant species (*P. guajava*) in the MNP does not necessarily ensure their consumption by *C. thous* and *L. gymnocercus*. A comparable behaviour was observed in *L. vetulus*, whose diet exhibited minimal consumption of bromeliad fruits, despite the presence of fruiting plants in a context of a scarce supply of other edible plant species (Dalponde and De Souza Lima 1999).

The implications of these findings for the conservation of both species and the knowledge of their biology are significant. The high niche overlap and remarkable dietary plasticity of *C. thous* and *L. gymnocercus* confirm their capacity as generalist and opportunistic predators. This dietary flexibility provides them with high adaptability to resource availability, a key trait for their survival in complex, dynamic environments such as the Mburucuyá National Park, as well as in habitats that may be altered by human activities. The confirmation of an active foraging behavior, especially for key resources like palm fruits during periods of scarcity, underscores the importance of conserving these specific plant species and their habitats to ensure the foxes' food security. Finally, this study not only contributes new records of trophic relationships but also validates and expands knowledge on the mechanisms of coexistence between these foxes, which is fundamental for formulating effective management and conservation strategies in the Iberá region and in other areas where these species are sympatric.

Conclusions

In this study, we assessed the diet, overlap, and the selection of resources by *C. thous* and *L. gymnocercus* in a comparative context within the MNP. The research aimed to investigate the dimensions of their ecological niche, evaluating potential overlaps in trophic resource utilization and the mechanisms that facilitate their coexistence. The results confirm the high dietary similarity between the two species, both of which function as generalist predators. Their diet is highly variable, composed of common items such as fleshy fruits, invertebrates, and small mammals, which contributed over 90% of the consumed biomass. The composition of food categories exhibited seasonal variations, suggesting that foraging patterns are influenced by changes in the availability of resources throughout the year.

The study also confirmed a strong opportunistic behavior by the foxes in response to the availability of certain fruits, particularly during summer. However, their diet was not solely dependent on resource abundance; for example, they actively foraged for palm fruits (*S. romanzoffiana*) in the winter when other options were scarce, but they avoided abundant fruits like *P. guajava*. These findings highlight that coexistence between the two species is not maintained through strict dietary partitioning but rather through their flexible feeding strategies, which respond to the dynamic availability of resources in a complex, multi-habitat environment.

Acknowledgements

We would like to express our sincere gratitude to the reviewers for their constructive and detailed suggestions, which substantially improved the quality of the manuscript. We also wish to express our sincere gratitude to our field assistants and collaborators for their invaluable help, especially González CA, Vanzetti A, Pombo J and González E. We also thank the National Parks Administration (APN) and the park rangers of the Mburucuyá National Park for their work permits and logistical support, particularly Raviculé S, Vallejos A, Hervás JM, Gómez C, Fleitas A, Sotelo V, Müller G and Losada P. The research was approved by the authorities of the Mburucuyá National Park (APN 2014 - 2015) and the National University of the Northeast (EXA – DOC – 2012 - 18). This study constitutes a segment of a comprehensive Ph.D. project on carnivores in Mburucuyá National Park, undertaken by one of the authors (Romero VL), who was supported by a doctoral grant from CONICET-UNNE, Argentina (2014 - 2017).

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Associated editor: Gabriel P. Andrade Ponce
Submitted: June 2, 2025; Reviewed: November 11, 2025
Accepted: December 8, 2025; Published on line: December 16, 2025

THERYA, 2026, Vol. 17(1):45-58 DOI: 10.12933/therya.2026.6227 ISSN 2007-3364

Bat diversity (Order: Chiroptera) in a suburban area belonging to the Mexican Plateau

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Bats are the second most diverse order of mammals. There is evidence that bats assemblages are influenced by urbanization, exhibiting changes in species diversity. Some species show a strong degree of adaptation to urban habitats or are even favored by them. Our aim was to characterize the bat species composition present in the suburban park "Ecoparque Centenario"located on the Mexican Plateau, using two different methods of species identification. Over the course of one year, mist nets were set up, and echolocation pulses were recorded using an ultrasonic microphone. Species were identified based on their morphological characteristics and echolocation calls. Species accumulation curves were generated, and diversity indices were calculated based on both morphological and acoustic analyses. In total, 28 bat species belonging to four families were identified using both methods: Vespertilionidae (20 spp.), Molossidae (6 spp.), Mormoopidae (1 sp.) and Phyllostomidae (1 sp.). The family Vespertilionidae was more represented, and the diversity indices indicated moderated diversity without species dominance. In general, suburban areas have been shown to support higher bat diversity and activity due to an increase in potential prey availability, benefiting both generalist and specialist species. Most of the species identified are listed as Least Concern according to the IUCN, except *Choeronycteris mexicana* which is classified as Near Threatened. Considering this, Ecoparque Centenario represents an important area for bat conservation within a semiarid landscape.

Keywords: acoustic monitoring, echolocation calls, Ecoparque Centenario, semiarid landscape, species accumulation curves, Zacatecas.

Los murciélagos son el segundo orden más diverso de mamíferos. Existe evidencia de que los ensamblajes de murciélagos están influenciados por la urbanización, mostrando cambios en la diversidad de especies. Algunas especies presentan un alto grado de adaptación a los hábitats urbanos, o incluso se ven favorecidas por ellos. Nuestro objetivo fue caracterizar la composición de especies de murciélagos presente en el parque suburbano Ecoparque Centenario, ubicado en la Meseta Mexicana, utilizando dos métodos diferentes de identificación de especies. A lo largo de un año, se colocaron redes de niebla y se registraron pulsos de ecolocación mediante un micrófono ultrasónico. Las especies fueron identificadas con base en sus características morfológicas y en los llamados de ecolocación. Se generaron curvas de acumulación de especies y se calcularon índices de diversidad a partir de los análisis morfológicos y acústicos. En total, utilizando ambos métodos, se identificaron 28 especies de murciélagos pertenecientes a cuatro familias: Vespertilionidae (20 spp.), Molossidae (6 spp.), Mormoopidae (1 sp.) y Phyllostomidae (1 sp.). La familia Vespertilionidae fue la mejor representada, y los índices de diversidad indicaron una diversidad moderada sin dominancia de especies. En general, se ha demostrado que las áreas suburbanas mantienen una mayor diversidad y actividad de murciélagos debido al incremento en la disponibilidad de presas potenciales, lo que beneficia tanto a especies generalistas como especialistas. La mayoría de las especies identificadas están categorizadas como de Preocupación Menor según la UICN, excepto *Choeronycteris mexicana*, que está clasificada como Casi Amenazada. Considerando lo anterior, el Ecoparque Centenario representa un área importante para la conservación de murciélagos dentro de un paisaje semiárido.

Palabras clave: ambiente semiárido, curvas de acumulación, Ecoparque Centenario, llamados de ecolocalización, monitoreo acústico, Zacatecas.

Diversity patterns (henceforth, DPs) exist in all ecosystems around the planet and are constantly changing due to the interaction of abiotic and biotic factors in ecosystems (Chesson 2000; Dirzo and Raven 2003; Brown *et al.* 2004; Sibly *et al.* 2012; Villalobos and Rangel 2014). These changes are generally reflected in the fluctuations in abundance, diversity, or richness of the species distributed in a given area. A clear example occurs in urban areas (*i.e.* geographic spaces with human activity and presence, *sensu* Weeks) (Weeks 2010), where these types of environments may alter the habitat and therefore, the species composition and dynamics (Rosenzweig 1995; Challenger and Dirzo 2009; Faeth *et al.* 2011).

Bats are cosmopolitan, they are vagile and present different functional traits, which allows them to be distribute in different ecosystems, including urban environments where they constitute a key component of the mammalian fauna (Van der Ree and McCarthy 2005). It has been observed that in urban environments richness decreases for most bat species, whereas abundance increase only for some groups that are able to adapt to the new characteristics of the environment (generalist species) (Segura *et al.* 2007; Jung and Kalko 2011; Clavel *et al.* 2011; Threlfall *et al.* 2012; Büchi and Vuilleumier 2014; Jung and Threlfall 2016). However, within the urban matrix, suburban areas (*i.e.* areas of lower human population density located

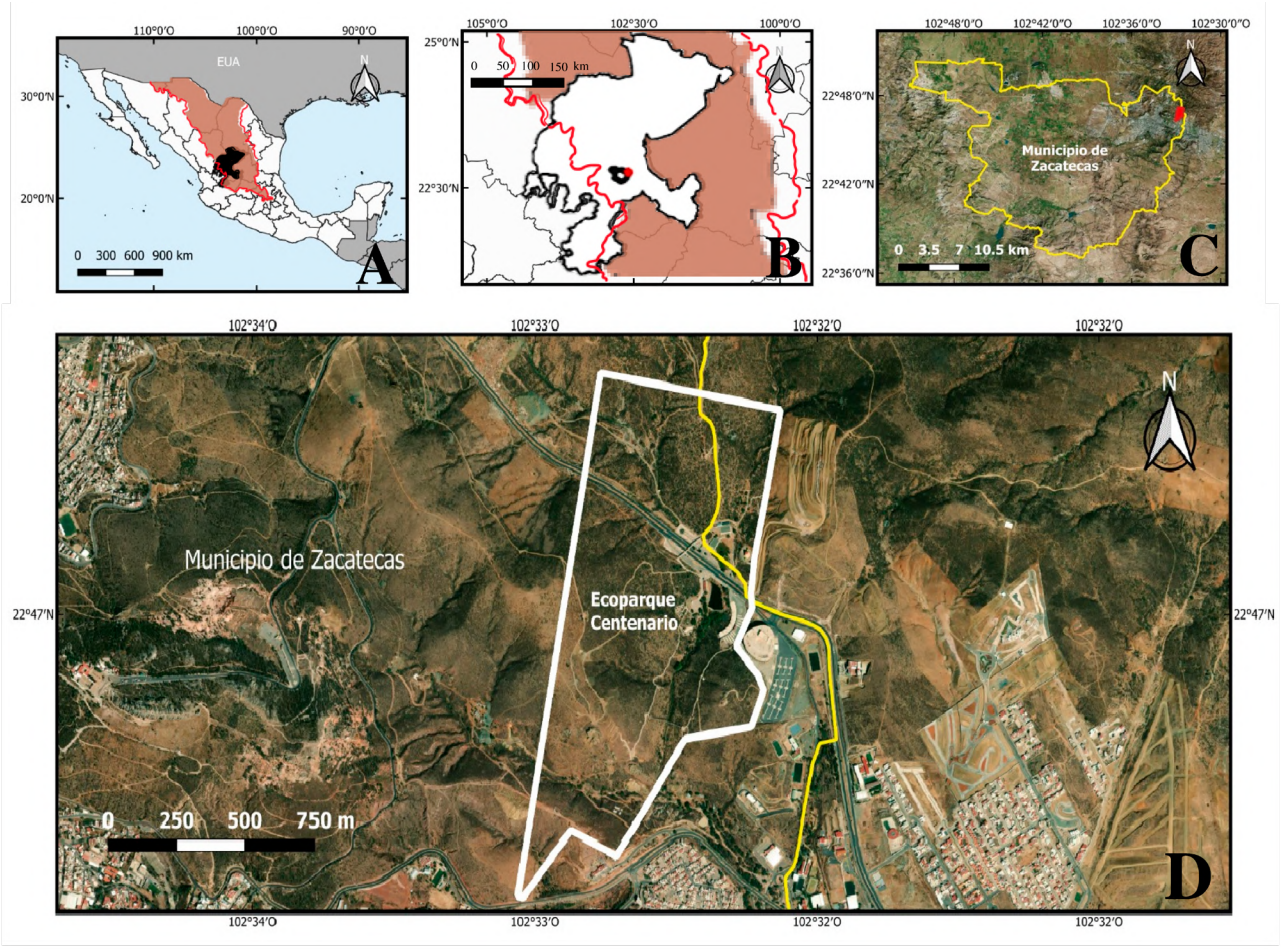

Figure 1. Geographic location of the "Ecoparque Centenario" (*ECO*) area. The Mexican Plateau is shown as the red silhouette. The state of Zacatecas is shown as black outline. The municipality of Zacatecas is shown as bold black line (b) and yellow line (c-d). The Ecoparque Centenario is shown as red dot (b-c) and white line (d)

on the periphery of cities or urban areas) have shown a higher bat species richness and abundance compared to urban areas sensu Weeks (2010).

The presence of bats could be related to the availability of food, shelter and foraging sites typical of urban environments (Violle *et al.* 2007). Furthermore, the modification of these sites and their characteristics can alter bat diversity and consequently generate certain diversity patterns (Russo and Ancillotto 2015). In general, suburban areas have been shown to support higher bat diversity and activity due to increase of potential prey's number, for generalist and specialist species equally (Shochat *et al.* 2004; Coleman and Barclay 2012; Luck *et al.* 2013).

Three hypotheses may explain this phenomenon: i) the heterogeneity hypothesis, ii) the intermediate disturbance hypothesis, and iii) the habitat productivity hypothesis. Together they describe how richness and abundance vary with the disturbance frequency and intensity. Disturbance creates heterogeneity in the environment that, combined with the addition of anthropogenic organic matter, increases primary productivity and provides a greater number of available resources (Connell 1978; Shochat *et al.* 2004; Shochat *et al.* 2006; McKinney 2008; Gaston and Gaston 2010; Threlfall *et al.* 2011). Such DPs have been observed in some vertebrate groups, such as bats (Duchamp *et al.* 2004).

In Mexico most studies aimed at characterizing bat diversity in urban or suburban environments, have focused on tropical regions (Medellín 1993; Arita 1993), even though, more than 50% of the national territory has a dry or semi-dry climate type, where such studies are scare (SEMARNAT 2015). The city of Zacatecas is characterized by semi-dry climate, and the only available information about bat diversity comes from the company URSAMEX (2014), which was the responsible for the construction of the suburban park "Ecoparque Centenario" (*ECO*), our study area. They report 5 bat species: *Mormoops megalophylla*, *Leptonycteris nivalis*, *Myotis auriculus*, *M. planiceps* and *Dermanura azteca*, although 3 species distribution (*L. nivalis*, *M. planiceps* and *D. azteca*) does not correspond to what has been previously reported (Medellín *et al.* 2008; Ortega *et al.* 2022). In addition, neither the identification methods nor the sampling effort is presented, so the information is incomplete and inaccurate. The *ECO* is surrounded by active mines, and this site was recognized as a natural protected area (URSAMEX 2014); it has been suggested that mines can be used by different species of bats as perching sites, which could favor their diversity. On the other hand, in addition to the recognition, the area requires constant and exhaustive diversity studies. Therefore, our aims were to determine the bat diversity using 2 identification methods

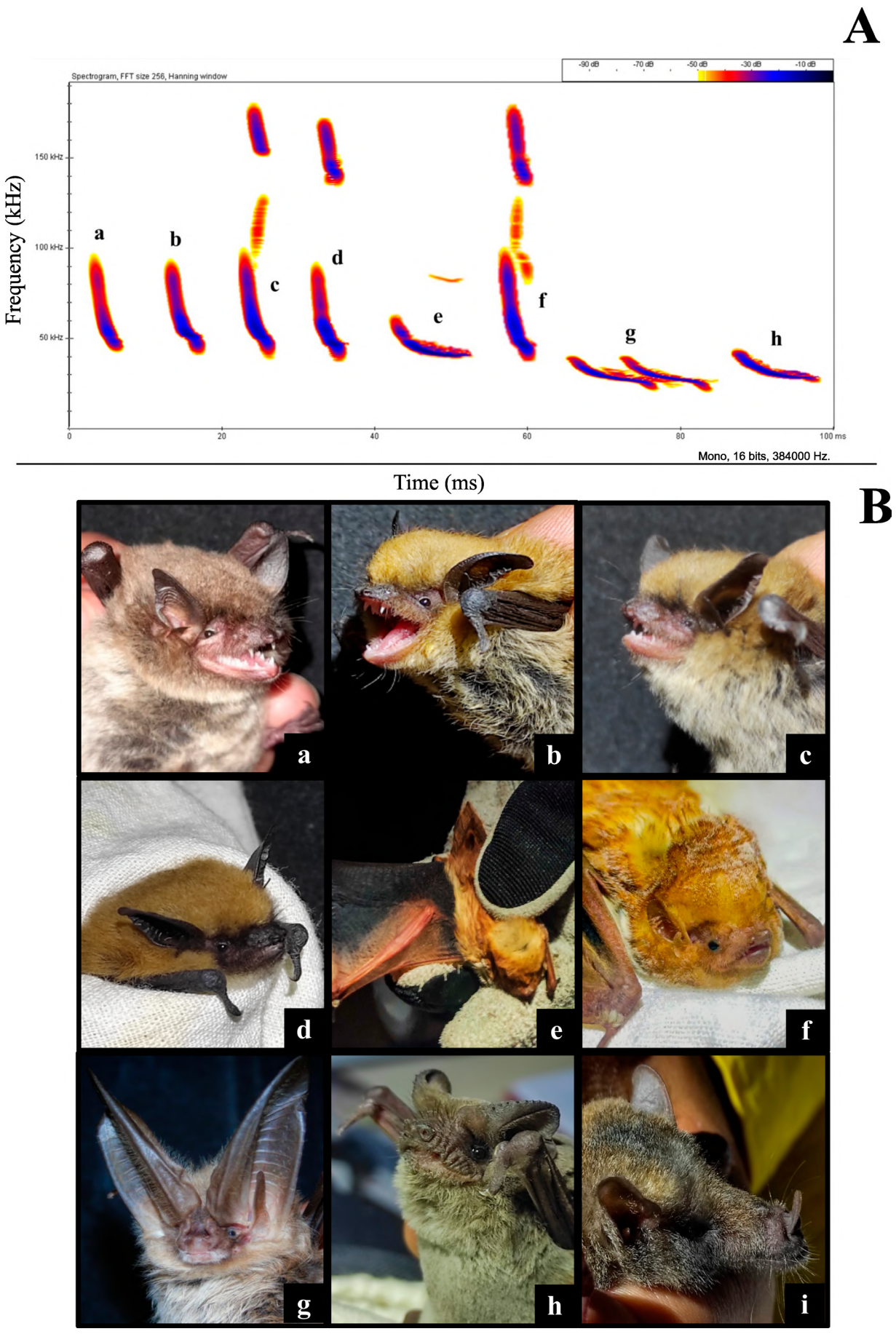

Figure 2. Echolocation calls (A) and photographs (B) of bat species captured. a) *M. yumanensis*, b) *M. californicus*, c) *M. volans*, d) *M. ciliolabrum*, e) *L. ega*, f) *L. frantzii*, g) *C. townsendii*, h) *T. brasiliensis,* and i) *C. mexicana*.

Table 1. List of bat species identified.

Family	Genus	Specie	Morphologic presences	Individuals captured	Acoustic presences	Acoustic records	Total presences	Total detections
Vespertilionidae	*Antrozous*	*pallidus*	0	0	4	6	4	6
	Baeodon	*alleni*	0	0	8	21	8	21
	Corynorhinus	*mexicanus*	0	0	1	1	1	1
		townsendii	4	6	8	23	11	29
	Eptesicus	*fuscus*	0	0	16	94	16	94
	Lasiurus	*cinereus*	0	0	32	330	32	330
		ega	1	1	8	48	9	49
		frantzii	1	1	14	36	14	37
		intermedius	0	0	41	623	41	623
		xanthinus	0	0	3	8	3	8
	Myotis	*auriculus*	0	0	22	127	22	127
		californicus	8	11	32	237	34	248
		fortidens	0	0	18	79	18	79
		ciliolabrum	3	3	19	53	20	56
		velifer	0	0	19	67	19	67
		volans	3	3	28	262	28	265
		yumanensis	6	10	31	341	35	351
	Neoeptesicus	*brasiliensis*	0	0	12	52	12	52
	Parastrellus	*hesperus*	0	0	4	9	4	9
	Rhogeessa	*parvula*	0	0	2	2	2	2
Mormoopidae	*Mormoops*	*megalophylla*	0	0	1	2	1	2
Molossidae	*Molossus*	*nigricans*	0	0	13	26	13	26
	Nyctinomops	*aurispinosus*	0	0	20	43	20	43
		femorosaccus	0	0	8	13	8	13
		laticaudatus	0	0	30	200	30	200
		macrotis	0	0	11	24	11	24
	Tadarida	*brasiliensis*	1	1	32	341	32	342
Phyllostomidae	*Choeronycteris*	*mexicana*	1	1	0	0	0	1
Total	**14**	**28**	**28**	**37**	**437**	**3068**	**449**	**3105**

(acoustic and morphologic) and to estimate diversity index in order to establish the patterns present in a suburban area "Ecoparque Centenario" belonging to the Mexican plateau during an annual cycle.

Materials and Methods

The Mexican plateau is located between the Western and Eastern Sierras, and, in the south, it is limited by the transversal volcan axis. This is an extensive area characterized by altitudes near 2000 m asl. The predominant type of vegetation is xeric scrub, pine-oak forest and isolated patches of low deciduous forest (Rzedowski 2006). The *ECO,* protected natural area belonging to the Mexican plateau, is located in the Arroyo de la Plata micro-watershed, between the Central Mesa and Western Sierra Madre physiographic regions. The *ECO* is located at coordinates: 22º 46' 49.14" N, 102º 32' 37.96"W (Figure 1), between Zacatecas, Vetagrande and Guadalupe municipalities; the park is in the border area of the Zacatecas city, at an altitude of 2448 m asl, with an average annual rainfall of 400 mm to 450 mm. The climate type corresponds to *BS1kw* (dry or semi-dry with temperate regions and an average annual temperature that ranges between 12 and 18 ºC; García 2004).

The predominant vegetation is induced grassland, riparian vegetation, xerophytic scrub and *Opuntia* spp. scrub (Rzedowski 2006). The tree density is composed by pirul (*Schinus molle*) and mesquite (*Prosopis* sp.) along the stream banks (URSAMEX 2014). Six sampling points were selected near to water bodies and alternated according to the annual season to increase the probability of bat capture during the dry season. Four mist nets (2.5 × 6 m) were placed alternately at the 6 points (Bell 1980; Kurta and Kunz 1988; MacSwiney *et al.* 2008; Gilley and Kennedy 2010). Mist nets were sampled for 5 days, each month, for one year, from April 2022 to May 2023. They were opened during 5 hours, after sunset with monitoring every 30 minutes (Holloway and Barclay 2000; MacSwiney *et al.* 2008; Coleman and Barclay 2012; Barboza-Marquez *et al.* 2014).

Table 2. Characterization of echolocation pulses (EPs) from bat species identified.

Family	Genus	Specie	Maxium frequency (kHz)	Minium frequency (kHz)	Peak frequency (KHz)	Duration (ms)	Bandwidth (kHz)	n
Vespertilionidae	*Antrozous*	*pallidus*	59.1 ± 17.3	27.8 ± 2.8	36.7 ± 6.7	9.3 ± 2.6	31.3 ± 14.6	6
	Baeodon	*alleni*	98.5 ± 4.4	33.3 ± 2.1	46.5 ± 1.3	5.1 ± 0.6	65.2 ± 5.3	21
	Corynorhinus	*mexicanus*	46.9 ± 0.0	21.6 ± 0.0	33.3 ± 0.0	12.3 ± 0.0	25.3 ± 0.0	1
		townsendii	40.1 ± 2.4	23.9 ± 1.9	31.2 ± 1.3	11.7 ± 2.9	16.1 ± 2.6	23
	Eptesicus	*fuscus*	51.4 ± 5.3	27.7 ± 1.9	33.5 ± 1.9	9.8 ± 1.8	23.7 ± 5.8	94
	Lasiurus	*cinereus*	49.8 ± 6.9	25.6 ± 1.9	32.7 ± 2.6	11.9 ± 2.4	24.2 ± 6.8	330
		ega	55.8 ± 11.7	34 ± 4.8	38.2 ± 5.4	9.7 ± 4.5	21.7 ± 8.2	48
		frantzii	84.6 ± 13.9	37.3 ± 2.4	45.5 ± 2.3	5.9 ± 1.9	47.3 ± 15.3	36
		intermedius	41.2 ± 6.6	23.4 ± 1.5	28.2 ± 1.6	12.7 ± 2.2	17.9 ± 6.7	623
		xanthinus	72.9 ± 7.3	32.8 ± 1.0	39.1 ± 1.2	7.8 ± 0.7	40.1 ± 7.9	8
	Myotis	*auriculus*	86.6 ± 8.9	32.2 ± 2.2	43.9 ± 1.8	6.2 ± 1.0	54.4 ± 9.5	127
		californicus	94.9 ± 5.3	41.8 ± 2.3	54.3 ± 2.3	5.1 ± 2.7	53.1 ± 6.2	237
		fortidens	98.7 ± 4.6	42.9 ± 1.6	55.9 ± 1.2	4.4 ± 0.6	55.7 ± 5.1	79
		ciliolabrum	100.6 ± 5.2	39.7 ± 2.5	54.9 ± 3.6	4.6 ± 0.9	60.8 ± 5.2	53
		velifer	83.5 ± 11.3	37.8 ± 1.7	46.1 ± 2.9	5.7 ± 0.6	45.6 ± 11.1	67
		volans	93.1 ± 6.0	37.5 ± 2.7	47.7 ± 2.7	5.5 ± 0.7	55.6 ± 6.5	262
		yumanensis	96.9 ± 5.6	42.7 ± 2.8	54.6 ± 3.9	4.7 ± 0.6	54.2 ± 6.0	341
	Neoeptesicus	*brasiliensis*	53.7 ± 4.1	30.8 ± 1.9	37 ± 2.0	10.9 ± 1.7	22.9 ± 4.5	52
	Parastrellus	*hesperus*	69.9 ± 4.4	41.9 ± 0.9	47.1 ± 1.8	6.2 ± 2.3	27.9 ± 3.7	9
	Rhogeessa	*parvula*	87.9 ± 0.6	41.7 ± 1.5	53.9 ± 0.0	3.8 ± 0.2	46.2 ± 2.1	2
Mormoopidae	*Mormoops*	*megalophylla*	57.6 ± 1.2	41.5 ± 0.7	54 ± 0.2	6.4 ± 1.0	16.1 ± 1.8	2
Molossidae	*Molossus*	*nigricans*	35.1 ± 3.7	25.7 ± 1.6	30.1 ± 1.3	13.3 ± 2.3	9.4 ± 3.4	26
	Nyctinomops	*aurispinosus*	36.2 ± 6.1	19.7 ± 0.8	26.4 ± 1.8	14.3 ± 1.7	16.4 ± 6.3	43
		femorosaccus	32.9 ± 4.7	18 ± 1.0	23.9 ± 0.6	13.2 ± 1.9	14.9 ± 4.7	13
		laticaudatus	35.4 ± 6.6	20.7 ± 1.0	25.6 ± 1.8	13.8 ± 2.2	14.7 ± 6.8	200
		macrotis	27.7 ± 3.3	17.4 ± 2.8	21.5 ± 1.9	14.9 ± 2.3	10.3 ± 4.7	24
	Tadarida	*brasiliensis*	40.7 ± 8.6	24.1 ± 2.3	29.5 ± 3.7	12.8 ± 2.6	16.5 ± 7.3	341
Total								**3068**

For acoustic monitoring, the Echo Meter Touch 2 Pro ultrasonic microphone (connected to a tablet Lenovo Xiaoxin Pad 2022) and Echo Meter software (Wildlife Acoustics, Inc. Maynard, Massachusetts) were used to record EPs. The detection range of echolocation calls was set to a minimum frequency of 15,000 *Hz* with a sampling rate of 384 *kHz* (Pettersson 2004). Acoustic monitoring was semi-active and was conducted by walking the trail from net to net and lasted as long as the mist nets remained open (five hours per day; MacSwiney *et al.* 2020).

BatSound V4.1 software (Pettersson 2004) was used to characterize search phases of the EPs, as they are relatively constant compared to other types of bat vocalizations (*e.g.* social pulses, feeding buzzes) (Fenton and Bell 1981; O'Farrell and Miller 1999; Barclay 1999; Papadopoulos and Allen 2007; Agranat 2013). EPs with an intensity less than 30 dB were not considered for characterization, since it has been determined that frequencies less than this value tend to attenuate at short distances, therefore higher intensity frequencies can travel farther in the environment and consequently, be recorded by ultrasonic microphones (Surlykke and Kalko 2008). These parameters (measured in *kHz*) were: maximum frequency (Fmax), minimum frequency (Fmin), peak frequency, and bandwidth (the difference between Fmax and Fmin), whereas intensity was expressed in *dB*, and duration (*DUR*) in milliseconds (*ms*) (Corben 2004; Miller 2004). The values of each pulse were checked against the "Compendio de Llamados de Ecolocalización de los murciélagos insectívoros mexicanos" (Ortega *et al.* 2022) and the SONOZOTZ echolocation call library (Zamora-Gutiérrez *et al.* 2020). A species was assigned under the concept of sonospecies in the case of meeting the above assumptions, particularly Fmin, peak frequency and DUR (Thomas *et al.* 1987). Morphological bat identifications were made using the field keys in Medellín *et al.* (2008), according to the diagnostic morphological characteristics (Martínez-Rodríguez *et al.* 2024).

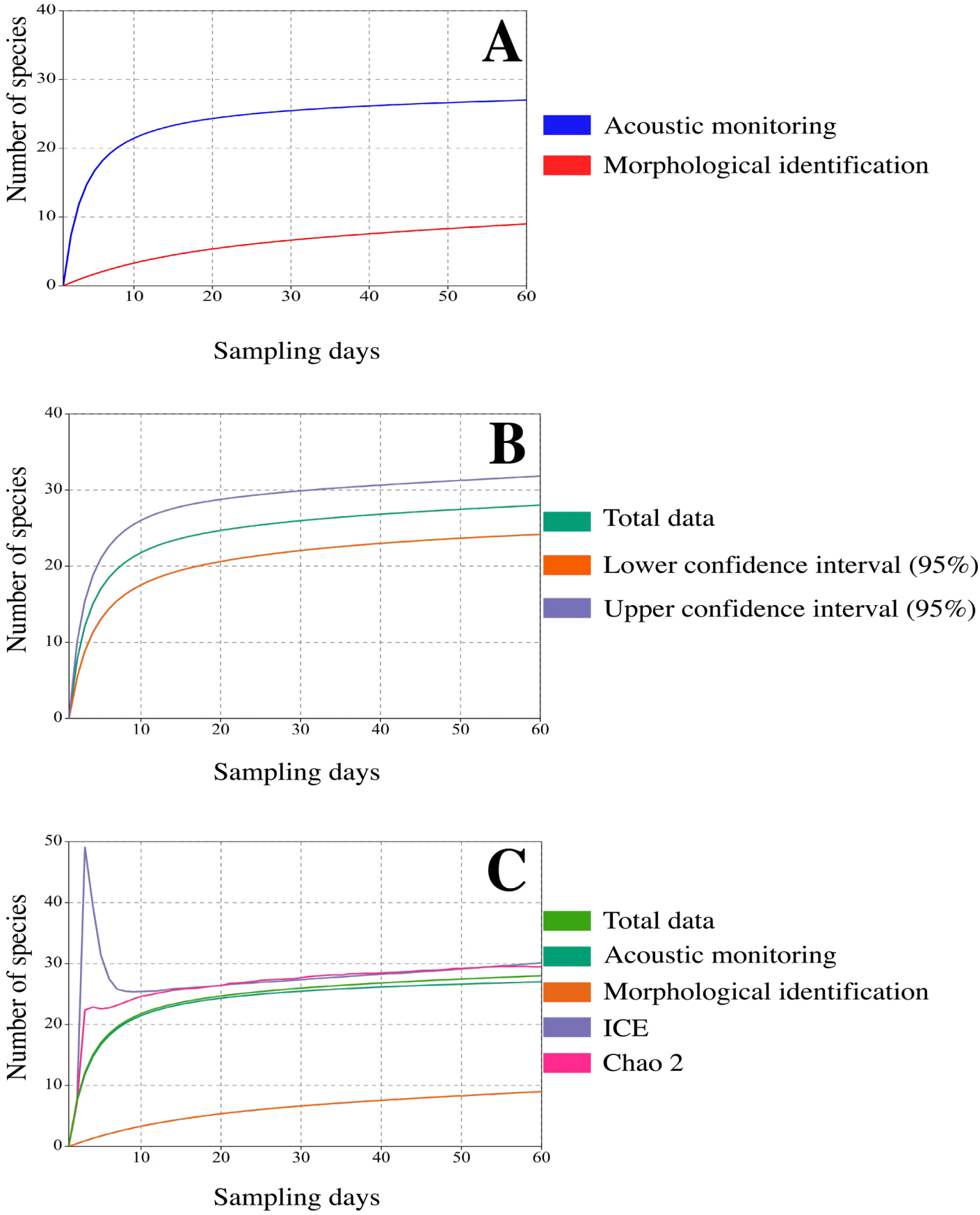

Figure 3. Species accumulation curves and diversity estimators. (A) shows the identified species by acoustic monitoring (blue line = 27 spp.) and morphological identification (red line = 9 spp.). (B) shows the total presences data with both methods (green line = 28 spp.), upper confidence interval at 95% (blue line = 31.83) and lower confidence interval at 95% (orange line = 24.17 spp.). (C) shows the total data (green line = 28 spp.), acoustic monitoring (cian line = 27 spp.), morphological identification (orange line = 9 spp.), ICE estimator (purple line = 30.14 spp.) and Chao 2 estimator (pink line = 29.47 spp.).

Presence/absence data matrices were constructed for the species identified by both methods. The diversity estimators *ICE* (Incidence-based Coverage Estimators) and *Chao 2* (Lee and Chao 1994) were calculated using the EstimateS v 9.1 software (Colwell 2013). Species accumulation curves were generated using the PAST v 4.17 software (Hammer and Harper 2001) with i) acoustic and morphological data, ii) the total observed data for the number of species and 95% confidence intervals, and iii) the non-parametric estimators (*ICE* and *Chao 2*), acoustic, morphological and the total data observations with both methods (Chao *et al.* 2009). Shannon, Margalef, Gini-Simpson and Berger-Parker indices were calculated (Margalef 1972; Moreno 2001; Magurran 2007; Magurran *et al.* 2019) in order to elucidate the diversity patterns in the suburban area.

Results

The total sampling effort was 59 days, 17,700 net-hours and 10,215 acoustic recordings of which 3,068 met the characteristics described in the methodology. Twenty-eight species, 14 genera and 4 families were identified (Table 1). Only 19 species were identified by analysis of their EPs (Table 2); 8 species were identified using both methods and one species was identified only by taxonomic keys (*Choeronycteris mexicana;* Figure 2). The family Vespertilionidae was the most represented with 9 genera and 20 species (71.4 %), with 7 species correspond to the genus *Myotis* (25 %) and 5 to the genus *Lasiurus* (17.8 %). Three genera and 6 species were included in the Molossidae family (21 %), and the most represented genus was *Nyctinomops* with 4 species (14.2 %). Only one species was recorded in the families Mormoopidae and Phyllostomidae, *Mormoops megalophylla* and *C. mexicana*, respectively (7.1 %).

In the context of acoustic monitoring, the species with highest number of occurrences were *Lasiurus intermedius* (n = 630), *Myotis yumanensis* (n = 341), *Tadarida brasiliensis* (n = 341), *L. cinereus* (n = 330), *M. volans* (n = 262), *M. californicus* (n = 237), and *N. laticaudatus* (n = 200). The species with the fewest recorded occurrences were *Corynorhinus mexicanus* (n = 1), *Rhogeessa parvula* (n = 2), and *M. megalophylla* (n = 2). We also recorded *L. ega* (Figure 2 B, e), which has not been previously registered in the north-central region of the country.

For the morphological analysis, 37 specimens were captured which corresponded to 3 families, 5 genera and 9 species; 35 specimens and 7 species belong to the Vespertilionidae (94.5%): *M. yumanensis* (n=10), *M. californicus* (n=11), *M. ciliolabrum* (n=3), *M. volans* (n=3), *L. ega* (n=1), *L. frantzii* (n=1), and *C. townsendii* (n=6). In the families Molossidae and Phyllostomidae only 1 species was captured: *T. brasiliensis* (n=1) and *C. mexicana* (n=1), respectively. The photographs and EPs corresponding to each species are shown in Figure 2, except for *C. mexicana* (Figure 2 B, i) considered a "whispering" species due to its *EP's* characteristics (low intensity and high frequency).

The first accumulation curve (Figure 3 A) shows the differences in the number of species recorded between the identification methods used. While acoustic monitoring (blue line = 27) tends to asymptote, morphological identification (red line = 9) shows no signs of saturation. Figure 3 B shows the total data (green line = 28), which indicates that both methods cover 87.96% of diversity according to the upper confidence interval (blue line = 31.83). However, figure 3 C shows that the sampling effort was satisfactory according to the diversity estimators *ICE* (blue line = 30.12) and *Chao 2* (pink line = 29.47), which suggest that between 92.89 % and 95 % of the richness was recorded in the study area. Acoustic monitoring (cyan line = 27) recovered between 89.58% (*ICE*) and 91.6% (*Chao 2*) of the richness. Morphological identification (orange line = 9) recorded between 29.86% (*ICE*) and 30.5% (*Chao 2)* of the richness.

The value for Margalef specific diversity index was R = 4.421, which indicates a moderate diversity. The Shannon diversity index had a value of H' = 3.073, which also indicates moderate diversity in the study area. For the Gini-Simpson index, a value of $1\text{-}D$ = 0.9474 was obtained; this value indicates that there is a high probability of obtaining two different species from a random sample, thus indicating that there is no species dominance in the study area.

Finally, the value obtained for the Berger-Parker index was D = 0.09131, indicating that the species with the highest proportion of occurrences in the sample represents 9.1% of the recorded richness, therefore, there is no indication of dominance of any species in the study area. With these values, together with the bat diversity composition, we can infer that the *ECO* complies with the hypothesis of intermediate disturbance.

Discussion

The most represented bat family in our sample was Vespertilionidae (20 spp.), followed by the Molossidae (6 spp.). The diversity composition recorded in this study is consistent with previously described diversity patterns in arid and semiarid climates of the Mexican plateau and the south of Arizona (USA) (e.g. Ortega and Arita 1998; López-González *et al.* 2015; Segura-Trujillo *et al.* 2016; Bazelman 2016; Dwyer 2021; Segura-Trujillo *et al.* 2022; Ramos-H *et al.* 2024). Because the study site is nearby to the transitional zone between the Nearctic and Neotropical biogeographic regions, it is possible to find elements of Neotropical origin, such as species of the families Molossidae, Mormoopidae, and Phyllostomidae (Ortega and Arita 1998; López-González *et al.* 2015). In this study these elements represent only 8 species (*i.e.* 28.5% of the total sample).

In relation with the generalist and specialist bat species, previous studies have determined that at least 10 of the 28 identified species in this study are generalists, whose presence is correlated with suburban environments (*T. brasiliensis, E. fuscus, M. yumanensis, M. californicus, M. velifer, M. volans, N. macrotis, L. xanthinus, L. intermedius* and *M. megalophylla*) (Avila-Flores and Fenton 2005;

Dixon 2012; Bazelman 2016; Rodríguez-Aguilar *et al.* 2017; Adams 2021; Dwyer 2021; Dwyer *et al.* 2024; Briones-Salas *et al.* 2024). However, it has been reported that the species *N. femorosaccus, C. townsendii, N. brasiliensis, M. auriculus, P. hesperus* and *C. mexicana* have specialized habits, with relatively low activity levels in urban areas (Husar 1976; Arroyo-Cabrales *et al.* 1987; Bazelman 2016; Rodríguez-Aguilar *et al.* 2017; Dwyer 2021; Dwyer *et al.* 2024). In addition to having specialist habits, *C. mexicana* has been classified as near threatened by the IUCN (Solari 2018). Therefore, this work contributes to generating information to make decisions about conservation strategies for species that can inhabit suburban habitats in the Mexican plateau.

Evidence suggests that species of the genus *Lasiurus* (*L. frantzii, L. ega, L. cinereus* and *L. intermedius*) tolerate intermediate levels of urbanization. However, given their foraging and refuge site characteristics, they tend to avoid such environments. The exception is *L. xanthinus*, which has not been reported reduce its presence in habitats due to increased urbanization (Aguilar *et al.* 2013; Dwyer 2021).

According to Moreno and Halffter (2001), it is necessary to recover a minimum of 90 to 95 % of the bat diversity to ascertain that the sampling effort was sufficient. We recovered between 92.89 % (ICE) and 95 % (Chao 2), indicating a satisfactory sampling effort. This is due to the use of two identification methods, which have been deemed optimal in suburban environments or areas characterized by minimal vegetation cover, such as xerophytic scrub vegetation (Rautenbach *et al.* 1996; Kuenzi and Morrison 1998; Rydell *et al.* 2002; Berry *et al.* 2004; MacSwiney *et al.* 2020). Furthermore, these methods are complementary to each other, and their efficiency varies depending on habitat characteristics and the trophic guild to which the bat species belong (*i.e.* open space aerial foragers, closed space aerial foragers, surface foragers, and edge space foragers). For instance, numerous authors (*e.g.* O'Farrell and Miller 1999; Kalko *et al.* 2008; MacSwiney *et al.* 2008) have mentioned that acoustic monitoring has been found to be the most efficient in recording species that forage in open spaces, while mist nets have been determined to be optimal for capturing species that forage in closed or surface spaces (La Val 1970; Kunz 1973; Kunz and Brock 1975; Kuenzi and Morrison 1998; Rydell *et al.* 2002; Larsen *et al.* 2007). This distinction is evident in the accumulation curves of each method and the feeding habits reported in previous studies (Mora-Villa *et al.* 2014; Segura-Trujillo *et al.* 2016). Acoustic monitoring recorded 13 genera and 27 species (96.4% of the sample), of which six molossid species belonged to the guild of open-space aerial foragers and at least 17 species of Vespertilionids belonged to the guild of edge-space foragers.

In the other hand, the morphological identification recorded 9 spp. of which 8 were also identified through their EPs, and which mostly belong to the guild of closed-space foragers and edge-space foragers. The only exception was *C. mexicana* which feeds on pollen and nectar from Agavaceae flowers and has EPs that are complicated to record, like other Phyllostomids species, but they are relatively easy to capture with mist nets (Kunz and Kurta 1988; Simmons and Voss 1998; Clarke *et al.* 2005; MacSwiney *et al.* 2008; Pérez-Hernández and Martínez-Coronel 2023). In addition, the combined methods allowed us to identify acoustically and morphologically the species *L. ega*, which some authors have reported that it is distributed mainly on the slope of the Gulf of Mexico (Medellín *et al.* 2008; Barquez and Diaz 2016; Ortega *et al.* 2022), although others mention that its distribution includes the north-central region of the country (Kurta and Lehr 1995). Because of this uncertainty and the migratory habits of this species, it is imperative to provide information on the distribution of the species in the arid and semiarid regions of the country, where studies are limited.

In the context of acoustic characterization, it was observed that the Fmin values recorded for all the 27 species matched the values of the "Compendio de Llamados de Ecolocalización de los murciélagos insectívoros mexicanos" and the SONOZOTZ echolocation call library (Ortega *et al.* 2022). The recorded Fmax values differ in 5 species (*Lasiurus xanthinus, L. cinereus, M. megalophylla, Molossus nigricans* and *N. laticaudatus*). Conversely, peak frequency values differ in one species (*Corynorhinus townsendii*) and the recorded DUR values differ in 17 spp. (*Antrozous pallidus*, *Baeodon alleni*, *C. mexicanus*, *C. townsendii*, *Eptesicus fuscus*, *L. cinereus*, *L. ega*, *L. frantzii*, *M. ciliolabrum, M. auriculus*, *Parastrellus hesperus*, *R. parvula*, *N. aurispinosus*, *N. femorosaccus*, *N. laticaudatus*, *N. macrotis* and *T. brasiliensis*).

The parameters that exhibited the most discrepancy was Fmax and DUR, which may be associated with the capacity of bats to decrease Fmax and increase DUR in response to the climatic and structural characteristics of the site. This phenomenon can be a strategy employed by bats to mitigate atmospheric attenuation and avert the masking of their EPs by anthropogenic sounds in this context (Thomas *et al.* 1987; Wund 2006; Gillam *et al.* 2009).

The calculated diversity indices values suggest that the study area has a moderate bat diversity (H' = 3.073, R = 4.421, 1-D = 0.9474 and, D = 0.09131). There is a possibility that this is due to the suburban park characteristics (*i.e.* moderate levels of urbanization, tree cover, water bodies and streetlights) and the presence of caves and abandoned mines in the vicinity of the *ECO*. Several studies have documented that these features may explain why the richness of bat species was higher in suburban parks or in suburban areas due the presence of available roost and foraging sites for different species of bats (Kurta and Teramino 1992; Gehrt and Chelsvig 2008; Loeb *et al.* 2009; Russo and Ancillotto 2015). In addition, we inferred from the calculated values of each index and the suburban characteristics of the environment, the diversity pattern present in the *ECO* polygon corresponds to the hypothesis of intermediate disturbance.

This hypothesis states that species can take advantage of moderately altered habitats and increase the richness and diversity in general (Connell 1978; Castro-Luna *et al.* 2007; Threlfall *et al.* 2011; Dodd *et al.* 2012). Our study is an example of how systematic and formal studies about diversity of bats using 2 methods of identification, can elucidate and provide information about the species distribution and their EPs characteristics in regions where these types of studies are limited. In addition, it is important to highlight the importance of its implementation to reduce bias regarding the diversity of bats present. In our case, for example, the company URSAMEX (2014) mentions that they identified five species of bats, but three species do not correspond to the reported distribution, moreover statistical methods were not used to evaluate the sampling effort, and only one identification method was used, which could have underestimated the diversity of bats present in the park. In fact, we only identified two of these mentioned species which correspond to *Myotis auriculus* and *Mormoops megalophylla*. This is an example of how the use of complementary methods for bat species identification can expand and provide accurate information about the actual knowledge of diversity at the local level in this type of environment which cover a Mexican plateau.

Conclusions

We registered 28 bat species with two methods of identification. Our study represents the first formal and systematic listing of bat species in a suburban environment in the Mexican plateau region and particularly in the state of Zacatecas. In addition, we registered the species *L. ega* which was not reported in the region. Finally, our sampling effort was satisfactory, and the bat diversity pattern identified in the *ECO* corresponds to the pattern observed in the north-central region of the country, that is, a greater representation of the families Vespertilionidae and Molossidae. Furthermore, according to the calculated diversity indexes values, it is inferred that the suburban characteristics of Park maintain a moderate diversity of chiropteran species and it is suggested that it corresponds to the pattern of the intermediate disturbance hypothesis.

Acknowledgments

We thank the reviewers of this work whose invaluable comments greatly improved the quality of this work. We also thank COZCyT who provided us with financial support to present our study in different forums, as well as the Secretaría Agua y Medio Ambiente (SAMA) for allowing us to carry out the study in the "Ecoparque Centenario".

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Associated editor: Sergio Solari
Submitted: August 21, 2025; Reviewed: November 15, 2025
Accepted: December 2, 2025; Published on line: December 17, 2025

THERYA, 2026, Vol. 17(1):59-70 DOI: 10.12933/therya.2026.6225 ISSN 2007-3364

Potential distribution and current records of Neotropical otter (*Lontra annectens*) in central Mexico

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The distribution of the Neotropical otter (*Lontra annectens*), recently recognized as a separate species after its taxonomic separation from *Lontra longicaudis annectens*, in central Mexico is poorly known. This study aimed to update its distribution in the State of Mexico using a potential distribution model and field validation to identify priority areas for conservation. The model was generated with the *kuenm* package in R, incorporating topographical, climatic, and ecological variables. The presence of the species was verified at nine model-predicted sites based on interviews and field trips carried out between April 2024 and March 2025, including expeditions in rivers in the municipality of Temascaltepec. The model predicted an approximate potential distribution of 5300 km of suitable water bodies, representing only a fraction of the state territory. Field validation confirmed the presence of the species in the Telpintla, Grande, and Chilero rivers and documented the Vado River, Temascaltepec, for the first time. Additionally, interviews confirmed its presence in Malinalco and adjacent areas. These findings update the distribution of *L. annectens* in the State of Mexico and identify areas with high ecological suitability that should be prioritized for its conservation.

Keywords: State of Mexico, indirect evidence, distribution models, new records, Temascaltepec.

La distribución de la nutria neotropical (*Lontra annectens*), recientemente reconocida como especie independiente tras su separación taxonómica de *Lontra longicaudis annectens,* es poco conocida en el centro de México. Este estudio tuvo como objetivo actualizar su distribución en el Estado de México mediante un modelo de distribución potencial y su validación en campo, con el fin de identificar áreas prioritarias para la conservación. El modelo se generó con el paquete *kuenm* en R, utilizando variables topográficas, climáticas y ecológicas. La presencia de la especie se verificó en nueve sitios predichos por el modelo a partir de entrevistas y recorridos de campo realizados entre abril de 2024 y marzo de 2025, incluyendo exploraciones en ríos del municipio de Temascaltepec. El modelo predijo una distribución potencial aproximada de 5,300 km de cuerpos de agua adecuados, lo que representa solo una fracción del territorio estatal. La validación en campo confirmó la presencia de la especie en los ríos Telpintla, Grande y Chilero, y registró por primera vez el río Vado, Temascaltepec. Adicionalmente, las entrevistas corroboraron su presencia en Malinalco y áreas cercanas. Estos resultados permiten actualizar la distribución de *L. annectens* en el Estado de México e identifican zonas con alta idoneidad ecológica que deben considerarse prioritarias para su conservación.

Palabras clave: Estado de México, evidencias indirectas, modelos de distribución, nuevos registros, Temascaltepec.

Otters are regarded as of great ecological importance because they are top predators in aquatic ecosystems and are sensitive to drastic environmental changes (Gómez-Nísino 2006; Sánchez *et al.* 2007; Monroy-Vilchis and Mundo 2009; Ramos-Rosas *et al.* 2012). The Neotropical otter, also known as "water dog", has recently been recognized as a separate species. Recent analyses of nuclear genome data have revealed a marked genetic separation between trans-Andean Neotropical otters, *Lontra annectens* (Mayor 1897), and the other cis-Andean otters (de Ferran *et al.* 2024).

In Mexico, this species thrives in various aquatic ecosystems, including rivers, streams, lagoons, lakes, mangroves, and reservoirs. It shows a wide and varied geographical distribution, ranging from sea level in the Pacific and Gulf of Mexico coasts and mangrove areas, to altitudes above 1000 m a.s.l. in the Mexican plateau, parts of the Trans-Mexican Volcanic Belt and Sierra Madre del Sur, as well as in mountainous areas and upper river basins in the states of Oaxaca, Chiapas, Puebla, Veracruz (Gallo-Reynoso 1989; 1997), Sonora and Chihuahua (Gallo-Reynoso *et al.* 2019).

Despite its broad distribution and its status as a bioindicator species (Gómez-Nísino 2006; Sánchez *et al.* 2007; Monroy-Vilchis and Mundo 2009; Arellano Nicolás *et al.* 2012), the Neotropical otter faces various threats that have adversely impacted its populations and distribution in some regions of Mexico. The main threats include pollution of rivers and lakes, deforestation, habitat degradation,

poaching, and infrastructure development such as river canals and hydroelectric facilities (Gallo-Reynoso en Chehébar 1990; Gallo-Reynoso 1997; Rheingantz *et al.* 2017; 2021). In general, the threats facing *Lontra longicaudis* have been identified. However, given its reassignment to *Lontra annectens*, these have not yet been fully determined, making it necessary to characterize these threats, its potential distribution, and its state of conservation. The International Union for Conservation of Nature (IUCN) has listed this species as "Near Threatened" and reports a decreasing population trend (Rheingantz *et al.* 2021). In Mexico, it is listed as endangered in the short to mid term under the Mexican Standard NOM-059-SEMARNAT-2010 (DOF 2019).

The geographical distribution of the Neotropical otter proposed by the IUCN does not include the central region of Mexico. However, the presence of this species in the State of Mexico was documented in 1576, when the hunting of a "water dog" was recorded in the Santa Cruz Coacalco Lagoon (Gallo-Reynoso 1989). Later, in 1981, the species was recorded in Malinaltenango (Gallo-Reynoso 1989). In the southern area of the State of Mexico, its presence was documented in rivers and streams in Villa Guerrero, Santo Tomás, Temascaltepec, Zacazonapan, and Bejucos through indirect evidence, including tracks, feeders, and interviews with inhabitants and fishers in the region (Gallo-Reynoso 1989). Then, the presence of the species in the municipality of Temascaltepec was confirmed in 1998 (Brito-Cruz *et al.* 1998).

The latest records of the species in the State of Mexico correspond to the Temascaltepec region (Simón-Martínez 2003; Monroy-Vilchis and Mundo 2009; Guerrero-Flores *et al.* 2013). However, this area, like many others in the state, is undergoing an accelerated landscape transformation associated with the expansion of agriculture and livestock raising (SEMARNAT 2016), which has transformed the riparian ecosystems and reduced the availability of shelters and resources, jeopardizing the quality and availability of suitable habitats for the species (Gallo-Reynoso 1997).

In Michoacán, there are confirmed records of otter in the Balsas River basin, particularly in areas with clean rivers and well-conserved riparian vegetation, as well as in mountainous regions of the eastern part of the state and the Pacific coast (Monterrubio-Rico and Charre-Medellín 2014). Similarly, its presence has been documented in the Sierra Norte de Puebla (Ramírez-Bravo 2010) and the Huasteca Hidalguense (Aguilar-López *et al.* 2015; Hernández-Silva *et al.* 2024), where aquatic systems remain well preserved. In contrast, there are no recent records from Tlaxcala and Morelos, although its presence has been inferred from basin connectivity with adjacent regions, such as Puebla and the State of Mexico (Sierra-Huelsz and Vargas-Contreras 2002).

At the local scale, the use of species distribution models has been instrumental in identifying areas with optimal conditions for reintroducing locally extinct species or for establishing new protected areas (Guisan and Simmermann 2000). These models enable the integration of ecological information into the design of conservation strategies based on scientific evidence (Franklin and Miller 2010). Additionally, these models can be useful for anticipating areas at risk of invasion or conflict between humans and wildlife (Peterson *et al.* 2011).

As information on the distribution and state of the habitat of this species in central Mexico is scarce and outdated, the main objective of this study was to produce a potential distribution model for the Neotropical otter in the State of Mexico, supplemented with verification field work to determine its presence and distribution, aiming to identify the most suitable areas for conservation. This research not only contributes to the preservation of an emblematic species but also supports the conservation of aquatic biodiversity by providing a scientific basis for decision-making in environmental policy and education.

Materials and methods

Study area. The State of Mexico, located in the central region of the country, covers 22 351 km^2 (Figure 1). It is part of the Trans-Mexican Volcanic Belt, characterized by mountainous areas, valleys, and riparian and lake basins with elevations ranging from 300 m to more than 4600 m a.s.l. at the highest peak of the state, Nevado de Toluca (INEGI 2020). The regional climate is temperate-subhumid and cold in the mountains and warm-subhumid in the southern region. The mean annual temperature ranges from 12 °C to 22 °C. Annual precipitation ranges from 600 to 1500 mm, with most falling from May to October (CONABIO 2008). The predominant vegetation types are fir forest, mountain cloud forest, pine forest, pine-oak forest, oak forest, oak-pine forest, low deciduous forest patches, and induced grassland (CONABIO 2011).

The state is located in the "Lerma-Chapala-Santiago" hydrological basin in the central-western zone, which encompasses approximately 2900 km of rivers and streams (CAEM 2023). The Lerma River, its main tributary, is heavily polluted as it flows through the Toluca Valley industrial zone (Carreño de León *et al.* 2018). The "Alto Pánuco" area — the driest in the state — is located in the north, with annual precipitation below 600 mm, creating semiarid conditions (CAEM 2023). In contrast, the southern region comprises the Balsas River basin, where high precipitation and local topography result in approximately 230 interconnected rivers and streams totaling more than 5300 km (CAEM 2023), which can be key to the presence of otters. The Temascaltepec sub-basin originates at the peak of Nevado de Toluca, at 4595 m a.s.l. The stream flows to 800 m a.s.l. before reaching the Tingambato Hydroelectric Power Plant, in the state of Michoacán. It then joins the Tilostoc River, a tributary of the Cutzamala River, which, farther down, joins the Balsas River. The Temascaltepec River is the main watercourse in the region and plays a key role in aquatic connectivity and habitat availability for otters (Manzo Delgado and López García 1997).

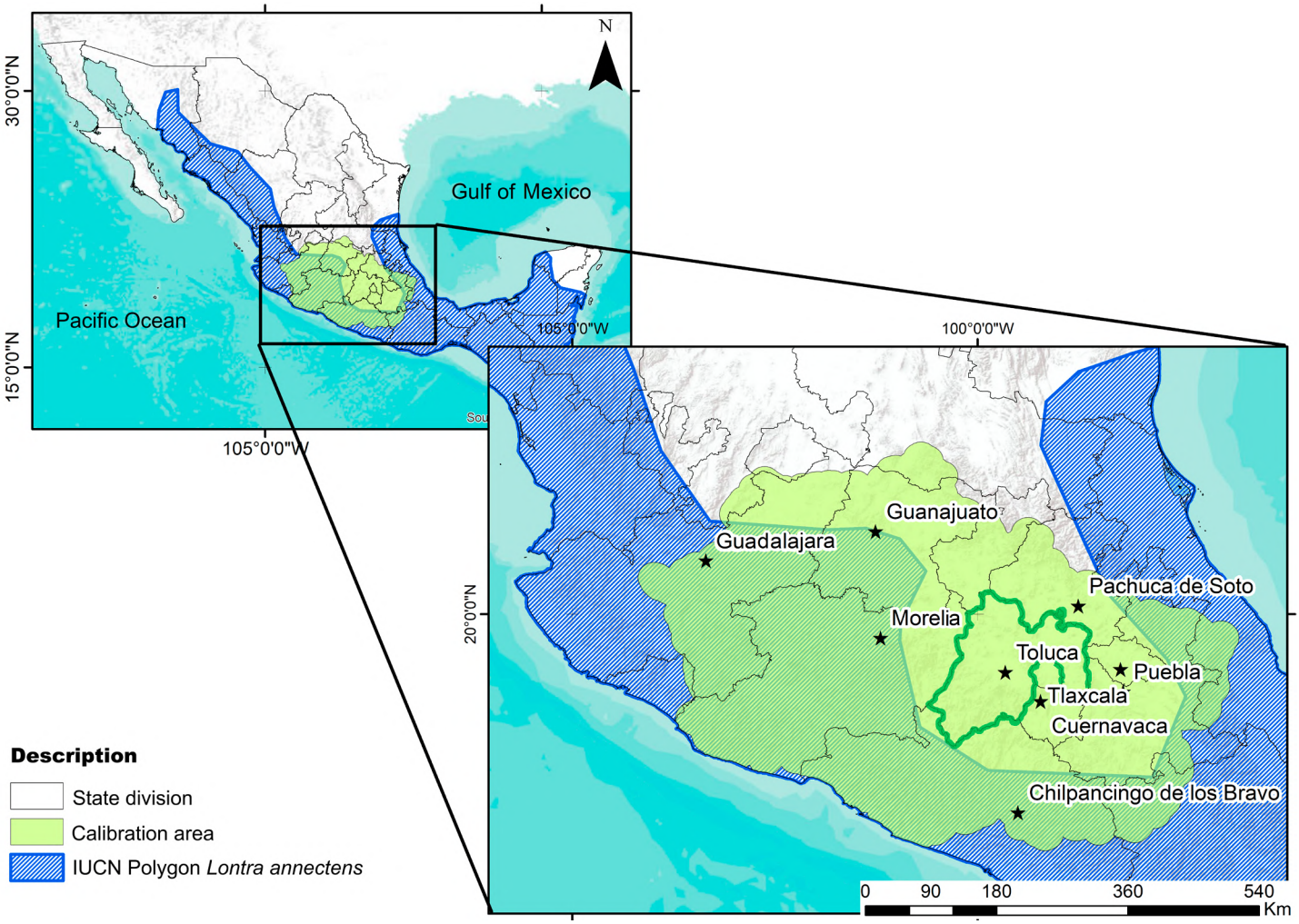

Figure 1. Study area showing the historical accessibility hypothesis (M area) delimited in green and its intersection with the distribution polygon proposed by IUCN for *Lontra annectens*. The State of Mexico is highlighted.

There are 84 protected natural areas of various categories, including the Nevado de Toluca Flora and Fauna Protection area; the Valle de Bravo-Tilostoc-Temascaltepec Basin Natural Resources Protection Area; and the Sierra de Nanchititla State Flora and Fauna Protection Area (CEPANAF 2023). These regions can play a fundamental role in Neotropical otter conservation by providing more stable environmental conditions and low anthropogenic disturbance, being an object of spatial evaluation and interpretation of results in distribution models (CONABIO 2023), in addition to supplying water to nearby communities and the Cutzamala System of Mexico City.

Potential distribution map. A calibration area was delimited based on the map of terrestrial ecoregions of Mexico (SD1, INEGI-CONABIO-INE 2008), focused on the central region of the country. This area represents the historical accessibility hypothesis for the Neotropical otter, known as the *M area* within the conceptual framework of the BAM diagram (Figure 1; Soberón *et al*. 2017).

We conducted a comprehensive literature survey and review of records of *L. annectens* for Mexico, particularly in the State of Mexico and neighboring states, considering the calibration area (Figure 1; SD2). We considered records for the period 1987–2024, which corresponds to the time interval used for constructing the environmental variables, to ensure that the model adequately reflects the ecological conditions of the species and avoid bias when estimating its potential distribution (Araújo and Peterson 2012).

Records lacking explicit geographic coordinates were georeferenced using the localities described in the original sources that included information on the municipality, the locality, or proximity to a water body. To this end, we used topographic maps at a 1:250 000 scale. To minimize spatial bias and avoid overfitting in areas with higher sampling effort, duplicate records were excluded using a 1 km spatial filter between points, implemented with the spThin package in R (Aiello-Lammens *et al*. 2015). This distance was considered adequate, given the spatial resolution of the environmental layers used. This process yielded 105 presence records, which were randomly divided into two subsets: 70% for calibration (74 records) and 30% for evaluation (31 records).

Topographic, climatic, and ecological variables were examined based on ecological factors relevant to Neotropical otters (Table 1). These layers were either included or excluded from the variance inflation factor (VIF)

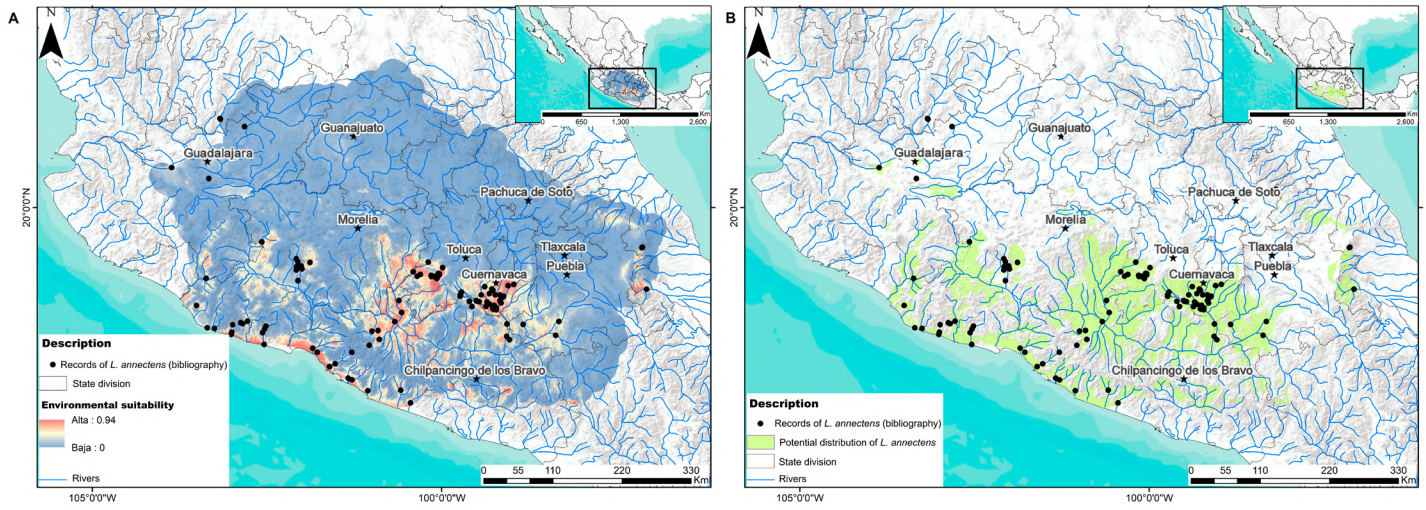

Figure 2. Potential distribution of *Lontra annectens* in central Mexico, showing the literature records obtained and the predicted suitable areas. Each record in the map represents a sampling locality. a) Continuous model; b) binary model.

to reduce collinearity between environmental variables. This process enabled the identification of highly correlated variables that may be redundant in explaining the distribution of the species (Aiello-Lammens *et al.* 2015). VIF values were calculated considering a VIF exclusion threshold >10. Variables that exceeded this value were excluded, and only those with low or moderate collinearity (VIF < 10) were selected for the final modeling of the potential distribution (SD3). All environmental variables were standardized to a spatial resolution of 1 km and delineated according to the defined calibration area.

Candidate models were created using the kuenm package in R (Cobos *et al.* 2019). This package implements the MaxEnt algorithm (Phillips *et al.* 2006; Phillips and Dudik 2008), enabling evaluation of various sets of environmental layers across multiple configurations. In Maxent, we explored 10 values of the regularization multiplier (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2) and 31 combinations of features (l, q, p, t, h, lq, lp, lt, lh, qp, qt, qh, pt, ph, th, lqp, lqt, lqh, lpt, lph, lth, qpt, qph, qth, pth, lqpt, lqph, lqth, lpth, qpth, lqpth).

The regularization multiplier controls model complexity by penalizing overfitting; larger values tend to produce more generalized models that are less fitted to the training data, whereas smaller values produce more specific and fitted models (Merow *et al.* 2013; Morales *et al.* 2017). On the other hand, features determine the relationship between potentiality and environmental variables, thereby influencing the flexibility of the model in representing complex distributions and ecological niches (Phillips and Dudik 2008; Elith *et al.* 2011). In this sense, the joint evaluation of both hyperparameters facilitates balancing fit and predictive capacity, thereby increasing the robustness and transferability of the resulting models (Warren and Seifert 2011).

The performance and selection of the best model were evaluated based on the statistical significance of the partial ROC curve (Cobos *et al.* 2019), omission rates (OR),

Table 1. Environmental variables used to model the potential distribution of *Lontra annectens*.

Variables	Source
Topographic variables	
Digital Elevation Model (DEM)	Mexican Elevation Continuum MEC – INEGI (2013)
Slope	EarthEnv (2010)
Climatic variables	
<hurs> Near-surface relative humidity hurs_max / hurs_mean / hurs_min / hurs_range	Chelsa BioClim+ (1981–2010)
<rsds> Downward surface shortwave radiation rsds_max / rsds_mean / rsds_min / rsds_range	
<pet> Potential evapotranspiration pet_max / pet_mean / pet_min / pet_range	
<cmi> Climatic moisture index cmi_max / cmi_mean / cmi_min / cmi_range	
<swb> Site water balance	
<npp> Net potential primary productivity	
Bio 1. Mean annual temperature	
Bio 2. Mean diurnal range	
Bio 3. Isothermality	
Bio 4. Temperature seasonality	
Bio 5. Maximum temperature of the warmest month	
Bio 6. Minimum temperature of the coldest month	
Bio 7. Annual temperature range	
Bio 10. Mean temperature of the warmest quarter	
Bio 11. Mean temperature of the coldest quarter	
Bio 12. Annual precipitation	
Bio 13. Precipitation of the wettest month	
Bio 14. Precipitation of the dryest month	
Bio 15. Precipitation seasonality	
Bio 16. Precipitation of the wettest quarter	
Bio 17. Precipitation of the dryest quarter	
Ecological variables	
Landsat Normalized Difference Vegetation Index (NDVI)	INEGI (2013)
Landsat Index of Surface Quality Water from Space	INEGI (2013)
Anthropogenic variables	
Ecosystem Integrity Index (EII)	CONABIO (2018)

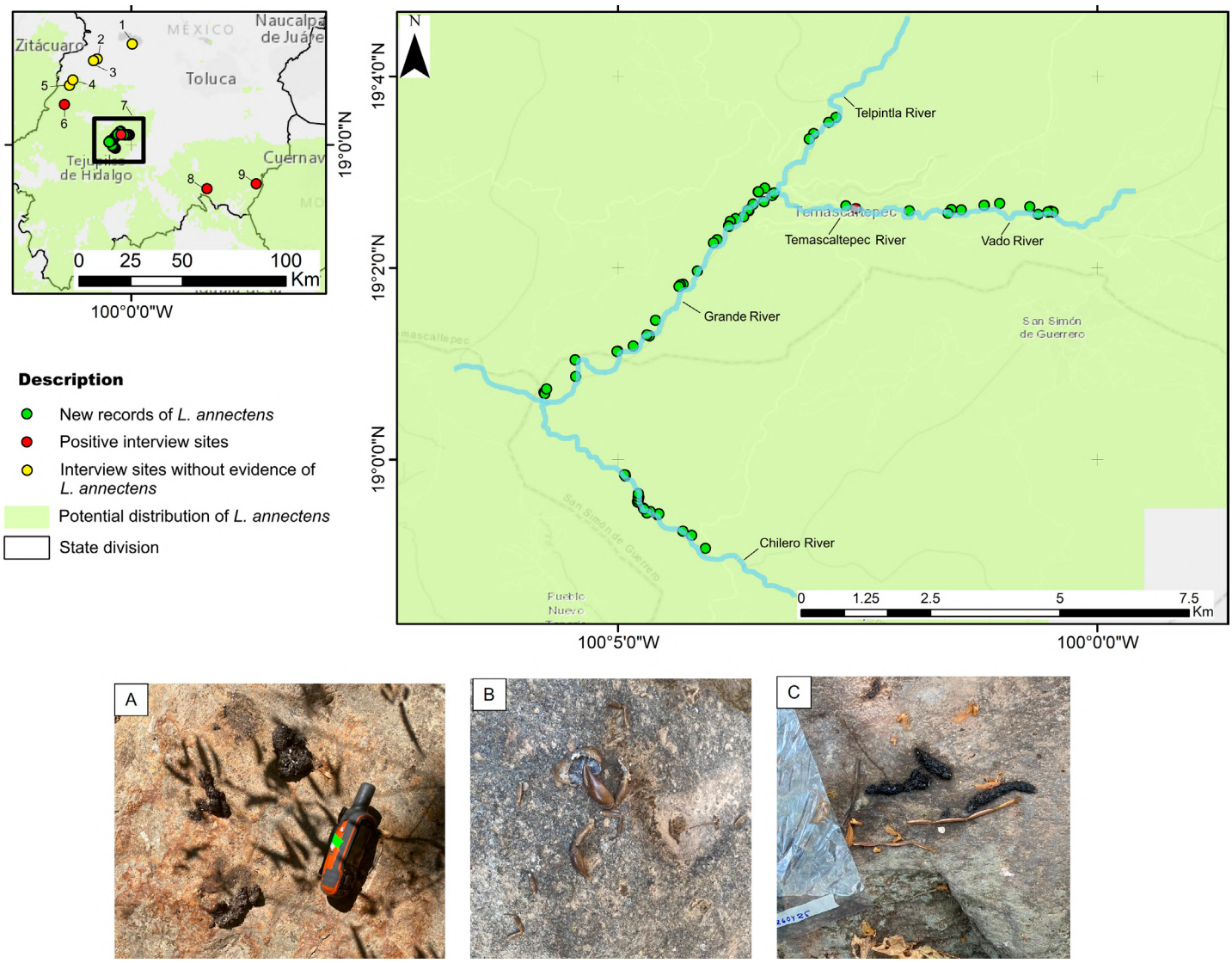

Figure 3. New records of *L. annectens* in the Temascaltepec region and interviews in potential sites in the State of Mexico. Yellow dots mark interviewed localities with no evidence of the presence of otters; red dots indicate sites with confirmed Neotropical otter presence; and green dots are the records obtained during our sampling in the Temascaltepec region. Localities: 1) Villa Victoria; 2) San José Villa de Allende, Villa de Allende; 3) La Peña, Villa de Allende; 4) Parque el Salto Chihuahua, Ixtapan del Oro; 5) Casas Largas tourist corridor, Ixtapan del Oro; 6) Santo Tomás; 7) Temascaltepec; 8) Tonatico y 9) El Platanar, Malinalco. Records: a) spraints in the Vado River, b) feeder in the Chilero River, and c) spraints in the Grande River.

and the Corrected Akaike Information Criterion for small sample sizes (AICc). A 10% omission was considered, and 10 replicates per bootstrap were applied. From the final model, a binary model was generated from the tenth percentile as a cut-off threshold to discriminate between suitable and unsuitable areas for the species. All analyses were performed in ArcMap 10.3 (Esri 2014) in R version 4.2.2 (R Core Team 2018).

Field confirmation of Neotropical otter presence. The presence of otters was confirmed through field interviews and transects. To this end, we selected nine sites based on habitat suitability predicted by the potential distribution model in the State of Mexico. These nine sites were visited during 2024 and 2025 to confirm the presence of *Lontra annectens*. The local inhabitants were interviewed in each site to obtain information about possible sightings or indirect evidence of the species. At each site, records were georeferenced using a GPS receiver (Garmin GPSmap 64s) and fed into a database.

Results

Potential Neotropical otter distribution. A total of 310 candidate models were evaluated, whose parameters reflect all combinations of 10 regularization multiplier configurations, 30 fitting-model combinations, and 15 environmental variables (SD4 and SD5). Of this set, only one model (M_2_F_lp) met the established performance parameters.

The potential distribution area for otters in the calibration region corresponds to 23.97% (Figure 2). Specifically in the State of Mexico, the potential distribution area covers approximately 6162 km^2. However, considering the semiaquatic behavior of the species, we estimate that the area effectively usable for the Neotropical otter corresponds to nearly 5300 km of water bodies, approximately equivalent to the total length of the rivers running across the state, which represents only a small fraction of the state territory.

According to the potential distribution model, suitable regions for otters include the Temascaltepec sub-basin in

the southwest and the Malinalco region in the southeast of the State of Mexico. In turn, the Temascaltepec sub-basin is part of the "Valle de Bravo, Malacatepec, Tilostoc, and Temascaltepec River Basins" Natural Resource Protection Area (APRN, in Spanish) (CEPANAF 2023), which includes the municipalities of Amanalco, Donato Guerra, Ixtapan del Oro, Otzoloapan, San Simón de Guerrero, Santo Tomás, Temascaltepec, Valle de Bravo, Villa de Allende, Villa Victoria, and Zinacantepec (Figure 2).

Field confirmation of the presence of the Neotropical otter. In 2024 and 2025, field interviews and field trip were conducted at nine sites to verify the potential distribution model for *Lontra annectens* in the State of Mexico. During fieldwork, the Telpintla, El Vado, Chilero, Temascaltepec, and Grande rivers were sampled by walking along their banks in search of direct and indirect evidence of the species, including sightings, footprints, spraints, and latrines (Figure 3). In other sites, we verified the existence of rivers that showed channel transfer, such as Arroyo Grande, Malacatepec River, and Ixtapan del Oro-Santo Tomás de los Plátanos River, all within the Cutzamala Plan (Figure 3).

We confirmed the presence of the Neotropical otter in the State of Mexico, particularly in the municipality of Temascaltepec (Table 1). We found that the species is preferentially associated with river stretches that maintain good water quality, riparian vegetation coverage, and food availability, even in areas where fish farming is practiced (Table 2).

Table 2. Characteristics of sampled rivers in the Temascaltepec region with evidence of the presence of *Lontra annectens.*

River	Sampling effort (km)	Amount and type of records	Site characteristics	River depth (m)	River width (m)
Chilero	6	12 spraints (scats) and one feeder	Clear water Big rocks Presence of pools Rugged river channels	0.2–1.1	3–4
Telpintla	3	7 spraints	Narrow and rocky Small rocks Turbid water Scarce pools	0.3	2
Vado	3	10 spraints	Clear water Big rocks Presence of pools Rugged river channels	0.2–0.4	4
Grande	7	7 spraints	Clear water Presence of pools Big rocks Rugged river channels	0.3–0.7	5–10
Temas-caltepec*	2	7 spraints and one feeder	Sewage odor Scarce pools Turbid water Scarce water flow Presence of garbage Abundant small rocks	0.3	4

The evaluated sites have favorable conditions for maintaining local subpopulations, including clear water, riverbank vegetation, rocky substrates that form pools and waterfalls that provide shelter and facilitate movement, and abundant aquatic prey, such as trout (*Oncorhynchus mykiss*), which can sustain local subpopulations. Together, these conditions constitute highly favorable habitats for the establishment and persistence of *L. annectens* in the area (Table 2).

During field trips, we obtained records from previously explored areas in which no evidence of Neotropical otter presence had been found, such as the Vado River (Simón-Martínez 2003). Based on indirect evidence, including interviews, spraints, and feeders, we confirmed the presence of the species in the municipalities of Santo Tomás de los Plátanos, Ixtapan de la Sal, Tonatico, and Temascaltepec, and possible presence in Malinalco and its surroundings. Similarly, we found no evidence of its presence in the area comprising Villa Victoria, Villa de Allende, Donato Guerra, and Ixtapan del Oro (numbers 1, 2, 3, 4; Figure 3).

Discussion

The modeling identified areas that are highly suitable for the presence of the Neotropical otter (*Lontra anectens*) in the State of Mexico and surrounding regions. In addition, field sampling confirmed its presence in places where it had not been previously recorded, such as the Vado River in Temascaltepec or the Platanar region in Malinalco, in addition to areas with historical records such as Malinaltenango, confirming its permanence over time (Simón-Martínez 2003; Monroy-Vilchis and Mundo 2009; Guererro Flores *et al.* 2013).

Our results clarify the distribution of the Neotropical otter (*Lontra annectens*) in central Mexico, which had been underestimated by the IUCN (Rheingantz *et al.* 2021). The persistence of the species in this region has been questioned due to anthropogenic impact on its habitat (Gallo-Reynoso 1997, Moreno Barrera *et al.* 2025). This has led to the omission of documented records in entities such as the State of Mexico and other areas where multiple recent sightings and suitable habitats have been confirmed (Sierra-Huelz and Vargas-Contreras 2002).

Although progress has been made in understanding the distribution of *Lontra annectens* in Mexico, the studies conducted to date are scarce and geographically limited. These studies have focused on specific regions, such as the Apatlaco-Tembembe basin, in the state of Morelos (Cirelli Villanova 2005), the Yucatan Peninsula (Ortega-Padilla *et al.* 2022), the Huicicila River hydrological basin, in Nayarit (Luna Aranguré 2015), and the state of Michoacán (Monterrubio-Rico and Charre-Medellín 2014). In these studies, the presence of otters has been associated with variables such as altitude, vegetation type, and hydrological characteristics. The results indicate that the Neotropical otter is present mainly in perennial rivers with habitat continuity and that the protected areas that host the species have limited coverage.

The limited geographic coverage and the lack of comprehensive studies at the regional and national scales

make it difficult to fully understand the distribution and ecology of the species (Gallo-Reynoso 1989; 1997; Gallo-Reynoso and Meiners 2018). Furthermore, fragmentation of aquatic habitats, urbanization, and topography may be leading to basin-differentiated population structure (Guerrero-Flores 2014, Hernández-Romero *et al*. 2018; Latorre-Cardenas *et al*. 2021), suggesting that populations in the State of Mexico may be locally genetically isolated (Rivera-Ortíz *et al*. 2014).

The combination of environmental variables, geographical barriers, and hydrography promotes population divergence in *L. annectens*, with important genetic and morphological implications (Hernández-Romero *et al*. 2017). Alterations to the natural landscape, such as dam construction and the loss of riparian vegetation, disrupt the dispersal of Neotropical otter populations, reducing genetic connectivity between them (Latorre-Cardenas *et al*. 2021). Therefore, otters inhabiting the center of the country may be genetically closer to central populations of Oaxaca, Guerrero, Jalisco, or Veracruz than to populations from the northern Pacific or the Atlantic slope (Guerrero *et al*. 2015).

The absence or scarce presence of otters in the area of the northern State of Mexico adjacent to the state of Hidalgo, as suggested by the potential distribution model, is explained by the lack of suitable perennial aquatic habitats, unfavorable altitude and climate, and environmental degradation of riverbanks essential for Neotropical otter survival (Botero-Botero *et al*. 2017). Although otters have been recorded in mountainous areas of Mexico and Colombia at altitudes of up to 2000 and 3000 m a.s.l., these populations are very localized and sparse (Andrade-Ponce and Angarita-Sierra 2017; Esparza-Carlos *et al*. 2022). Otters require perennial rivers with high dissolved oxygen levels and adequate prey availability (Casariego Madorell *et al*. 2006). On the other hand, mountainous areas of the eastern State of Mexico have cold ecosystems and high altitudes (2500–3000 m a.s.l.), with intermittent or very small waterways that lack dense riparian vegetation required by otters for shelter and food (Lavariega *et al*. 2020).

In the northern State of Mexico, hydrological systems are characterized by low flows and fragmented waterways, which preclude the establishment of stable Neotropical otter populations (CAEM 2025). In addition, river piping and the Cutzamala plan have eliminated channels through which otters could move (CAEM 2025). Anthropogenic effects such as urbanization, agriculture, and pollution have degraded riverbanks in mountainous volcanic areas, thereby preventing otters from establishing burrows and reducing food availability (Gallo-Reynoso 1997).

The Temascaltepec region, in which the greatest evidence of the presence of otters in the State of Mexico has been reported, still shows adequate riparian vegetation and rivers with permanent water flows that sustain local Neotropical otter populations (Arellano Nicolás *et al*. 2012). We documented the presence of the Neotropical otter at a new location in the area and confirmed that it occurs in the Vado River, where no previous evidence had been found (Guerrero-Flores *et al*. 2013). Furthermore, we confirmed previous records of the species in the Telpintla, Grande, and Chilero rivers (Simón-Martínez 2003; Monroy-Vilchis and Mundo 2009; Guerrero-Flores *et al*. 2013) 19 years after the last evidence was reported.

The Neotropical otter populations in Temascaltepec live in highly modified environments where their diet consists mainly of trout (*Oncorhynchus mykiss*; Monroy-Vilchis and Mundo 2009), a species introduced to central Mexico, in contrast to less disturbed populations elsewhere in the country. The Amacuzac River, which runs through the state of Morelos, partly derived from the Lerma River and flowing into the Balsas River through its tributary through the Chontalcoatlán River that crosses the Cacahuamilpa Caves, is an important system of basins that could have historically facilitated the mobility of otters; however, they are currently fragmented by dams and human alterations (Arellano Nicolás *et al*. 2012; González-Christen *et al*. 2013; Guerrero-Flores *et al*. 2013; Lavariega *et al*. 2020).

The potential distribution model identified adequate habitats in localities near Temascaltepec, including Cañadas de Nanchititla, within the Sierra de Nanchititla State Park in the southwestern area of the State of Mexico. This region is covered by natural vegetation and crossed by major waterways suitable for otter populations (CEPANAF 2023). Although these areas have been identified as potentially adequate for the species, field validation is necessary. However, access is limited by the insecure conditions associated with organized crime, a significant obstacle to fieldwork.

The otter population living in the Temascaltepec region may move to, or be linked to, populations in the southern parts of the state, adjacent to Tingambato, Michoacán, due to the flow of waterways, although there are no records of otters in Tingambato (Monterrubio-Rico and Charre-Medellín 2014). Therefore, it is essential to continue the search for *L. annectens* in all rivers and streams that connect with the Temascaltepec River and other main rivers, such as Tilostoc, to determine the mobility or migration of otters across the sub-basin.

The distribution of the Neotropical otter in the State of Mexico and central Mexico is poorly known. Although its presence is mentioned in the list of fauna of the "Valle de Bravo, Malacatepec, Tilostoc and Temascaltepec River Basins" Natural Resources Protection Area, there are no management plans or conservation strategies for the species. In this context, the present study is a valuable contribution to planning the monitoring and conservation of the Neotropical otter in central Mexico, by identifying areas with high ecological suitability that can host undocumented populations.

The results of the present study set the basis for defining priority areas for conservation and shelter determined by the potential distribution model. Monitoring programs must consider environmental variables that affect the presence of the species, such as the pollution of water bodies and the

conservation status of riparian vegetation (Botero-Botero *et al*. 2016; Lavariega *et al*. 2020). Therefore, management and monitoring strategies aimed at protecting *L. annectens* must adopt a comprehensive approach that integrates spatial analysis with detailed ecological and environmental assessments to ensure the effectiveness of long-term conservation actions. Finally, the lack of genetic studies on otters inhabiting the State of Mexico makes it difficult to evaluate connectivity between otter populations, a key factor in understanding the dynamics and viability of the species in this entity.

Acknowledgements

This work was supported by the National Council of Humanities, Sciences, and Technologies (Conahcyt) through the 2022–2 National Scholarship Program (Traditional), under CVU No. 868097. We also extend our gratitude to Mr. Guadalupe, a resident of Temascaltepec; to the social service students of the Environmental Biology undergraduate program at the Universidad Autónoma Metropolitana (Lerma Unit); and to A. L. Bussy for their invaluable assistance in the field. We are grateful to the communities of Temascaltepec, Malinalco, and the Villa Victoria region for granting access to their territories and for sharing their knowledge of the local fauna.

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Associated editor: Sergio Solari
Submitted: August 8, 2025; Reviewed: October 30, 2025
Accepted: December 12, 2025; Published on line: January 30, 2026

Supplementary material

This article contains Supplementary Material or Data Files, which can be downloaded from the journal website.

SD1. Terrestrial ecoregions of Mexico included in the calibration area polygon (M) constructed for *Lontra annectens*.

SD2. States, localities, and rivers with records of Neotropical otter in central Mexico collected for the present study.

SD3. Results of the Variance Inflection Factor (VIF) analysis applied to the environmental variables to minimize the environmental noise in the resulting models.

SD4. Estimates of the relative contributions of environmental variables to the resulting model.

SD5. Performance statistics of candidate models. The final model is highlighted.

THERYA, 2026, Vol. 17(1):71-80 DOI: 10.12933/therya.2026.6240 ISSN 2007-3364

The complete mitochondrial genome and phylogenetic position of the Commissaris's long-tongued bat *Glossophaga commissarisi*

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Well resolved phylogenetic relationships are fundamental to understanding species' evolutionary history, but inferring robust phylogenies can be challenging. The mitochondrial genome has resulted a valuable resource to achieve higher phylogenetic resolution and support. The Commissarisis's long-tongued bat (*Glossophaga commissarisi*) is a widely distributed nectar-feeding bat in the Americas, ranging from Mexico to Colombia and Peru. In this study, we sequenced and assembled the complete mitochondrial genome of the species to characterize its genomic structure, codon usage, and patterns of selection; and to determine its phylogenetic position within Phyllostomidae. We confirmed its phylogenetic position within the genus *Glossophaga* and the family Phyllostomidae. The mitogenome was a circular molecule with a total length of 16,648 bp, containing 13 protein-coding genes (PCGs), two ribosomal genes, 22 transfer RNA genes, and one D-loop or control region (CR). The overall nucleotide composition was A = 32.18%, T = 29.63%, C = 23.97%, and G = 14.22%, with A + T content = 61.81% and G + C content of 38.19%. The phylogenetic tree reconstructed using the 13 PCGs included 61 taxa and recovered *G. commissarisi* as a sister species of *G. leachii* and a fully-supported clade (bv = 100) containing the genus *Glossophaga*. Our study provides a crucial genomic resource for the study of these bats and demonstrates the utility of complete mitogenomes in achieving well-resolved phylogenies for rapidly diversifying mammalian groups.

Keywords: Chiroptera, genomic resources, mitochondrial evolution, molecular phylogenetics, New World bats, selective constraints

Las relaciones filogenéticas bien resueltas son fundamentales para comprender la historia evolutiva de las especies; sin embargo, es un reto obtener filogenias concluyentes. El genoma mitocondrial ha resultado ser un recurso valioso para obtener mayor resolución en las relaciones filogenéticas. El murciélago lengüetón de Commissarisi (*Glossophaga commissarisi*) es un murciélago nectarívoro ampliamente distribuido en las Américas, con un rango de distribución que va desde México hasta Colombia y Perú. En este estudio, secuenciamos y ensamblamos el genoma mitocondrial completo (mitogenoma) de la especie para caracterizar su estructura genómica, uso de codones y patrones de selección; y para determinar su posición filogenética dentro de la familia Phyllostomidae. Confirmamos su posición filogenética dentro del género *Glossophaga* y la familia Phyllostomidae. El mitogenoma fue una molécula circular con una longitud total de 16,648 pb, que contenía 13 genes codificantes de proteínas (PCG), dos genes ribosomales, 22 genes de ARN de transferencia y una región D-loop o región control (CR). La composición nucleotídica general fue A = 32.18%, T = 29.63%, C = 23.97% y G = 14.22%, con un contenido A + T = 61.81% y un contenido G + C de 38.19%. El árbol filogenético reconstruido utilizando los 13 PCG incluyó 61 taxones y recuperó a *G. commissarisi* como una especie hermana de *G. leachii* y un clado con soporte completo (bv = 100) que contenía al género *Glossophaga*. Nuestro estudio proporciona un recurso genómico crucial para el estudio de estos murciélagos y demuestra la utilidad de los mitogenomas completos para obtener filogenias bien resueltas en grupos de mamíferos que se diversifican rápidamente.

Palabras clave: Chiroptera, evolución mitocondrial, filogenética molecular, murciélagos del Nuevo Mundo, recursos genómicos, restricciones selectivas

Phylogenetic relationships are fundamental to understanding evolutionary history, biogeography, and the evolution of ecological traits. However, inferring robust phylogenies can be challenging, often yielding incongruent results between studies that use different datasets (e.g., morphological vs. molecular data) or limited molecular markers (Dávalos *et al.* 2012; Dumont *et al.* 2012). Such phylogenetic incongruence can stem from factors like convergent evolution, incomplete lineage sorting, or the limited phylogenetic signal of individual genes (Rokas and Carroll 2005; Degnan and Rosenberg 2009). The integration of genomic-scale data has emerged as a powerful approach to resolve these conflicts, providing a more comprehensive and statistically robust view of evolutionary relationships by aggregating signals from thousands of independent loci (McCormack *et al.* 2013; Reddy *et al.* 2017).

Among genomic resources, the complete mitochondrial genome (mitogenome) offers a particularly valuable tool for phylogenetic inference at intermediate taxonomic levels (Gissi *et al.* 2008). Mitogenomes provide a set of 37 linked genes that evolve at different rates, combining fast-evolving regions useful for recent divergences with highly

conserved protein-coding genes informative for deeper nodes (Boore 1999). This, coupled with features like rare gene rearrangements, codon usage bias, and patterns of evolutionary selection, makes mitogenomic data superior to single mitochondrial genes for achieving higher phylogenetic resolution and support, especially within rapidly diversifying clades (Cameron 2014; Tan *et al.* 2015).

The New World leaf-nosed bats (family Phyllostomidae) represent a classic example of an adaptive radiation, exhibiting extraordinary ecological diversity – from insectivory and carnivory to frugivory, nectarivory, and even sanguivory – within a relatively recent evolutionary timeframe (Dumont *et al.* 2012; Rojas *et al.* 2016). This rapid diversification has sometimes resulted in unresolved or conflicting phylogenetic hypotheses, particularly within subfamilies (Baker *et al.* 2012; Dávalos *et al.* 2014). The subfamily Glossophaginae (nectar-feeding bats) is a key component of this radiation. Within it, the genus *Glossophaga* is widespread and species-rich, yet the phylogenetic relationships among its species remain partially unresolved due to limited genomic data (Hoffmann *et al.* 2019). The Commissaris's long-tongued bat, *Glossophaga commissarisi* (Gardner 1962), is a widely distributed nectar-feeding bat found from Mexico to Peru, also acting as a facultative insectivore (Sánchez-Casas and Alvarez 2000). Despite its abundance, no complete mitogenome was available for this species, creating a gap in resources needed for phylogenetic analyses of the genus.

To address this gap, we sequenced, assembled, and annotated the first complete mitochondrial genome of *G. commissarisi* with the following objectives: (1) to characterize its genomic structure, codon usage, and patterns of selection; and (2) to determine its phylogenetic position within Phyllostomidae using a phylomitogenomic approach. We predicted that the mitogenome of *G. commissarisi* would exhibit structural conservation and strong purifying selection typical of functional mitochondrial genomes. Furthermore, we predicted that the use of complete mitogenomic data would provide high statistical support for resolving its position as sister to *G. leachii* and for clarifying relationships within Glossophaginae, offering a superior resource compared to single-gene studies.

Materials and methods

Mitogenome sequencing and assembly. Muscle tissue from an adult male of *G. commissarisi* (Figure 1) was collected in the Ejido Loma Bonita, shore of the Lacantun River, municipality Ocosingo, Chiapas, Mexico (Latitude 16.1014583° N, Longitude -91.00196° W), in March 2017 and preserved as a standard museum voucher specimen (skin and skeleton) following established practices (Sikes *et al.* 2011). The tissue was stored at the Mammal tissue collection of El Colegio de la Frontera Sur (ECO-SC-M 8490). Genomic DNA was extracted using a modified phenol-chloroform protocol (Sambrook and Russell 2001). DNA quality was assessed with a Nanodrop 2000 and a quantified Qubit 4 fluorometer. A pair-end (PE, 150 bp) shotgun library was constructed and sequenced on an Illumina NovaSeq 6000 platform at Novogene (Sacramento, CA). 68,005,078 reads in FASTQ format were generated and utilized for *de novo* assembly of the mitochondrial genome (Illumina 2020).

To characterize the genomic structure and codon usage of the mitochondrial genome of *G. commissarisi* we obtained a *de novo* assembly with GetOrganelle v1.7.7.0 with k-mer sizes of 21, 45, 65, 85, and 105, using the mitochondrial genome (seed file) of congeneric *Glossophaga mutica* (Genebank: OR263465.1) (Jin *et al.* 2020). To guarantee a high-quality genome, we analyzed the depth of coverage. The annotation of the complete mitochondrial genome was performed using the web server MITOS2 hosted in Galaxy with the vertebrate code (usegalaxy.org; Donath *et al.* 2019). Nucleotide composition and skew curves of the complete mitogenome and the control region were estimated using a custom R script (R Core Team 2022). We visualized the circular genome using Proksee (Grant *et al.* 2023). Using the Codon Usage web server (vertebrate mitochondrial code), we analyzed PCG codon usage, then calculated amino acid frequencies and RSCU with Ezcodon on the Ezmito web server (http://ezmito.unisi.it/ezcodon; Cucini *et al.* 2021). Mitochondrial tRNA secondary structures, predicted by MiTFi, were visualized using FORNA (Jühling *et al.* 2012; Kerpedjiev *et al.* 2015).

To characterize the patterns of selection in each mitochondrial PCG, we estimated the nonsynonymous (Ka) and synonymous (Ks) substitution rates for each mitochondrial PCG using KaKs_Calculator v2.0.1 to assess selective constraints. The resulting Ka/Ks ratio (ω) for each gene, calculated from pairwise comparisons with *G. leachii* and *G. mutica*, indicated neutral evolution ($\omega = 1$), purifying selection ($\omega < 1$), or positive selection ($\omega > 1$). Calculations employed the γ-MYN model to account for variable mutation rates across sequences (Wang *et al.* 2020).

The control region was examined for microsatellites using the Microsatellite Repeats Finder (Bikandi *et al.* 2004) and for tandem repeats using Tandem Repeats Finder (Benson 1999). The secondary structure of this region was subsequently predicted with FORNA (Kerpedjiev *et al.* 2015).

Phylomitogenomics of Glossophaga commissarisi and family Phyllostomidae. To determine the phylogenetic position of *G. commissarisi* within Phyllostomidae, we used a dataset comprising 55 complete mitochondrial genomes. The ingroup consisted of 51 mitogenomes representing 46 species from the family Phyllostomidae, including our newly sequenced *G. commissarisi*. For the outgroup, we selected four representative species from families closely related to Phyllostomidae, based on recent molecular phylogenies: *Mystacina tuberculata* Mystacinidae), *Myotis nigricans* (Vespertilionidae), *Noctilio leporinus* (Noctilionidae), and *Pteronotus rubiginosus* (Mormoopidae) (Shi and Rabosky 2015).

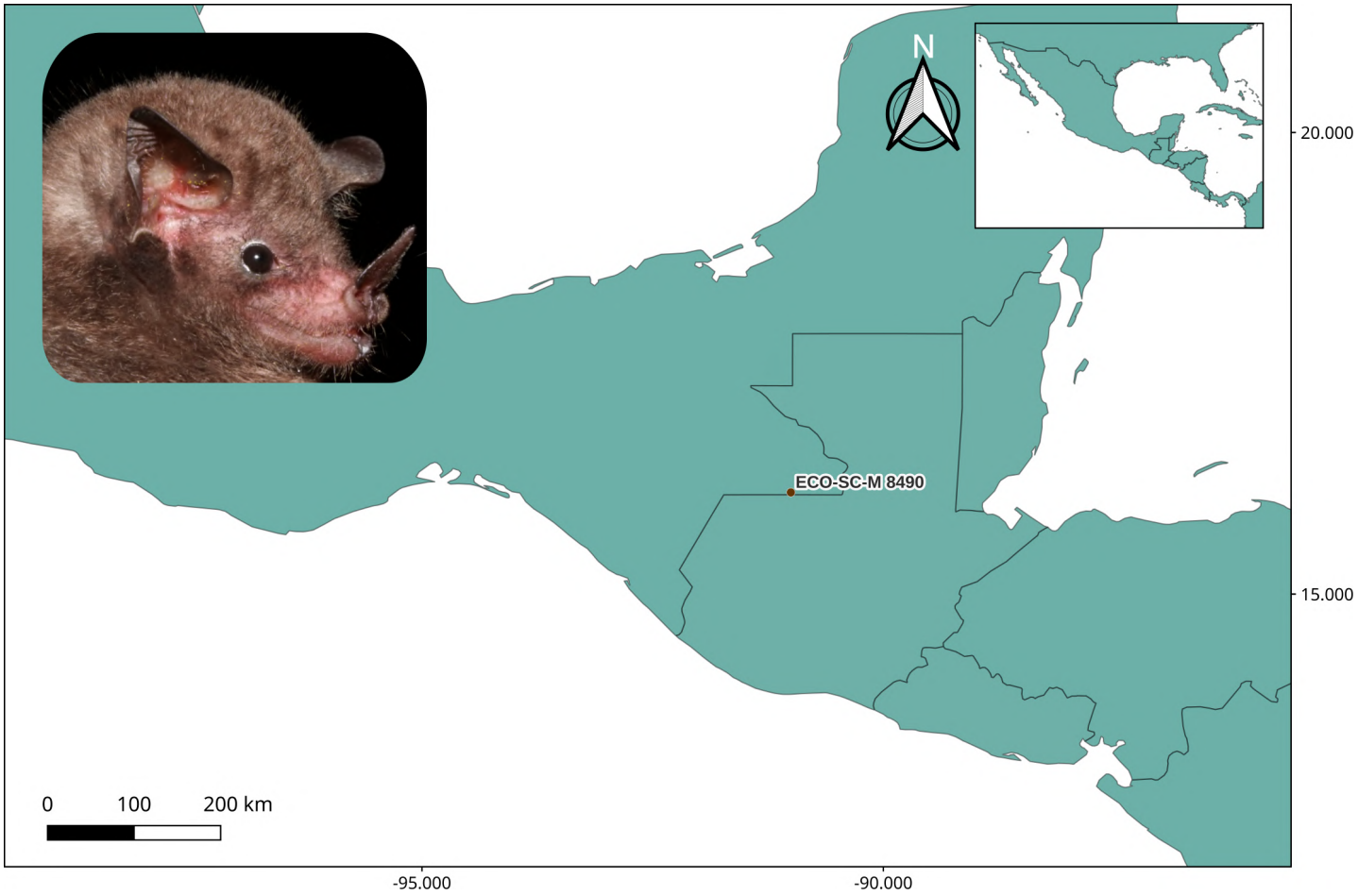

Figure 1. Image of *Glossophaga commissarisi* and its sampling location within the distribution of the species. Collection voucher is denoted in the coordinate label (ECO-SC-M 8490). Photo by Juan Cruzado, used with permission.

A maximum-likelihood (ML) phylogeny was reconstructed using the MitoPhAST v3.0 pipeline (Tan *et al.* 2015). This tool automatically extracted and generated a concatenated and partitioned amino acid alignment from the 13 protein-coding genes (PCGs) across all taxa. This final alignment was then used to build the ML tree with IQ-TREE (Nguyen *et al.* 2015), which automatically selected the best-fit model of protein evolution. The topological robustness of the inferred tree was evaluated with 1,000 bootstrap replicates (Felsenstein 1985).

Results

The mitochondrial genome of Glossophaga commissarisi

The mitogenome (Genbank: PX387959) of Commissaris's long-tongued bat *Glossophaga commissarisi* is 16,648 bp in length (average coverage of 1,684 x) and encodes 37 genes, 13 PCGs, 22 tRNA genes, and two rRNA (rrnS and rrnL) genes, and a 1,202 bp long non-coding region (Table 1; Figure 2; Supplementary Figure 1). Most of the PCGs and tRNAs are encoded on the heavy (H) strand, while the NAD6 gene and eight tRNAs (trnaQ, trnaA, trnaN, trnaC, trnaY, trnaS2, trnaE, and trnaP) are encoded in the light strand (L) (Table 1).

The Control Region in *Glossophaga commissarisi* spans 1,202 bp in length with an A + T content of 59.65%. Microsatellites Repeat Finder analysis identified eight repeats in the CR including five di-nucleotide motifs (AT, TA, CT, CC) and one tri-nucleotide motif (CCA) (Supplementary Table 4). Likewise, Tandem Repeat analysis found a tandem repeat region in the CR spanning positions 776-898 in approximately 10 copies. The motif sequence is CGTATACGCCTA, with base composition A = 24, C = 33, G = 17, T = 25 (Supplementary Table 5).

All tRNA genes exhibit anticodon, acceptors, DHU, and TΨC stems (Supplementary Figure 5) and display a cloverleaf secondary structure, except for tRNA-Serine 1, which usually lacks the DHU arm.

The studied rrnS (12s) and rrnL (16s) genes in *G. commissarisi*'s mitochondrial genome are 971 bp long and 1,573 bp long respectively (see Table 1 of main text). The rrnS gene is positioned between trnaF and trnaV genes, while rrnL is located between trnaV and trnaL2 genes. These genes exhibit an AT composition of 59.94% (12S) and 60.20% (16S).

The nucleotide composition of the positive DNA strand of the mitochondrial genome was as follows: A = 32.18%, T = 29.63%, C = 23.97%, and G = 14.22%, resulting in an overall A + T content of 61.81% and G + C content of 38.19%. The AT skew we observed in the mitogenome is 0.041 (Supplementary Table 1).

The amino acids in each of *Glossophaga commissarisi*'s PCGs are encoded by at least two different codons

Table 1. Characteristics of the mitochondrial genome of *Glossophaga commissarisi*. Continuity refers to the number of overlapping nucleotides between consecutive features: positive values indicate gene overlap, negative values indicate intergenic gaps, and zero indicates adjacent features. Stop codons that are shown with parentheses denote incomplete stop codons.

Feature	Type	Start	End	Strand	Length (bp)	Start codon	Stop codon	Anticodon	Continuity
Phe	tRNA	1	68	+	68			GAA	0
12S rRNA	rRNA	69	1039	+	971				0
Val	tRNA	1040	1107	+	68			TAC	0
16S rRNA	rRNA	1108	2680	+	1573				0
Leu	tRNA	2682	2756	+	75			TAA	1
NAD1	PCG	2759	3715	+	957	ATG	TAA		2
Ile	tRNA	3715	3783	+	69			GAT	-1
Gln	tRNA	3781	3853	-	73			TTG	-3
Met	tRNA	3853	3921	+	69			CAT	-1
NAD2	PCG	3922	4965	+	1044	ATT	TAG		0
Trp	tRNA	4966	5033	+	68			TCA	0
Ala	tRNA	5038	5106	-	69			TGC	4
Asn	tRNA	5108	5180	-	73			GTT	1
OL		5183	5213	-	31				2
Cys	tRNA	5213	5278	-	66			GCA	-1
Tyr	tRNA	5279	5344	-	66			GTA	0
COX1	PCG	5346	6890	+	1545	ATG	TAA		1
Ser	tRNA	6888	6958	-	71			TGA	-3
Asp	tRNA	6962	7028	+	67			GTC	3
COX2	PCG	7029	7712	+	684	ATG	TAA		0
Lys	tRNA	7716	7784	+	69			TTT	3
ATP8	PCG	7786	7989	+	204	ATG	TAA		1
ATP6	PCG	7947	8627	+	681	ATG	TAA		-43
COX3	PCG	8627	9410	+	784	ATG	T(AA)		-1
Gly	tRNA	9411	9480	+	70			TCC	0
NAD3	PCG	9481	9828	+	348	ATT	TAA		0
Arg	tRNA	9829	9896	+	68			TCG	0
NAD4L	PCG	9897	10193	+	297	ATG	TAA		0
NAD4	PCG	10187	11564	+	1378	ATG	T(AA)		-7
His	tRNA	11565	11632	+	68			GTG	0
Ser	tRNA	11633	11691	+	59			GCT	0
Leu	tRNA	11693	11762	+	70			TAG	1
NAD5	PCG	11763	13592	+	1830	ATA	TAA		0
NAD6	PCG	13567	14094	-	528	ATG	TAA		-26
Glu	tRNA	14096	14164	-	69			TTC	1
CYTB	PCG	14174	15313	+	1140	ATG	AGA		9
Thr	tRNA	15314	15380	+	67			TGT	0
Pro	tRNA	15380	15446	-	67			TGG	-1
OH		15746	16019	+	274				299
CR	D-loop	15447	16648		1202				

(Supplementary Table 2), with a preference for codons ending in adenine or thymine, while codons ending in guanine are less frequently used. Relative synonymous codon usage (RSCU) and amino acid frequency are summarized (Supplementary Figure 2). The most frequently used codons include ATA (Ile), TTT (Phe), and AAA (Lys), whereas CGG (Arg) and GCG (Ala) are among the least frequently used codons.

Analysis of Ka/Ks ratios for all mitochondrial PCGs (Supplementary Figure 3; Supplementary Table 3) revealed values below 1 ($P < 0.001$). CYTB, COX1, COX2, and NAD4L exhibited the lowest Ka/Ks ratios, whereas ATP8, NAD1, and NAD6 showed relatively higher values. The average Ka/Ks across all 13 PCGs ranged from 0.016 to 0.529, depending on the gene and comparison.

Phylomitogenomics of Glossophaga commissarisi. The ML phylogenetic analysis recovered the expected relationships within Phyllostomidae with high bootstrap support (Figure 3). Within the nectarivorous Glossophaginae, *Glossophaga* sequences clustered

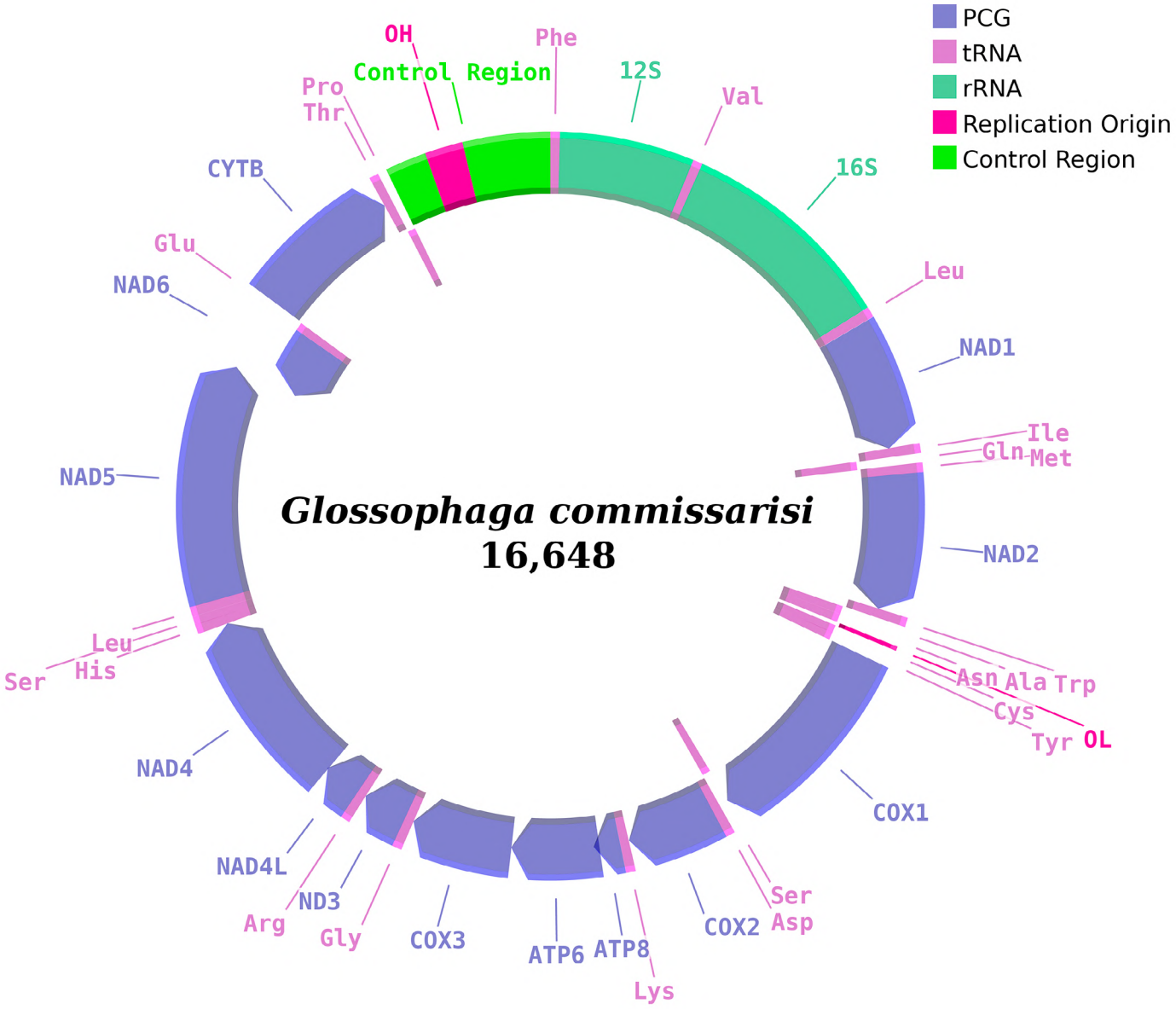

Figure 2. Circular representation of the mitochondrial genome of *Glossophaga commissarisi*. Colors indicate the composition and arrangement of genes. The origins of replication of the light strand (OL) and heavy strand (OH) are annotated in the figure.

together (bv = 100), with *G. soricina* sister to the *G. mutica* clade, and *G. leachii* and *G. commissarisi* forming a strongly supported subclade (bv = 99.3). *Leptonycteris* species were sister to Glossophaginae (bv = 99.9). Other subfamilies, including Phyllostominae, Stenodermatinae, and Lonchorhininae, were recovered with variable support among nodes (bv = 99.6–100).

Discussion

The mitochondrial genome of Glossophaga commissarisi. The gene arrangement in the *Glossophaga commissarisi* mitochondrial genome is similar to those previously reported for phyllostomids and species of the subfamily Glossophaginae (Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025). The Control Region is moderately shorter than congeneric species in *Glossophaga* (Supplementary Figure 4) (Rocamontes-Morales *et al.* 2025). The length and structural features of the tRNA genes resemble those observed in congeneric *Glossophaga*, other phyllostomids and beyond (Meganathan *et al.* 2012; Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025).

The overall nucleotide composition is consistent with the range observed in congeneric *Glossophaga* species, and other Phyllostomids (Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025). Likewise, the observed codon usage patterns have been observed in other congeneric species and other Phyllostomid bats (Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025).

Analysis of evolutionary pressures shows that the mitogenome of *Glossophaga commissarissi* has evolved

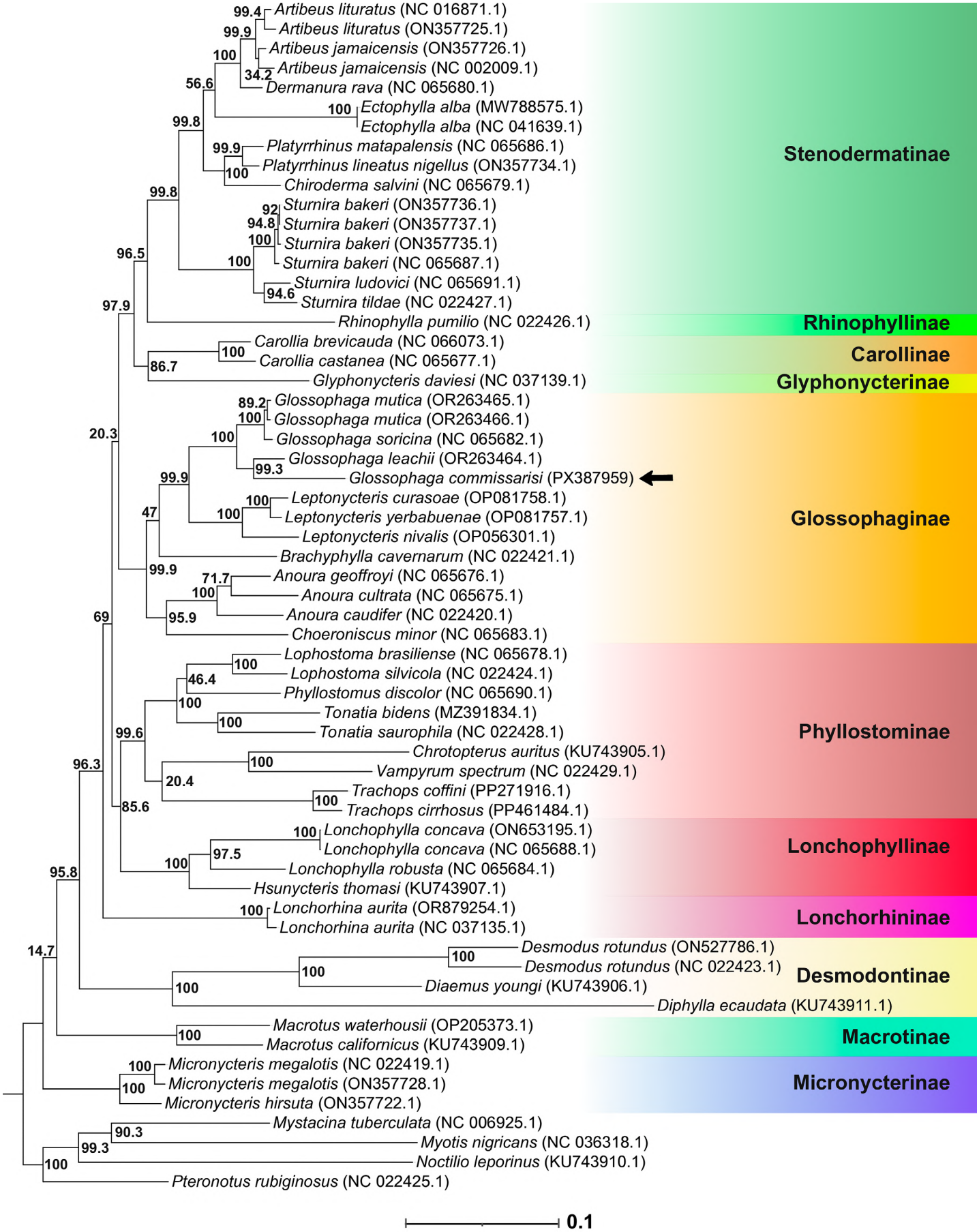

Figure 3. Maximum-likelihood phylogeny of the Phyllostomidae based on mitochondrial genomes. The newly assembled *Glossophaga commissarisi* genome from this study is highlighted with an arrow. Accession numbers are provided in parentheses for all species. Node support is indicated by bootstrap values (shown above/below branches). Species from the Mystacinidae, Vespertilionidae, Noctilionidae, and Mormoopidae families were used as an outgroup.

under strong purifying selection, as evidenced by Ka/Ks ratios below 1 for all 13 protein-coding genes. This finding is consistent with patterns documented across other phyllostomid bats, supporting the conserved functional role of these mitochondrial genes (Vivas-Toro *et al.* 2021; Baeza *et al.* 2022; Barrera *et al.* 2023; Camacho et al. 2022; Vargas-Trejo *et al.* 2023; Rocamontes-Morales *et al.* 2025).

Phylomitogenomics of Glossophaga commissarisi. Our phylogenetic results are largely congruent with previous studies based on concatenated mitochondrial genes (e. g., CYTB, ND2) and multi-locus nuclear datasets, which consistently recover *Glossophaga* as a monophyletic group and place *G. commissarisi* as sister to *G. leachii* (Hoffmann *et al.* 2019; Rocamontes-Morales *et al.* 2025). However, the use of complete mitogenomes in this study provided substantially higher nodal support across the phylogeny, particularly for deeper relationships within Phyllostomidae. For example, the clade containing *G. commissarisi* and *G. leachii* received a bootstrap value of 99.3, and monophyly of Glossophaginae was recovered with full support (bv=100). This contrasts with some earlier studies using fewer mitochondrial markers, where support for these same models was moderate or variable (e.g., Hoffmann *et al.* 2019). The increased signal provided by the concatenated alignment of all 13 protein-coding genes likely contributed to this improved resolution, underscoring the utility of phylomitogenomic approaches in resolving relationships within rapidly diversifying groups like the phyllostomid bats.

Conclusions

In this study, we assembled and annotated the complete mitochondrial genome of *G. commissarisi*. A phylogenetic tree was reconstructed based on all translated PCGs and supports *G. commissarisi* as sister to *G. leachii,* also forming a well-supported clade with *Glossophaga* and subfamily Glossophaginae. These findings provide genetic resources for future studies on the complex and seldom-studied *Glossophaga* genus. We recommend that future research sequence remaining congeners to resolve the comprehensive phylogenetic relationships within the entire genus.

Ethical approval

The material used in this study does not involve ethical conflicts. Tissue samples were donated by the Mammal Tissue Collection of El Colegio de la Frontera Sur, Unidad San Cristóbal de Las Casas, managed by Consuelo Lorenzo. Tissue was collected with collection permit SGPA/DGVS/01186/17 of Consuelo Lorenzo. *G. commissarisi* is a non-endangered species, and all procedures followed the ethical standards outlined in the ARRIVE guidelines and the American Society of Mammalogists (Sikes *et al.* 2011). The protocol for this research project was reviewed and approved by the ECOSUR Ethics Committee for Research (CEI-05-07-2023b).

Acknowledgements

We thank the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCyT) for funding this project (FORDECYT-PRONACES No. 15307), and the Instituto Politécnico Nacional (Project SIP 20251003). We also wish to thank Maricela García Bautista for her technical support during DNA extractions at the Laboratorio Institucional de Genética ECOSUR, Unidad San Cristóbal de Las Casas. We thank anonymous reviewers for their comments that helped improve this manuscript.

Data availability statement

The mitochondrial genome sequence can be accessed via accession number PX387959 in the GenBank of NCBI. The associated BioProject and SRA numbers are PRJNA1021227 and SAMN51504582. The tissue for *G. commissarisi* (ECO-SC-M 8490) was stored at the ECOSUR, Unidad San Cristobal de Las Casas mammal tissue collection managed by Consuelo Lorenzo (clorenzo@ecosur.mx).

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Associated editor: Ella Vázquez Domínguez
Submitted: October 22, 2025; Reviewed: November 25, 2025
Accepted: January 6, 2026; Published on line: January 30, 2026

THERYA, 2026, Vol. 17(1):81-98 DOI: 10.12933/therya.2026.6236 ISSN 2007-3364

Exploring species boundaries of the spotted ground squirrel (*Xerospermophilus spilosoma*) complex

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Xerospermophilus spilosoma exhibits notable geographic and morphological variation, prompting a debate over its taxonomic status. Currently, it is unclear whether it represents a single highly variable species or a complex of cryptic species that includes *X. perotensis*. Although the latter has a larger body size and a distinctive dorsal pattern, current genetic analyses do not support its recognition as a separate species. This study aimed to delimit potential evolutionary units within the *X. spilosoma* complex. Twenty-four sequences of the mitochondrial *cytochrome b* gene were analyzed using 690-bp fragments from *X. spilosoma* and *X. perotensis* specimens collected from eight locations. Phylogenetic and divergence time inferences were estimated using Maximum Likelihood and Bayesian Inference, along with analyses of genetic distances and haplotype networks. Three species delimitation methods (ABGD, PTP, and GMYC) were applied, and the ecological uniqueness and areas of overlap within and between species of the *X. spilosoma* complex were assessed. Four lineages comprising 22 unique haplotypes were identified, with interpopulation genetic distances ranging from 3 % to 6 %. Species delimitation methods suggested between one and four potential species. Meanwhile, the comparison of ecological niches revealed limited overlap. Genetic and environmental evidence indicate that *X. spilosoma* comprises at least three evolutionarily independent lineages. The complex originated in the Miocene, more than 5 million years ago, with divergence events concentrated between 3.5 and 1.5 million years ago, in accordance with geographical barriers such as Río Grande and the Nazas River and the Trans-Mexican Volcanic Belt. These results highlight the need to conserve these populations as independent evolutionary units, particularly the Perote population, given its isolation and ecological and genetic uniqueness.

Keywords: Chihuahuan Desert; *cytochrome b*; Río Grande; Taxonomy.

Xerospermophilus spilosoma presenta una notable variación geográfica y morfológica, lo que ha generado debate sobre su estatus taxonómico. Actualmente se discute si representa una única especie o un complejo de especies crípticas que incluiría a *X. perotensis*. Aunque esta última presenta mayor tamaño corporal y un patrón dorsal distintivo, los análisis genéticos actuales no respaldan su reconocimiento como especie válida. El objetivo de este trabajo fue delimitar las posibles unidades evolutivas dentro del complejo *X. spilosoma*. Se analizaron 24 secuencias del gen mitocondrial *citocromo b,* usando fragmentos de 690 pb de *X. spilosoma* y *X. perotensis,* de ocho localidades distintas. Las inferencias filogenéticas y de tiempo de divergencia se estimaron mediante Máxima Verosimilitud e Inferencia Bayesiana, junto con como análisis de distancias genéticas y redes de haplotipos. Se aplicaron tres métodos de delimitación de especies (ABGD, PTP y GMYC), además de evaluarse el nicho ecológico por especie y sobreposicionamiento entre especies del complejo *X. spilosoma*. Se identificaron cuatro linajes con 22 haplotipos únicos con distancias genéticas interpoblacionales entre el 3 y el 6%. Los métodos de delimitación de especies sugieren entre una y cuatro especies potenciales. Por su parte, la comparación de los nichos ecológicos mostró un bajo solapamiento de áreas. La evidencia genética y ambiental obtenida sugiere que *X. spilosoma* corresponde a un complejo de al menos tres linajes evolutivamente independientes. El complejo se originó en el Mioceno, hace más de 5 Ma, con eventos de divergencia concentrados entre 3.5 y 1.5 Ma, en concordancia con barreras geográficas como los ríos Grande y Nazas y la Faja Volcánica Transmexicana. Estos resultados, resaltan la necesidad de conservar estas poblaciones como unidades evolutivas independientes, especialmente la de Perote, por su aislamiento y singularidad ecológica y genética.

Palabras clave: *Citocromo b*; Desierto Chihuahuense; Río Grande; Taxonomía.

Establishing the boundaries between species is a difficult task, especially in taxa for which speciation processes have not resulted in an evident morphological differentiation (Goldstein and De Salle 2011; Fišer *et al.* 2018). This can be explained by phenomena such as convergence (Losos 2008, 2011), stabilizing selection (Gould 2002; Hansen and Houle 2004), or a recent speciation process (Gittenberger 1991; Rundell and Price 2009). A major problem in taxonomy is that species boundaries vary widely depending on the species concept employed (De Queiroz 2007). Therefore, a unified concept based on their evolutionary origin has been proposed, defining them as evolutionarily independent lineages. These lineages are identified by evaluating secondary characteristics, e.g., ecological, morphological,

and genetic traits, that reflect some degree of evolutionary independence (Simpson 1961; Wiley 1978; Bock 2004; Hey 2006; De Queiroz 2007).

With more than 2600 species, the order Rodentia is the most diverse group of mammals worldwide (D'Elía *et al.* 2019). This diversity is partly due to their high evolutionary rates and rapid radiation processes, often associated with evolutionary convergences, which has produced taxonomic complexities that still require resolution (Triant and De Woody 2006; Fabre *et al.* 2012; Burgin *et al.* 2018). Within this order, squirrels of the family Sciuridae stand out for their diversity, with about 300 species distributed in a wide range of ecosystems, from deserts to tropical forests, and from sea level to more than 4500 meters above sea level. This family exhibits great morphological variation, including arboreal, terrestrial, and gliding forms, with solitary or social patterns (Koprowski *et al.* 2016; Rocha *et al.* 2016). In addition, their biological characteristics, such as low dispersal capacity, short life cycles, and varied reproductive strategies, allow for a detailed analysis of the differentiation and evolutionary isolation processes, making squirrels ideal models for genetic and evolutionary studies (Rocha *et al.* 2016; Flores-Manzanero and Vázquez-Domínguez 2019; Waterman *et al.* 2021).

The current distribution of most squirrel species in North America is explained by allopatric speciation processes, where geographic barriers such as mountain systems and water bodies have limited gene flow (Harrison *et al.* 2003; Ge *et al.* 2014; Zelditch *et al.* 2015). For example, the Snake River, in the northwestern United States, has influenced the diversification of small-eared ground squirrel species of the genus *Urocitellus* (McLean *et al.* 2025). In this context, several current species have been isolated in valleys due to geological events that occurred during the Quaternary period, such as fluctuating glaciations or volcanic activity (Harrison *et al.* 2003; Van Tuinen *et al.* 2008; Menéndez *et al.* 2021). A peculiar case is that of the Mojave ground squirrel, *Xerospermophilus mohavensis*, whose possible origin involved allopatric speciation in a small isolated refuge within the Mojave Desert, delimited by the Sierra Nevada (Bell *et al.* 2010).

However, geographic isolation does not always imply sufficient ecological or genetic divergence to justify the separation into distinct species (Barton 2020; Kulmuni *et al.* 2020). An example is the population of Mearns squirrels *(Tamiasciurus mearnsi)* in Baja California, for which genetic studies indicate a relatively recent separation and a limited ecological differentiation despite being geographically isolated from *Tamiasciurus douglasii*, leading to the question of whether *T. mearnsi* should really be considered a distinct species (Arbogast *et al.* 2001; Pecnerová and Martinova 2012; Hope *et al.* 2016). Therefore, examining the niches of species in the environmental space and projecting them in the geographical space constitutes an additional line of evidence to evaluate whether populations, in addition to being physically separated, have occupied and exploited different environments, which could indicate processes of ecological differentiation that support or facilitate the delimitation of species (Peterson *et al.* 2000; Kozak and Wiens 2006; Pahad *et al.* 2019). Similarly, niche modeling studies contribute to understanding the relationship between genetic variation patterns and the environmental parameters that limit the distribution of a species (Ashrafzadeh *et al.* 2018; Calixto-Pérez *et al.* 2018; Luna-Aranguré and Vázquez-Domínguez 2020).

A taxonomic review of the genus *Spermophilus* based on morphology and sequences of the *cytochrome b* (*cytb*) mitochondrial gene revealed its paraphyletic condition, which led to the reassignment of several species to new genera. Thus, *Xerospermophilus* was recognized as a genus that includes *X. mohavensis*, *X. tereticaudus*, *X. spilosoma,* and *X. perotensis* (Helgen *et al.* 2009). However, the phylogenetic relationship between *X. spilosoma* and *X. perotensis* remains a matter of debate, particularly with regard to their recognition as separate species (Harrison *et al.* 2003; Helgen *et al.* 2009; Fernández 2012).

Xerospermophilus spilosoma is the most divergent species that is distributed over a wide geographic range, from Wyoming, South Dakota, and Nebraska to central Mexico (Lacher *et al.* 2016; Álvarez-Castañeda 2024). This wide distribution has favored a high morphological diversity within the species, including variation in coat coloration from cinnamon to dark smoky-gray shades, through various shades of brown. In addition, it has a series of spots on the back and flanks whose shape and intensity vary depending on the environment, probably in response to local ecological conditions (Cothran *et al.* 1977; Álvarez-Castañeda 2024). As a result of this morphological variation, 13 nominal subspecies have been described (Helgen *et al.* 2009; Mammal Diversity Database 2025).

The taxonomic classification of *X. spilosoma* is currently a subject of debate. It is not clear whether it is a single species with high population variability that includes several subspecies (Helgen *et al.* 2009; Fernández 2012), including *X. perotensis*, or if the possible paraphilia observed between the subspecies of *X. spilosoma* and *X. perotensis* indicates that it could be a complex of species (Harrison *et al.* 2003).

Although *X. perotensis* has a larger body size, differences in coloration with a distinctive pattern of dorsal spots and distinct vocalizations, the genetic analyses available to date, based on nuclear (GHR and IRBP) and mitochondrial (Cytb and 12S rRNA) genes, suggest that genetic divergence would not be sufficient to support its recognition as a separate species (Fernández 2012). However, it is worth noting that these conclusions were based on an limited number of specimens, namely 3 individuals of *X. spilosoma* and 4 of *X. perotensis*. Therefore, the objective of this study was to evaluate the differentiation of populations within the *X. spilosoma* complex and their possible status as species using four distinct lines of evidence: (1) phylogenetically delineating the evolutionary units; (2) estimating genetic distances between populations to quantify their

divergence; (3) applying species delimitation methods that allow the identification of separate evolutionary lineages; and (4) comparing the degree of ecological differentiation between populations.

Materials and methods

Sample collection. A total of 23 tissue samples were processed, from specimens collected in the field (n = 12) and from specimens obtained from different national and international scientific collections (n = 11). The field collection consisted of ectomization of the third phalanx from *X. perotensis* and *X. spilosoma* specimens captured in Perote and Mapimí, respectively. Sample collection followed the recommendations of Romero-Almaraz *et al.* (2007) and the guidelines of the Committee on Animal Care and Use of the American Society of Mammalogists (Sikes *et al.* 2016). The corresponding collection permits were issued by the Board of the Environment and Natural Resources (SPARN/DGVS/04074/23 and SPARN/DGVS/08359/23). The tissues were fixed separately in 96% ethanol and stored at -70 °C until processing.

Genomic DNA (gDNA) was extracted from the tissues using the DNeasy Blood and Tissue Kit (QIAGEN, USA) according to the manufacturer's instructions. Subsequently, the concentration of the genetic material was quantified in a Nanodrop spectrophotometer (model DS-11, Denovix). To perform the amplification of *cytb* fragments by Polymerase Chain Reaction (PCR), a new pair of oligonucleotides was designed in PerlPrimer v1.1.21: Xeros_Fw (5'YSAYTTACMYGCACCYTCC-3') and Xeros_Rv (5'GGRTATWCAACRGGTTGYCMTC 3'), which amplifies a 981-bp fragment. This primer pair was evaluated *in silico* using SnapGene v.8.1 to verify its specificity, hybridization efficiency, and absence of secondary structures.

The PCR reactions were run in a final volume of 25 µL per sample, containing 2 µL of gDNA (with concentrations between 20 and 200 ng/µL), 14.25 µL of nuclease-free H_{2O}, 6.25 µL of Master mix DreamTaq (QIAGEN, USA), and 2 µL of each primer at 10 µM. One positive control and one negative control were included in each run to ensure procedural reliability and to rule out contamination. The amplification protocol consisted of an initial denaturation step at 94 °C for 5 minutes, followed by 40 denaturation cycles at 94 °C for 30 seconds, alignment at 56 °C for 30 seconds, extension at 72 °C for 70 seconds, and a final elongation step at 72 °C for four minutes. The PCR tests were performed on a BioRad T100 thermal cycler. PCR products were visualized on 2 % agarose gels, stained with red gel, and examined under UV light using a transilluminator. The PCR products were then purified using the Qiaquick Gel Extraction Kit (QIAGEN, USA) according to the manufacturer's instructions. The purified products were sent for bidirectional sequencing to Macrogen, South Korea.

In total, 24 *Xerospermophilus* samples were considered for subsequent analyses. Of these, 12 were generated in this study (7 for *X. spilosoma* and 5 for *X. perotensis*), and 12 were recovered from GenBank. The sequences were edited manually, then aligned using the MUSCLE algorithm, and visually inspected using Geneious Prime and MEGA X (Kumar *et al.* 2018). The final alignment included 14 sequences for *X. spilosoma*, 10 for *X. perotensis* (Appendix 1), and one for *Ictidomys mexicanus* as an outgroup (Harrison *et al.* 2003; Guevara-Chumacero *et al.* 2006; Fernández 2012).

Phylogenetic analyses and genetic diversity. From the global alignment, the best substitution model was selected through an exhaustive search in JModelTest v2.1.10 (Darriba *et al.* 2012). Phylogenetic inference was performed under the Maximum Likelihood (ML) criterion using the previously selected model (GTR + G), with estimated branch support across 10 000 ultrafast bootstrap replicates (Hoang *et al.* 2018) in IQ-TREE v1.6.12 (Nguyen *et al.* 2015). Bayesian Inference (BI) was performed in MrBayes v.3.2.7 (Ronquist *et al.* 2012) with the MCMC algorithm and the previously calculated substitution model (GTR + G + I); to this end, two separate runs were performed with three hot chains and one cold chain with 10 million generations and a 25 % burn-in. Chain convergence and good sampling (ESS > 200) were assessed in TRACER v1.7 (Rambaut *et al.* 2018). The consensus trees generated in each inference were visualized and edited using FigTree v1.4.4. (Rambaut 2010).

Subsequently, a haplotype file was generated in DnaSP v6 (Rozas *et al.* 2017), from which the number of haplotypes was identified, and haplotype diversity (Hd) and nucleotide diversity (Pi) were calculated. With this information, a haplotype network was constructed using the TCS criterion in PopArt v1.7 (Leigh *et al.* 2015). Additionally, an analysis of intra- and interclade genetic distances was performed using uncorrected pairwise sequence distances (p-distances) using the *ape* library (Paradis *et al.* 2004). Based on these data, a heat map was drawn up with the *adegenet* and *split* libraries (Jombart 2008; Ezard *et al.* 2009), both in R v4.4.1 (R Core Team 2020). Genetic distances within the genus *Xerospermophilus* were evaluated using 15 sequences of *X. tereticaudus* and 10 sequences of *X. mohavensis* (Harrison *et al.* 2003; Bell *et al.* 2010; Fernández 2012).

Divergence times. With the alignment constructed as described above, divergence times were inferred in BEAST v2.6 (Bouckaert *et al.* 2019), considering a non-correlated lognormal relaxed molecular clock and replacing *I. mexicanus* for *Cynomys ludovicianus* (Appendix 1) as the outgroup (Castellanos-Morales *et al.* 2014). GTR substitution models with the gamma distribution and the Bayesian Coalescent Skyline plot model were established.

A secondary calibration based on four nodes was performed. The first node was calibrated based on the fossil record of *Cynomys rafinesque*, with a maximum age of 1.8 Ma (Ge *et al.* 2019), representing the divergence between *Cynomys* and the *X. spilosoma* complex. The second node corresponds to the separation between the Kansas, Texas, and New Mexico populations, in the USA, and those of Arizona and Mexico, attributed to the formation of the Rio Grande, whose average consolidation is estimated to have

occurred 2.6 Ma ago (Morgan and Golombek 1984; Repasch 2017). The third node reflects the divergence between the populations of Arizona, USA, and Mapimí, Mexico, in contrast to the populations of the Central Plateau of Mexico, associated with the Nazas River, whose origin dates back to the Pliocene and with its current configuration estimated to have occurred 1.5 Ma ago (Petersen 1976; Hafner and Riddle 2011). Finally, the fourth node represents the isolation of the Perote population, in Mexico, attributable to the culmination of the formation of the Trans-Mexican Volcanic Belt, completed approximately 1.6 Ma ago (Ferrari *et al.* 1999, Ferrari 2000; Gámez *et al.* 2017).

All nodes were assigned a lognormal distribution as a prior, as this model reflects the observation that evolutionary divergence typically precedes both the first fossil record and the complete establishment of a geographic event. Under this perspective, speciation does not occur at the same time that a fossil appears or a barrier is formed; instead, these elements represent only minimum age limits (Ho 2007). MCMC analyses were performed with two independent runs of 10 million generations each, sampling every 1000 generations. The convergence, stability, and adequate sampling of the results were evaluated with Tracer v1.7.1 (Rambaut *et al.* 2018), applying a 25 % burn-in. The final phylogenetic tree that included the divergence time intervals was generated using TreeAnnotator v2.6.2 (Bouckaert *et al.* 2019). The graphs were generated using the *deeptime* (Gearty 2025) and *ggplot2* (Wickham 2016) packages in R.

Species delimitation. Boundaries between species within the *X. spilosoma* complex were evaluated using three delimitation methods: the Automatic Barcode Gap Discovery (ABGD), which is based on the identification of a barcode corresponding to the natural discontinuity between intraspecific (minor) and interspecific (major) genetic variability. ABGD groups sequences according to this discontinuity, without the need for an *a priori* hypothesis about the number of species (Puillandre *et al.* 2012). The Poisson Tree Processes (PTP) method, based on a maximum likelihood model, assesses differences in substitution rates between clades to detect clusters that are consistent with putative species (Zhang *et al.* 2013), while the Generalized Mixed Yule Coalescent (GMYC) method uses an ultrametric tree to analyze branch lengths and determine whether they correspond to intraspecific or interspecific processes (Fujisawa and Barraclough 2013).

For ABGD, the previously calculated genetic distance matrix was used. The configuration of the parameters was (i) a minimum intraspecific distance (Pmin) of 0.001 and a maximum intraspecific distance (Pmax) ranging from 0.02 to 0.1; (ii) a Barcode Gap Width of 1.5; and (iii) the Jukes-Cantor model (JC69); for the analysis, we used the command line in the ABGD program (Puillandre *et al.* 2012). For PTP, we used the ML tree generated with 100 000 MCMC generations and a 10 % burn-in on the PTP web server (http://species.h-its.org/). For GMYC, we used an ultrametric guide tree of the genus *Xerospermophilus* generated in MEGA X (Kumar *et al.* 2018). A multiple method was applied using a $\lambda = 5$ to fit the molecular-clock model and evaluated confidence intervals (0 - 2); the model was performed using the *splits* package in R (Ezard *et al.* 2009).

Niche modeling. In addition to the genetically analyzed samples, we included 404 records of the presence of *X. spilosoma* and *X. perotensis* from GBIF (2024). These records were organized according to the identification of terrestrial ecoregions proposed by Olson *et al.* (2001) corresponding to each clade defined in the phylogenetic analyses and the delimitation of potential geographical barriers between records: the Rio Grande between clades A and B, the Nazas River between clades B and C, and a minimum convex polygon for clade D, subsequently intersected with the aforementioned ecoregions. A 6 km buffer was established for each polygon delimited by these ecoregions and barriers. This distance was set given the low mobility reported for these squirrels, whose mean dispersal distance is approximately 1.5 km (maximum 2.8 km) (Montero-Bagatella and González-Romero 2014). For clades A and D, the delimitation was obtained directly with this procedure. For clade B, a northern limit was established considering a 30 km buffer with respect to the clade A polygon and applying a 21 km reduction to the south. For clade C, a 21 km reduction to the north was applied, maintaining the previous delimitation to the south, which delineated a 30 km strip between the clades A, B, and C polygons. Based on these polygons, records were classified and grouped for modeling by clades, excluding those located within the 30 km strips or outside the polygons; this procedure was carried out in ArcMap v.10.5.

The ecological niche models were constructed using the 19 bioclimatic variables available in the WorldClim platform (WorldClim 2024), which are in raster format with a 30 arcsec (1 km^2) resolution. The models were generated using the Maximum Entropy algorithm, implemented in MAXENT v3.4.1 (Phillips *et al.* 2017). First, a general model was constructed for each clade to identify the most representative variables. To this end, we used the contribution and permutation percentage table, as well as the Jackknife test, both available in the MAXENT outputs, to assess the relative importance of the explanatory variables. Based on the variables selected by clade, a consensus was performed, selecting those relevant to the four clades.

Subsequently, a Pearson correlation analysis was performed on the previously selected variables. Highly correlated variables ($r > 0.85$) were excluded to avoid collinearity and reduce redundant information (Segurado *et al.* 2006). We selected seven correlated variables with a simpler environmental interpretation that do not combine humidity and temperature data: BIO1 (mean annual temperature, °C), BIO2 (mean diurnal temperature range), BIO4 (temperature seasonality, %), BIO10 (mean temperature of the warmest quarter, °C), BIO11 (mean temperature of the coldest quarter, °C), BIO15 (seasonality

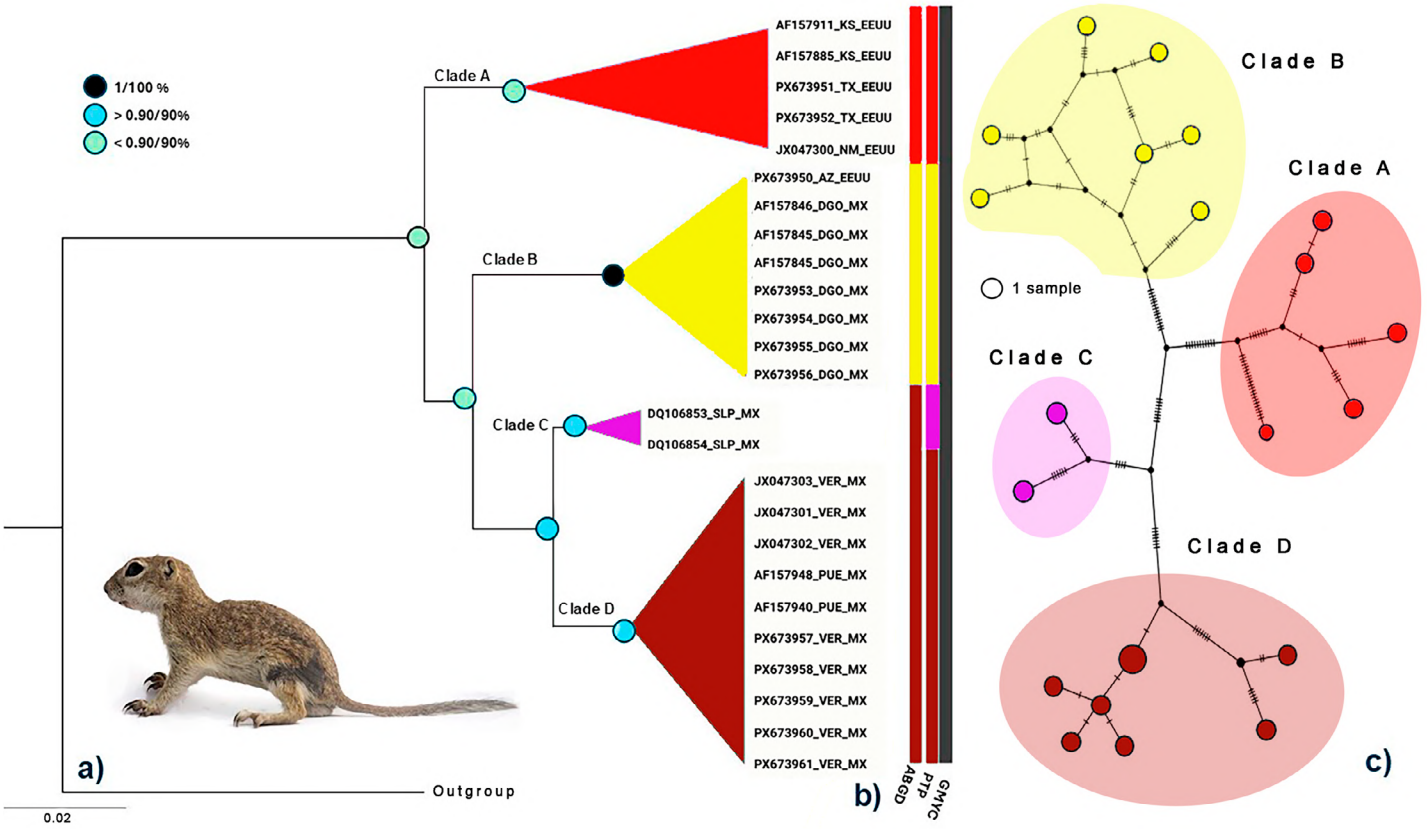

Figure 1. Inferred phylogenetic tree for the *Xerospermophilus spilosoma* complex based on mitochondrial *cytochrome b* gene sequences (690 bp) using the BI and ML methods (a). Circles represent post-bootstrap probability values. (b) Results of species delineation analyses suggesting the presence of between 1 and 4 potential species within the *X. spilosoma complex*. (c) Haplotype networks observed for the different populations analyzed; lines perpendicular to the main branches indicate the estimated number of evolutionary steps.

of precipitation, variability index), and BIO16 (precipitation of the wettest quarter, mm).

Once the clade-based groups were defined, we performed a 1 km spatial filtering of the final records (clade A, 151; clade B, 72; clade C, 37; clade D, 37) using the *Wallace* package (Kass *et al.* 2018). Then, the models for each group were calibrated using the accessibility or mobility area with 20 000 randomly selected background pixels. This selection represents a hypothesis about the area to which the species has or has had access to disperse (Barve *et al.* 2011). Subsequently, a spatial partitioning of occurrence data was performed to train and validate the models. The method implemented was the chessboard, considering an aggregation factor of 2 (Muscarella *et al.* 2014). Models with linear, quadratic, and combined functions were fitted using regulation multipliers between 0.5 and 3 (0.5 intervals), selecting the best models according to the AIC (Burnham and Anderson 2002). From these, binary (presence/absence) distribution maps were generated according to the biogeographic provinces of Olson *et al.* (2001).

Additionally, niches were characterized in a multivariate space using a Principal Component Analysis (PCA), performing the ordination through a correlation matrix and using Mahalanobis distance approximations (Broennimann *et al.* 2012). The degree of overlap or divergence between the ecological niches of the candidate species was evaluated using similarity and equivalence statistical tests implemented in the *ecospat* v4.2.1 package (Di Cola *et al.* 2017). In both cases, the overlap between niches was quantified using Schoener's-*D* index, whose values range from 0 (completely discordant niches) to 1 (identical niches) (Schoener 1970; Warren *et al.* 2008).

The niche similarity analysis assesses whether the niches of two species are more similar than expected by chance, accounting for the environmental context in which they occur. In this test, a *P*-value < 0.05 suggests that niches are more similar than expected by chance (Broenniman *et al.* 2012). In contrast, in the niche equivalence test, the observed *D*-value is compared to a null distribution constructed from random reassignments of occurrences of each species pair (Brown and Carnaval 2019). In this test, a *P*-value < 0.05 indicates that the niches are more different than expected by chance. For each test, 100 replicates were performed to generate a null distribution of overlap scores, which was compared to the observed values.

Results

The final alignment comprised 690 bp, with 146 variable sites, 81 of which were parsimony-informative. The ML and BI topologies were consistent, recovering the same phylogenetic relationships and with support values greater than 0.85 / 85 %, respectively. Four clades were identified (Figure 1a): clade A includes sequences from the USA populations (Kansas, Texas, and New Mexico); clade B, from Arizona, USA, and Durango, Mexico; clade C only includes sequences from San Luis Potosí, Mexico; finally, clade D

	I. mexicanus	Clade A	Clade B	Clade C	Clade D	*X. tereticaudus*	*X. mohavensis*
I. mexicanus							
Clade A	0.10						
Clade B	0.11	0.06					
Clade C	0.10	0.05	0.05				
Clade D	0.10	0.06	0.05	0.03			
X. tereticaudus	0.10	0.09	0.09	0.08	0.09		
X. mohavensis	0.10	0.08	0.09	0.08	0.09	0.04	

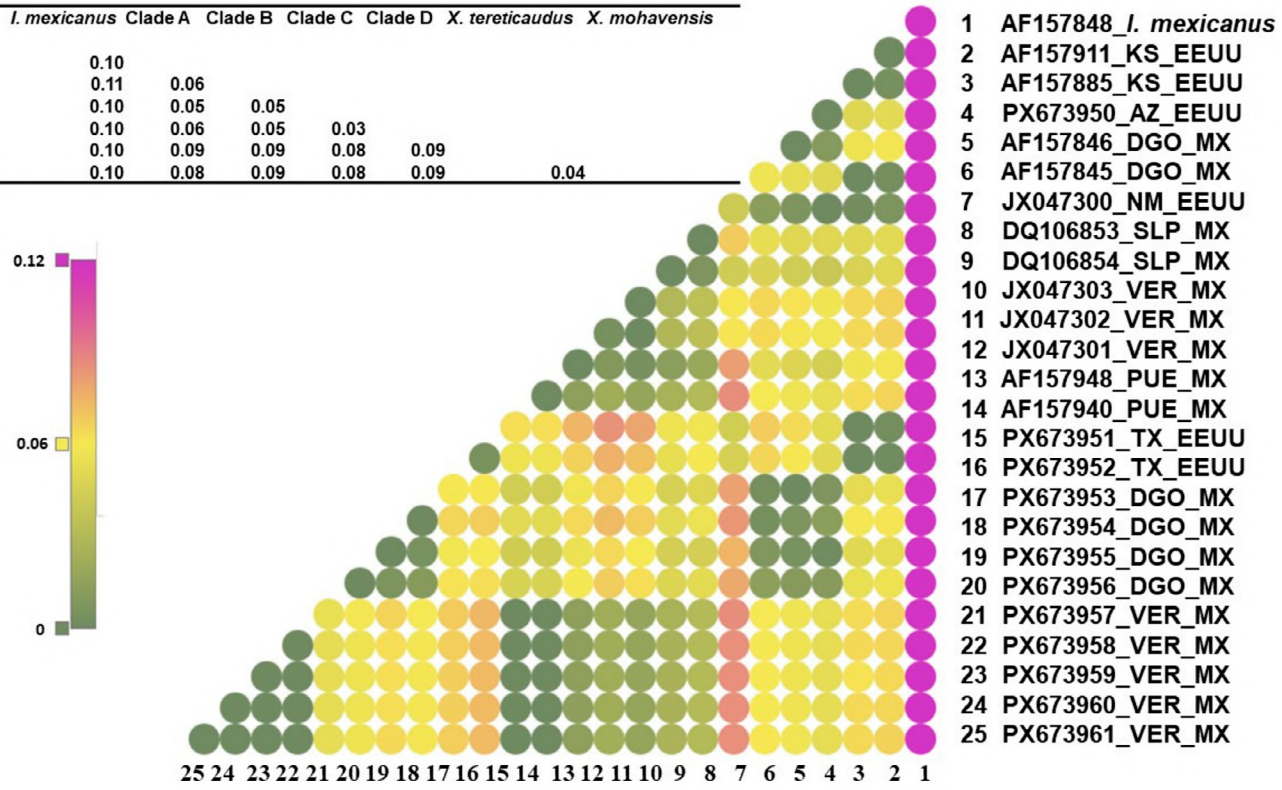

Figure 2. Heatmap showing genetic distances (p) for the *Xerospermophilus spilosoma* complex. Interspecific genetic distances >0.10 are marked in pink, and intraspecific distances ≤0.6 are marked in green, according to the scale. The table presents the genetic distances (p) between the clades and the species close to *X. spilosoma*.

includes sequences from the Perote valley in Puebla and Veracruz, Mexico.

A total of 22 unique haplotypes were recovered, with a haplotype diversity (Hd) of 0.989 and a nucleotide diversity (Pi) of 0.04360. The haplotype network showed four clusters corresponding to the clades observed in the phylogenetic relationships (Figure 1c). These clusters were separated by different numbers of mutational steps (7-14), indicating clear genetic differences between them. Likewise, a high number of mutational steps were observed between the Arizona and Mapimí populations, as well as between the New Mexico and Texas–Kansas populations. In addition, several hypothetical haplotypes were identified that connect the various clusters. On the other hand, the genetic distances between *I. mexicanus* and the remaining sequences analyzed were low (10–11 %). In *X. spilosoma*, intrapopulation genetic variability ranged from 1 % to 3 %, whereas interpopulation variability ranged from 3 % to 6 % (Figure 2).

The estimated divergence times suggest that the *X. spilosoma* complex originated toward the end of the Miocene, just over 5 Ma ago (Figure 3). Most divergence events are concentrated between 3.5 and 1.5 Ma, with a marked increase in the divergence process during the Pliocene.

Of the eight partitions generated in the ABGD analysis, the three-species option yielded the highest statistical significance (P = 0.007; Figure 1b). On the other hand, the PTP analysis identified between 2 and 6 potential species; however, only the four species with support values close to 0.5 were considered. Finally, the GMYC clustering analysis identified only one evolutionary entity, with a likelihood ratio of 19.12 (P = 7.04e-05).

Ecological niche analyses using PCA indicate consistent ecological segregation across most clades, except for clades A and B, which show a smaller Mahalanobis distance between their environmental centroids (Figure 4). The overlap between clades is low to moderate, suggesting possible ecological differentiation (Figure 5). In contrast, clade A and clade B showed a partial differentiation with some overlap (D = 0.23). The comparisons of clades B–C and B–D showed the least separation, with overlap approaching 1 in both comparisons (D = 0.92). The analysis indicated no evidence of similarity greater than expected by chance (P > 0.05), with the exception of clades B vs. D (P

Table 1. Results of the similarity and ecological niche equivalence analyses and their interpretation. The asterisk (*) indicates a significant difference.

Comparison	Similarity (D) *P*-value	Equivalences (D) *P*-value	Interpretation
A vs B	(0.07) 0.45	(0.03) 0.16	Dissimilar, equivalent
A vs C	(0.01) 0.57	(0.005) 0.97	Dissimilar, equivalent
A vs D	(0) 1	(0) 1.00	Dissimilar, equivalent
B vs C	(0.10) 0.12	(0.07) 0.94	Dissimilar, equivalent
B vs D	(0.21) 0.03*	(0.14) 0.88	Similar, equivalent
C vs D	(0) 0.34	(0.06) 0.51	Dissimilar, equivalent

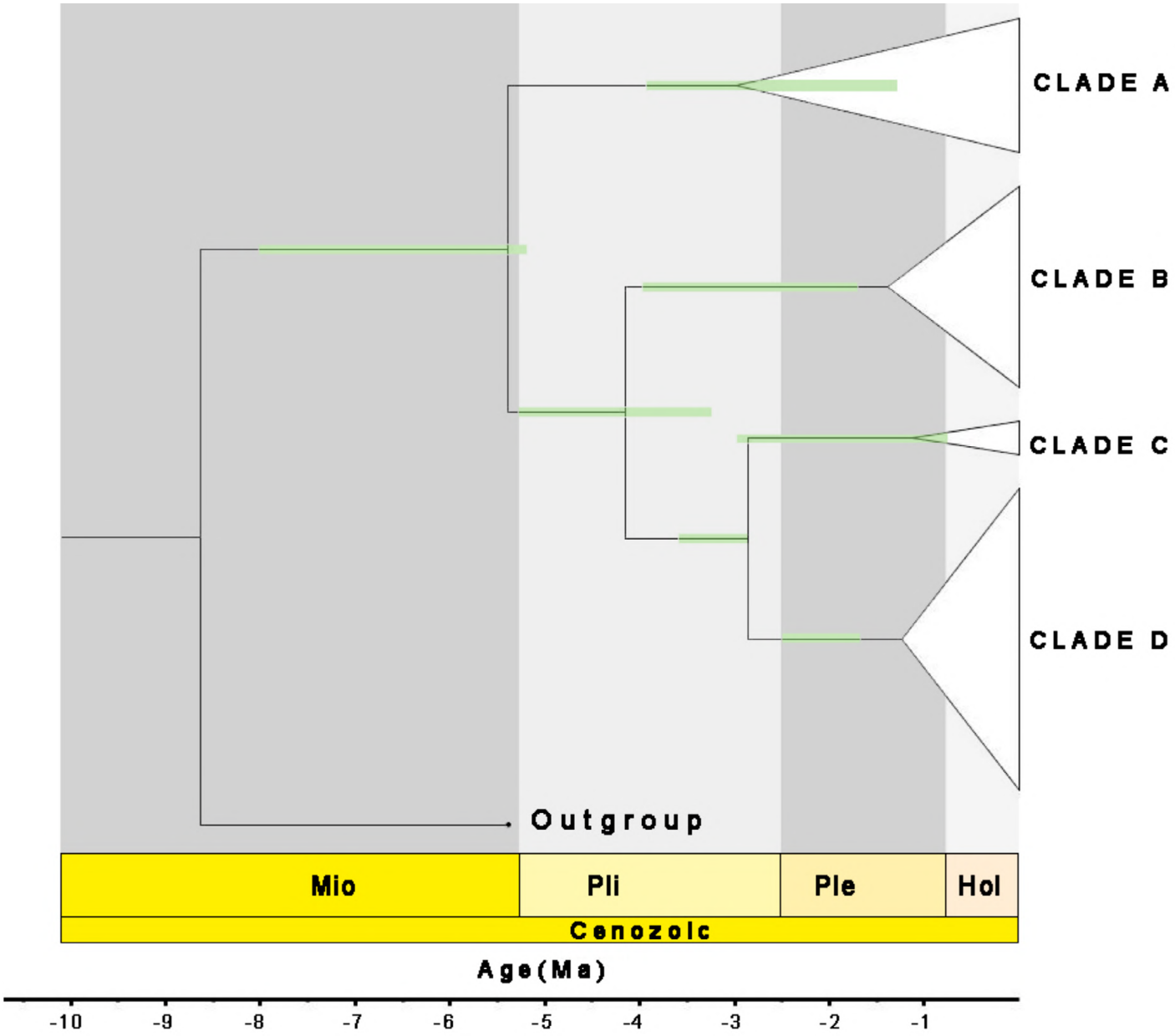

Figure 3. Phylogenetic relationships of the *Xerospermophilus spilosoma* complex calibrated with temporal divergence estimates. Nodes represent lineage-divergence events, and horizontal bars indicate 95% confidence intervals for estimated dates, scaled to the late Cenozoic era.

= 0.03; Table 1). On the other hand, in the equivalence test, none of the comparisons showed significant differences ($P < 0.05$), indicating that the niche models generated for each clade pair did not differ in their niches within the shared environmental space, with *D*-index values ranging from 0 to 0.14 (Table 1).

Discussion

The results suggest that *Xerospermophilus spilosoma* comprises a complex that hosts four evolutionarily independent lineages, a hypothesis supported by clear phylogenetic separation, consistent haplotype distributions across environmentally segregated populations, and differentiation of ecological niches among these lineages.

The estimated divergence times differ from those proposed by Fernández (2012), who suggested that the *X. spilosoma* complex is approximately 2.69 Ma old and that the last common ancestor of the San Luis Potosí and Perote lineages existed 0.74 Ma ago. In contrast, our results indicate that the *X. spilosoma* complex emerged toward the end of the Miocene, just over 5 Ma ago. Most divergence events are concentrated between 3.5 and 1.5 Ma and are consistent with the estimated ages of the geographical barriers that influenced their diversification.

Clade A is the first to diverge, possibly favored by the geographical barrier represented by the Rio Grande (Figure 6). This river, with a length of approximately 3,050 km, flows through a large portion of the southwestern United States, emptying into the Gulf of Mexico (Kelley 1952). It was formed during the Late Miocene and Pliocene between 6.9 and 2.5 Ma (Morgan and Golombek 1984; Gamez *et al.* 2017), consistent with the divergence time estimated in

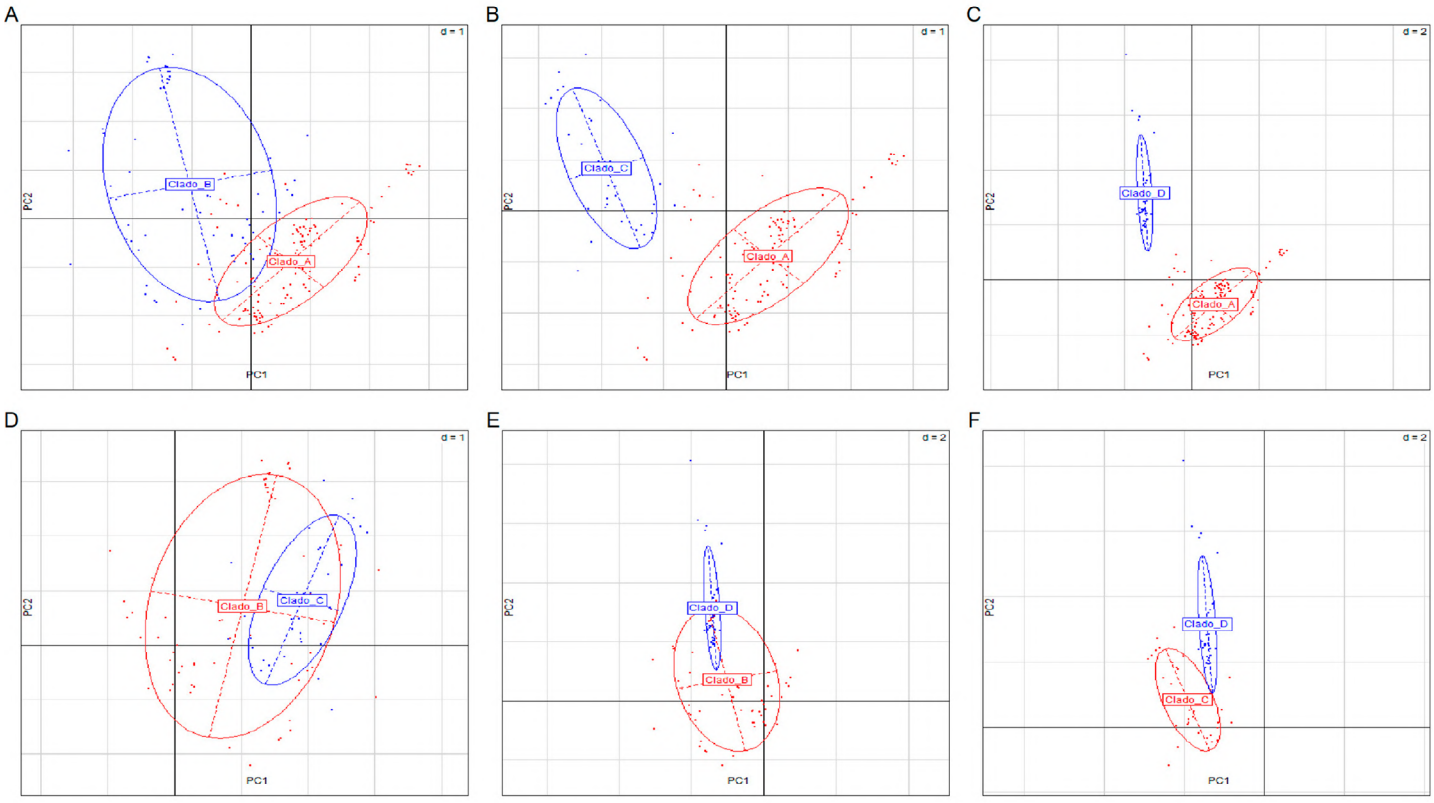

Figure 4. Ellipses indicate 95 % confidence areas around the means of each group; comparison between clades: A) A and B; B) A and C; C) A and D; D) B and C; E) B and D; and F) C and D.

this work. The importance of this water body as a driver of speciation processes has been documented for other rodents, such as *Chaetodipus* and *Eutamias* (Sullivan 1985; Neiswenter *et al.* 2019).

Clade B is distributed in the Trans-Pecos and Bolsón de Mapimí, while clade C is represented by the population inhabiting the Central Plateau in San Luis Potosí, Mexico. These three zones are subregions of the great Chihuahuan Desert, considered among the most diverse arid regions worldwide (Dinerstein *et al.* 2000; Edwards *et al.* 2001). The geological and climatic events of the Miocene and Pliocene, along with the cyclical climatic changes of the Pleistocene, had a profound effect on the genetic structure and distribution of various species that live in this desert (Raymo and Ruddiman 1992; Hafner and Riddle 2005; Loera *et al.* 2017; Scheinvar *et al.* 2020). These events possibly favored divergence between clades, which is supported by the divergence times estimated in this study, similar to the reports regarding the diversification between *Cynomys mexicanus* and *C. ludovicianus* (Castellanos-Morales *et al.* 2016), as well as about the genetic patterns of *Perognathus* and *Chaetodipus* mice (Riddle *et al.* 2000; Neiswenter and Riddle 2010) and the differentiated distributions of grasshopper mice of the genus *Onychomys* (Riddle 1995).

On the other hand, the divergence between clades B and C could be related to the Nazas River**,** whose formation is estimated to have occurred between mid- and late Pliocene (Petersen 1976; Hafner and Riddle 2011), a period estimated in the present work. This river has been identified as a possible biogeographic barrier for several species, including the rats *Neotoma albigula* and *N. leucodon* (Edwards *et al.* 2001), the cactus mouse *Peromyscus eremicus* (Riddle *et al.* 2000), gophers of the genus *Cratogeomys* (Hafner *et al.* 2008), and the black-tailed hare *Lepus californicus* (Lorenzo *et al.* 2021).

On the other hand, clade D is restricted to the Eastern Basin, a high-mountain region, formed mostly during the Pleistocene (<1.6 Ma) as part of the uplift of the Trans-Mexican Volcanic Belt (Ferrari *et al.* 1999). The emergence of this mountain range could have favored the divergence of this clade, as documented for other rodents, such as *Dipodomys phillipsi* and *Peromyscus bullatus* (Peterson *et al.* 2000; González-Ruíz *et al.* 2005; Sánchez-Cordero *et al.* 2005; Fernández *et al.* 2012).

The interspecific genetic distances observed within the *X. spilosoma* complex were slightly below the threshold proposed by Baker and Bradley (2006) to recognize entities as distinct species. However, these distances are similar to those accepted for other closely related genera, such as *Spermophilus*, when supported by additional evidence, such as genetic structure and multilocus analysis (Simonov *et al.* 2024). On the other hand, these divergence levels are consistent with evolutionarily independent lineages, as proposed for squirrel species of the genus *Tamias*, which supports their possible recognition as distinct species (Ge *et al.* 2014). The genetic diversity patterns found in *X. spilosoma*

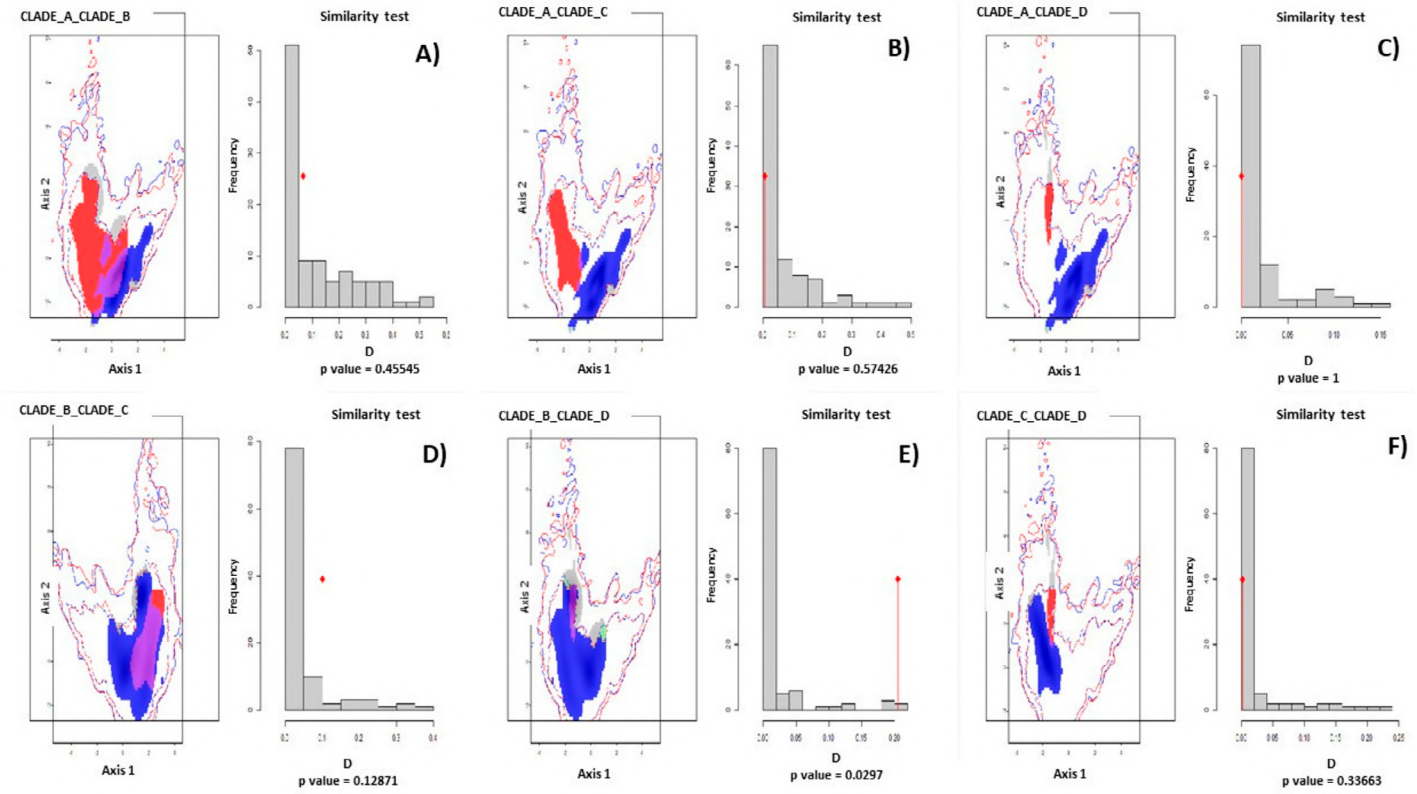

Figure 5. Comparisons between the environmental datasets of clades: A) A and B; B) A and C; C) A and D; D) B and C; E) B and D; and F) C and D, using a principal component analysis (PCA), showing the distribution of populations in the space defined by the first two principal components (PC1 and PC2). Bars in the similarity test histograms represent null models, and the red line represents the observed *D*-value.

in the present study reinforce this interpretation. A high Hd = 0.99 and a considerable Pi = 0.04 were observed, which are within the upper range reported for other rodents, such as *Neotoma mexicana* (Hd = 0.97; Pi 0.03–0.05) and *Reithrodontomys chrysopsis* (Hd = 0.99; Pi = 0.03) (Hernández-Canchola *et al.* 2021; León-Tapia *et al.* 2023).

On the other hand, the different species delimitation methods yielded inconsistent results, as they identified different numbers of evolutionary entities. Such discrepancies between methods are common, as each approach is based on different assumptions and models (Feijó *et al.* 2019; Martínez-Borrego *et al.* 2023). The partial concordance between ABGD and PTP suggests some degree of genetic structure within *X. spilosoma*, although the low resolution of GMYC could indicate recent diversification or insufficient mitochondrial differentiation, which warrants further analysis.

In addition, correspondence between genetic structure and ecological niche modeling was observed. Clades A, C, and D exhibited marked ecological differentiation, with minimal or no niche overlap among them. In contrast, we found evidence of partial or complete niche overlap between clade B and all other clades. This overlap can be explained by the niche breadth of clade B, associated with the marked climatic heterogeneity of the Mapimí region, which offers a wide range of environmental conditions (Van Devender and Burgess 1985; García-Arévalo and Nocedal 2008). Although the overall pattern is a positive relationship between niche breadth and geographic range (Morin and Lechowicz 2013; Slatyer *et al.* 2013; Moulatlet *et al.* 2025), niche breadth does not necessarily correspond to a larger potential distribution area, since the conditions that make up that niche may be unequally represented in geography (Dallas and Ten Caten 2025). For example, clade A has a potential distribution area of 637 867 km^2, more than twice the estimated area for clade B (309 468 km^2), with a smaller environmental niche breadth; this highlights the importance of the environmental heterogeneity of the Mapimí region and is related to the distribution of clade B (García-Arévalo and Nocedal 2008).

Statistically, the ecological niche of clade B showed similarity only with the niche of clade D. Niche similarity between clades B and D could be explained by a non-typical niche phylogenetic conservatism, in which species maintain environmental similarities regardless of their genetic distances (Wiens and Graham 2005: Losos 2008). It is also possible that both clades are preserving a niche close to a midpoint within the environmental space occupied by the clade set (Wiens *et al.* 2010; Peixoto *et al.* 2017). However, the geographical distance between populations, genetic evidence, and the limited dispersal capacity of squirrels suggest that clades B and D are distinct groups despite their ecological proximity. The equivalence test was not significant, implying that the compared niches cannot be considered identical. This is consistent with the fact that the equivalence test is more conservative

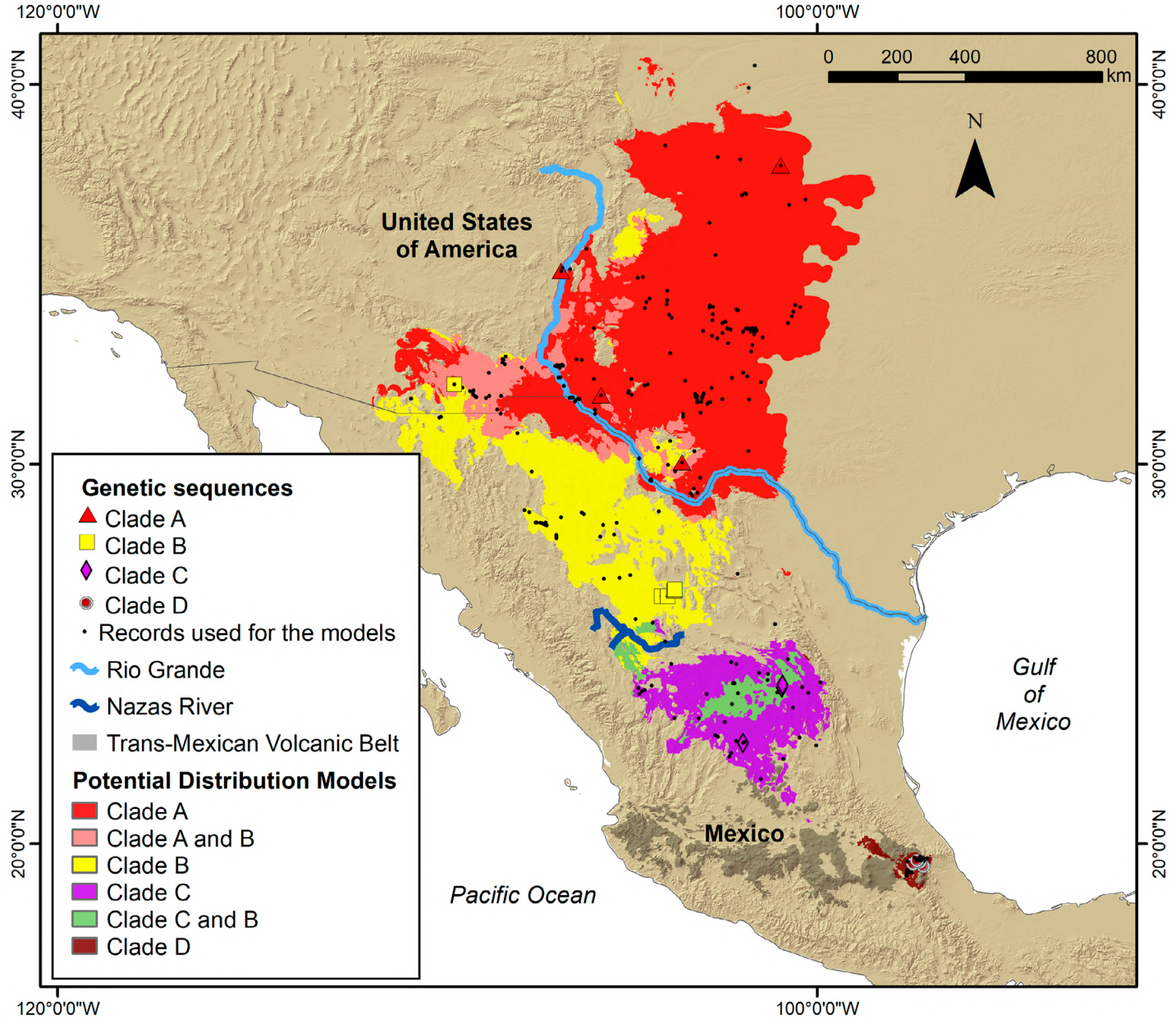

Figure 6. Potential distribution map of phylogenetic clades A, B, C, and D generated by ecological niche modeling using the MAXENT algorithm. The colored areas indicate the estimated environmental suitability for each clade, and symbols (triangle, square, rhombus, and circle) represent collection locations.

than the similarity test, as it assesses only whether two niches are indistinguishable from actual locations, without incorporating the surrounding environmental space (Brown and Carnaval 2019).

Taken together, the lines of evidence discussed here highlight the importance of considering both evolutionary history and local ecological conditions in order to understand diversification processes within the *X. spilosoma* complex. The evidence from the present study suggests that this complex can be divided into four lineages. Since species delimitation should not be based solely on absolute genetic distances, it is essential to integrate other factors such as monophyly, phylogenetic structure, and the gradual nature of allopatric speciation, the pace of which may vary according to the ecological, genetic, and geographical conditions involved (Carstens *et al.* 2013). In addition, it is important to recognize that genetic divergence can advance even in the absence of differentiated ecological selective pressure (Wiens 2004; Nosil 2012).

In the case of clade D (*X. perotensis*), the data support its recognition as an evolutionarily distinct lineage characterized by geographic isolation, a unique genetic structure, monophyly, and ecological differentiation from the other clades. These elements support its recognition as a sister species of *X. spilosoma* under the unified species concept framework. However, inconsistencies between the delimitation methods and the molecular evidence, limited to a mitochondrial gene fragment, preclude a definitive taxonomic conclusion. An alternative interpretation is that *X. perotensis* is at an advanced stage of the allopatric speciation continuum.

Although only a partial region (690 bp) of the *cytochrome b* gene was analyzed, our results provide solid evidence on genetic structuring and ecological differentiation in squirrels of the genus *Xerospermophyllus*. A more complete and robust reconstruction of the evolutionary history of this taxonomic complex warrants the incorporation of additional mitochondrial and nuclear markers, along with other types of evidence (e.g., morphological, behavioral, or genomic). It will also be essential to increase the sample size and include additional populations, such as those inhabiting the Great Tamaulipas Desert, which could not be included in this study due to insufficient data. Finally, a major limitation in the reconstruction of ecological niche models is the lack of biotic data, such as ecological interactions, resource availability, or predator pressure, which restricts the power of models to more accurately discriminate between ecological niches of different clades (Peng *et al.* 2025).

While the taxonomy of this squirrel complex is being resolved, it is important to recognize that the identification of genetically differentiated clades and distinct ecological niches underscores the need to conserve each genetic lineage as a significant evolutionary unit. This is particularly relevant for the Perote population, the southernmost and most isolated squirrel population, which, due to its distinctive characteristics, is essential for preserving the genetic and ecological diversity of this group.

Acknowledgements

We thank J. Ramos-Luna and E. López-Tello Mera for their support during fieldwork, and C. Flores for supporting the primer design. We also thank the curators for facilitating access to tissue samples for genetic analysis, including T. Lee from the Natural History Collection of the Christian University of Abilene (ACUNHC), F. Cervantes and Y. Hortelano from the National Collection of Mammals, Universidad Nacional Autónoma de Mexico (CNMA), C. López and D. García from the Mammal Collection of the CIIDIR. Secretariat of Science, Humanities, Technology and Innovation (SECIHTI), for the doctoral scholarship number 4001596 awarded to Sergio Albino Miranda.

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Associated editor: Mónica Díaz
Submitted: September 23, 2025; Reviewed: November 26, 2025
Accepted: December 12, 2025; Published on line: January 30, 2026.

Appendix 1

Appendix 1. Geographical data of the specimens used and National Center for Biotechnology Information (NCBI) access number. In the species column, X = *Xerospermophilus*; I = *Ictydomys*; and C = *Cymomys*.

Species	Country	State	Latitude	Longitude	Collection ID	NCBI #	References
X spilosoma	USA	Kansas	37.872	-100.964		AF157885	Harrison *et al.* 2003
X spilosoma	USA	Kansas	35.084	-106.74		AF157911	Harrison *et al.* 2003
X spilosoma	USA	New Mexico	35.084	-106.738		JX047300	Fernández 2012
X spilosoma	USA	Arizona	32.1080556	-109.555278	ACUNHC 566	PX673950	This study
X spilosoma	USA	Texas	30.0487278	-103.551506	ACUNHC 1376	PX673951	This study
X spilosoma	USA	Texas	31.8174644	-105.688989	ACUNHC 2180	PX673952	This study
X spilosoma	Mexico	Durango	26.523	-104.089		AF157845	Harrison *et al.* 2003
X spilosoma	Mexico	Durango	26.524	-103.929		AF157846	Harrison *et al.* 2003
X spilosoma	Mexico	Durango	2953720	624503	AGR01	PX673953	This study
X spilosoma	Mexico	Durango	2950540	623514	AGR03	PX673954	This study
X spilosoma	Mexico	Durango	2953955	624710	UNAM EY1194	PX673955	This study
X spilosoma	Mexico	Durango	2953955	624710	UNAM MVA103	PX673956	This study
X spilosoma	Mexico	San Luis Potosí	24.125	-100.925		DQ106853	Chumacero *et al.* 2006
X spilosoma	Mexico	San Luis Potosí	24.2	-100.901667		DQ106854	Chumacero *et al.* 2006
X perotensis	Mexico	Puebla	19.49	-97.489		AF157840	Harrison *et al.* 2003
X perotensis	Mexico	Puebla	19.49	-97.489		AF157948	Harrison *et al.* 2003
X perotensis	Mexico	Veracruz	19.587	-97.33		JX047301	Fernández 2012
X perotensis	Mexico	Puebla	19.49	-97.489		JX047302	Fernández 2012
X perotensis	Mexico	Veracruz	19.572	-97.383		JX047303	Fernández 2012
X perotensis	Mexico	Veracruz	32.4166667	-97.8166667	AMS01	PX673957	This study
X perotensis	Mexico	Veracruz	2161070	678473	AMS02	PX673961	This study
X perotensis	Mexico	Veracruz	2161070	678473	AMS03	PX673960	This study
X perotensis	Mexico	Veracruz	2161070	678473	AMS04	PX673958	This study
X perotensis	Mexico	Veracruz	2161070	677627	AMS06	PX673959	This study
I mexicanus	Mexico	Edomex				AF157848	Harrison *et al.* 2003
C ludovicianus	Mexico	Chihuahua				JQ885590	Castellanos-Morales *et al.* 2014

THERYA, 2026, Vol. 17(1):99-110 DOI: 10.12933/therya.2026.6230 ISSN 2007-3364

Puma (*Puma concolor*) and bobcat (*Lynx rufus*) diet overlap in northern Chihuahua

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In carnivores, diet overlap is essential for understanding resource selection and competition in various environments. The objective of this study was to compare the diet composition and overlap between puma (*Puma concolor*) and bobcat (*Lynx rufus*) in northern Chihuahua. We expected greater overlap in disturbed environments. Puma and bobcat scats were collected from disturbed and non-disturbed environments in northern Chihuahua. Percentage of occurrence, dietary overlap, and differences in diet composition were calculated using Chi-square contingency tables. Twenty-three *Puma concolor* and 70 *Lynx rufus* scats were analyzed. The main prey consumed by both species were rodents, followed by lagomorphs. The consumption of plant materials, cattle, other carnivores, arthropods, and bats was observed. In disturbed environments, diet overlap was complete at two sites and partial at the other; in undisturbed sites, one site showed no overlap, and two showed partial overlap. Both felines share similar diets in disturbed areas, with substantial overlap in common prey such as lagomorphs and rodents. In undisturbed areas, their diets are more differentiated. In disturbed environments, their diets differed, and both species resorted to unusual sources (chiropterans, plant materials, and garbage). Therefore, in disturbed environments of the desert region of northern Chihuahua, changes in the diet of both felids occurred, along with increased competition for resources.

Key words: Competition; interspecific predation; percentage of occurrence; Pianka index; scat analysis; trophic plasticity.

La sobreposición de dieta entre carnívoros es clave para entender la selección y competencia por recursos en diversos ambientes. El objetivo fue comparar la composición y la sobreposición entre la dieta del puma (*Puma concolor*) y el gato montés (*Lynx rufus*) entre ambientes perturbados y no perturbados en el norte de Chihuahua. Se espera que la sobreposición sea mayor en ambientes perturbados. Se colectaron excretas de puma y gato montés en localidades perturbadas y no perturbadas del norte de Chihuahua. Se calculó el porcentaje de ocurrencia, la sobreposición de dieta y su diferencia por medio de tablas de contingencia de Chi-cuadrada. Se analizaron 23 excretas de puma y 70 de gato montés. Los roedores y lagomorfos fueron los principales alimentos de ambas especies. Destaca el consumo de materiales vegetales, ganado vacuno, otros carnívoros, artrópodos y murciélagos. En los ambientes perturbados la sobreposición de dieta fue completa en dos localidades y media en la otra, en cambio, en los no perturbados fue media en dos localidades y no hubo en la otra. Ambos felinos tienen dietas similares en los ambientes perturbados, con una sobreposición importante por lagomorfos y roedores. En los ambientes perturbados, sus dietas fueron diferentes, y ambas especies recurrieron a fuentes no comunes (quirópteros, materia vegetal y basura). Por lo tanto, en ambientes perturbados de la zona desértica del norte de Chihuahua se presentaron cambios en la dieta de ambos felinos y mayor competencia por los recursos.

Palabras clave: Análisis de excretas; competencia; depredación interespecífica; índice de Pianka; plasticidad trófica; porcentaje de ocurrencia.

The diet of carnivorous species is not only influenced by the abundance, composition, assemblage, energy requirements, and type of prey available, but also by environmental factors (Krebs *et al.* 1995; Carbone *et al.* 1999; Sinclair, 2003; Haswell *et al.* 2017), interspecific competition (Litvaitis and Harrison 1989; Hass 2009), and the hunting strategies of each species (Hernández 2015; Husseman *et al.* 2003). Dietary overlap between species is useful for assessing interactions by measuring the share or competition for food components (Elbroch and Kusler 2018).

The puma (*Puma concolor*) and the bobcat (*Lynx rufus*) coexist in North America from southwest Canada, in the region bordering the United States southward through the central part to the west coast and reaching northern and central Mexico (Koehler and Hornocker 1991; Hass 2009), with a 96 % geographical overlap in Mexico (Sánchez-Cordero *et al.* 2008). Both carnivores share prey, but differences in dietary preferences based on prey size, energy intake, and abundance influence the feeding patterns of each feline species (Hass, 2009).

Laundré *et al.* (2009) evaluated potential factors influencing puma abundance in the Chihuahuan Desert by comparing Sierra Rica and El Cuervo in Chihuahua. Sierra El Cuervo, with more inhabitants and easier access, showed a higher incidence of poaching, which could reduce prey abundance and, consequently, impact the puma population. Fischer *et al.* (2012) suggest that urbanization has altered trophic dynamics in McCormick County, South Carolina, by reducing top-down control (ecological control exercised by predators over lower trophic levels, regulating energy distribution) and increasing bottom-up control (control generated by energy and nutrient flow

over the number of primary consumers and predators that the system can sustain) due to increased availability of food produced by man.

In the desert region of northern Chihuahua, pumas and bobcats interact despite increased productive activities and land-use changes associated with urban growth, as observed in other regions (Lewis *et al.* 2015; Parsons *et al.* 2019). Large-scale spatio-temporal analyses have revealed that in carnivores, habitat preference exerts a greater influence than interactions among them (Jensen *et al.* 2024; Suraci *et al.* 2025). In contrast, at local scales, evasion patterns are evident, as in the case of bobcats that avoid coyotes (*Canis latrans*), which in turn avoid bobcats and pumas (Jensen *et al.* 2024). The co-occurrence of bobcats with dominant carnivores such as pumas and wolves (*Canis lupus*) is negatively affected by factors associated with human activities; in contrast, its coexistence with two subordinate carnivores, the red fox (*Vulpes vulpes*) and the gray fox (*Urocyon cinereoargenteus*), depends mostly on environmental factors such as precipitation and gross primary production (Hubbard *et al.* 2022).

Studies on the puma diet report ungulates as the primary prey (Prude and Cain III 2021; Iacono *et al.* 2024; Bender *et al.* 2025); on the other hand, bobcats mainly prey on lagomorphs and rodents, with variable preference (Romero and Cervantes 2014; Sánchez-González *et al.* 2018; Draper *et al.* 2022). The diet overlap of bobcats and pumas ranges between 0.22 and 0.56 according to the Pianka index (Luna-Soria and López-González 2005; Hass 2009); between puma and jaguar, it ranges from 0.46 (Flores-Turdera *et al.* 2021) to 0.77 (Ávila-Nájera *et al.* 2018) and is greater than 90 % between bobcat and coyote (Martínez-García 2014; Witczuk *et al.* 2015). This raises the question of whether there are differences in the degree of diet overlap of two feline species in disturbed (P) versus undisturbed (NP) environments. The degree of overlap in disturbed environments is expected to be greater. Therefore, the objective of this study was to analyze the degree of diet overlap of two feline species in disturbed and undisturbed environments in northern Chihuahua.

Materials and methods

The study was conducted at eight locations in the municipalities of Ascension and Juárez, in northern Chihuahua. Disturbed environments (P) were defined as sites with human activities, such as agriculture, materials extraction, peri-urban areas, and the presence of garbage dumps, while undisturbed sites (NP) lacked these characteristics. P localities were West Sierra Juárez (WSJ), UACJ campus (CU), Rancho Arantxa (RA), and Sierra Presidio (SP); NPs were Rancho Blanco (RB) and Microondas Las Dunas Microwave Antennas (MWD), in Ascension; Rancho El Lobo (REL), and Southern Sierra Samalayuca (SSS; Figure 1). The dominant landscapes in all of them are microphyllous desert scrub and sandy deserts with stabilized dunes (INEGI 2021; León-Pesqueira *et al.* 2024).

Several field trips were carried out between May 2022 and July 2024. Cross-country transects measuring 2 to 4 km were established for scat collection, accounting for dirt roads, cattle and wildlife trails, latrines, and paths between hills. Scats were photographed *in situ*, placing a *vernier* caliper on one side. These were identified based on the criteria of Halfpenny and Biesiot (1986) and Aranda (2012). The characteristics used to identify puma scats were large size (20 to 30 cm long by 2 to 3.5 cm wide), cylindrical shape, presence of constrictions, and characteristic odor. The associated footprints measure 7 cm by 10 cm long, with round, teardrop-shaped toe pads, absence of claws, and metacarpal pads straight or concave on the front and with three lobes on the back. In the case of bobcats, scats are cylindrical, between 10 and 15 cm long and 1.5 to 2.5 cm wide, with marked constrictions and a characteristic odor that differentiates them from canid scat. The associated footprints measured between 4.5 and 5 cm long by 4 to 5 cm wide. Although scats were determined using the traditional approach, it should be noted that, ideally, genetic determination of predators is the most convenient method, as in the work of Torres-Romero *et al.* (2019). Scats were transferred to the Laboratory of Ecology and Animal Biodiversity (LEBA) of the UACJ, under the collection permits SGPA/DGVS/02524/22 and SPARN/DGVS/05498/23.

Scats were processed according to Ackerman *et al.* (1984). Vertebrate remains and hair were identified by comparison with voucher specimens deposited in the Scientific Collection of Vertebrates (CCV) of the UACJ (CHI-VER 189-0806). Arthropods were identified using the key of Eaton and Kaufman (2007), and plants were identified with expert assistance.

The percentage of occurrence (PO) of each food type for each predator was calculated in general and by locality (Sperry 1933; Alanis-Hernández *et al.* 2023). Only localities with scat data for both feline species were compared. The degree of overlap between environments and localities was determined using Pianka's index (Pianka, 1973), where values close to 0 indicate no overlap and 1 indicates total overlap of the diet (Krebs, 1999). Finally, the difference in diet composition between the two felines in both P and NP localities was evaluated using Chi-square contingency tables (Siegel and Castellan, 1988).

Results

A total of 23 scats of *Puma concolor* and 70 of *Lynx rufus* from eight sites were analyzed. Pumas living in P environments (n = 9) consumed 24 food items in five categories, with an average of 4.9 ± 2.0 per scat (Table 1). In NP environments (n = 14), they consumed 30 food items in three categories, with an average of 4.9 ± 1.9. Rodents had the highest PO (50 %) in both environments (P and NP), followed by lagomorphs (18.18 % and 19.10 %, respectively). Bobcats in P environments (n = 51) consumed 54 food items in six categories, with an average of 4.2 ± 1.5 per scat; mammals recorded the highest PO (73.14 %), mainly composed of

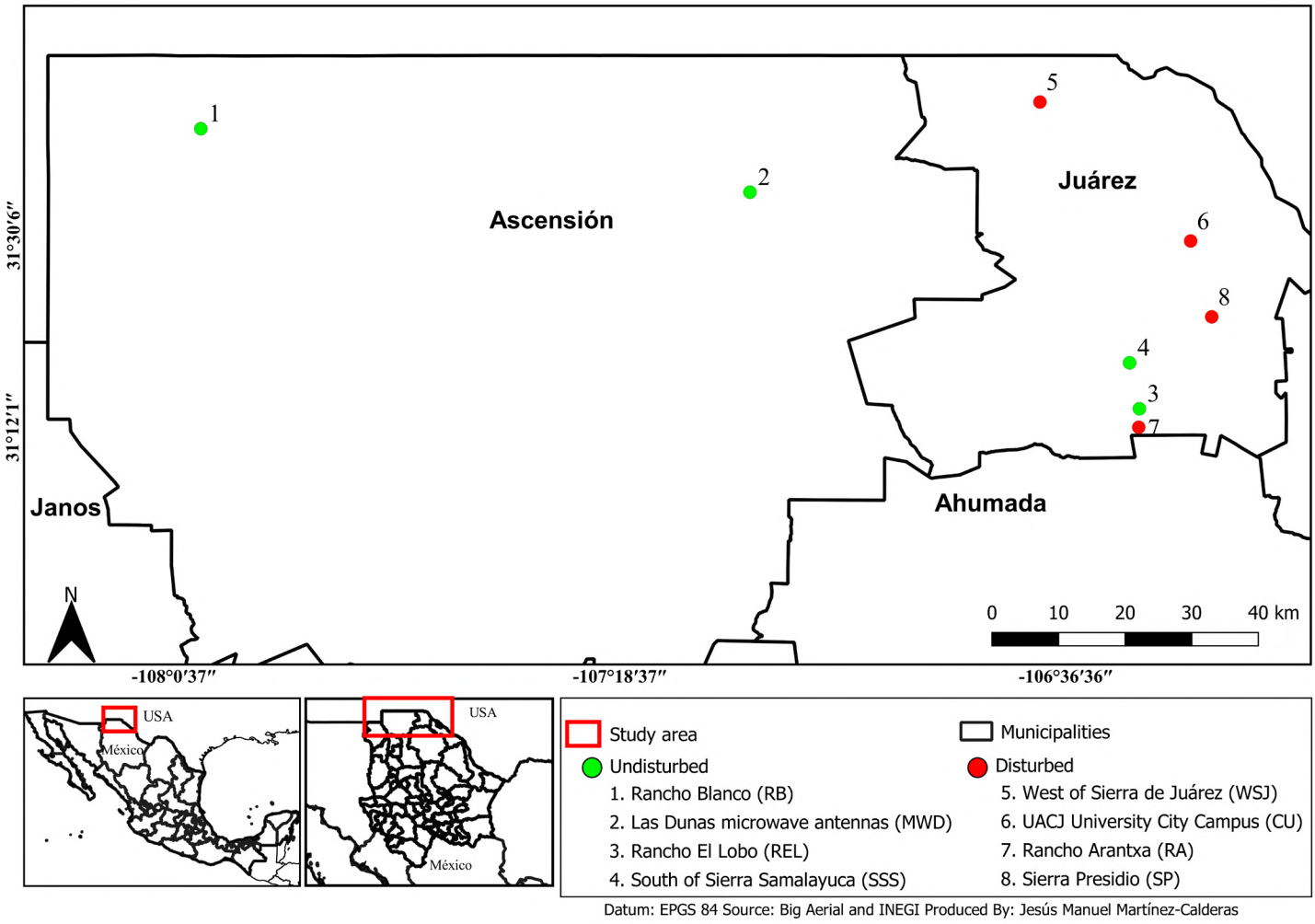

Figure 1. Map of the localities sampled in northern Chihuahua.

rodents (55 %) and plant matter (14.36 %) (Figure 2). In NP environments ($n = 19$), they consumed 41 food items in five categories, with an average of 4.5 ± 2.0 (Figure 3). Mammals accounted for 79.8 % of the diet, with rodents being the most frequently consumed prey (60.7 %), followed by lagomorphs (13.5 %). This study documented puma consumption of wild ungulates in both environments. In NP, we reported consumption of pronghorn (*Antilocapra americana*) at one locality (MWD) and mule deer (*Odocoileus hemionus*) at two localities (SSS and REL). In P environments, mule deer consumption was recorded at one locality (CU); also, livestock consumption was higher in P localities. Equine consumption by pumas was documented at WSJ; in NP localities, cattle consumption by pumas was recorded in SSS. Bobcats fed on cattle, pigs, goats, and horses in WSJ, and pigs were consumed in CU. In NP localities, bobcats fed on cattle in MWD.

Both feline species preyed upon mesocarnivores. In P localities, pumas preyed on northern fox (*Vulpes macrotis*); in NP environments, they consumed northern fox, raccoon (*Procyon lotor*), and skunk (*Conepatus leuconotus* and *Mephithis mephitis*). For its part, bobcats inhabiting P environments consumed foxes (*Urocyon cinereoargenteus* and *V. macrotis)* and skunks (*M. macroura* and *C. leuconotus*); in NP, they preyed on northern fox and skunks (*M. mephitis* and *M. macroura*). Consumption of dog (*Canis lupus familiaris*) by puma in P environments was recorded in CU.

The consumption of uncommon food items was also documented in P environments, where the puma (RA and WSJ) and the bobcat (CU and RA) preyed on bats; pumas also preyed on them in an NP location (REL). Arthropods recorded a higher PO in bobcats than in pumas in both environments, with insects yielding the highest PO values. The consumption of birds and reptiles was low. As for plants, pumas and bobcats consumed grasses, mesquite, and cactus fruits and seeds in P environments; in NP, both consumed grass, mesquite, and walnut fruits and seeds of the genus *Carya*. Bobcats consumed unidentified plant materials in both environments. Garbage consumption was recorded in two P localities: CU (puma) and WSJ (bobcat). Garbage materials consumed included food packaging (aluminum and plastic) in both species and animal leather (shoe and bag remains) in bobcats.

In general, Pianka's index between P and NP environments showed high overlap values (0.71). As for the localities, P exhibited partial overlap (0.50) in OSJ and complete overlap (1.00) in CU and RA. NP localities showed partial overlap in REL (0.57) and SSS (0.61), and low overlap in MWD (0.03).

Table 1. Frequency of occurrence (FO) and percentage of occurrence (PO) of the diet of puma (*Puma concolor*) and bobcat (*Lynx rufus*) in northern Chihuahua, in disturbed (P) and undisturbed environments (NP).

	***Puma concolor* (n = 23)**				***Lynx rufus* (n = 70)**			
	P		**NP**		**P**		**NP**	
Category/Components	FO	PO	FO	PO	FO	PO	FO	PO
Phylum Arthropoda								
Insects	0	0	0	0	1.96	0.46	5.26	1.12
Coleoptera	0	0	0	0	17.65	4.17	10.53	2.25
Cerambycidae	11.11	2.27	0	0	0	0	0.00	0.00
Orthopthera	0	0	0	0	5.88	1.39	0.00	0.00
Solifugae	0	0	0	0	0	0	5.26	1.12
Class Reptilia								
Unidentified reptiles	0	0	7.14	1.47	11.76	2.78	10.53	2.25
Sauria	0	0	0	0	1.96	0.46	5.26	1.12
Colubridae	0	0	0	0	0	0	5.26	1.12
Crotalus sp.	0	0	0	0	1.96	0.46	0.00	0.00
Class Aves								
Unidentified birds	11.11	2.27	0	0	3.92	0.93	10.53	2.25
Class Mammalia								
Antrozous pallidus	0	0	14.29	2.94	0	0	0.00	0.00
Tadarida brasiliensis	0	0	0	0	1.96	0.46	0.00	0.00
Myotis yumanensis	11.11	2.27	0	0	1.96	0.46	0.00	0.00
Eptesicus fuscus	11.11	2.27	7.14	1.47	0.00	0.00	0.00	0.00
Notiosorex crawfordii	0	0	0	0	1.96	0.46	0.00	0.00
Canis lupus familiaris	11.11	2.27	0	0	0.00	0.00	0.00	0.00
Urocyon cinereoargenteus	0	0	0	0	1.96	0.46	0.00	0.00
Vulpes macrotis	22.22	4.55	7.14	1.47	5.88	1.39	5.26	1.12
Procyon lotor	0	0	7.14	1.47	0.00	0.00	5.26	1.12
Taxidea taxus	0	0	0	0	1.96	0.46	0.00	0.00
Mephitis macroura	0	0	0	0	5.88	1.39	5.26	1.12
Mephitis mephitis	0	0	7.14	1.47	0.00	0.00	5.26	1.12
Conephatus leuconotus	0	0	7.14	1.47	1.96	0.46	0.00	0.00
Antilocapra americana	0	0	7.14	1.47	0.00	0.00	0.00	0.00
Odocoileus hemionus	11.11	2.27	28.58	5.89	0.00	0.00	0.00	0.00
Sus scrofa	0	0	0	0	7.84	1.85	0.00	0.00
Bos taurus	0	0	14.29	2.94	3.92	0.93	5.26	1.12
Capra aegagrus hircus	0	0	0	0	3.92	0.93	0.00	0.00
Equus caballus	33.33	6.82	0	0	5.88	1.39	0.00	0.00
Cratogeomys castanops	0	0	0	0	1.96	0.46	0.00	0.00
Geomys arenarius	0	0	7.14	1.47	1.96	0.46	0.00	0.00
Dipodomys merriami	33.33	6.82	28.58	5.89	31.37	7.41	52.63	11.24
Dipodomys ordii	33.33	6.82	14.29	2.94	17.65	4.17	15.79	3.37
Dipodomys spectabilis	0	0	0	0	7.84	1.85	5.26	1.12
Chaetodipus baileyi	0	0	0	0	1.96	0.46	5.26	1.12
Chaetodipus eremicus	33.33	6.82	35.71	7.35	9.80	2.31	10.53	2.25

Chaetodipus hispidus	0	0	0	0	5.88	1.39	5.26	1.12
Chaetodipus intermedius	0	0	14.29	2.94	15.69	3.70	15.79	3.37
Perognathus flavescens	0	0	0	0	0.00	0.00	5.26	1.12
Perognathus flavus	0	0	0	0	1.96	0.46	10.53	2.25
Perognathus merriami	0	0	7.14	1.47	1.96	0.46	5.26	1.12
Neotoma albigula	55.56	11.36	50	10.29	43.14	10.18	31.58	6.74
Neotoma mexicana	0	0	0	0	1.96	0.46	0.00	0.00
Neotoma micropus	0	0	0	0	7.84	1.85	5.26	1.12
Onychomys arenicola	0	0	0	0	3.92	0.93	5.26	1.12
Onychomys leucogaster	0	0	0	0	1.96	0.46	10.53	2.25
Peromyscus difficilis	0	0	0	0	3.92	0.93	0.00	0.00
Peromyscus eremicus	33.33	6.82	21.43	4.41	9.8	2.31	10.53	2.25
Peromyscus leucopus	0	0	0	0	3.92	0.93	0.00	0.00
Peromyscus maniculatus	11.11	2.27	28.58	5.89	27.45	6.48	26.32	5.62
Peromyscus truei	0	0	0	0	1.96	0.46	5.26	1.12
Reithrodontomys fulvescens	0	0	7.14	1.47	7.84	1.85	15.79	3.37
Reithrodontomys megalotis	11.11	2.27	7.14	1.47	1.96	0.46	0.00	0.00
Reithrodontomys montanus	0	0	0	0	0	0	5.26	1.12
Sigmodon fulviventer	0	0	0	0	1.96	0.46	5.26	1.12
Sigmodon hispidus	0	0	0	0	3.92	0.93	10.53	2.25
Sigmodon ochrognathus	0	0	0	0	1.96	0.46	0	0
Ammospermophilus interpres	11.11	2.27	7.14	1.47	0	0	0	0
Otospermophilus variegatus	11.11	2.27	7.14	1.47	3.92	0.93	10.53	2.25
Xerospermophilus spilosoma	11.11	2.27	7.14	1.47	7.84	1.85	10.53	2.25
Lepus californicus	44.44	9.09	57.14	11.77	11.76	2.78	36.84	7.87
Sylvilagus audubonii	44.44	9.09	35.71	7.35	19.61	4.63	26.32	5.62
Plant materials								
Unidentified plant material	0	0	0	0	23.53	5.56	10.53	2.25
Neltuma glandulosa	11.11	2.27	14.29	2.94	23.53	5.56	21.05	4.49
Poaceae	11.11	2.27	14.29	2.94	9.8	2.31	5.26	1.12
Cactaceae	0.93	0.03	0	0	3.92	0.93	0	0
Carya illinoinensis	0	0	7.14	1.47	0	0	5.26	1.12
Yucca sp.	0	0	7.14	1.47	0	0	0	0
Garbage								
Garbage	11.11	2.27	0	0	7.84	1.85	0	0
Total	489.78	100	485.71	100	423.49	100	468.42	100

Regarding the difference in diet composition between puma and bobcat by locality using the Chi square method, P environments showed a significant difference between the diets of both felines ($X^2 = 8.18$, d.f. = 1, $p = 0.004$), but not in NP localities ($X^2 = 1.13$, d. f = 1, $p = 0.288$). In these environments, significant differences were observed in WSJ ($X^2 = 12.19$, d.f. = 1, $p = 0.0005$), but not in RA ($X^2 = 0.37$, d.f. = 1, $p = 0.542$) and CU ($X^2 = 2.28$, d.f. = 1, $p = 0.132$). In NP environments, no differences were observed in MWD ($X^2 = 1.22$, d.f. = 1, $p. = 0.269$) and SSS ($X^2 = 0.79$, d.f. = 1, $p = 0.3751$), and a moderate difference was recorded in REL ($X^2 = 3.67$, d.f. = 1, $p = 0.055$).

Discussion

In both environments (P and NP), bobcats mainly preyed on rodents, while pumas mainly consumed rodents and lagomorphs. It is known that bobcats prefer lagomorphs and rodents (Leopold and Krausman 1986; Delibes and Hiraldo, 1987; Hass, 2009; López-Vidal *et al.* 2014; Romero and Cervantes 2014; Sánchez-González *et al.* 2018; Draper *et al.* 2022). On the other hand, it has been documented that pumas consume more ungulates in North America (Iriarte *et al.* 1990; Pierce *et al.* 2000; De la Torre and De la Riva 2009; Hass, 2009). Mule deer (*Odocoileus hemionus*)

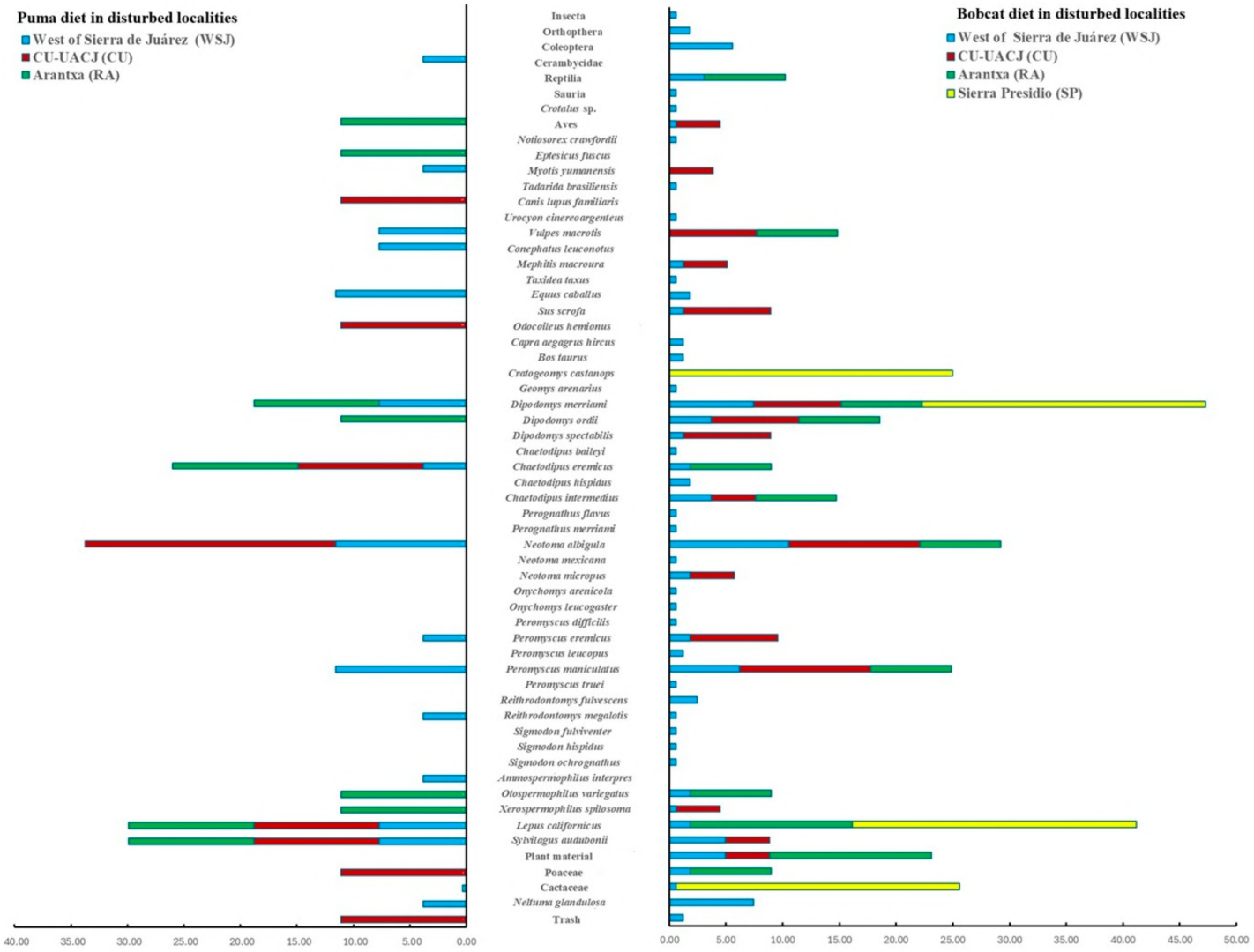

Figure 2. Percentage of occurrence of prey in the diet of puma (*Puma concolor*) and bobcat (*Lynx rufus*) in disturbed localities (P) in northern Chihuahua.

is the most consumed prey type by pumas in desert areas (Leopold and Krausman, 1986; Koehler and Hornocker, 1991; Cunningham *et al.* 1999; Logan and Sweanor, 2001; Prude and Cain III, 2021), although when its availability is low, the consumption of smaller prey increases by up to 50 %, consistent with the results of the present study. This finding confirms the plasticity of the puma diet and reaffirms its ability to persist in environments where its main prey decreases or is harder to capture, so its consumption becomes energetically non-profitable, as the cost and risk associated with its search and capture outweigh the benefits obtained (Leopold and Krausman 1986; Yañez *et al.* 1986; Iriarte *et al.* 1991; Donadio *et al.* 2010; Villepique *et al.* 2011; Pia 2013; Bender *et al.* 2025).

Regarding pronghorn consumption, there are no records of this species in the MWD area, so it is necessary to determine whether there are nearby populations. Distribution areas have been documented in the southwestern part of the municipality of Ascensión (Carreón-Hernández and Lafón-Terrazas 2014), 100 km from MWD. It has been reported that seasonal puma activity areas can be greater than 100 km^2 (Dellinger *et al.* 2018), so pumas may consume pronghorn in that area and leave evidence in MWD. Bernard *et al.* (2023) reported pronghorn consumption in northern New Mexico, which they considered infrequent. As for the consumption of cattle and domestic animals, we found evidence of puma having low consumption of horses in P environments and cattle in NP localities, as previously reported for the species (Ackerman *et al.* 1984; Luna-Soria and López-González, 2005; Rosas-Rosas *et al.* 2008; De la Torre and De la Riva 2009; Amador-Alcalá *et al.* 2013; Peña-Mondragón and Castillo 2013; Palmeira *et al.* 2015; Cassaigne *et al.* 2016; Prude and Cain III 2021; Guerisoli *et al.* 2021; Mesler and Jones 2022; Iacono *et al.* 2024; Racero-Casarrubia *et al.* 2024). Cattle, pig, goat, and horse consumption by bobcats is similar to that previously reported, being low relative to other food components (Aranda *et al.* 2002; Peña-Mondragón and Castillo 2013; Prude and Cain III 2021).

Predation of mesocarnivores by both felines was common. The species consumed were similar to those reported by Hass (2009) and Prude and Cain III (2021), who recorded seven and 11 carnivores in the puma diet,

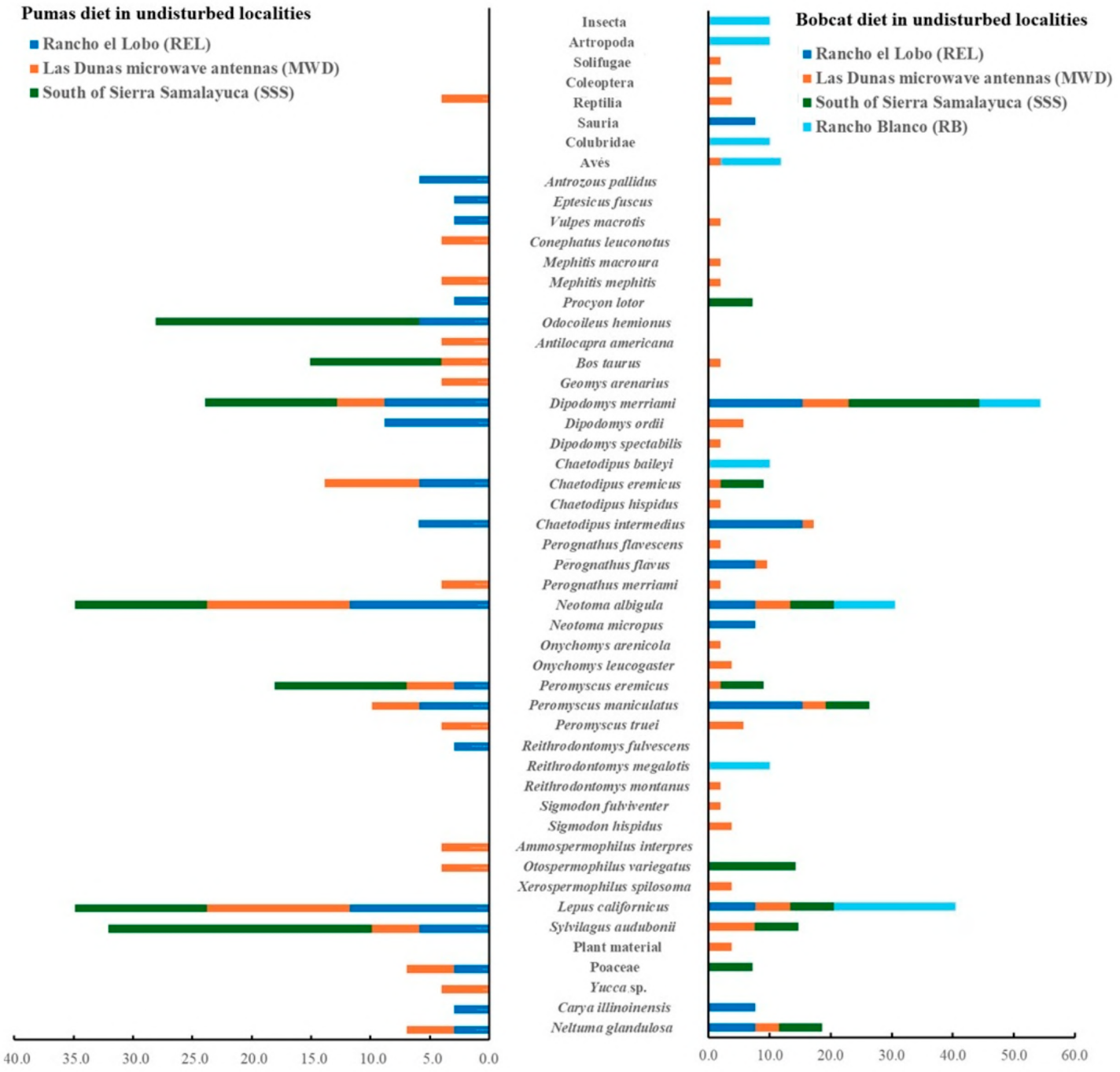

Figure 3. Percentage of prey occurrence in the diet of puma (*Puma concolor*) and bobcat (*Lynx rufus*) in undisturbed localities (NP) in northern Chihuahua.

respectively. Bobcat is known to have consumed gray fox and skunks (Hamilton and Hunter 1939; Litvaitis *et al.* 1981; Story *et al.* 1982; Trevor *et al.* 1989; Fedriani *et al.* 2000; Farias *et al.* 2005; Hass 2009; Draper *et al.* 2022; Landry *et al.* 2022). We found no studies reporting bobcat consumption of northern foxes or tlalcoyotes (*Taxidea taxus*). In addition, puma consumption of dogs (*Canis lupus familiaris*) in a P environment was recorded in CU. It has been reported that pumas commonly hunt dogs (Mazzolli, 2009; Buttler *et al.* 2014) and occasionally consume them (Farrell *et al.* 2000; Leberg *et al.* 2004; Villepique *et al.* 2011; Prude and Cain III 2021, Racero-Casarrubia *et al.* 2024). In Arizona, Wroe and Wroe (1982) reported bats preyed upon by bobcats. As for puma, only the presence of a non-identified bat has been reported in puma scats collected at Manu National Park, Peru (Emmons 1987). We found no reports of pumas hunting bats in North America, which is notable given the rarity of this prey, characterized by low energy value and great difficulty of capture due to its size and behavior. This occasional consumption suggests a high degree of opportunism and trophic plasticity in pumas, particularly in disturbed or ecologically changing environments, where traditional resources may be limited. Regarding plant materials, several authors mention that felines do

not consume plants because they are obligate carnivores (Morris *et al.* 2002; Sanquist and Sanquist 2002; Verbrugghe and Hesta 2017), since, despite their being present, they are not considered typical components in the diet of this group, so they have not been described or quantified. However, the opposite has been the subject of recent discussions (Yoshimura *et al.* 2020; Yoshimura *et al.* 2021). For example, Yoshimura *et al.* (2021) mention that in 361 studies of feline diet, only 37 % mention the frequency of occurrence of plant materials, and 7.3 % simply report plant material. Few studies include plant materials in the diet composition (Ackerman *et al.* 1986; Rocha-Mendes *et al.* 2010; Montalvo *et al.* 2020), and grasses (Ackerman *et al.* 1986; Gómez-Ortiz *et al.* 2011; Villepique *et al.* 2011; Franck and Farid 2020) consumed by pumas. For bobcat, several authors report the use of plant material, mainly grasses, mesquite fruits, *Yucca,* and cacti in desert environments (Litvaitis and Harrison 1989; Mckinney and Smith 2007; López-Vidal *et al.* 2014). The presence of pecan nuts (*Carya illinoinensis*) in feline scat in NP environments is associated with nearby farms cultivating this crop, a growing agricultural activity that is transforming the Chihuahuan desert. It is suspected that fiber-rich plant materials (unidentified plant material and grasses) were consumed seeking to improve digestion or excrete parasites (Yoshimura *et al.* 2021), while the consumption of fruits (cactus seeds, mesquite *Neltuma* sp., *Yucca* sp., and *Carya*) was due to diploendozoochory, a phenomenon that has been observed in both bobcats (Rubalcava-Castillo *et al.* 2021) and pumas (Sarasola *et al.* 2016).

Insects, reptiles, and birds have been documented in the bobcat diet in Mapimí (López-Vidal *et al.*, 2014), with insects at a lower percentage than that recorded in the present study. The puma consumed birds in NP environments, as previously documented (Prude and Cain III 2021). The presence of garbage in feline scat has been documented in P environments. In these areas, where the natural habitat of wild felines is altered by urban expansion, felines may lose their territories and be forced to adapt to anthropic environments (Bateman and Fleming 2012; Robins *et al.* 2019; Bartolucci *et al.* 2020; Riley *et al.* 2021), such as clandestine or unregulated garbage dumps, which are common in these areas. Under such conditions, these felines may resort to alternative food sources, including food scraps, garbage, and other anthropogenic wastes (Baruch-Moro *et al.* 2014; Plaza and Lambertucci 2017; Handler *et al.* 2020; Larson *et al.* 2020). Although garbage and anthropogenic organic waste are not commonly consumed by wild felines (Riley *et al.* 2021), human pressures and environmental pollution can increase the probability of felines encountering these wastes. Plastic consumption by puma is similar to that reported by Bartolucci *et al.* (2020), who identified two types of polyethylene. The consumption of garbage and human waste could harm the health of wild species, as it has been shown to affect domestic animals (Jensen and Nolte 2008; Prabhakar *et al.* 2012; Paraš *et al.* 2017).

A high dietary overlap was observed between bobcats and pumas in both environments, according to the Pianka index, indicating that both species share a large number of prey species. In P localities (CU and RA), both felines occupy nearly identical food niches, indicating high competition in these environments. In contrast, food overlap in NP localities was either low (MWD) or moderate (REL and SSS), suggesting that the species occupy very different niches, possibly due to differences in available resources or ecological strategies, which could indicate greater specialization or ecological segregation. This finding is similar to that observed by Hass (2009) in Tucson, Arizona, where an intermediate level of diet overlap between pumas and lynxes was reported. The observations in both studies are consistent with the idea that competition between species varies with regional ecological conditions and resource availability, which favors less competition between the two species and results in less overlap in their diets. The compositional difference analysis revealed that the diets of puma and bobcat are similar in P environments, possibly because environmental disturbances affect prey availability and force both feline species to consume resources not commonly observed in studies of diets in undisturbed environments. In contrast, in NP localities, the diets of puma and bobcat were different. It is likely that, in NP environments, each species has access to more varied and specific food resources, leading to differences in their feeding habits (Foster *et al.* 2010; Khorozyan *et al.* 2015; Ferretti *et al.* 2020). Therefore, studies examining predator and prey diets and abundances are necessary to evaluate the importance and interactions among the mechanisms that may be shaping trophic dynamics in urban and suburban areas (Fischer *et al.*, 2012).

In conclusion, prey consumption by bobcats and pumas differs between undisturbed and disturbed environments. There is substantial overlap in the diets of puma and bobcat in disturbed environments of northern Chihuahua, with greater consumption of common prey such as lagomorphs and rodents, highlighting competition between these felines. Competition for resources in areas near human settlements forces these felines to resort to unconventional food sources, such as garbage and plant materials. The findings in the present study underscore the importance of considering ecological interactions and the impact of habitat alteration on the diet and behavior of felines in desert environments.

Acknowledgments

To SEMARNAT for the collection permit SGPA/DGVS/02524/22. To the individuals who granted access for sample collection. To C. Muñoz Rivas, S. Ramos Guerra, D. Chávez, and E. Carrejo for their support during fieldwork, and to A. Zapata, P. Osorio, S. Vásquez, and K. Esparza for their assistance with sample processing. To Laura de León Pesqueira and Juan Rolando Rueda for their botanical support. We also thank the reviewers of this manuscript for their corrections and comments.

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Associated editor: Alina Gabriela Monroy-Gamboa
Submitted: September 4, 2025
Reviewed: December 15, 2025
Accepted: January 9, 2026
Published on line: January 30, 2026

THERYA, 2026, Vol. 17(1):111-124 DOI: 10.12933/therya.2026.6238 ISSN 2007-3364

Crónica sobre los métodos de control del murciélago vampiro común (*Desmodus rotundus*)

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El murciélago vampiro común (*Desmodus rotundus*) es un quiróptero de la familia Phyllostomidae, subfamilia Desmodontinae que habita las regiones tropicales y subtropicales de América Latina, se alimenta exclusivamente de sangre y es el transmisor de la rabia paralítica bovina, enfermedad que produce grandes pérdidas económicas en la ganadería. Las poblaciones de *D. rotundus*, aumentaron después de la introducción del ganado europeo al Continente Americano por los colonizadores, ya que representó una fuente de alimento abundante y de fácil acceso para estos quirópteros. Con el aumento del tamaño de las poblaciones de *D. rotundus* consecuentemente los casos de rabia paralítica bovina incrementaron. En los años setenta del siglo XX, se iniciaron estudios en México para controlar las poblaciones de *D. rotundus*, que derivaron en varios métodos basados en el envenenamiento con anticoagulantes. Durante más de 50 años en América Latina se ha utilizado este método; sin embargo, estudios actuales han demostrado que el uso de anticoagulantes químicos no ha reducido los casos de rabia paralítica bovina, por el contrario, han aumentado y avanzado geográficamente. En esta revisión se analizan los métodos de control de las poblaciones de *D. rotundus* que se han utilizado tradicionalmente, se discute su eficacia y se mencionan alternativas de investigación que podrían contribuir a resolver el problema, limitando a su vez el uso de métodos letales.

Palabras Clave: *Desmodus rotundus*, murciélago hematógafo, rabia, uso de anticoagulantes, vampiricida

The common vampire bat (*Desmodus rotundus*) is a bat of the family Phyllostomidae, subfamily Desmodontinae that inhabits the tropical and subtropical regions of Latin America. It feeds exclusively on blood and is the vector of bovine paralytic rabies, a disease that causes significant economic losses in livestock farming. *D. rotundus* populations increased after the introduction of European cattle to the Americas by colonizers, as these represented an abundant and easily accessible food source for these bats. With the increase in *D. rotundus* populations, cases of bovine paralytic rabies consequently increased. In the 1970s, studies began in Mexico to control *D. rotundus* populations, leading to several methods based on anticoagulant poisoning. This method has been used in Latin America for over 50 years. However, current studies have shown that the use of chemical anticoagulants has not reduced cases of bovine paralytic rabies; on the contrary, they have increased and spread geographically. This review analyzes the traditional methods used to control *D. rotundus* populations, discusses their effectiveness, and proposes alternative research approaches that could help solve the problem while limiting the use of lethal methods.

Keywords: Culling with anticoagulants, *Desmodus rotundus*, hematophagous bat, rabies, vampiricide

Es un hecho que el mito sobre los vampiros existía mucho antes de que se identificara plenamente en el Continente Americano a los únicos mamíferos que se alimentan exclusivamente de sangre: los murciélagos hematófagos, comúnmente llamados vampiros. Estos peculiares quirópteros, originales de la región Neotropical, dieron sustento real al mito ancestral del vampirismo reforzando el hecho de que Drácula se transforma en murciélago vampiro (Stoker 1987; Rydell *et al.* 2018; Nowik-Niton 2024). Estos murciélagos se distribuyen desde el norte de México hasta el norte de Argentina en América Latina y en algunas islas del Caribe. Están representados por tres géneros, cada uno con una sola especie: el vampiro común *Desmodus rotundus*; el vampiro de patas peludas *Diphylla ecaudata* y el vampiro de puntas de ala blancas *Diaemus youngi*. Estos tres géneros pertenecen a la subfamilia Desmodontinae, dentro de la familia Phyllostomidae (murciélagos con hoja nasal) (Hermanson y Carter 2020).

Diphylla eucaudata es un murciélago hematófago especializado en consumir sangre de aves grandes,

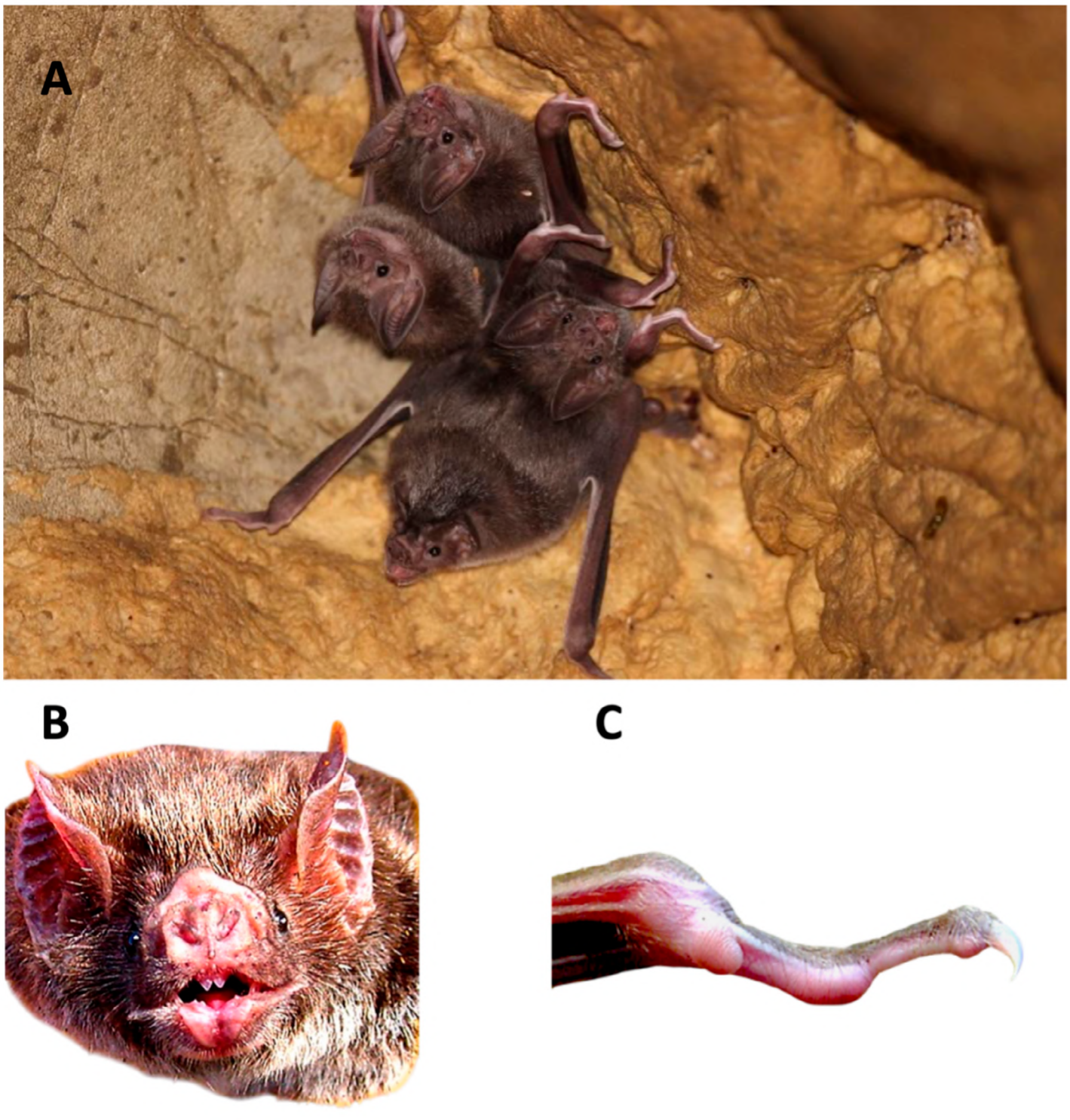

Figura 1. Características del vampiro común: A) aspecto general; B) labio en forma de V con incisivos afilados; C) pulgar desarrollado con tres cojinetes.

aunque ocasionalmente también puede consumir sangre de mamíferos. *D. youngi* se especializa también en consumir sangre de aves. El vampiro común, *D. rotundus* (Figura 1), consume principalmente sangre de mamíferos aunque puede hacerlo de cualquier vertebrado; es la más conocida y estudiada de las tres especies de vampiros y la de mayor distribución y abundancia. *D. rotundus*, posee una importancia capital en la economía ganadera de la región Neotropical, por lo que esta especie ha sido el blanco del desarrollo de los metodos de control a lo largo de la historia. En la época prehispánica, las evidencias apuntan a que las poblaciones del vampiro común no eran muy numerosas (Vos *et al.* 2011). Posteriormente, con la introducción del ganado en América por los conquistadores, las poblaciones de *D. rotundus* aumentaron, ya que el ganado introducido representa una fuente de alimento ilimitado y de fácil acceso para estos animales (Paniagua Pérez 2021).

Figura 2. Vaca cebú con mordeduras de murciélago vampiro escurriendo sangre en la espalda a nivel de la joroba y el cuello. Fotografía tomada en Yucatán, México.

Según las crónicas, la llegada del ganado europeo al Continente Americano comenzó con el segundo viaje de Cristóbal Colón en 1493. Durante este viaje, introdujo vacas, terneros, cabras, ovejas, cerdos y pollos desde las Islas Canarias al archipiélago de las Antillas, específicamente a la isla de La Española (actual República Dominicana y Haití), donde se establecieron los primeros criaderos. Posteriormente, alrededor de 1510, estos criaderos se extendieron a las islas de Puerto Rico, Jamaica y Cuba. Cuando el conquistador Hernán Cortés desembarcó en lo que hoy es México, solo llevaba consigo 14 caballos y algunos cerdos. Fue después de la conquista del Imperio Azteca (1521) que se trajo ganado al continente para su crianza y explotación. A partir de entonces, se generó todo un complejo de explotaciones ganaderas, que inicialmente abarcaron algunas zonas tropicales y subtropicales y las tierras altas semiáridas de la meseta central del actual México, desde donde se expandieron a las provincias del norte. Durante tres siglos, la Nueva España (México) abarcó gran parte de lo que hoy es Estados Unidos, desde Texas hasta California (Brand 1961; Villegas Durán *et al.* 2001). Cabe mencionar que, en el siglo XIX, la introducción del ganado cebú en América (Gómez 1972), resistente a las condiciones tropicales y subtropicales, consolidó aún más la ganadería en las áreas de distribución del murciélago vampiro común, favoreciendo además el aumento de sus poblaciones (Figura 2).

Por otro lado, el establecimiento de grandes hatos de ganado en América Latina y el consecuente aumento de *D. rotundus*, puso de manifiesto lo que en la actualidad se conoce como la Rabia Paralítica Bovina (RPB). Esta afección, es una de las enfermedades zoonóticas con mayor impacto sobre el ganado en América Latina, con una mortalidad de decenas de miles de cabezas de ganado por año y que se estima, genera pérdidas económicas anuales de 30 millones de dólares en la región neotropical del continente americano, sin contar la subnotificación que existe y los gastos recurrentes en vigilancia, diagnóstico y prevención (Benavides *et al.* 2020). Esta enfermedad es causada principalmente por las variantes V3, V5 y V11 del virus de la rabia que circulan en *D. rotundus* transmitidas al ganado, mediante la saliva, por la mordedura de esta especie de quirópteros (Velasco Villa *et al.* 2006).

La RPB, antes del siglo XX no se reconocía ni se relacionaba con la rabia clásica conocida ancestralmente en el Viejo Mundo, en donde era transmitida principalmente por cánidos (perros, lobos, zorros, etc.). La enfermedad transmitida por los vampiros, y que actualmente sabemos que es la rabia, en América Latina se conocía con diferentes nombres, por ejemplo, en México se le conocía como "derriengue", "güila" y "tronchado"; en Brasil, "peste das cadeiras", en Colombia y Costa Rica «hueguera» o «renguera» etc., y se le atribuyeron diferentes causas como intoxicaciones u otras afecciones que se relacionaban con la parálisis y los síntomas nerviosos que el virus de la rabia les provoca (Aréchiga-Ceballos *et al.* 2022). Fue hasta el siglo XX que el investigador brasileño Lima en 1934, vinculó esta afección con la rabia ancestral conocida en el Viejo Mundo, hecho verificado posteriormente por Pawan en 1936. En la revisión histórica realizada por Vos *et al.* (2011), es evidente que los primeros exploradores europeos del Continente Americano reportaron la existencia del murciélago vampiro común, ya que algunos de ellos y sus animales fueron mordidos por estos murciélagos y en algunos casos, la mordedura se relacionó con la presentación de una enfermedad mortal, pero sin los detalles que permitieran confirmar, con certeza, que se trataba de la rabia. Dada esta falta de información, resulta difícil cuantificar las pérdidas causadas por la rabia al inicio del establecimiento de la ganadería europea en AL.

Actualmente, se sabe que las principales especies afectadas son los bovinos. Para tener una idea de la proporción de las especies afectadas, en un análisis realizado por Ortega-Sánchez *et al.* (2022) en México se reportaron un total de 3,469 brotes en el periodo de 2010-2019, de los cuales el 89.1% ocurrieron en bovinos, 4.3% en caballos, 1.5% en ovinos, 0.6% en cabras, 0.01% en cerdos.

Una de las líneas de investigación que se iniciaron en México en los años setenta del siglo pasado, fue el estudio de diversos métodos para controlar las poblaciones de murciélagos vampiros, con miras a reducir los casos de RPB en el ganado. Desde aquel entonces, hace más de medio siglo, en América Latina, se siguen aplicando las técnicas de control de las poblaciones de los vampiros generadas en aquella época. Sin embargo, la RPB no se ha logrado controlar de forma consistente (Hayes y Piaggio 2018; Kraker-Castañeda *et al.* 2024; Olave-Leyva *et al.* 2025a). Cabe señalar que las investigaciones en la materia después de los años setenta y ochenta del siglo pasado han sido escasas. Es hasta el siglo XXI que han salido nuevas publicaciones analizando el problema (Osorio-Rodríguez y Saldaña-Vázquez 2019; Ávila-Vargas *et al.* 2025). En esta revisión, se exponen los métodos que se utilizan tradicionalmente en AL para el control de las poblaciones de los vampiros, argumentando su eficacia a la luz de investigaciones recientes y se mencionan alternativas de investigación que podrían contribuir a resolver el problema, limitando a su vez el uso de métodos letales.

Historia de los métodos de control del *D. rotundus*

Aunado a la leyenda negra que cargan los murciélagos, en la primera mitad del siglo XX, los productores, al ver afectado su ganado por las mordeduras de los vampiros y constatar que estos quirópteros pueden provocar la RPB a sus animales, han tratado de exterminarlos con prácticas drásticas, poco selectivas y perjudiciales, que no han sido avaladas por la comunidad científica. Se emplean gases venenosos para fumigar los refugios, insecticidas como el dicloro difenil tricloroetano (DDT), dieldrín y malathion, entre otros. También se utilizan explosivos o se incendian o saturan los refugios con humo, provocando así daños ecológicos irreversibles y la destrucción de especies concomitantes benéficas, además de contaminar el ambiente con sustancias tóxicas que afectan también al ser humano y otras especies (Lord 2018). Estas prácticas a toda costa deben de combatirse y ser eliminadas.

Los estudios científicos encaminados al control del vampiro común se iniciaron en México en los años setentas del siglo XX en el extinto Instituto Nacional de Investigaciones Pecuarias (INIP), bajo el auspicio del Gobierno Mexicano, de la Agencia para el Desarrollo Internacional de Estados Unidos (Agency for International Development, U.S. Department of State, USAID) y de la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO, Food and Agriculture Organization of the United Nations). En ese entonces investigadores pioneros como Raúl Flores Crespo, Samuel Linhart, Clay Mitchell y otros, realizaron las primeras observaciones sobre el comportamiento del vampiro común. Sus estudios revelaron varias características de los murciélagos hematófagos como su comportamiento social y hábitos alimenticios, que permitieron el diseño de varios "métodos selectivos" dirigidos al control del vampiro común. Estos métodos se basan en la utilización de sustancias anticoagulantes como la difenadiona y la warfarina que se utilizan habitualmente para matar roedores.

En un principio se propuso localizar los nichos donde se instalan las colonias de vampiros adentro de los refugios y untar el veneno anticoagulante con una brocha en las paredes del nicho (Flores Crespo *et al.* 1974a). Sin embargo, lo laborioso del método y su evidente inespecificidad condujeron al estudio de otras alternativas. Los estudios y resultados obtenidos condujeron a tres tratamientos o variantes de la utilización de anticoagulantes para matar a los murciélagos hematófagos:

i) Tratamiento tópico de D. rotundus con pomada vampiricida (anticoagulante en un vehículo como la vaselina). Esta técnica aprovecha la conducta de limpieza o acicalamiento individual y social de los vampiros y consiste en capturar algunos animales a los cuales se les unta la pomada vampiricida en su cuerpo (principalmente en el dorso) y después se les libera (Figura 3). Los animales tratados con la pomada regresan a sus refugios y durante las sesiones de acicalamiento social, ingieren el veneno

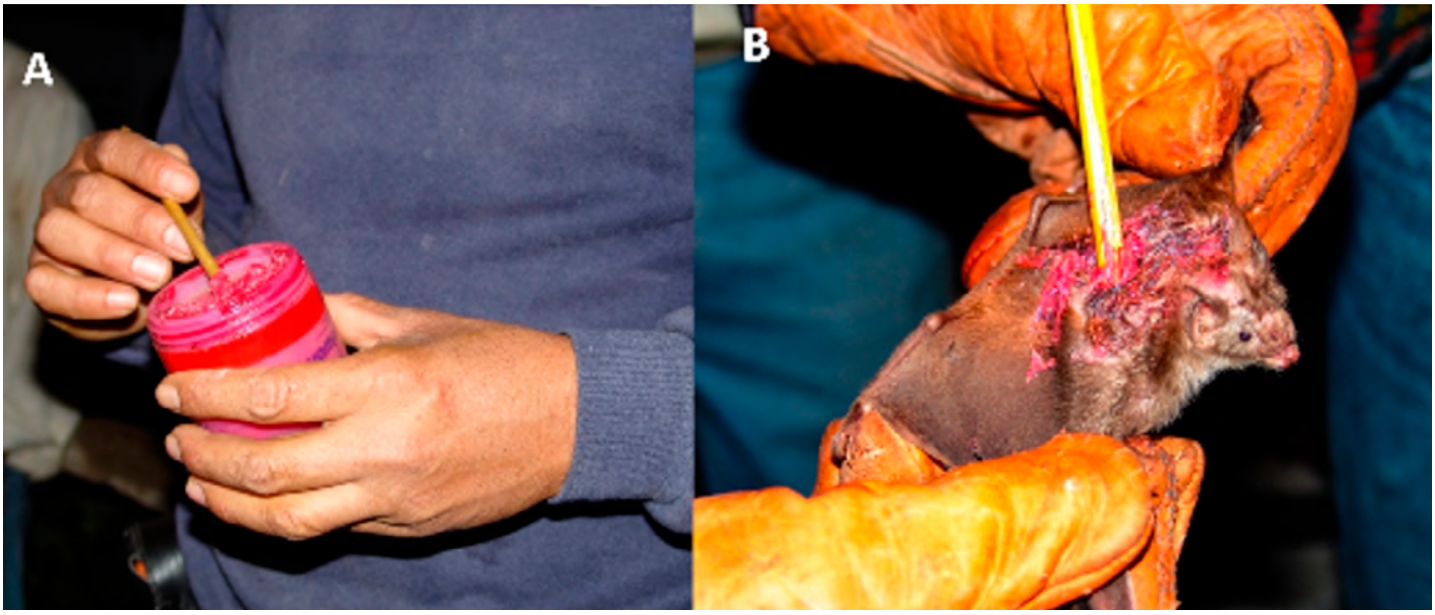

Figura 3. Aplicación tópica de veneno anticoagulante a un vampiro capturado. A) Tarro con vampiricida comercial; B) aplicación del producto en el lomo de un vampiro (*D. rotundus*) capturado.

ellos mismos y sus congéneres, provocando múltiples muertes en la colonia (Linhart *et al.* 1972).

ii) Tratamiento tópico del ganado mediante cobertura con pomada vampiricida de las mordeduras recientes de vampiros. Esta metodología aprovecha el hábito observado que tiene el vampiro común de alimentarse varias veces del mismo animal y en las mismas heridas, de esta manera al cubrir la herida con anticoagulante, al regresar, el vampiro ingiere el veneno y muere. Una variante de este método es untar una línea de pomada vampiricida a lo largo del dorso del animal, desde la cabeza hasta la cola con la intención de que los vampiros ingieran el veneno al morder al animal tratado (Flores Crespo *et al.* 1976).

iii) Tratamiento sistémico de los bovinos con anticoagulante. Esta metodología consiste en inyectar por vía sistémica (intramuscular o intrarruminal) a los bovinos expuestos al ataque de vampiros, con dosis bajas del anticoagulante (dosis supuestamente inocuas para los bovinos, pero letales para los vampiros). De esta manera, los vampiros que se alimentan de la sangre del bovino tratado sucumbirán al efecto del anticoagulante cuando se alimentan de ese animal, mientras que los bovinos tratados sobreviven ya que se demostró son menos susceptibles al efecto letal del anticoagulante (Flores Crespo *et al.* 1979).

De los tres métodos mencionados, el más eficaz y letal, es el primero (tratamiento i); es decir, el procedimiento tópico de algunos *D. rotundus* capturados con la pomada vampiricida, seguido de su liberación. Las otras opciones, tienen un efecto parcial y de menor duración, además de ser difíciles de implementar en un gran número de cabezas de ganado (Ávila-Vargas *et al.* 2025). Desde su implementación en los años setenta del siglo XX (hace más de medio siglo) hasta nuestra época, podemos decir que este es el método más utilizado en las campañas oficiales de varios países.

Eficacia de los métodos utilizados tradicionalmente para el control de *D. rotundus*

Es evidente que las prácticas para el control de las poblaciones de *D. rotundus* que como se mencionó, no tienen fundamento científico, como la fumigación de los refugios con veneno, la utilización de explosivos, fuego o humo en los mismos, a toda costa deben de evitarse y combatirse por los daños irreversibles que pueden provocar a la biodiversidad y al medio ambiente.

El tratamiento ii (cobertura de mordeduras de vampiro con pomada vampiricida o distribución del anticogulante en la piel del ganado), si bien puede tener cierta utilidad en hatos pequeños, presenta la dificultad de ser muy laborioso cuando se trata de un número grande de animales y la reducción de la población de vampiros es limitada. Hay evidencia de que el tiempo de contacto de los vampiros con su presa es muy variable y solo el 40% de los animales recibirían dosis letales del anticoagulante mediante esta metodología (Ávila-Vargas *et al.* 2025).

El método iii (tratamiento sistémico de los bovinos con anticoagulante) también presenta la limitación de ser laborioso cuando se trata de un número grande de animales, sin embargo, es necesario considerar que, aunque se utilicen bajas dosis de anticoagulantes supuestamente no letales en los bovinos, no se han hecho estudios a fondo de las consecuencias de este fármaco en la salud del ganado y en el consumo de los productos de este (Flores Crespo *et al.* 1979; Ávila-Vargas *et al.* 2025). Los tratamientos ii y iii, en ocasiones se aplican a individuos que presentan múltiples mordeduras ya que se ha observado que los vampiros muerden más a ciertos individuos en un hato; por ejemplo, se ha demostrado que individuos de la raza Holstein son más atacados que los de las razas Brahaman o Charolais, probablemente porque los primeros tienen un temperamento dócil y tranquilo, mientras que los de

las dos razas restantes tienen un temperamento nervioso (Flores Crespo *et al.* 1974b; Arellano-Sota 1988). Dado que el método i (tratamiento tópico con pomada vampiricida de individuos capturados y su liberación) ha sido el método más utilizado y fomentado en los países afectados desde hace más de medio siglo, en adelante nos centraremos en analizar eficacia de este método.

En primer lugar, es necesario señalar que, a pesar de su continua aplicación en muchos ranchos y regiones de América Latina, hasta el momento no se puede asegurar que este método sea una solución 100% eficaz para controlar la RPB ya que no se tiene noticia de una eliminación permanente de la enfermedad a largo plazo, en área alguna con o sin la aplicación de anticoagulantes (Viana *et al.* 2023; Ávila-Vargas *et al.* 2025). Por el contrario, si ha constado que la enfermedad actualmente se presenta en regiones en las que no había sido reportada con anterioridad, tal es el caso del Estado de Tamaulipas en México, en donde no se había reportado la RPB hasta la última década del siglo XX (1994), se presentó un brote en el municipio de Aldama (Martínez-Burnes *et al.* 1997).

Posteriormente, la RPB ha ido avanzando hacia el norte reportándose nuevos casos en municipios más cercanos a la frontera con EE. UU. (Hayes y Piaggio 2018; Olave-Leyva *et al.* 2025a). En el futuro, debido al cambio climático, se predice que la RPB podría llegar hasta EE. UU. (Hayes y Piaggio 2018; Olave-Leyva *et al.* 2025b). Es importante señalar como lo hacen Ávila-Vargas y colaboradores (2025), que todos los estudios de la eficacia del uso de los anticoagulantes se basan en constatar la reducción de las mordeduras del vampiro en el ganado y ninguno en la reducción de los brotes de rabia o de su avance territorial (Osorio-Rodríguez y Saldaña-Vázquez 2019). Viana *et al.* (2023) demostraron, mediante un modelo bayesiano, que el envenenamiento selectivo de vampiros por un periodo de dos años no logró reducir la propagación de la RPB al ganado, a pesar de reducir la densidad de la población de estos quirópteros. La secuenciación completa del genoma viral y los análisis filogeográficos demostraron, además que el sacrificio antes de la llegada del virus puede ralentizar la propagación espacial del virus, pero el sacrificio dentro de un brote establecido la acelera, lo que sugiere que los cambios en la dispersión de murciélagos inducida por su envenenamiento promueven la invasión viral (Viana *et al.* 2023).

Si bien con el método i (aplicación tópica del anticoagulante en el cuerpo de los murciélagos hematófagos), se nota una rápida y drástica reducción de las mordidas en el ganado por los vampiros (>95%) (Flores Crespo *et al.* 1976), actualmente se han señalado algunos inconvenientes de utilizar esta metodología. En primer lugar, si bien, este método parece tener cierta especificidad, los animales muertos o debilitados por la acción del anticoagulante podrían convertirse en presas fáciles de predadores como los mapaches (*Procyon lotor*), el cacomixtle (*Bassariscus astutus*), el coatí de nariz blanca (*Nasua narica*), los gatos domésticos (*Felis catus*), felinos silvestres, algunos mustélidos, sin contar aves rapaces tanto diurnas como nocturnas, y necrófagos especializados como los zopilotes (*Coragyps atratus*), etc. De esta manera, los animales que consumen a los murciélagos muertos o moribundos podrían resultar también dañados indirectamente con el anticoagulante (Pérez-Rivero *et al.* 2014; Ávila-Vargas *et al.* 2025).

Por otro lado, poco se sabe del efecto, en el sistema inmune de los murciélagos, de dosis no letales de los anticoagulantes utilizados para envenenarlos. Es lógico pensar que, durante las sesiones de acicalamiento, no todos los animales de la colonia van a consumir la misma cantidad del anticoagulante, algunos consumirán dosis suficientemente letales, pero otros solo alcanzarán dosis subletales que no llegan a matarlos, pero que sí pueden alterar su sistema inmune, haciéndolos más susceptibles a contraer infecciones como la rabia y facilitando así la diseminación de la enfermedad (Ávila-Vargas *et al.* 2025).

Al mismo tiempo, estudios realizados por Streicker *et al.* (2012), han demostrado que la aplicación tópica del anticoagulantes a murciélagos capturados y su liberación (tratamiento i), afecta sobre todo a los individuos adultos dejando una población juvenil que en principio es más susceptible a contraer la rabia, lo que propiciaría el avance del virus al encontrar una población sin barreras inmunológicas que lo detengan (Figura 4) (Streicker *et al.* 2012; Aréchiga-Ceballos *et al.* 2019; León *et al.* 2021). Eliminar a todos los vampiros de una colonia establecida en un lugar donde existe una oferta alimentaria de fácil acceso (*vg.* zonas ganaderas en regiones tropicales y subtropicales de América Latina), puede provocar que individuos de colonias aledañas se desplacen, ocupen y aprovechen el nicho que antes ocupaban los murciélagos envenenados, estableciendo una nueva colonia con individuos diferentes (Scheffer *et al.* 2014; Huguin *et al.* 2018; Benavides *et al.* 2020; Gonçalves *et al.* 2021; Rocke *et al.* 2023). De esta manera, si alguno o varios de los individuos que se instalen en el nicho desocupado están infectados con rabia, la enfermedad ocuparía esta nueva área junto con los nuevos invasores.

Finalmente, en ocasiones al aplicar el anticoagulante tópico, ha sucedido que se confunde al vampiro común con otras especies de la familia Phyllostomidae y en lugar de afectar al vampiro común, se afectan a otras especies que son benéficas (Uieda y Gonçalves de Andrade 2020; Kraker-Castañeda *et al.* 2024); es por ello que, es importante mencionar que esta metodología, en caso de utilizarse, sea aplicada por personal especializado capaz de diferenciar las especies benéficas de los murciélagos hematófagos.

Otras alternativas

Desde que surgieron los métodos de control de las poblaciones de vampiros con anticoagulantes, se señaló que la aplicación de los métodos de envenenamiento de las poblaciones de vampiros no era suficiente para detener la RPB y que estos métodos siempre deberían de acompañarse con la vacunación de los animales

Figura 4 . Vampiros juveniles abandonados tras la muerte de vampiros adultos que fueron tratados con vampiricida.

susceptibles (*vg*. ganado expuesto) contra la rabia. Como lo demostró Pasteur en sus estudios sobre la rabia "*el control de la enfermedad humana requiere intervenir en su reservorio animal*" (Sánchez-Paz *et al.* 2025).

Raúl Flores Crespo (1978), en una monografía sobre el control de los murciélagos hematófagos, hace las siguientes observaciones: *"Debido a que la única manera de prevenir la enfermedad es la vacunación, se recomienda hacerlo aún en aquellas zonas donde se efectúa el control de los vampiros; esto se debe a que no es posible, en el mejor de los casos, eliminar a todos con los métodos de control. Si se deja de vacunar, siempre existirá la posibilidad de que se presente la enfermedad y, por tanto, se recomienda insistentemente que se vacune contra la rabia a todos los bovinos…".* Desde aquel entonces, se hizo patente la imposibilidad de exterminar literalmente a *D. rotundus* en un área determinada, sin causar estragos graves colaterales al medio ambiente y por ende al ser humano. Por otro lado, el hecho de que la RPB no haya disminuido de manera considerable aplicando el envenenamiento o no de vampiros y que por el contrario haya avanzado geográficamente a zonas antes indemnes (Hayes y Piaggio 2018; Ávila-Vargas *et al.* 2025; Olave-Leyva *et al.* 2025a; Olave-Leyva *et al.* 2025b) nos obliga continuar con el estudio de otras alternativas que a continuación mencionamos.

La vacunación oral contra la rabia

En la primera mitad del siglo XX después de que se redujo drásticamente la rabia del perro mediante la vacunación de estos animales, en Europa Occidental constataron que los brotes de rabia en ganado y excepcionalmente en el humano lo provocaban ya no los perros, sino los zorros rojos (*Vulpes vulpes*), animal muy difícil de vacunar en su medio ambiente silvestre. Entonces, se pretendió controlar la rabia mediante el sacrificio de estos carnívoros silvestres, del mismo modo que actualmente se pretende eliminar la RPB mediante el sacrificio de *D. rotundus* en América Latina (Freuling *et al.* 2013). De esta manera en muchos lugares, el gobierno ofrecía recompensas por cada cola de zorro rojo presentada a las autoridades o hasta se llevaban a cabo campañas sistemáticas de envenenamiento de estos animales.

Pronto se dieron cuenta que el exterminar al zorro rojo de un área, no eliminaba la rabia de ese lugar. En efecto, se observó que los lugares en donde los zorros habían sido eliminados, se repoblaban rápidamente por zorros de áreas aledañas en donde no se habían exterminado y después de un tiempo, el problema recomenzaba (Jiguet 2020).

Por otro lado, se tenía la experiencia del éxito de la vacunación de los perros para la eliminación de la rabia

en esta especie ya que después de que se aplicaron las campañas de vacunación, ningún caso de rabia transmitida por este animal doméstico se había presentado ya. En los años setenta del siglo pasado, un grupo de investigadores europeos se planteó la posibilidad de vacunar a los zorros rojos en su ámbito silvestre. En aquella época esto parecía una utopía. Sin embargo, el Profesor Paul Pierre Pastoret y sus colaboradores en Bélgica, iniciaron el trampeo de zorros rojos, su vacunación con jeringas como se hace con los perros y su liberación al medio ambiente (Brochier *et al.* 1985). El trabajo que esto implicaba era realmente extenuante y no se lograba una inmunidad de rebaño de 80% de los individuos.

La confluencia de varios factores hicieron posible la vacunación de los zorros en su ámbito natural; la ingeniería genética permitió la obtención de vacunas resistentes al medio ambiente y con base a los estudios hechos por Georges Baer, que en aquel entonces trabajaba en el CDC (*Centers for Diseases Control and Prevention*) en EE. UU., se vio la posibilidad de aplicar la vacuna por vía oral, en lugar de tenerla que aplicar con una jeringa por vía parenteral como se hace con los perros (Baer 1988). Una vacuna oral, hecha con un virus resistente al medio ambiente, unida a un cebo atractivo para los zorros fue la solución (Wiktor *et al.* 1988). Los zorros ingerían la vacuna, atraídos por el cebo, logrando con esto la inmunización, con la ventaja de que los animales vacunados defendían su territorio e impedían la introducción invasores que podían portar la enfermedad. Es decir, se creaban barreras inmunes en ese territorio que impedían la introducción del virus. Utilizando estas vacunas orales de nueva generación, gran parte de la población de zorros rojos de Europa occidental ha sido vacunada a la fecha, con la consecuente eliminación de la rabia en aquella región (Brochier *et al.* 1991).

Esta historia de éxito animó a nuestro grupo a especular que podría hacerse lo mismo con el murciélago hematófago o vampiro. En los años ochenta del siglo pasado, con un proyecto financiado por la Unión Europea, nuestro grupo que incluía investigadores del Instituto Nacional de Investigaciones Pecuarias (INIP), del Instituto Mexicano del Seguro Social (IMSS) de México, y de la Universidad de Lieja (Bélgica), inició la vacunación de vampiros con la misma vacuna que se utilizaba para vacunar a los zorros en Europa occidental (la vacuna V-RG Raboral). La vacuna se aplicó a los vampiros principalmente por la vía oral, y posteriormente se ensayaron otras vías como la escarificación y por medio de aerosoles. Los resultados obtenidos fueron positivos ya que se logró, en todos los casos, la producción de anticuerpos y la protección de los animales contra el virus patógeno (Aguilar-Setién *et al.* 1998; Aguilar-Setién *et al.* 2002).

Estos experimentos no estuvieron exentos de críticas, arguyendo que era un "despropósito vacunar al enemigo". A pesar de dichas críticas, otros autores como Almeida y su grupo en Brasil encontraron también ventajas en esta aproximación e iniciaron por su parte experimentos de vacunación de vampiros (Almeida *et al.* 2008). Más aún, actualmente un grupo de investigación en Wisconsin, EE. UU. en el que participa la investigadora mexicana Elsa Cárdenas Canales, ha reiniciado experimentos de vacunación de vampiros con miras a establecer una metodología eficaz que impida la circulación del virus de la rabia en estos animales (Cárdenas-Canales *et al.* 2022).

La mayoría de las publicaciones sobre la vacunación de los vampiros, están basadas en la administración por la vía oral, aplicada como los venenos anticoagulantes de manera tópica en el cuerpo del animal para que lo consuman durante sus sesiones de acicalamiento; además se ensayó la aplicación de la vacuna por medio de aerosoles (Aguilar-Setién *et al.* 2002; Tesoro Cruz *et al.* 2006; Tesoro Cruz *et al.* 2008). Se consideró que la vacuna en aerosoles podría aplicarse en los refugios, como se hacía con los insecticidas y venenos, por medio de un nebulizador. Este sistema tendría la ventaja de que no solamente los vampiros quedarían vacunados contra la rabia, sino también los quirópteros benéficos que conviven con los vampiros en el mismo refugio, creando barreras más amplias contra el virus. Sin embargo, debemos tomar en cuenta que la aplicación de los aerosoles en refugios sería una tarea laboriosa y ser consciente de que se requieren más investigaciones al respecto.

Vacunas que se diseminan automáticamente entre los murciélagos vampiros.

Tomando en cuenta las experiencias expuestas sobre la vacunación de los vampiros, Griffiths *et al.* (2023) proponen el desarrollo de una "vacuna transmisible" que puede definirse como una biotecnología emergente que ofrece perspectivas para eliminar patógenos de poblaciones silvestres. En dichas vacunas se recurre a la ingeniería genética para modificar un virus no patógeno de origen natural, «vector viral», para que sea capaz de expresar antígenos de virus patógenos, conservando al mismo tiempo su capacidad de transmisión y su inocuidad (Griffiths *et al.* 2023). En concreto, Griffiths *et al.* (2023), proponen utilizar un virus herpes llamado *Desmodus rotundus betaherpesvirus* (DrBHV) que se disemina muy fácilmente entre los vampiros sin afectar a otras especies de murciélagos. A este virus DrBHV se le harían modificaciones genéticas para que expresara los antígenos del virus de la rabia. De tal manera que los vampiros que se infecten con este virus construido quedarían inmunizados contra el virus de la rabia y al mismo tiempo transmitirían el virus a sus congéneres susceptibles que conviven con ellos, los que a su vez quedarían también vacunados y con la posibilidad de contagiar el virus a otros individuos que no lo han adquirido, diseminando la vacuna entre la población.

Lord (2018) recomendó la vacunación de los vampiros como un método adecuado para el control de la rabia porque: *"... un animal inmunizado es doblemente valioso porque no solo no puede mantener la epizootia, sino también porque continúa ocupando su nicho de hábitat, defendiéndolo de los invasores..."*. Incluso si se controla la rabia vampírica,

existe la posibilidad de que el virus encuentre otras especies de murciélagos susceptibles.

La ventaja de la vacunación de una especie determinada es que se forman barreras inmunes que impiden la circulación del virus en esa especie y como no se mueren, la población permanece activa y defiende su territorio contra otros individuos, enfermos o susceptibles que pretendan apropiarse del área.

La cuestión que queda pendiente en este caso es la de que los individuos vacunados ya no podrán trasmitir la RPB, pero sí podrán seguir mordiendo al ganado, lo cual dependiendo de la óptica y los estudios que se hagan, podría ser una situación tolerable por los ganaderos. La investigación constante de estos temas tendrá la respuesta.

Vacuna contra la saliva del murciélago vampiro

Delpietro *et al.* (2021) publicaron un estudio en el que inmunizaron, con saliva de *D. rotundus*, grupos de ovejas previamente mordidas por murciélagos vampiro y grupos no mordidos, con el objetivo de inducir la producción de anticuerpos contra los anticoagulantes salivales de estos animales (anticoagulantes salivales de vampiro, ASV). Esto sugiere la utilidad de desarrollar un método alternativo para el control de los murciélagos vampiro, basado en la inducción de una fuerte respuesta inmunitaria del ganado contra los ASV, mediante la clonación y expresión de antígenos vacunales salivales «antivampiro» apropiados. En teoría, este producto biológico podría promover la coagulación sanguínea en el ganado inmunizado (debido a su resistencia adquirida contra el ASV), lo que dificulta la ingestión adecuada de sangre por parte del murciélago, así como su correcta digestión y eliminación del exceso de agua. Se supone que sería difícil para los murciélagos vampiros sobrevivir alimentándose de presas altamente resistentes al ASV, considerando las severas demandas que impone la hematofagia, como la gran cantidad de sangre que deben ingerir (en promedio 20 ml diarios por individuo) para cubrir sus necesidades energéticas. Efectivamente, estos autores, reportan acumulación de sangre en el sistema digestivo de los animales que se alimentaron de las ovejas inmunizadas.

Control de las poblaciones de vampiros mediante reducción de la fertilidad.

Actualmente se sabe que los métodos letales no siempre son eficaces, debido a la necesidad de aplicaciones repetidas, cubriendo amplias áreas geográficas (Massei *et al.* 2024). Hay que agregar el daño que los venenos utilizados pueden producir en otras especies silvestres o domésticas, incluyendo al ser humano y al medio ambiente en general. De hecho, en varios países el uso de estos venenos anticoagulantes está siendo cada vez más limitado (Jacob y Buckle 2017; Quinn *et al.* 2019). El control de la fertilidad de las plagas de mamíferos, que actúa reduciendo los nacimientos más que aumentando la mortalidad, se ha propuesto como una alternativa menos drástica que los métodos letales, con la ventaja de que una población regulada mediante la disminución de su fertilidad podría mantener mejor el equilibrio ecológico.

En el caso de los vampiros, como se ha mencionado, sacrificar a los individuos de una colonia con cualquiera de los métodos mencionados, si bien reduce el número de ganado mordido, podría propiciar el avance de un brote de rabia al promover el desplazamiento de las colonias y generar poblaciones de juveniles susceptibles (Streicker *et al.* 2012; Scheffer *et al.* 2014; Huguin *et al.* 2018; Aréchiga-Ceballos *et al.* 2019; Benavides *et al.* 2020; Gonçalves *et al.* 2021; León *et al.* 2021; Rocke *et al.* 2023).

Bajo esta óptica, Investigadores del IMSS y de la Universidad Autónoma Metropolitana (UAM) en México, a principios de este siglo experimentaron con la utilización del coumestrol aplicado por vía oral, como anticonceptivo para vampiros. El coumestrol es un fitoestrógeno que se une a receptores estrogénicos en los mamíferos y al cual se le ha encontrado la capacidad de reducir la testosterona y de afectar la espermatogénesis en ratas. Los resultados obtenidos al administrar el coumestrol por vía oral a vampiros adultos, indicaron que provoca alteraciones histológicas relacionadas con infertilidad, en los testículos de los machos y en los ovarios de las hembras tratados (Pérez-Rivero *et al.* 2004; Pérez-Rivero *et al.* 2014). El coumestrol podría administrarse a los vampiros, del mismo modo que se hace para administrarles los venenos anticoagulantes, sin embargo, esto debería ser realizado por personal altamente capacitado, que pueda diferenciar a las especies benéficas del vampiro común.

Otras posibilidades

Desde tiempos ancestrales, las barreras físicas como mallas, mosquiteros, redes, etc., se han utilizado y se han revelado eficaces para proteger a las personas contra las mordeduras de *D. rotundus*. Lord (2018), menciona que los indios Guajira de Venezuela, usan en sus hamacas cubiertas de tejido que los protegen de las mordeduras de los vampiros y de los piquetes de los mosquitos (Lord 2018). El uso de mosquiteros en las ventanas y orificios de las habitaciones humanas ha demostrado también ser una medida eficaz para protegerse (Lord 2018). Este tipo de protección solo podría implementarse en el caso de animales estabulados. La mayoría del ganado en las zonas tropicales y subtropicales deambula libremente en los potreros, por lo que este tipo de barreras resultaría difícil de implementar en dichas condiciones.

Hace tiempo se pensó que se podía proteger al ganado de las mordeduras de los vampiros manteniendo los corrales iluminados ya que son nocturnos y prefieren no salir con luna llena (Flores Crespo *et al.* 1972). Sin embargo, el vampiro común es un mamífero sumamente adaptable e inteligente y si bien en un principio el número de mordeduras puede disminuir en un corral iluminado, con el tiempo estos animales se acostumbran a la luz y regresan a su actividad normal.

Un campo que se ha abordado poco y que puede ser prometedor, es el estudio de sustancias atrayentes o repelentes en el caso de *D. rotundus*. En el caso de repelentes, Thompson *et al.* (1982) demostraron que los vampiros rechazan la sangre a la que se le ha agregado quinina. Recientemente, Ziegler y Behrens (2021) demostraron la sensibilidad de los receptores al sabor amargo de la quinina en los vampiros. Un repelente a base de quinina podría ser utilizado para proteger al ganado.

Consideramos que es muy importante destacar que actualmente controlar la rabia transmitida por vampiros representa un reto complejo en la medida de que se corre el riesgo de que cualquier acción desencadene un mayor número de agresiones a humanos y a fauna silvestre, favoreciendo brincos a otras especies o "*spill overs*" que pueden dar lugar a casos humanos o en animales que normalmente no son parte de la dieta habitual de *D. rotundus* como el caso de brote de rabia en capibaras (*Hydrochoerus hydrochaeris*) en Brasil (Mori *et al.* 2024; Soinski *et al.* 2024). En este sentido, nuestro grupo fue testigo en los años setenta del siglo pasado, como en Tejupilco, Estado de México, zona que se dedicaba a la cría de ganado que cambió repentinamente al cultivo de la caña de azúcar por razones económicas, se empezaron a reportar aumentos de mordeduras de vampiro en las personas como consecuencia de la ausencia repentina de su presa habitual: los bovinos (datos no publicados). Hay que considerar también las adaptaciones a otras especies que puedan actuar como reservorios del virus, conocidos como "*host switchings*", dando lugar a la emergencia de nuevas especies reservorio. Por lo que las acciones deben ser analizadas a nivel local y no asumir que la misma estrategia va a ser eficiente a lo largo de la distribución de *D. rotundus* (Gonçalves *et al.* 2021).

Conclusiones

El método de control del vampiro común que tradicionalmente se han utilizado en América Latina es principalmente la aplicación tópica del ungüento en los murciélagos vampiros, el cual contiene un anticoagulante que en un principio (desarrollado en la década de los años 70) contenía warfarina y actualmente contiene bromadiolona.

En México, el control de las poblaciones de murciélagos vampiros se realiza de acuerdo con la Norma Oficial Mexicana NOM-067-ZOO-2007, "Campaña nacional para la prevención y control de la rabia en bovinos y especies ganaderas", en donde se especifica que los productos vampiricidas que se utilicen en la campaña deben ser los elaborados con sustancias anticoagulantes. Sus vehículos, dosificación, así como el mismo vampiricida deben contar con el registro oficial de la Secretaría de Agricultura y Desarrollo Rural (SADER). Su aplicación se realiza conforme a la vía de administración y dosis indicada por el laboratorio fabricante.

La aplicación tópica del ungüento con anticoagulante en *D. rotundus* requiere de esfuerzo de muestreo en la captura de los especímenes, ya sea en corral y/o en refugio, y no se ha logrado demostrar que esta práctica reduzca los casos de RPB; empero sí se ha demostrado puede tener implicaciones en el mantenimiento y diseminación del virus rábico al perturbar y modificar las poblaciones de los murciélagos (Streicker *et al.* 2012).

Las alternativas al empleo de anticoagulantes que se han descrito y que ameritan mayor estudio son:

i).- La vacunación de los vampiros contra la rabia. Se ha demostrado que la vacunación de los animales que transmiten la rabia que son al mismo tiempo reservorios y vectores del virus (domésticos: perros y gatos o silvestres: zorro, mapache, coyote, etc.), ha sido una medida eficaz para reducir los casos de rabia en el ser humano y en los animales domésticos (Maki *et al.* 2017; Rupprecht *et al.* 2024). Los resultados obtenidos a la fecha en diversos estudios indican que igualmente, se podría lograr la reducción de los casos de RPB mediante la vacunación de *D. rotundus* (Cárdenas-Canales *et al.* 2022; Knuese *et al.* 2025). Los esfuerzos de investigación en este sentido son promisorios y deben continuarse.

ii).- Vacunación del ganado susceptible con la saliva de D. rotundus (ASV). En teoría, esta vacuna, podría promover la coagulación sanguínea en el ganado inmunizado (debido a la neutralización de los anticoagulantes que contiene la saliva de los vampiros). Se supone que sería difícil para los murciélagos vampiros sobrevivir alimentándose de presas resistentes al ASV. Sin embargo, hay que considerar que, el esfuerzo de vacunar al ganado contra la ASV sería el mismo que vacunarlo contra la rabia, que es a la fecha la medida más eficaz para reducir los casos de RPB. Si se llegara a comprobar que la vacunación del ganado contra la ASV influye disminuyendo las poblaciones de *D. rotundus*, entonces, la vacunación simultánea del ganado contra el virus de la rabia y contra la ASV tendría, en principio, doble beneficio. Son necesarios más estudios al respecto.

iii).- Métodos que reduzcan la fertilidad de D. rotundus. El control de la fertilidad de las plagas de mamíferos que actúa reduciendo nacimientos más que aumentando la mortalidad, se ha propuesto como una alternativa menos drástica que los métodos letales. Esta medida tendría la ventaja de que una población estable, mantiene un equilibrio ecológico y defiende su territorio contra intrusos que pueden estar infectados. Serían necesarios más estudios que nos permitan conocer cómo se podría mantener una población estable administrando compuestos que reduzcan la fertilidad, determinar las dosis, la vía de administración y diseñar métodos de evaluación de la reducción de la misma.

iv).- El estudio de repelentes o atrayentes de D. rotundus. Este es un tema escasamente abordado y que valdría la pena explorar; se sabe que sustancias amargas como la quinina hacen que los vampiros rechacen la sangre que se les ofrece y existen pocos sobre las feromonas que podrían atraerlos. La utilidad de las sustancias repelentes es evidente, los atrayentes podrían por otro lado utilizarse para

alejar o desviar a los murciélagos hematófagos del ganado y aplicar otros métodos de control y/o de vacunación en donde se congreguen por el atrayente.

El control de la rabia paralítica en especies ganaderas se ha realizado a través de la aplicación de la vacuna antirrábica, las cuales deben ser las elaboradas con virus activo modificado o con virus inactivado. Su aplicación se realiza conforme a la vía de administración y dosis indicada por el laboratorio fabricante, el manejo de la vacuna debe realizarse por un Médico Veterinario Zootecnista, certificado para tal actividad. La vacunación antirrábica de las especies ganaderas es obligatoria en el área enzoótica y en aquellos lugares donde se presenten casos clínicos y/o confirmados por laboratorio (NOM-067-ZOO-2007); la vacunación se debe aplicar al 100% de los animales y se debe establecer un calendario y las estrategias de vacunación para mantener la inmunidad de hato.

La aplicación de la vacuna a todos los animales de un hato, representa un esfuerzo arduo y oneroso, principalmente en las condiciones de potrero de las explotaciones de la región Neotropical de América Latina. Esfuerzo que muchos ganaderos no están dispuestos a realizar como medida preventiva. Cuando sí lo realizan, suele ser hasta que se presenta un brote, momento en el ya son evidentes las muertes del ganado por RPB, lo que disminuye la efectividad de la vacunación. Por ello, es necesario fomentar en las explotaciones la cultura de la prevención.

El estudio de las interacciones de las poblaciones de los mamíferos y su medio ambiente es complejo, más aún si agregamos factores que influyen en la epidemiología de las enfermedades. Desafortunadamente, los recursos para estudiar y experimentar en estas interacciones se han reducido considerablemente en la actualidad. Esperamos que los tomadores de decisiones, hoy como ayer, apoyen más investigaciones en este sentido.

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Editor asociado: Jesús Alonso Panti May
Sometido: Octubre 14, 2025; Revisado: Noviembre 22, 2025
Aceptado: Enero 7, 2026; Publicado en línea: Enero 30, 2026