Therya

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La portada

El jaguar (*Panthera onca*) es una especie distribuida desde el sur de los Estados Unidos hasta el norte de Argentina. Este individuo fue registrado por una cámara trampa en San Luis Potosí, México. Fotografía de Jorge Castro Urbiola.

Issue cover

The jaguar (*Panthera onca*) is a species distributed from the southern United States to northern Argentina. This individual was recorded in a camera trap in San Luis Potosí, México. Photograph by Jorge Castro Urbiola.

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. Al signo decimoprimero en la cosmogonía mexica. "Ozomatli" es una representación pictórica de los mono arañas (*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 visajes a la gente. Arrojan piedras y palos. Su cara es casi como la de una persona, pero tienen mucho pelo."

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Therya and Ammac

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DERECHOS DE AUTOR Y DERECHOS CONEXOS, año 16, No. 2, mayo-agosto del 2025, es una publicación cuatrimestral editada por la Asociación Mexicana de Mastozoología A. C. Calle 78 entre 13-1 y 128 Núm. 578 Colonia Residencial Pensiones VI Etapa Mérida, Yucatán. C.P. 97217. Distrito Federal, México. Telefono (228) 8471512, www.mastozoologiamexicana.org, therya@cibnor.mx. Editores responsables: Dra. Sonia Gallina Tessaro, Dr. Jorge Ortega Reyes y Dra. María Cristina Mac Swiney González. Reservas de Derechos al Uso Exclusivo No. 04-2022-032919260800-102, ISSN: 2007-3364 ambos otorgados por el Instituto Nacional de Derechos de Autor. Responsable de la última actualización de este número, Unidad de informática de la Asociación Mexicana de Mastozoología A. C. Dra. Sonia Gallina Tessaro, Instituto de Ecología A.C. fecha de la última modificación 29 mayo 2025.

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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.

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Editorial

Una nueva etapa en Therya

Si hoy preguntáramos a la IA (Inteligencia Artificial) cuál es la primera sociedad científica que reconocemos en Occidente, nos indicaría a la Royal Society (fundada en 1660), y sabríamos que su revista se creó pocos años después (1665) con el nombre de *Philosophical Transactions of the Royal Society.* Esta revista surgió con la intención de satisfacer la necesidad de los miembros de la sociedad de dar a conocer su trabajo, para que el resto de la comunidad lo pudiera estudiar, examinar y, si fuera el caso, refutar. La ciencia se basa en ciclos interminables de hechos y teorías que se confirman o no y en ese proceso las publicaciones científicas desempeñan un papel fundamental. En primera instancia se difunden resultados inéditos, los cuales fueron sometidos a una revisión por pares. También permiten marcar en la historia las fechas de los descubrimientos y a la par consolidan el concepto de autoría científica. Todo esto ha sido esencial en el desarrollo de la ciencia moderna desde el lanzamiento de esa primera revista hasta nuestros días.

Nuestra Asociación Mexicana de Mastozoología A.C. (AMMAC), como cualquier sociedad científica, deseaba contar con una revista que apoyara la difusión de las investigaciones sobre mamíferos en México y el mundo. Hace 15 años inició el camino de Therya, un intento exitoso por crear una publicación científica de la AMMAC, gracias al esfuerzo de muchas personas dentro de la sociedad que visualizaron un futuro prometedor para esta revista.

Tras 15 volúmenes con tres números por año y más de 250 artículos científicos sobre diversos aspectos de los mamíferos, su Editor General, el Dr. Sergio Ticul Álvarez Castañeda, y parte de su equipo, cierran un ciclo y se despiden de la revista. Todos los que formamos parte de la AMMAC lo reconocemos y agradecemos profundamente.

El trabajo y dedicación del Editor General y su equipo fueron fundamentales para la creación, consolidación y fortalecimiento de una revista que hoy es reconocida en el campo de la mastozoología, no solo en México o Latinoamérica, sino a nivel mundial. Therya está actualmente indexada en bases de datos bibliográficas como Scopus, Redalyc, SciELO, entre otras, y cada vez más mastozoólogos, dentro y fuera de México, la consideran una opción valiosa para dar a conocer sus hallazgos.

Este nuevo volumen, el número 16 de nuestra revista Therya, marca un importante cambio en su dinámica editorial, ya que tres destacados colegas en el campo de la mastozoología asumirán el papel de editores: la Dra. Sonia A. Gallina Tessaro, la Dra. María Cristina Mac Swiney González y el Dr. Jorge Ortega Reyes. Todos ellos son científicos de prestigio internacional que, de manera generosa y altruista, han aceptado encabezar nuestra revista con el compromiso de mantener e incrementar sus indicadores. Estamos seguros de que los nuevos editores, y todo su equipo de trabajo —editores asistentes, editores asociados, revisores, diseñadores— y por supuesto los autores, contribuirán a que la revista siga fortaleciéndose como hasta ahora.

Sin duda, el futuro de las revistas científicas es desafiante. Existen preguntas complejas: ¿Cómo conciliar el uso de la inteligencia artificial con la originalidad de los artículos científicos? ¿Cómo lograr revisiones ágiles y de calidad en un contexto donde cada vez es más difícil contar con revisores? ¿Cómo competir en un mercado de publicaciones científicas que genera 11,600 millones de dólares anuales y que pone en desventaja a las revistas de sociedades científicas, cuyo único sustento es el compromiso de sus miembros?

Estamos seguros de que, si todos colaboramos y contribuimos al esfuerzo colectivo, la revista Therya seguirá consolidándose como una de las mejores opciones de publicación para mastozoólogos.

GERARDO SANCHEZ ROJAS
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Mesa Directiva de la AMMAC

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Living on the city: Records of night monkeys (*Aotus* spp.) in urban and peri-urban forests of Colombia

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Urbanization has transformed natural habitats by concentrating human populations in urban areas, with important consequences for biodiversity. In Colombia, several mammals, including primates, have occupied urban areas in different regions of the country. The objective of this study is to compile information on night monkeys (*Aotus* spp.) in urban and peri-urban forests in Colombia, based on field records and literature review. We compiled our own and literature records of night monkeys from urban and peri-urban forests in Colombia between 2018 and 2024. We categorized these records by type and grouped them by night monkey species, geographic location, type of population center (departmental capital city, municipality, corregimiento and vereda), and proximity to urban infrastructure. 36 localities were identified (23 new and 13 from the literature), distributed in 11 departments, ranging from the capital city to the veredas, where night monkeys have been recorded in urban and peri-urban areas. The most abundant species was *A. lemurinus*, and we report the first urban and peri-urban records of *A. brumbacki*, *A. griseimembra* and *A. vociferans*. About 33 % of the records of night monkeys in urban and peri-urban forests were found in capital cities with a population of more than 100000 people. Additionally, we report the use of 3 artificial sleeping sites in urban and peri-urban forests by *A. brumbacki, A. griseimembra* and *A. vociferans* groups. Records were also found in educational institutions such as schools and universities, suggesting the possibility of establishing long-term studies with these night monkeys as flagship species. The monitoring of these primates in urban and peri-urban environments is crucial for their conservation and to guide public policies towards sustainable development, especially in the management of urban threats such as electrocution, which remains a significant problem.

La urbanización ha transformado los hábitats naturales al concentrar poblaciones humanas en áreas urbanas, con importantes consecuencias para la biodiversidad. En Colombia, varios mamíferos, incluyendo primates, han ocupado áreas urbanas en diferentes regiones del país. El objetivo de este estudio es recopilar información sobre monos nocturnos (*Aotus* spp.) presentes en bosques urbanos y periurbanos de Colombia, con base en registros de campo y revisión bibliográfica. Recopilamos entre 2018 y 2024 registros propios y de literatura de monos nocturnos de bosques urbanos y periurbanos en Colombia. Categorizamos estos registros según su tipo y los agrupamos de acuerdo con la especie de mono nocturno, la ubicación geográfica, el tipo de centro poblado (ciudad capital departamental, municipio, corregimiento y vereda) y la proximidad a infraestructuras urbanas. Se identificaron 36 localidades (23 nuevas y 13 de la literatura), distribuidas en 11 departamentos y ubicadas desde ciudades capitales hasta veredas donde se han registrado monos nocturnos en zonas urbanas y periurbanas. La especie más abundante fue *A. lemurinus*, y reportamos los primeros registros urbanos y periurbanos de *A. brumbacki, A. griseimembra* y *A. vociferans*. Aproximadamente el 33 % de los registros de monos nocturnos en bosques urbanos y periurbanos se encontraron en ciudades capitales con poblaciones superiores a 100000 habitantes. Adicionalmente, reportamos el uso de tres dormideros artificiales en bosques urbanos y periurbanos por grupos de *A. brumbacki, A. griseimembra* y *A. vociferans*. Se encontraron registros en instituciones educativas como escuelas y universidades, lo que sugiere la posibilidad de establecer estudios a largo plazo con estos monos nocturnos como especies banderas. El monitoreo de estos primates en entornos urbanos y periurbanos es crucial para su conservación y para orientar políticas públicas hacia el desarrollo sostenible, especialmente en la gestión de amenazas urbanas como la electrocución, que sigue siendo un problema importante.

Keywords: Andean region; Aotidae; educational institutions; electrocution; Primates; sleeping sites; urbanization.

Introduction

The percentage of natural cover worldwide has been transformed by anthropogenic activities by more than 75 % (IPCC 2019). In Colombia, although it is estimated that about 52 % of the territory is covered by natural forest, there is an annual deforestation rate of 0.62 % (Armenteras *et al.* 2013; IDEAM 2020). Especially in this country, the greatest transformation of forests has occurred as a result of the expansion of crops and the agricultural frontier, the establishment of pastures for livestock and the construction of infrastructures and urbanizations (Etter and Wyngaarden 2000; Etter *et al.* 2008).

Urbanization is the process through which dispersed human populations living in small rural settlements grow and concentrate in dense urban cores. This occurs alongside industrial and agricultural development necessary to provide services, resulting in alteration of the natural environment (McIntyre 2011; Hussain and Imitiyaz 2018). The changes in land use and intervention of natural land cover often result in a mixture of human infrastructures and forest remnants within urban areas or in surrounding areas (Wear 2013; Salbitano et al. 2016). In general, urban and peri-urban forests present alterations in their structure and composition due to the constant pressures of urbanization, as not all species are able to adapt to these changes (Wear 2013).

Colombia is recognized as the sixth country with the highest diversity of mammals due to its strategic location in the tropics, where different geographical regions converge (Ramírez-Chaves *et al.* 2016). This diversity is also represented in urban and peri-urban forests, where numerous mammal species among different orders have been recorded, such as Chiroptera (Ballesteros and Racero-Casarrubia 2012; Rosero-Taramuel *et al.* 2023), Didelphimorphia (Barrera-Niño and Sánchez 2014; Guimarães *et al.* 2023), Carnivora (González-Maya *et al.* 2017; Sánchez-Londoño *et al.* 2023) and Primates (Poveda and Sánchez-Palomino 2004; Soto-Calderón *et al.* 2016; Montilla *et al.* 2018, 2020; Bustamante-Manrique *et al.* 2021; Grajales-Suaza *et al.* 2021). Among the primates recorded in urban and peri-urban areas of Colombia are night monkeys of the species *A. lemurinus*, which is characterized, as well as the other species of the genus, by its ability to adapt to different forest types (Montilla *et al.* 2018, 2020; Bustamante-Manrique *et al.* 2021; Grajales-Suaza *et al.* 2021). Although night monkeys inhabit a variety of forest environments, most species in Colombia are threatened due to loss of habitat for urbanization, agricultural activities, illegal trade as pets, and biomedical research purposes (Maldonado *et al.* 2023; Shanee *et al.* 2023a).

Records of night monkeys in urban and peri-urban areas provide opportunities for implementation of conservation tools in nearby veredas, corregimientos, municipalities, and capital cities where these charismatic species can serve as flagship species to promote the conservation of entire communities of flora and fauna. In this context, the aim of this study is to provide new records and compile historical data on the presence of some species of the genus *Aotus* like *A. brumbacki, A. griseimembra, A. lemurinus*, and *A. vociferans* in urban and peri-urban forests in Colombia. Additionally, we recorded the use of artificial sleeping sites by groups of *A. brumbacki, A. griseimembra* and *A. vociferans* in peri-urban areas.

Materials and methods

We compiled records of night monkeys between 2018 and 2024 in urban and peri-urban forests from several research and conservation projects involving these primates in Colombia. Furthermore, we reviewed published scientific literature for records of night monkeys in urban and peri-urban forests. For each record obtained we determined the following aspects: the species of night monkey recorded, exact geographic location (department, municipality, locality, coordinates and altitude), date of observation, type of nearby population center (departmental capital city, municipality, corregimiento, or vereda), and source and type of record (either daytime observation in sleeping site, nocturnal observation when night monkeys are active, or electrocuted individuals). Moreover, for the records directly obtained by us, we calculated (using satellite images) the minimum distance between the observed night monkeys and the buildings that are part of the population center.

We included as urban and peri-urban records any within a minimum distance of 105 m, which corresponds to the 3.5 ha radius of the average home range reported for different groups of night monkeys in Colombia (between 1.1 and 8 ha; (Guzmán *et al.* 2016; Bustamante-Manrique *et al.* 2021; Montilla *et al.* 2021a). Based on the categorization by Balk *et al.* (2018), we defined as urban records all those where the area of 3.5 ha had a coverage of at least 50 % of buildings that are part of the population center, and as peri-urbans, those where the coverage of buildings was less than 50 %. The measurements of the area and the estimations of the percentage of coverage were made with the circular ruler tool of Google Earth Pro.

Considering the scarce morphological differentiation among night monkeys' species, the identification of each record was based mainly on the geographic distribution, following the proposals of Henao-Diaz *et al.* (2020) and Shanee *et al.* (2023b). Consequently, all sightings in the Orinoquía region were attributed to *A. brumbacki*, those in the Magdalena Medio Valley to *A. griseimembra*, those in the Amazonia to *A. vociferans*, and those in the Andes to *A. lemurinus.* The record located in northern Antioquia, which could be on the boundary between the distribution of *A. lemurinus* and *A. griseimembra*, was assigned to *A. lemurinus* according to the potential distribution map by Henao-Diaz *et al.* (2020).

Results

In total, we documented the presence of night monkeys in urban and peri-urban areas of 36 different localities in Colombia. Of these, 23 are new records, while 13 are records previously published in scientific literature. Sightings of night monkeys in urban and peri-urban forests are distributed in 20 municipalities belonging to 11 departments, at altitudes ranging from 93 to 2,258 masl (Table 1; Figure 1). Most of these records occurred in municipalities (16 records), followed by capital cities (12 records), corregimientos (4 records) and veredas (4 records). 12 of the records are located in urban areas, while 24 correspond to peri-urban forests. The most frequently species of night monkey in urban and peri-urban forests was *A. lemurinus*, with 26 records, followed by *A. brumbacki* and *A. griseimembra* with 4 records each, and *A. vociferans* with 2 records. Most of the records were obtained by nocturnal observation (19 records), followed by daytime observations at sleeping sites (9 records), and those documented after electrocution events (6 records). On one occasion, the presence of night monkeys in peri-urban forests was recorded through museum specimens and interviews. From the new records, we identified distances between 7 and 84 meters (mean: 28.73; ± 21.90) between night monkey observations and buildings that are part of population centers (see Table 1).

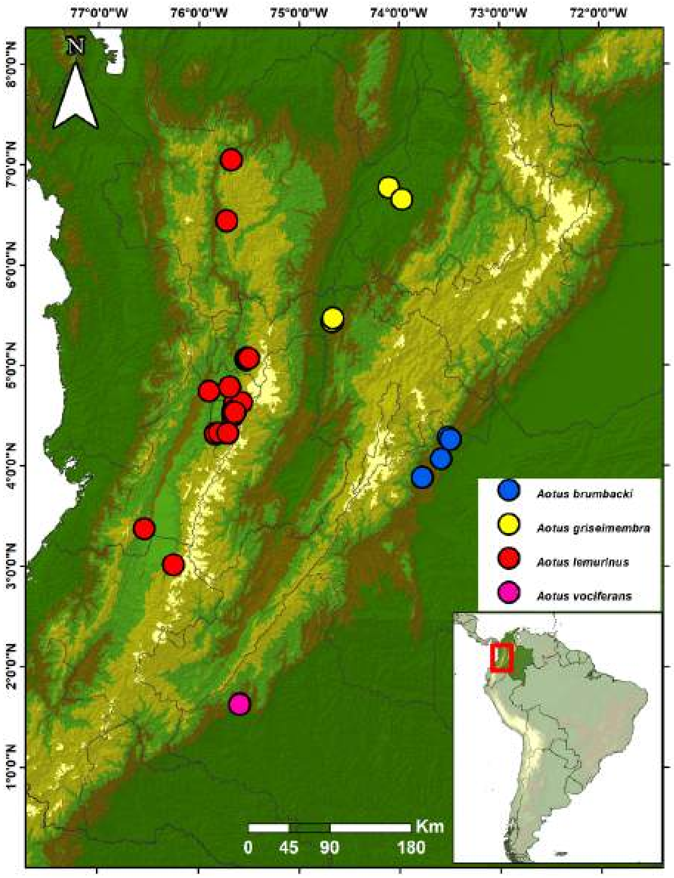

Figure 1. Geographic location of night monkey (*Aotus* spp.) records in urban and peri-urban forests in Colombia.

In three of the localities where we recorded the presence of night monkeys through diurnal observations, we identified artificial sleeping sites used by social groups of *A. brumbacki, A. griseimembra* and *A. vociferans*. In the first locality, situated in the municipality of Guamal, Meta, we recorded a group of four individuals of *A. brumbacki* using as a sleeping site a small "house" built with wooden planks and a galvanized roof. This structure was specifically constructed by the sector's inhabitants to serve as a sleeping site for the night monkeys (Figure 2). At the second locality, in Puerto Parra, Santander, we recorded a group of *A. griseimemebra* consisting of three individuals sleeping during the day on a corrugated zinc sheet rolled up from the base of the tree (Figure 3). This zinc sheet was unintentionally placed by the owner of a nearby house on the tree where the night monkeys were sleeping, and after a few days they began to use it as a sleeping site. In the third locality, situated in Florencia, Caquetá, we sighted a group of three *A. vociferans* individuals using a hole in a metal structure that serves as a door jamb of a country restaurant as a sleeping site. According to the owners of the restaurant, when they began construction of the door, they noticed that the main beam was being inhabited by night monkeys and instead of chasing them away, they decided to allow them to stay there (Figure 4).

Discussion

We provide new records of the presence of *A. lemurinus* and the first records of *A. brumbacki, A. griseimembra* and *A. vociferans* in urban and peri-urban areas of Colombia, through diurnal, nocturnal and by means of observations of electrocuted individuals. Other records of night monkeys in urban and peri-urban areas have been made in the state of Rondônia, Brazil for individuals of *A. nigriceps*, which have also been observed consuming exotic flowers in urban trees (Chaves *et al.* 2021). In Colombia, other studies have documented the presence of different primate species in urban and peri-urban forests. Among them are: *Alouatta seniculus* (Cañate *et al.* 2019), *Saguinus leucopus* (Poveda and Sánchez-Palomino 2004; Soto-Calderón *et al.* 2016), *Saimiri cassiquiarensis* (Alfonso 2017; Buitrago and Ceballos 2018), *Sapajus apella* (Alfonso 2017) and *Plecturocebus ornatus* (Alfonso 2017; Ortiz-Moreno *et al.* 2022).

About 72 % of the records of night monkeys in urban and peri-urban forests in Colombia correspond to the species *A. lemurinus*, which may be related to the distribution of this species in the Andean region of the country. This species is present on both slopes of the 3 Andean Mountain ranges (Henao-Diaz *et al.* 2020; Shanee *et al.* 2023b), where most urban settlements in Colombia are concentrated and where about 70 % of the national population resides (Defler 2010; Armenteras *et al.* 2011). This region is also characterized by one of the highest rates of landscape transformation in Colombia, as it estimated 80 % of the natural vegetation having been modified (Etter *et al.* 2008). The Andean region of Colombia also includes the Middle Mag-

Table 1. Records of night monkey's species (*Aotus* spp.) in urban and peri-urban forests in Colombia. Species (Sp), Department (Dept), Municipality (Mun), Locality (Loc), Latitude, longitude (Lat/Lon), Altitude (Alt), Urban or peri-urban (U/P), Population center (PopC), Distance from population center (m) (DistPop (m)), Source, type of record and date (Source/Record/Date).

Sp	Dept	Mun	Loc	Lat/Lon	Alt	U/P	PopC	DistPop (m)	Source/Record/Date
A. brumbacki	Meta	Cumaral	El Paraíso pools	4.2692, -73.4942	417	P	Municipality	12	Daytime observation (sleeping site)*, during September and November of 2021
			Inspección de Guacavía	4.2908, -73.5197	459	P	Corregimiento	21	Daytime observation (sleeping site)*, March 3, 2022
		Guamal	Vereda Santa Bárbara	3.8896, -73.7721	536	U	Vereda	7	Daytime observation (sleeping site)*, June 4, 2022
		Villavicencio	Universidad de Los Llanos	4.0751, -73.5845	400	P	Vereda	-	Alfonso 2017. Registered by interview
A. griseimembra	Caldas	La Dorada	Las Delicias neighborhood	5.4450, -74.6697	177	U	Municipality	11	Daytime observation (sleeping site)*, September 2, 2023
	Cundinamarca	Puerto Salgar	Comando Aéreo de Combate"	5.4774, -74.6624	174	U	Municipality	11	Daytime observation (sleeping site)*, May 11, 2023
	Santander	Puerto Parra	Vereda Agualinda	6.6614 -73.9712	143	P	Vereda	23	Daytime observation (sleeping site)*, August 18, 2024
			Vereda Bocas del Carare	6.7806 -74.1012	93	U	Vereda	33	Daytime observation (sleeping site)*, August 18, 2024
A. lemurinus	Antioquia	San Jerónimo	Los Cedros residential unit	7.0557, -75.6739	747	P	Municipality	40	Daytime observation (sleeping site)*, May 13, 2023
		Toledo	Corregimiento of El Valle de Toledo - Biomax service station	6.4476, -75.7181	484	P	Corregimiento	12	Electrocuted individual*, October 3, 2023
	Caldas	Manizales	Campohermoso neighborhood	5.0833, -75.5166	1900	P	Capital city	-	Bustamante-Manrique *et al.* 2021. Nocturnal observation
			La Francia neighborhood	5.0725, -75.5302	2007	U	Capital city	-	Montilla *et al.* 2020. Electrocuted individual
			Nogales neighborhood	5.0590, -75.5235	2029	U	Capital city	-	Montilla *et al.* 2020. Electrocuted individual
			Northwest of Manizales	5.0833, -75.5166	2100	P	Capital city	-	Bustamante-Manrique *et al.* 2021. Nocturnal observation
			Ecoparque Monteleón	5.07916, -75.4986	2258	P	Capital city	-	Museum specimens MHN-UCa1503, UCa504, UCa3288 cited in Marín 2023
	Cauca	Toribío	Corregimiento of Tacueyó - La Guaca Hostel	3.0206, -76.2447	1703	P	Corregimiento	10	Nocturnal observation*, February 14, 2024
	Quindío	Armenia	Museo del Oro Quimbaya	4.5705, -75.6483	1615	P	Capital city	-	Montilla *et al.* 2018. Nocturnal observation
			Parque de la Vida	4.5469, -75.657	1517	P	Capital city	63	Nocturnal observation*, May 23, 2023
			Sena Agropecuario	4.5712, -75.6415	1590	P	Capital city	-	Montilla *et al.* 2021b. Nocturnal observation
		Calarcá	La Bombonera synthetic fields	4.5390, -75.6367	1557	P	Municipality	57	Electrocuted individual*
			Colegio San José	4.5081, -75.6587	1473	P	Municipality	-	Montilla *et al.* 2021b. Nocturnal observation
		Circasia	Colegio Libre	4.6199, -75.6423	1746	P	Municipality	-	Montilla *et al.* 2021b. Nocturnal observation
		Pijao	Las Casuarinas avenue	4.3349 -75.7050	1666	U	Municipality	-	Montilla *et al.* 2020. Electrocuted individual
			La Playita neighborhood	4.3310 -75.7051	1635	U	Municipality	-	Montilla *et al.* 2020. Electrocuted individual
			Laureano Gómez neighborhood	4.3325, -75.7065	1660	U	Municipality	7	Nocturnal observation*, November 12, 2022
			Corregimiento of Barragán	4.3371, -75.792	1106	U	Corregimiento	8	Nocturnal observation*, March 15, 2018
		Salento	Alto de la cruz Viewpoint	4.6401, -75.566	2015	P	Municipality	57	Nocturnal observation*, January 13, 2024
	Risaralda	Pereira	Universidad Tecnológica de Pereira	4.7928, -75.6885	1459	U	Capital city	48	Daytime observation (sleeping site)*, May 8, 2018
	Valle del Cauca	Caicedonia	La Ciudadela neighborhood	4.3346, -75.8203	1145	P	Municipality	33	Nocturnal observation*, April 5, 2023
			Bellavista Lakes	4.3317, -75.8321	1167	P	Municipality	84	Nocturnal observation*, January 27, 2023
			Las Carmelitas neighborhood	4.3251, -75.8342	1191	P	Municipality	54	Nocturnal observation*, February 20, 2024
			La Isabela neighborhood	4.3243, -75.8322	1194	P	Municipality	23	Nocturnal observation*, during November, 2023
		Cali	Universidad del Valle	3.3772, -76.5344	981	U	Capital city	17	Nocturnal observation*, June 2, 2022
		Cartago	Ortéz creek	4.7521, -75.8945	945	P	Municipality	-	Grajales-Suaza *et al.* 2021. Nocturnal observation
A. vociferans	Caquetá	Florencia	La Florida neighborhood	1.6293, -75.5864	400	P	Capital city	14	Nocturnal observation*, June 20, 2020
			La Ceiba restaurant	1.6342, -75.5841	475	P	Capital city	16	Nocturnal observation*, June 22, 2022

*New records

Figure 2. Artificial sleeping site used by a group of our individuals of *A. brumbacki* recorded in urban forests of Guamal, Meta.

dalena Valley, where four more records of night monkeys in urban and peri-urban forests were presented, specifically for the species *A. griseimembra*. As for *A. brumbacki*, this was another species with a high number of records with a total of four occurrences, all of them restricted to the foothill subregion of the Orinoquía. This specific area concentrates approximately 80 % of the entire population of the Orinoquía region, where the largest regional infrastructure is located (Jiménez 2012; Devia and Piñeros 2021).

Nearly 33 % of night monkey records in urban and peri-urban forests in Colombia occurred in capital cities categorized as special (population over 500,001 inhabitants), as in the case of Cali, as well as in first category cities (with a population over 100,001 inhabitants), such as Armenia, Manizales, Pereira and Villavicencio. Of these cities, Armenia, Manizales y Pereira are the capitals of the three departments that make up the Eje Cafetero, a subregion with a landscape that has approximately 19.8 % crop cover and 25.3 % forest cover, according to Otero *et al.* (2005). This landscape is structurally complex, composed of agroforestry and silvopastoral systems, which allows the presence of native fauna and acts as biological corridors, contributing to some extent to the maintenance of environmental services (Weibull *et al.* 2003; Pérez-Torres *et al.* 2009). In the Eje Cafetero region, we have recorded night monkeys of the species *A. lemurinus* and *A. griseimembra* in urban and peri-urban forests. Although these species are listed in the vulnerable to extinction (VU) category of the IUCN (International Union for Conservation of Nature), they are considered to be adapted to transformed landscapes (Defler 2010; Montilla *et al.* 2018; Link *et al.* 2021a, 2021b).

The record of *A. lemurinus* from the Universidad del Valle in the urban area of Cali should be interpreted with caution due to uncertainty as to whether this population is introduced and arose from individuals released or escaped after biomedical research, or whether it represents a native population. In 1979, the Colombian National Health Institute (INS) established a colony of night monkeys in Bogotá for biomedical research purposes, from 57 specimens captured in San Marcos, Sucre (Caribbean region; Umaña *et al.* 1984). Subsequently, some individuals from this colony were transferred to several research centers, including the Instituto de Inmunología of the Universidad del Valle, in Cali, where several investigations on the development of vaccines against malaria were carried out using these primates as a model (López *et al.* 1997; Jordan-Villegas *et al.* 2005; Herrera-Valencia 2002, 2005). Particularly of the individuals at the Universidad del Valle, there are no clear reports on their final fate, and it has been suggested that some individuals may have escaped or been released, establishing social groups on the university campus. On the other hand, it is also possible that the night monkeys at the Universidad del Valle correspond to native individuals, since wild night monkeys have also been reported in the vicinity of the city of Cali, both to the south in the rural area of the corregimiento of Pance and to the north in the corregimiento of Dapa in the municipality of Yumbo (Hirche *et al.* 2017; Wolovich *et al.* 2023). Further genetic analyses are necessary to confirm the provenance of the night monkeys at the Universidad del Valle by comparing their genetic data to those of native populations present in rural areas of Cali and Valle del Cauca.

Two of the new records of night monkeys in peri-urban forests involved individuals of *A. lemurinus* that were victims of electrocution events. Additional incidents include one reported by Castaño *et al.* (2010) in a forest at the middle Cauca River basin, as well as two others reported by Saavedra-Rodríguez *et al.* (2013) in a rural area at the department of Valle del Cauca, and 10 more reported by Montilla *et al.* (2020) along the Cordillera Central. All of these records underestimate the true magnitude of the serious threat posed by power grids to night monkeys, especially for populations inhabiting urban and peri-urban forests. According to Kumar and Kumar (2015) and Slade (2016), it is estimated that only 31 to 36 % of electrocutions of primates are reported.

Figure 3. Artificial sleeping site used by a group of three individuals of *A. griseimembra* recorded in peri-urban forests of Puerto Parra, Santander.

In addition, six of the records of night monkeys in urban and peri-urban areas have been documented in educational institutions, such as the Colegio Libre in Circasia, Quindío, the Colegio San José in Calarcá, Quindío, the Gimnasio Militar FAC "TC. Flavio Angulo Piedrahita" in Puerto Salgar, Cundinamarca, the Universidad de Los Llanos in Villavicencio, Meta, the Universidad del Valle in Cali, Valle del Cauca, and the Universidad Tecnológica de Pereira in Pereira, Risaralda. These circumstances open the possibility for these institutions to advance in research processes through continuous long-term monitoring of night monkeys, which could lead to implementation of conservation strategies where these primates are considered as flagship species for these institutions.

Regarding the use of artificial sleeping sites by groups of *A. brumbacki, A. griseimembra* and *A. vociferans* in urban and peri-urban forests, several studies have focused on describing the behavior of sleeping sites used by night monkey species. However, none of these studies report these primates sleeping site in human-made structures (Aquino and Encarnación 1986; García and Braza 1993; Puertas *et al.* 1995; Savagian and Fernandez-Duque 2017; González-Hernández *et al.* 2020; Montilla *et al.* 2024). In the case of *Saguinus bicolor*, another Neotropical primate species, tests were conducted with artificial sleeping sites with different characteristics, and the individuals showed a preference for wooden boxes that provided them with greater security from predators (Ahsmann 2022). Due to the constant noise pressures and the presence of people near the urban and peri-urban forests where night monkeys live, it is possible that they perceive sleeping sites as safe places within environments altered by human activity.

Recording night monkeys in urban and peri-urban areas is key for research and opens opportunities for conservation. It is crucial to further understand the adaptive capacity of primates to these environments, the viability of these populations, and the effects of habitat degradation, loss, and fragmentation in these particular landscapes. Such knowledge will allow us to articulate compelling arguments for the inclusion of these charismatic and threatened species (in the case of *A. brumbacki*, *A. griseimembra* and *A. lemurinus*) habitat in public policies related to land use. Moreover, this will be useful to consider them in effective ecological development plans for municipalities, as well as monitoring and conservation of protected areas (*e. g.* water protection zone). Furthermore, this is also an opportunity to promote the development of these localities through specialized tourism (ecotourism and scientific tourism), conservation education, and the use of these species as a flagship for cultural activities. However, urban landscapes still pose threats to these primates that need to be managed in collaboration with local institutions and environmental authorities, such as run overs, electrocution, and illegal trade.

New records of night monkeys in urban and peri-urban areas of Colombia reveal the adaptability of these species to environments modified by human activities. These findings highlight the importance of conducting research to understand how human presence and landscape changes affect the distribution and behavior of wildlife. In addition, the identification of threats such as electrocution by power grids underscores the need to implement effective conservation measures to protect these species in urbanized environments. The possible perception by night monkeys of artificial sleeping sites as safe havens also raises questions about the interaction between wildlife and human infrastructure in these environments. Ultimately, these records are important as a basis for future research and conservation actions specific to primates near urban areas.

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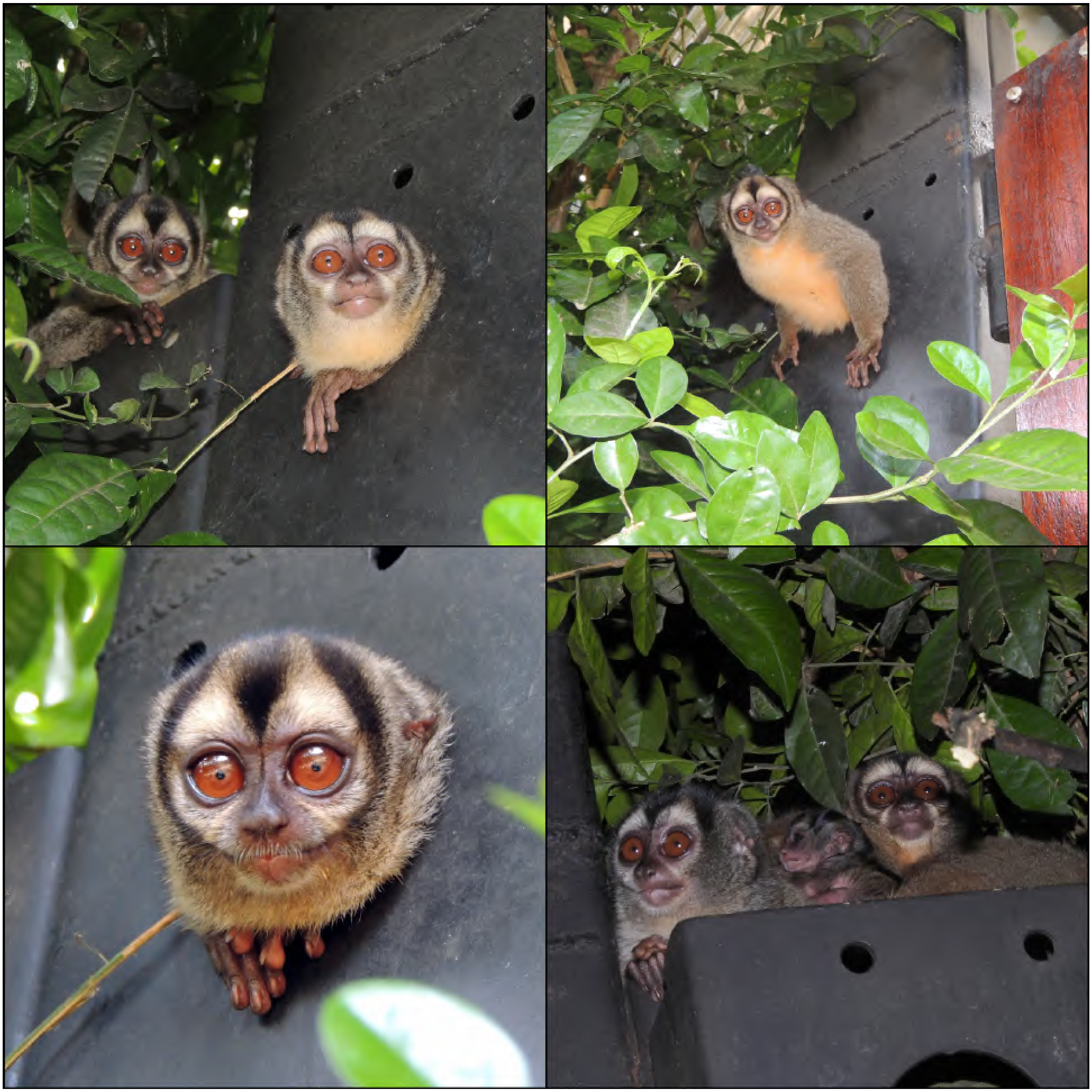

Figure 4. Artificial sleeping site used by a group of of three individuals of *A. vociferans* recorded in peri-urban forests of Florencia, Caquetá.

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Diversity and activity patterns of medium-and large-sized mammals in an intraurban vegetation fragment in the city of Mérida, Yucatán, Mexico

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Urban sprawl, a type of horizontal urban growth that characterizes many Latin American cities, is associated with low-density developments that encroach upon large natural or agricultural areas, fragmenting and isolating surrounding landscapes. Mérida is a typical example of this urbanization pattern, which has led to habitat fragmentation and significantly affected biodiversity. This study focused on medium- and large-sized mammals in an unprotected urban vegetation fragment, evaluating biodiversity, activity patterns, and the influence of feral fauna. Camera traps were installed across a 38-ha vegetation fragment in Mérida, collecting data during the dry and wet seasons. Photographs were analyzed to identify species, calculate the Relative Abundance Index (RAI), and estimate diversity and activity patterns. Nine wild mammal species were recorded; *Urocyon cinereoargenteus* and *Odocoileus virginianus* were the most abundant. We observed significant diurnal activity, especially in *Odocoileus virginianus* and *Nasua narica*, while *Sylvilagus yucatanicus* and *Urocyon cinereoargenteus* were most active at dawn and dusk, and *Spilogale yucatanensis* focused its activity at nighttime. We observed an overlap in activity between feral fauna and wild mammals. Despite the pressure from urbanization, the vegetation fragment studied harbors a significant diversity of wild mammals. However, feral fauna, including dogs and cats, adversely impacts wild species, highlighting the importance of implementing management and conservation strategies in urban fragments to protect biodiversity.

El crecimiento urbano horizontal, característico de muchas ciudades latinoamericanas, se asocia con desarrollos de baja densidad que invaden grandes áreas naturales o agrícolas, fragmentando y aislando los paisajes circundantes. Mérida es un ejemplo típico de este patrón de urbanización, que ha provocado la fragmentación de hábitats y afectado significativamente la biodiversidad. Este estudio se centra en los mamíferos medianos y grandes de un fragmento de vegetación urbana no protegida, evaluando su biodiversidad, patrones de actividad y la influencia de la fauna feral. Se instalaron cámaras trampa en un fragmento de 38 ha en Mérida, recolectando datos durante las temporadas seca y lluviosa. Las fotografías obtenidas se analizaron para identificar especies, calcular el Índice de Abundancia Relativa (IAR) y estimar la diversidad y los patrones de actividad. Se registraron 9 especies de mamíferos, siendo *Urocyon cinereoargenteus* y *Odocoileus virginianus* las más abundantes. Se observó que existe una importante actividad diurna especialmente en *O. virginianus* y *Nasua narica*. Mientras que especies como *Sylvilagus yucatanicus* y *Urocyon cinereoargenteus* presentan actividad diurna, pero concentrada sobre todo al amanecer y al anochecer. *Spilogale yucatanensis* concentró su actividad durante la noche. Además, se detectó una superposición en la actividad entre la fauna feral y los mamíferos silvestres. A pesar de la presión ejercida por la urbanización, el fragmento de vegetación conserva una diversidad significativa de mamíferos silvestres. Sin embargo, la presencia de fauna feral, como perros y gatos, ejerce un impacto negativo sobre estas especies, subrayando la importancia de implementar estrategias de manejo y conservación en fragmentos intraurbanos para proteger la biodiversidad.

Keywords: biodiversity; camera trap; feral fauna; interactions; urban area.

Introduction

Urban environments are among the terrestrial ecosystems most heavily altered by man, leading to direct and indirect effects on biodiversity (McKinney 2002; Fahrig 2003). Latin American cities are usually characterized by a compact urban core surrounded on the outskirts by uncontrolled urban sprawl, resulting in fragmented habitats and dysfunctional urban matrix (Monkkonen 2011; Inostroza *et al.* 2013; Duque *et al.* 2019). This growth pattern is associated with low-density human settlements spread over large surrounding rural or agricultural areas, characterized by being irregular, dispersed, and fragmented (European Environment Agency 2006). Frequently, housing developments are discontinuous, leaving fragments of natural vegetation or cropland that gradually become isolated patches within the urban matrix. This urbanization pattern affects ecological connectivity and restrains the movement of species across patches, disrupting dispersal corridors and creating habitat "islands" (Davis and Glick 1978; Olejniczak *et al.* 2018). Consequently, richness and abundance of wild species within fragments are severely affected (Crooks 2002; Davis and Glick 1978).

In Mexico, this type of horizontal expansion is common on the urban outskirts, in which housing developments grow at the expense of surrounding agricultural and native vegetation areas (Monkkonen 2012). Mérida, the capital city of the state of Yucatán, is the largest city in the Yucatán Peninsula and an emblematic case of this phenomenon. In recent decades, Mérida has experienced vigorous growth, from 269,000 inhabitants in 1979 to more than 1.2 million in 2020, resulting in the urbanization of 24,215 ha of tropical dry forest (SEDATU 2023; Carrillo-Niquete *et al.* 2022). Although the city has a well-defined and developed downtown core, it is currently surrounded by large and numerous horizontal urban housing developments called "*fraccionamientos*" (Pérez-Medina and López-Farfán 2015). This growth has been facilitated by the local flat topography, low vegetation that is easily cleared, access to groundwater, and poor urban regulation enforcement (Bolio-Osés 2000). As a result, the loss of these natural areas has interrupted dispersal corridors and fragmented habitats, in addition to producing isolated vegetation patches that function as temporary shelters for some species.

Medium- and large-sized mammals are key indicators of ecosystem health and play major ecological roles, such as seed dispersal, population control, and regulation of food webs (Savard *et al.* 2000; Fahrig 2001). Despite the limitations imposed by urbanization, Mexico and other Latin American cities have recorded several species of mammals in vegetation fragments located in urban areas. Species such as *Didelphis virginiana*, *Philander opossum*, *Dasypus novemcinctus*, *Cuniculus paca*, *Canis latrans*, *Leopardus pardalis*, *Herpailurus yaguaroundi*, or *Eira barbara,* among others, have been recorded in parks and vegetation fragments around urban areas, showing the ability of some mammals to adapt to these environments (Bernardo *et al.* 2013; Borges *et al.* 2014; Mella-Méndez *et al.* 2019; Coronel-Arellano *et al.* 2021; García-Padilla *et al.* 2021; Ramos-Luna *et al.* 2023; Gordillo-Chávez *et al.* 2024). However, their coexistence with feral fauna, such as *Canis familiaris*, and *Felis catus*, poses new challenges. These domestic species often reach high densities in urban areas due to their adaptability and opportunistic behavior, adversely affecting native species through competition, predation, and disease transmission (Loss *et al.* 2013; Vanak *et al.* 2014).

In Mérida, the loss of natural areas associated with urban growth has generated isolated vegetation fragments where many native species are trapped and exposed to the pressure of feral fauna and other impacts derived from urban sprawl. Not only do these fragments represent refuges for biodiversity, but their conservation can be key to mitigating the adverse impacts of urbanization, such as the formation of urban heat islands, and to maintaining the ecosystem services associated with native vegetation (Carrillo-Niquete *et al.* 2021; Villanueva-Solís and Torres-Pérez 2023).

Due to their potentially detrimental effects on native species, it is important to investigate and report the behavior of invasive species, particularly in protected areas (Doherty *et al.* 2015). Feral dogs and cats can be effective competitors and predators in natural ecosystems (Vanak and Gompper 2009). Thus, in urban areas, feral fauna can play an important role as stressors for native wild mammals and affect their persistence and survival (Parsons *et al.* 2016; Zapata-Ríos and Branch 2016). Therefore, it is imperative to evaluate the spatial and temporal overlap between native mammals and wildlife in patches of natural vegetation within urban areas to establish feral wildlife management plans and control programs to promote the establishment and maintenance of wild mammal populations in these areas (Coronel-Arellano *et al.* 2022). Although the effects of feral fauna on mammals in protected areas and urban parks have been studied in Mexico and Latin America (Bernardo *et al.* 2013; Borges *et al.* 2014; Mella-Méndez *et al.* 2019; Coronel-Arellano *et al.* 2021), the impact of these interactions in urban areas remains poorly understood and is considered a priority for wildlife conservation in urban areas (Hortelano-Moncada *et al.* 2024). This is greatly important in isolated fragments associated with urban sprawl, which favor the entry of feral fauna and its interactions with native species (Coronel-Arellano *et al.* 2021).

In this context, the overall objective of this study was to evaluate the activity patterns of medium-sized mammals in an unprotected fragment of urban vegetation in Mérida. Specifically, we answered the following questions: (a) Which mammal species occur in this fragment? (b) What are their activity patterns? (c) Is there temporal or spatial overlap in activity between feral and wild fauna? These questions will enable the identification of key interactions between species and generate valuable information for biodiversity management and conservation in urban areas under horizontal expansion.

Materials and methods

Study area. The study area is an isolated vegetation patch of approximately 38 ha in Gran San Pedro Cholul, located in northeast Mérida, Yucatán, Mexico (21°1′ N, 89°32′ W; Figure 1). The vegetation fragment is currently unprotected, isolated by housing developments (clusters of individual houses, roads, shopping centers, service areas, sports fields, schools, and others) as a result of urban expansion over the past 20 years. The vegetation patch is located in the primary urban growth zone established in the Municipal Urban Development Program (Ayuntamiento de Mérida 2024a), less than 2 km in straight line from kilometer 1 of the Mérida-Motul federal highway and the Licenciado Manuel Berzunza city peripheral highway. The local topography is mainly flat, with an elevation between 5 m and 10 m asl. The climate is warm sub-humid with summer precipitation, with annual precipitation between 800 mm and 1000 mm (INEGI 2017). This study identified two climatic seasons: dry (15 October 15 to 25 May) and wet (26 May to 14 October; de la Barreda *et al.* 2020). The original vegetation was tropical dry forest; however, anthropogenic activities such

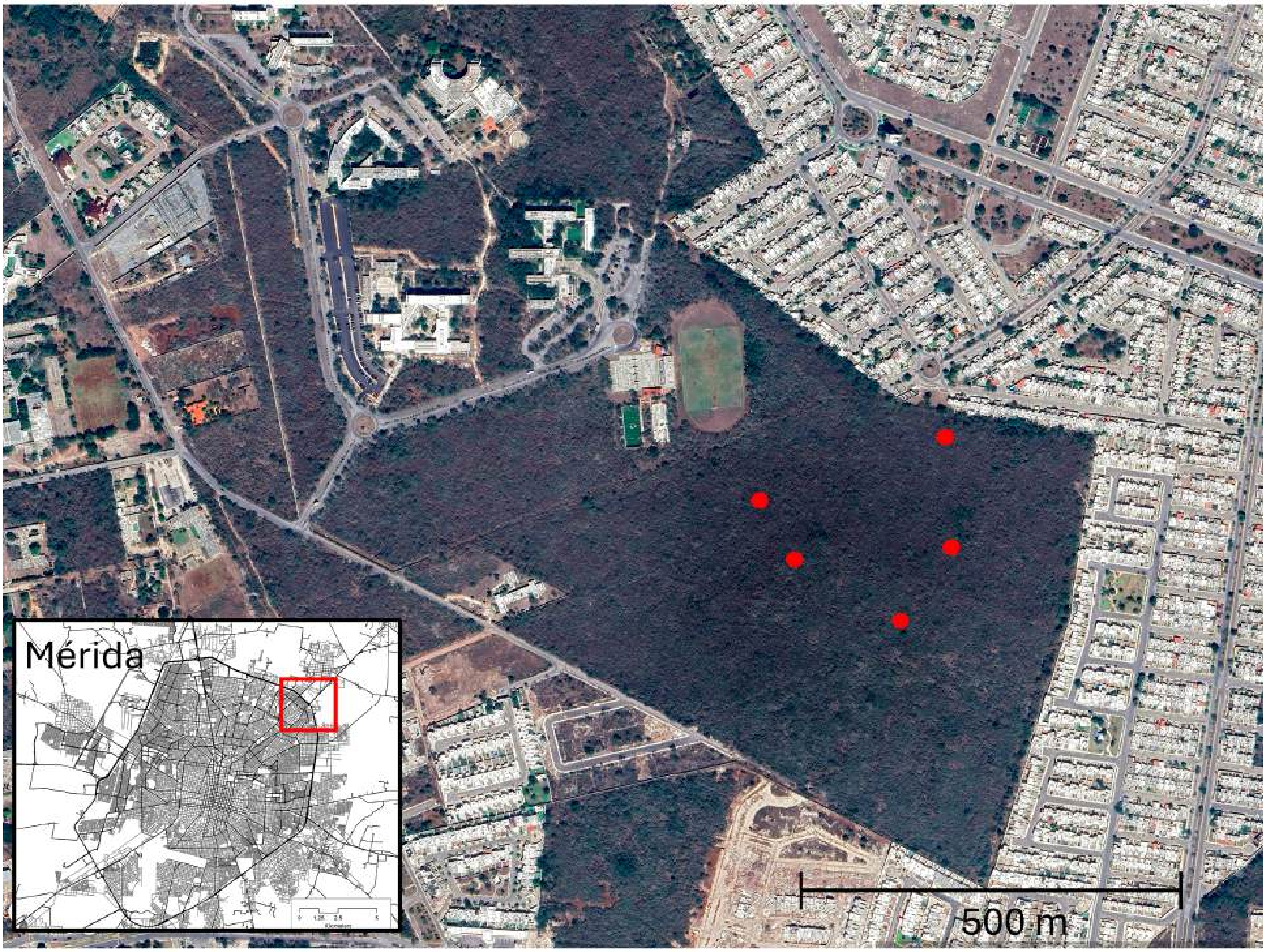

Figure 1. Location of camera trap stations in an isolated vegetation fragment located in the northeast of the city of Mérida, Yucatán, Mexico. Google Earth satellite image from February 2024. The red dots in the image mark the location of camera traps.

as agriculture, livestock raising, and, mainly, Mérida's urban sprawl modified the vegetation matrix from tropical dry forest to secondary successional stages with different ages of abandonment (Ellis *et al.* 2017; Palafox-Juárez *et al.* 2021; Carrillo-Niquete *et al.* 2022).

Mammal sampling. Five stations were established over the isolated vegetation fragment (Figure 1). Each station consisted of a camera trap (Brownig Dark Ops Pro X 1080; Browning, Morgan, Utah) that operated continuously between 30 May 2023 and 4 April 2024, covering the 2023 wet season (30 May to 14 October 2023) and the 2023–2024 dry season (15 October 2023 to 4 April 2024). Camera traps were placed along paths that delimit the properties into which the vegetation fragment was divided. The cameras were strapped to trees approximately 50 cm above the ground and set to work 24 h per day, capturing 3 photographs for each recorded event and with a 1-minute delay between events. Cameras were checked every 45 days to verify their operation, replace the batteries, and make a backup of the photographs captured.

Data analysis. The captured photographs were stored and processed using Camera Base (Tobler 2015). Specimen images were identified according to Reid (2009). Only photographs of medium- and large-sized domestic and wild mammals (i.e., with a mean weight of at least 0.5 kg) and with terrestrial or semi-arboreal habits were analyzed (Reid 2009). Since the photographs did not allow us to identify the taxonomic characteristics that differentiate opossum species of the genus *Didelphis* (*i.e., D. virginiana* and *D. marsupialis*), the data for these specimens were grouped as *Didelphis* sp. Once the list of medium- and large-sized mammals was created, it was supplemented with the conservation status of each species according to the Mexican legislation (SEMARNAT 2010) and the Red List of the International Union for Conservation of Nature (IUCN 2024).

The number of independent records for each species of medium- and large-sized mammals captured at each photo-trapping station was obtained. A record of a species at a given station was considered independent of another record of the same species at the same station when the time interval between photographs exceeded 60 minutes (Tobler *et al.* 2008). To calculate the sampling effort, we obtained the number of trap days that each camera trap station operated in the field from the time the camera was active to the date when the last photograph was captured. A trap day was considered to be a 24-hour period. The total sampling effort was obtained by adding the number of days that each camera trap station operated during the study.

The Relative Abundance Index (RAI) was estimated for each treatment per season as a measure of the rate of visits (O'Brien 2011). The RAI was calculated by dividing the total number of independent records of each species per season by the camera trap effort at each station for each site per season, multiplied by 100.

Diversity was estimated for equally large (common sample size) or equally complete (common sample coverage) standardized samples for wild mammals in the vegetation fragment based on the integrated rarefaction and extrapolation sampling curves of true diversity indices for q = 0 and 1 (Chao and Jost 2012; Colwell *et al.* 2012; Chao *et al.* 2014). To this end, asymptotic diversity profiles were obtained based on the statistical estimation of Hill numbers of orders q = 0 and q = 1 (Chao and Jost 2015). We assumed that species were present in the vegetation fragment throughout the sampling period and that the seasonal presence or absence of any of them was due to differences in activity and catchability. Therefore, the diversity analysis was not divided by seasons, and it was carried out using all data recorded during the sampling. Species accumulation curves and true diversity analyses were performed using the iNEXT package in R (Hsieh *et al.* 2016).

A Kernel density estimator was used (Ridout and Linkie 2009) to generate seasonal activity patterns for domestic species and those wild species for which at least 25 independent records were captured during each season. To compare the activity patterns of domestic and wild species between the two seasons, treatment pairs were compared using the R package overlay (Meredith and Ridout 2014). The overlap coefficient (Δ) was calculated with 95 % confidence intervals (CI) using a bootstrap of 1000 samples. Following Meredith and Ridout (2014), Δ1 was used as the overlap estimator if the smallest sample had less than 50 photographic records; otherwise, Δ4 was used. The estimated overlap coefficient ranges from 0 (no overlap) to 1 (complete overlap). Activity was classified as (1) daytime, from 1 hour after sunrise to 1 hour before sunset; (2) nighttime, from 1 hour after sunset to 1 hour before sunrise; and (3) twilight, from 1 hour before to 1 hour after sunrise and sunset (Foster *et al.* 2013).

Table 1. Number of independent seasonal records, number of seasons in which the species was recorded, and relative abundance index (RAI) of medium- and large-sized mammals recorded in an isolated vegetation fragment in the city of Mérida, Yucatán, Mexico. Dry season: 15 October 2023 to 4 April 2024. Wet season: 30 May to 14 October 2023

Species	Dry season			Rainy season		
	Number of records	Number of stations	RAI	Number of records	Number of stations	RAI
Didelphimorphia						
Didelphis sp.	18	1	1.20			
Cingulata						
Dasypus novemcinctus				5	4	0.33
Lagomorpha						
Sylvilagus yucatanicus	55	4	3.65	25	3	1.66
Rodentia						
Sciurus yucatanensis	17	3	1.13	1	1	0.07
Carnivora						
Felis catus	285	5	18.94	49	4	3.26
Canis familiaris	147	5	9.77	78	5	5.18
Urocyon cinereoargenteus	796	5	52.89	210	5	13.95
Spilogale yucatanensis	33	3	2.19	31	2	2.06
Nasua narica	20	5	1.33	6	3	0.40
Procyon lotor	3	1	0.20			
Artiodactyla						
Odocoileus virginianus	103	5	6.84	29	5	1.93

Results

During 1545 trap nights (860 in the dry season and 685 in the wet season), 1352 records of medium- and large-sized mammals were captured (1045 in the dry season and 307 in the wet season; Table 1). The captured mammals belonged to 11 species: 9 native wild species (8 in the dry season and 7 in the wet season) and 2 domestic mammals. Of the native species, *Didelphis* sp., *Dasypus novemcinctus*, and *Procyon lotor* were recorded in only one season, while the other 6 species were present in both seasons (Table 1; Figure 2). The most abundant native species in both seasons was *Urocyon cinereoargenteus*, followed by *Odocoileus virginianus* in the dry season and *Spilogale yucatanensis* in the wet season. The domestic species *Felis catus* and *Canis familiaris* were recorded in both seasons; *F. catus* was more abundant in the dry season and *C. familiaris* in the wet season. The photographs showed both collared dogs and cats and collarless individuals whose ownership could not be established. In the case of *C. familiaris*, 15 photographs were captured showing that dogs form groups of between 2 and 5 adult individuals. Over the course of the study, no photographs of people unrelated to the project were captured, even though the vegetation fragment includes free-access areas and indicators of human presence, such as litter, construction debris, and fires, are common along its borders.

The diversity analysis in the fragment revealed a sample coverage of 0.999 for wild mammals, indicating that the sampling was adequate. For the expected species richness ^{0}D (Figure 3), for the maximum value of independent records (1.351), ^{0}D was 9.0 (confidence interval = 0.27), while Shannon's exponential ^{1}D was 2.67 (confidence interval = 0.240).

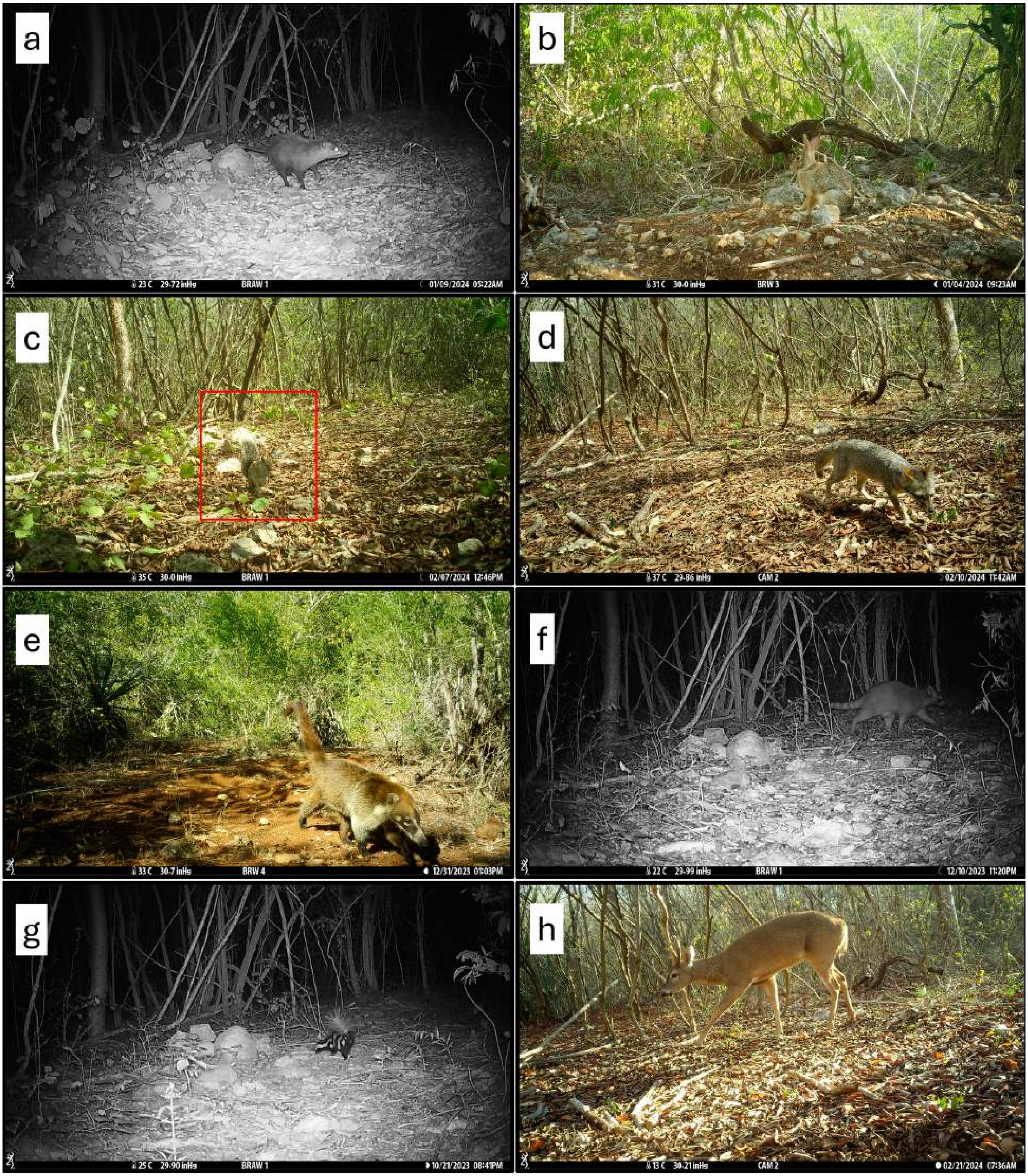

Figure 2. Photographs of wild mammals captured by camera traps in an isolated vegetation fragment in Mérida, Yucatán, Mexico. a) *Didelphis* sp.; b) *Sylvilagus yucatanicus*; c) *Sciurus yucatanensis*; d) *Urocyon cinereoargenteus*; e) *Nasua narica*; f) *Procyon lotor*; g) *Spilogale yucatanensis*; h) *Odocoileus virginianus*.

Activity patterns were analyzed for 6 species (4 native and 2 domestic) having sufficient records (Table 2; Figure 4). *Sylvilagus yucatanicus*, *O. virginianus*, *U. cinereoargenteus*, *Spilogale yucatanensis*, and *F. catus* showed consistent patterns between seasons, although with slight variations. *S. yucatanicus*, *U. cinereoargenteus*, and *F. catus* had both daytime and nighttime activity, with peaks at dawn and dusk; *O. virginianus* was mainly diurnal and *Spilogale yucatanensis* nocturnal. In contrast, *C. familiaris* was the only species that showed seasonal variations in Δ, being

Table 2. Temporal overlap Δ between medium- and large wild mammal species with domestic species in an isolated vegetation fragment in the city of Mérida, Yucatán, Mexico. The number corresponds to the value of Δ. The figure in parentheses corresponds to the 95 % confidence interval.

Species 1	Species 2	Dry season	Rainy season
Canis familiaris	*Urocyon cinereoargenteus*	0.357 (0.297-0.421)	0.771 (0.674-0.861)
	Odocoileus virginianus	0.403 (0.323-0.480)	0.561 (0.399-0.718)
	Sylvilagus yucatanicus	0.346 (0.251-0.442)	0.707 (0.562-0.842)
Felis catus	*Urocyon cinereoargenteus*	0.860 (0.803-0.915)	0.776 (0.650-0.874)
	Sylvilagus yucatanicus	0.783 (0.670-0.891)	0.688 (0.522-0.825)
	Spilogale yucatanensis	0.562 (0.433-0.683)	0.581 (0.438-0.723)

predominantly diurnal in the wet season and concentrating its activity at dawn in the dry season. Species with insufficient records for the kernel density analysis, such as *Didelphis* sp., *D. novemcinctus*, and *P. lotor*, were exclusively nocturnal, while *Nasua narica* showed daytime activity in both seasons.

In the dry season (Figure 5), *C. familiaris* had a low overlap in activity patterns with wild species with sufficient records (*U. cinereoargenteus*, *O. virginianus*, *S. yucatanicus*, and *Spilogale yucatanensis*). However, in the wet season (Figure 6), the activity pattern of *C. familiaris* broadly coincided with those of *U. cinereoargenteus* and *S. yucatanicus*, but not with those of *O. virginianus* due to the diurnal activity of the latter.

Regarding *F. catus*, its activity patterns overlapped significantly with those of *U. cinereoargenteus* and *S. yucatanicus* in both seasons (Figure 5, 6), which could facilitate negative interactions or induce pathogen exchange. On the other hand, the overlap with *Spilogale yucatanensis* was low because the latter is mainly nocturnal, while *F. catus* has high crepuscular and diurnal activity (Figure 5, 6).

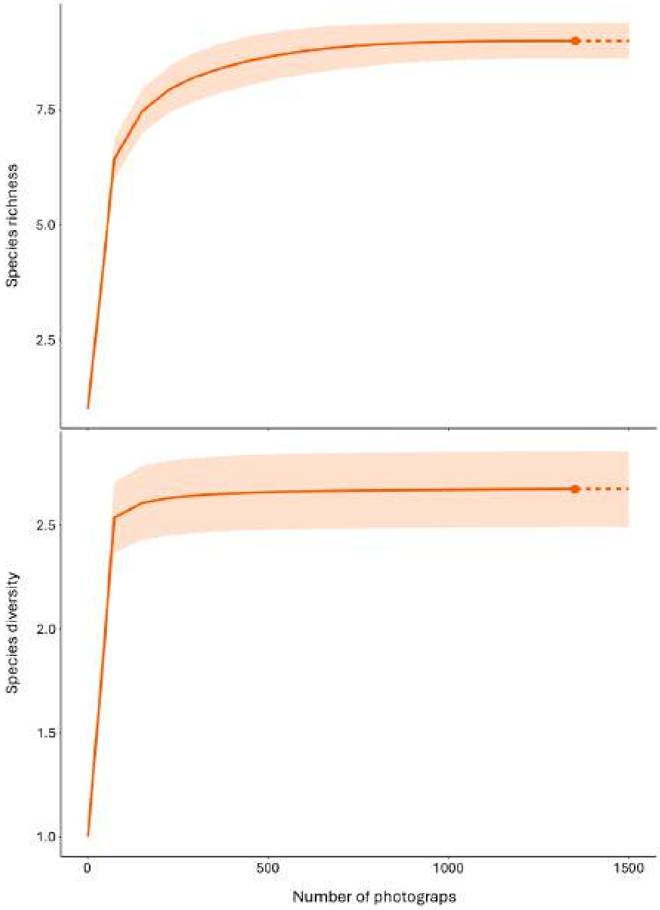

Figure 3. Species accumulation curves and confidence intervals for medium- and large-sized mammals captured in an isolated vegetation fragment in the city of Mérida, Yucatán, Mexico. These curves were constructed using diversity indices at two levels: ^{0}D (species richness) and ^{1}D (Shannon's exponential). The solid line refers to the interpolated data, and the dotted line refers to the extrapolated data.

Discussion

Vegetation fragments are common in the interior of the city of Mérida due to population growth and urban expansion. Despite the magnitude of urban impact, the studied fragment is home to several species of wild mammals typical of low deciduous forests in northern Yucatán (Sosa-Escalante *et al.* 2017). The number of species found (9) represents 21 % of the 42 species of medium- and large-sized terrestrial mammals recorded in the state (Sosa-Escalante *et al.* 2014), which is remarkable considering the size of the fragment (38 ha). However, species such as *Herpailurus yaguaroundi*, *Galictis vittata*, and *Canis latrans*, reported in the nearby Cuxtal Ecological Reserve (Sosa-Escalante *et al.* 2017), were not detected in the fragment, possibly due to the habitat and home range requirements of these species (Caso 2013). An isolated fragment such as the one studied here can hardly support multiple species of medium- and large-sized mammals, since small fragments are rarely suitable to sustain a population, or even an individual, of species such as *H. yaguaroundi*, whose minimum home range is 10 km^2 (Caso 2013). However, it cannot be ruled out that individuals of this or other species, such as *C. latrans*, may occasionally transit through the fragment during dispersal, as recently reported for the suburban area of Mérida in the media (Diario de Yucatán 2023; El Universal 2024).

Species such as *Urocyon cinereoargenteus*, *Procyon lotor*, *Didelphis* sp., and *Dasypus novemcinctus* have been commonly recorded in urban environments in Mexico (Mella-Méndez *et al.* 2019; Coronel-Arellano *et al.* 2021; García-Padilla *et al.* 2021; Ramos-Luna *et al.* 2023), but this study reports for the first time the presence of *Nasua narica* and *Odocoileus virginianus* in an urban area of the country. The presence of *O. virginianus* is notable, as it is the largest wild species (approximately 30 kg) recorded in urban areas in Mexico (Contreras-Moreno *et al.* 2021); it is remarkably abundant in the vegetation fragment studied, being the second-most abundant species in the study. We identified at least 4 distinct males, recognizable by their

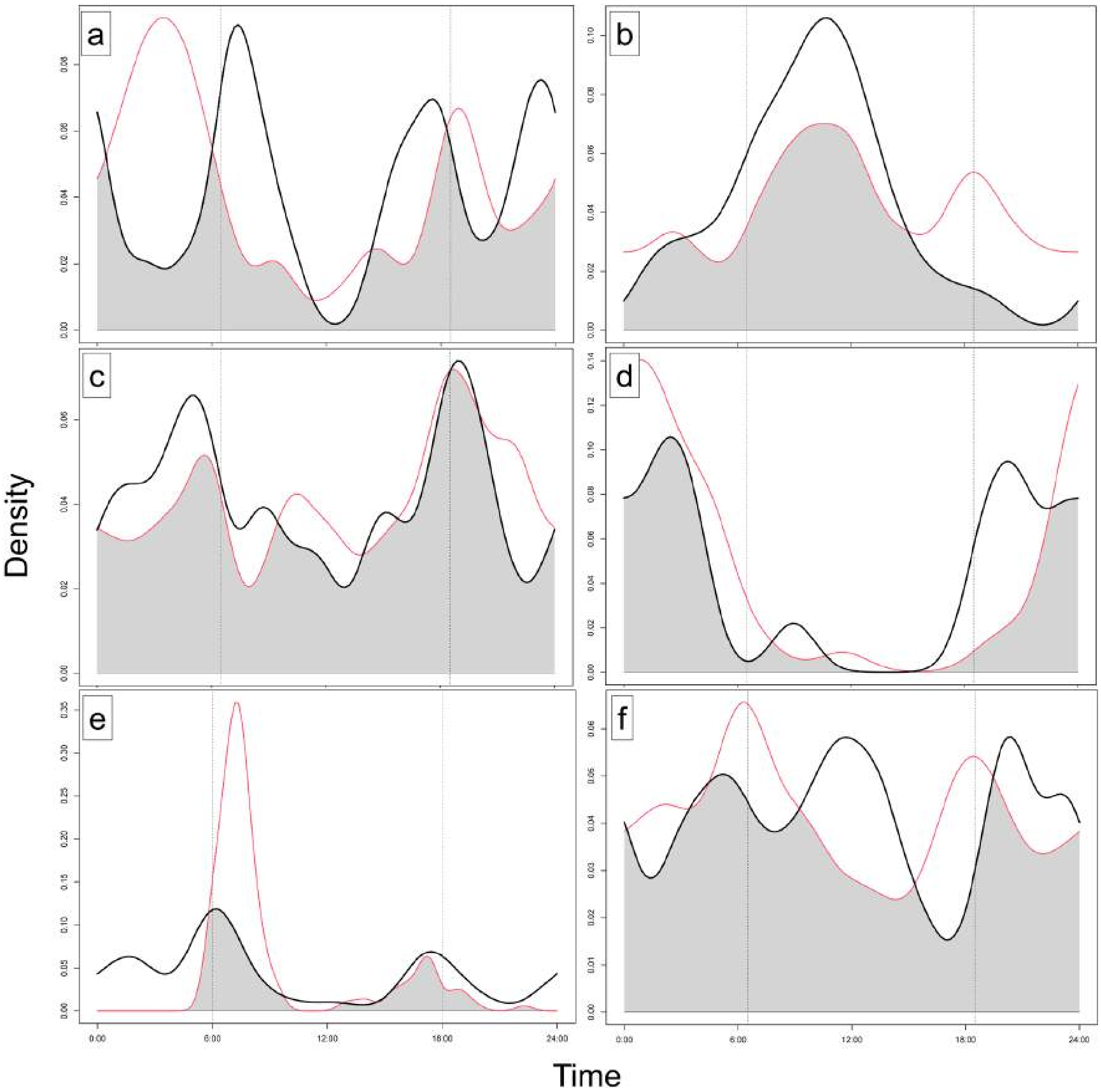

Figure 4. Seasonal activity patterns of medium- and large domestic and wild mammals were recorded in an isolated vegetation fragment in the city of Mérida, Yucatán, Mexico. a) *Silvilagus yucatanicus*; b) *Odocoileus virginianus*; c) *Urocyon cinereoargenteus*; d) *Spilogale yucatanensis*; d) *Canis familiaris*; e) *Felis catus*. The black line corresponds to the wet season (30 May to 14 October 2023). The red line corresponds to the dry season (15 October 2023 to 4 April 2024). The shaded gray space indicates the areas of overlap between the two seasons. The vertical line indicates the approximate time of sunrise (06:00 h) and sunset (18:00 h).

antlers, as well as probably several females that could not be identified individually. In Campeche wetlands, the home range of white-tailed deer covers 12.67 ha to 21.57 ha in females and 37.31 ha to 90.16 ha in males, depending on the season, water availability, and flood levels (Contreras-Moreno *et al.* 2021). In the Yucatán region, this species is subject to intense pressure from subsistence poachers, which has led to significant reductions in its populations (Burgos-Solís *et al.* 2023). The presence of this species in unprotected urban vegetation fragments within Mérida could reflect the difficulties of poaching in urban areas due to vigilance by police and neighbors.

Some of the native species recorded in this study, such as *Didelphis* sp., *P. lotor*, and *U. cinereoargenteus*, are classified by McKinney (2002) as adapted to rural, suburban, and urban areas. This group includes medium-sized species with a generalist and opportunistic diet that take advantage of food and other resources subsidized by man in suburban spaces, forest patches, and surrounding green spaces. Open dumps, where organic waste is usually disposed of, are common in the surroundings of the fragment. These may benefit mainly *U. cinereoargenteus*, since several photographs showed individuals carrying bags that probably contained food remains. *Didelphis* sp.

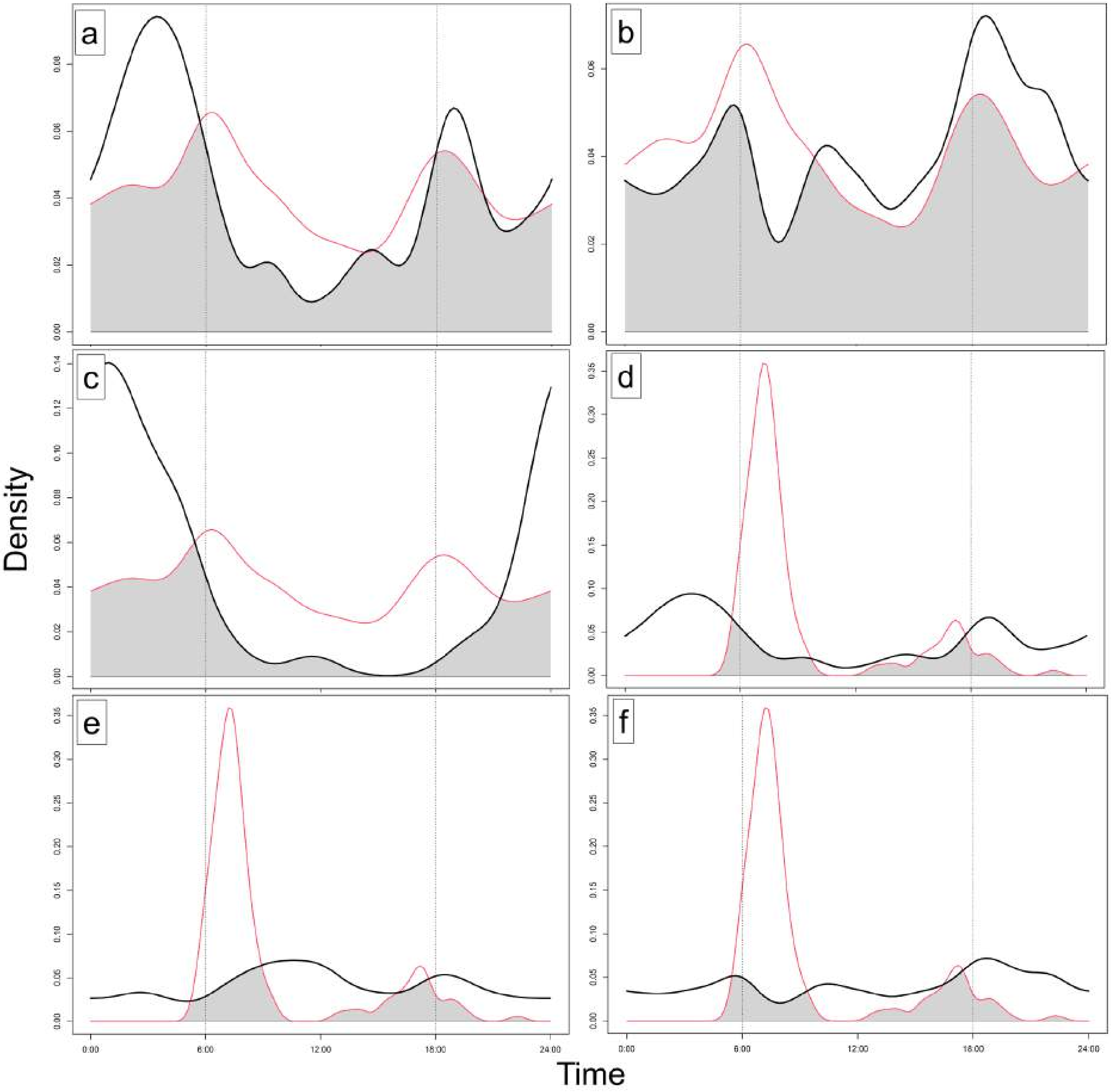

Figure 5. Comparison of the activity pattern of medium- and large-sized mammals with the activity pattern of domestic mammals during the dry season (15 October 2023 to 4 April 2024) in an isolated vegetation fragment in the city of Mérida, Yucatán, Mexico. Panels a, b, and c correspond to the comparison between *Felis catus* and a) *Silvilagus yucatanicus*; b) *Urocyon cinereoargenteus*; c) *Spilogale yucatanensis*. Panels d, e, and f correspond to the interaction of *Canis familiaris* with d) *Silvilagus yucatanicus*; e) *Odocoileus virginianus*; f) *Urocyon cinereoargenteus*. The red line corresponds to the domestic mammal, and the black line corresponds to the wild mammal. The shaded gray space indicates the areas of overlap between the two species. The vertical line indicates the approximate time of sunrise (6:00 h) and sunset (18:00 h).

and *P. lotor* were not abundant in the interior of the studied fragment; in the particular environment of Mérida, they may not be as abundant as in other urban areas (e. g., Coronel-Arellano *et al.* 2021; García-Padilla *et al.* 2021). However, it is worth mentioning that in the present study *Didelphis* sp. was observed on numerous occasions (living and roadkill animals) in urban areas surrounding the vegetation fragment, even though the species is not abundant inside it. *Didelphis* sp., and particularly *Didelphis virginiana,* are highly adaptable to anthropized and urban environments in Mexico, where they are most abundant species (Cruz-Salazar *et al.* 2016; Coronel-Arellano *et al.* 2021; García-Padilla *et al.* 2021). There is no clear explanation for the low abundance of *Didelphis* sp. inside the fragment studied. Possible hypotheses could be associated with the high abundance of wild species such as *U. cinereoargenteus,* which is a potential competitor and predator, or that *Didelphis* sp. uses mostly human resources, and it uses the

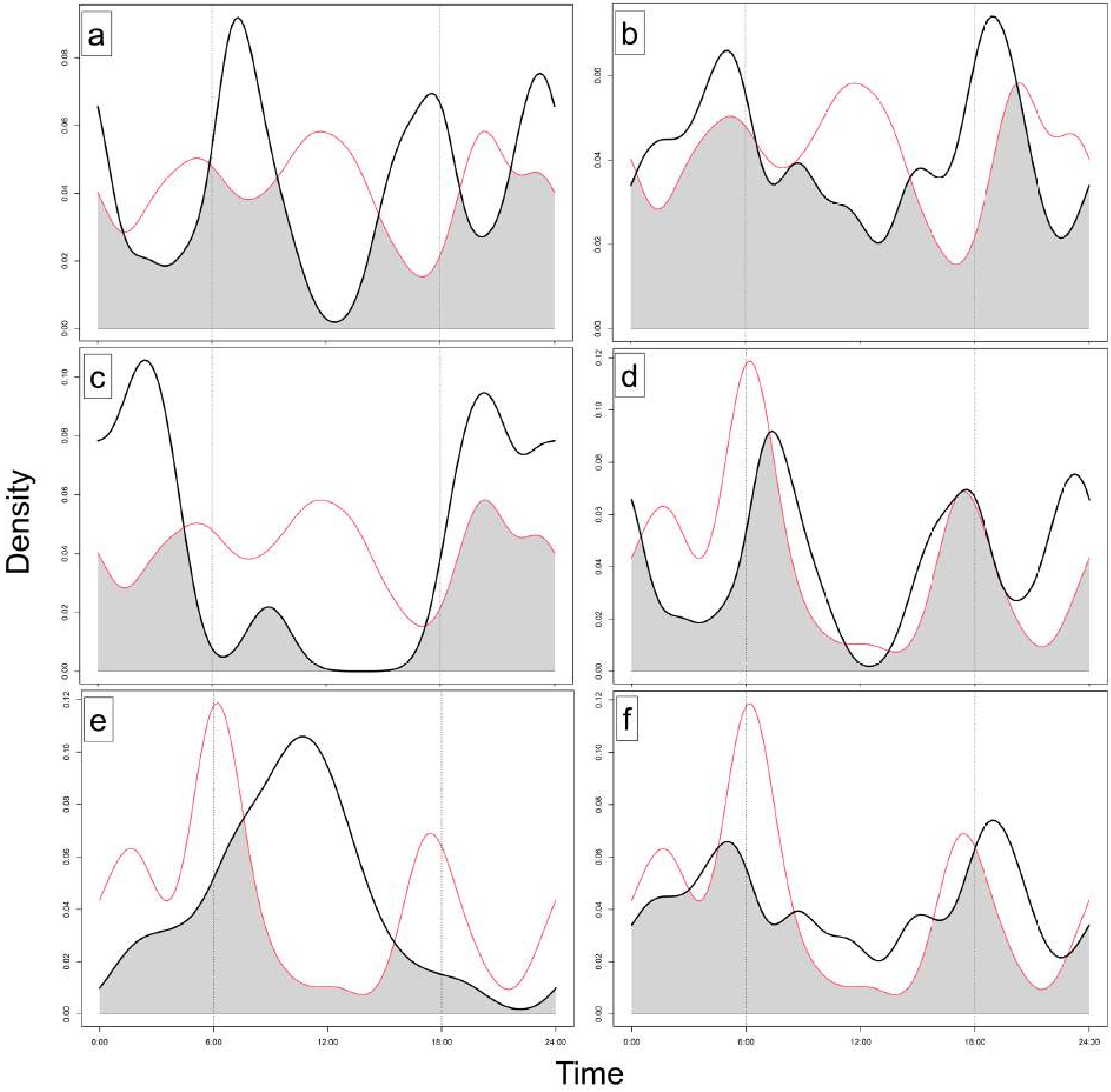

Figure 6. Comparison of the activity pattern of medium- and large-sized mammals with the activity pattern of domestic mammals during the wet season (30 May to 14 October 2023) in an isolated vegetation fragment in the city of Mérida, Yucatán, Mexico. Panels a, b, and c correspond to the comparison between *Felis catus* and a) *Silvilagus yucatanicus*; b) *Urocyon cinereoargenteus*; c) *Spilogale yucatanensis*. Panels d, e, and f correspond to the interaction of *Canis familiaris* with d) *Silvilagus yucatanicus*; e) *Odocoileus virginianus*; f) *Urocyon cinereoargenteus*. The red line corresponds to the domestic mammal, while the black line corresponds to the wild mammal. The shaded gray space indicates the areas of overlap between the two species. The vertical line indicates the approximate sunrise time (6:00 h) and sunset time (18:00 h).

forest cover to a lesser extent (Rodríguez *et al.* 2021).

Reports in the literature state that most species of wild mammals observed in urban environments tend to display nocturnal activity patterns, which have been commonly related to avoidance of human activity (e.g., Mella-Méndez *et al.* 2019; Coronel-Arellano *et al.* 2021). In the present study, the wild mammals found in Mérida showed significant diurnal activity, especially *O. virginianus* and *N. narica*, likely due to the limited access of people to the vegetation fragment studied. For their part, *S. yucatanicus* and *U. cinereoargenteus* showed diurnal activity, but concentrated especially at dawn and dusk, similar to their behavior in Mexico City or Xalapa (Mella-Méndez *et al.* 2019; Coronel-Arellano *et al.* 2021). The strictly nocturnal species found in the vegetation fragment of Mérida were *Spilogale yucatanensis*, *D. novemcinctus*, *Didelphis* sp., and *P. lotor*, which, except for the first one, had already been reported as mainly nocturnal species in other urban areas of Mexico

(Mella-Méndez *et al.* 2019). Regarding domestic species, cats were active during the day and night, as previously reported for this species in urban areas (Coronel-Arellano *et al.* 2021; Horn *et al.* 2011). In the case of dogs, activity in Mérida is concentrated at dawn and dusk, with very little activity at night. This finding contrasts observations in other areas, where dogs tend to be diurnal in a rural-urban gradient (Wang *et al.* 2015) and nocturnal within cities (De Andrade Silva *et al.* 2018; Mella-Méndez *et al.* 2019; Coronel-Arellano *et al.* 2021).

The results showed that during the dry season, the activity period of dogs overlaps slightly with those of *U. cinereoargenteus*, *O. virginianus,* and *S. yucatanicus*. In the wet season, the activity patterns of dogs are similar to those of *U. cinereoargenteus* and *S. yucatanicus* but differ from those of *O. virginianus*. In the dry season, dogs are predominantly diurnal, being most active at dawn, while *U. cinereoargenteus* and *S. yucatanicus* are active throughout the day, and *O. virginianus* is mainly diurnal, with greater activity at noon. *U. cinereoargenteus* and dogs share hours of activity in urban areas, which can lead to the death of *U. cinereoargenteus* due to this interaction (Mella-Méndez *et al.* 2019). In Mérida, no interactions were observed between these species, although the high overlap during the wet season suggests the possibility of interactions. The peaks of activity of dogs at dawn and dusk reduce this possibility.

Odocoileus virginianus shows significant twilight and nighttime activity in the dry season in warm areas of southern Mexico, probably to reduce hydric stress (Hidalgo-Mihart *et al.* 2024). However, in an isolated vegetation fragment in Mérida, despite comparable temperatures and water scarcity, *O. virginianus* was most active at noon. The avoidance of interactions with dogs could explain the peaks of activity at times of high temperatures. In the study, two series of photographs showed dogs running one minute after an *O. virginianus* passed, suggesting a chase and a negative interaction that *O. virginianus* would avoid by being active when dogs are not.

Cats were observed to be active both day and night, with a great overlap with *U. cinereoargenteus* and *S. yucatanicus* during the dry and wet seasons, but not with *Spilogale yucatanensis*, which is mostly nocturnal in the vegetation fragment. In urban areas, *U. cinereoargenteus* and other Sylvilagus species coincide in their hours of activity with cats (Coronel-Arellano *et al.* 2021), as observed in observed in S. yucatanicus in the vegetation fragment in Mérida. Cats are highly active during S. *yucatanicus* activity peaks, suggesting that cats could be important predators of this species, although no predation events were recorded (Loyd *et al.* 2017). In the case of *U. cinereoargenteus*, four series of photographs showing close interactions with cats were recorded. In all cases, both species seem to detect each other, but they stay away and evade encounters, which does not rule out the possibility of negative interactions. However, the frequent observation of interactions between these species suggests the possibility of pathogen exchange, posing the potential risk of transmission of infectious diseases between wild and domestic species (Clifford *et al.* 2006). Finally, the activity of *Spilogale yucatanensis* has been recorded mostly at night (Pérez-Irineo *et al.* 2020), consistent with our observations in the vegetation fragment in Mérida. The reduced temporal interaction with cats is likely due to its nocturnal habits rather than to its active avoidance of cats.

The case of the vegetation fragment in Mérida illustrates that urban patches of natural vegetation, despite their isolation and the pressures of urbanization, can host important communities of medium- and large-sized mammals. This fact highlights the importance of implementing urban conservation strategies that consider interactions between wild and domestic species while mitigating the impacts of urban sprawl. The protection of these fragments must be a priority, recognizing them as strategic areas for ecological restoration, intra-urban biological corridors, or archipelago-model protected natural areas (Carrillo-Niquete *et al.* 2021; Sosa-Escalante and González-Herrera 2022).

The present study also underlines the need to update land-use planning and urban development instruments in Mérida, such as Urban Development Programs (PDU, in Spanish) and Local Ecological Land-Use Planning (OETL, in Spanish), to include specific guidelines to promote the conservation of intra-urban natural vegetation fragments (Sosa-Escalante 2024). These efforts would prevent biodiversity loss, mitigate urban heat islands, and reduce the risk of zoonotic diseases by implementing nature-based solutions and green infrastructure (Carrillo-Niquete *et al.* 2021; Villanueva-Solís and Torres-Pérez 2023; Ayuntamiento de Mérida 2016; Ayuntamiento de Mérida 2024b).

In a broader context, the case of Mérida reflects the challenges and opportunities associated with urban sprawl in Mexico and Latin America. Cities with similar urbanization patterns face common problems of habitat fragmentation, loss of ecological connectivity, and pressure on biodiversity. However, the implementation of public policies focused on the conservation of intra-urban fragments and the improvement of the urban landscape can contribute significantly to the sustainable coexistence between humans and wildlife, serving as a model for other urban contexts in the region.

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Habitat use of *Myocastor coypus* in the Ciervo de los Pantanos National Park, Buenos Aires, Argentina

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The present research was carried out in the two main wetlands of the Ciervo de los Pantanos National Park (PNCP), Province of Buenos Aires, Argentina: Laguna Grande (LG) and Juncal del Río Luján (JRL). The objectives were: 1) To corroborate the presence of the coypu, *Myocastor coypus* (Molina, 1782) within the park, so far only confirmed for the coast of the Luján River, and to study the variability between seasons, 2) To evaluate the habitat use of the coypu and the potential variables that determine it through macro and microhabitat scales analysis, and 3) To conduct studies of the spatial disposition of the signs of presence of the species. The last two are fundamental to understanding possible underlying processes, such as food availability or response to the historical hunting pressure. We not only sought to answer which environments of each wetland they use, but also how they use them. The hypotheses were: 1) Sites with signs presence vary between seasons; 2) Sites where signs are present depend on the type of vegetation cover; and 3) Spatial arrangement of signs is clustered. Four transects were surveyed at LG and three at JRL using the transect line method. In each, sites with and without signs were georeferenced, and cover variables of different plant species (%) and water depth (cm) were measured. Generalized linear mixed models (GLMM) with presence/absence as response variable were used for both, macro and microhabitat analysis. For the spatial analysis, a count of signs per quadrant was performed in each wetland and their distribution was evaluated by means of goodness-of-fit tests to Poisson and negative Binomial distributions. At JRL, coypus use both identified environments: Edge of the Marsh (EM) and Inner Marsh (IM). There is no seasonal variation with the exception of spring where there was total absence of signs. No cover variables were detected to predict presence. In the LG they only use the Middle Marsh (MM) environment, and not those of Edge (E) and Open Waters (OW). The probability of detection increases during autumn and winter and *Schoenoplectus californicus* was the main predictor species for the presence of signs. In both wetlands, signs were found in a clustered disposition. These results confirm the presence of coypu in the CPNP, with the JRL being a potential pathway into the park, and the MM of the LG being a key point of establishment. The environments used and the spatial arrangement of signs could be the result of the sustained food availability through seasons within the used environments, and protection from poaching and predation pressure. The seasonal variation partially coincides with the reproductive peaks previously observed in the wild for the species.

La presente investigación se desarrolló en los dos principales humedales del Parque Nacional Ciervo de los Pantanos (PNCP), Provincia de Buenos Aires, Argentina: Laguna Grande (LG) y Juncal del Río Luján (JRL). Los objetivos fueron: 1) Corroborar la presencia del coipo o falsa nutria, *Myocastor coypus* (Molina, 1782) dentro del parque, hasta el momento solo confirmada en la costa del Río Luján, y estudiar la variabilidad entre estaciones del año, 2) Evaluar el uso de hábitat del coipo y las variables potenciales que lo determinan mediante análisis a escalas de macro y microhábitat, y 3) Realizar estudios de disposición espacial de los signos de presencia de la especie. Estos dos últimos resultan fundamentales para comprender posibles procesos subyacentes, como la disponibilidad de alimento o respuesta a la histórica presión de caza. No solo se buscó responder cuáles ambientes de cada humedal utilizan, sino de qué manera lo hacen. Las hipótesis fueron: 1) Los sitios con presencia de signos varían entre estaciones, 2) Los sitios con presencia de signos dependen del tipo de cobertura vegetal, y 3) La disposición espacial de signos es agrupada. Se relevaron cuatro transectas en LG y tres en JRL mediante el método de línea transecta. En cada una se georeferenciaron sitios con y sin signos, se midieron variables de cobertura de diferentes especies vegetales (en %) y profundidad del agua (en cm). Tanto para macro como microhábitat se plantearon modelos lineales generalizados mixtos (GLMM) con presencia/ausencia como variable respuesta. Para el análisis de espacialidad se realizó un recuento de signos por cuadrante en cada humedal y se evaluó su distribución mediante pruebas de bondad de ajuste a distribución de Poisson y Binomial negativa. En JRL los coipos usan los dos ambientes identificados como Borde del Juncal e Interior del Juncal. No hay variación estacional, a excepción de la primavera donde hubo ausencia de signos. No se detectaron variables de cobertura que permitan predecir la presencia. En la LG solo utilizan el ambiente de Juncal Interior, y no los de Borde y Aguas Abiertas. La probabilidad de detección aumenta durante el otoño e invierno y la totora o junco, *Schoenoplectus californicus*, fue la principal especie predictora de la presencia de signos. En ambos humedales los signos se encuentran agrupados. Estos resultados confirman la presencia del coipo en el PNCP, siendo el JRL una potencial vía de ingreso al parque, y el Juncal Interior de la LG un punto clave de establecimiento. Los ambientes utilizados y la disposición espacial de signos podría ser resultado de la sostenida disponibilidad de alimento en donde fueron detectados, y de protección ante la presión por caza furtiva y depredación. La variación estacional coincide parcialmente con los picos reproductivos, observados previamente en estado silvestre para la especie.

Keywords: multi-scale study; Paraná River; populations; Rodentia; wetlands.

Introduction

The coypu, *Myocastor coypus* (Molina, 1782) is considered of special value by society for being a representative and conspicuous species of the wetlands (Administración de Parques Nacionales 1999). This species is a semi-aquatic rodent native to South America with gregarious habits (Guichón *et al.* 2003a). Its peak of activity occurs during crepuscular hours when it spends approximately 60 % of its time foraging (Salas *et al.* 2022) with a diet consisting mostly of rooted and floating vegetation present in the wetlands (Borgnia *et al.* 2000; Guichón *et al.* 2003b), although in some cases they may also consume grasslands vegetation relatively close to bodies of water (Galende *et al.* 2013; Hong *et al.* 2016). In terms of reproduction, it requires a minimum water level for copulation, and as a polyestrous species, multiple reproductive events can be detected throughout a year (Spina *et al.* 2009; Courtalon *et al.* 2011; 2015; with litters averaging between 3 - 6 individuals, and a range of 1 - 12 (Gosling 1981; Courtalon *et al.* 2015; Porini *et al.* 2019). Individuals can be detected directly by visualization, or indirectly by the presence of signs such as platforms, trails, caves, footprints, feces or chewed vegetation. The platforms are built with vegetation, which they weave for resting, but the females also use them to give birth. Trails are spaces left between the floating and rooted vegetation, as a coypu swims (Porini *et al.* 2019).

Although categorized as Least Concern by the Secretaría de Ambiente y Desarrollo Sustentable de la Nación (SAyDS) and Sociedad Argentina para el Estudio de los Mamíferos (2019), coypu populations have been under strong hunting pressure within its distribution in Argentina, being commercially the main wild mammal species for the value of its fur and meat (Colantoni 1993; Bó *et al.* 2006, 2013). Several authors have studied habitat use and population parameters of the coypu in areas under hunting pressure to better understand how the species may be affected (Arias *et al.* 2005; Guichón and Cassini 2005; Bó *et al.* 2006; Cruz Pinzón and Courtalon 2017). How this species responds to that pressure, however, is still a question pending to be answered, and studies involving explicit spatial data, such as this one, may help to elucidate.

Manly *et al.* (1993), proposes that habitat use may vary among different spatial scales. Therefore, it is important to define them clearly. The term "macrohabitat" is used to define the area where organisms carry out their biological functions and where their area of action is included. At this scale, different types of environments are often considered, such as forests, grasslands or flooded areas, among others (Maitz and Dickman 2001). The "microhabitat", on the other hand, refers to the physical and biotic conditions that influence the development of organisms and is expressed in the distribution of their activities within the

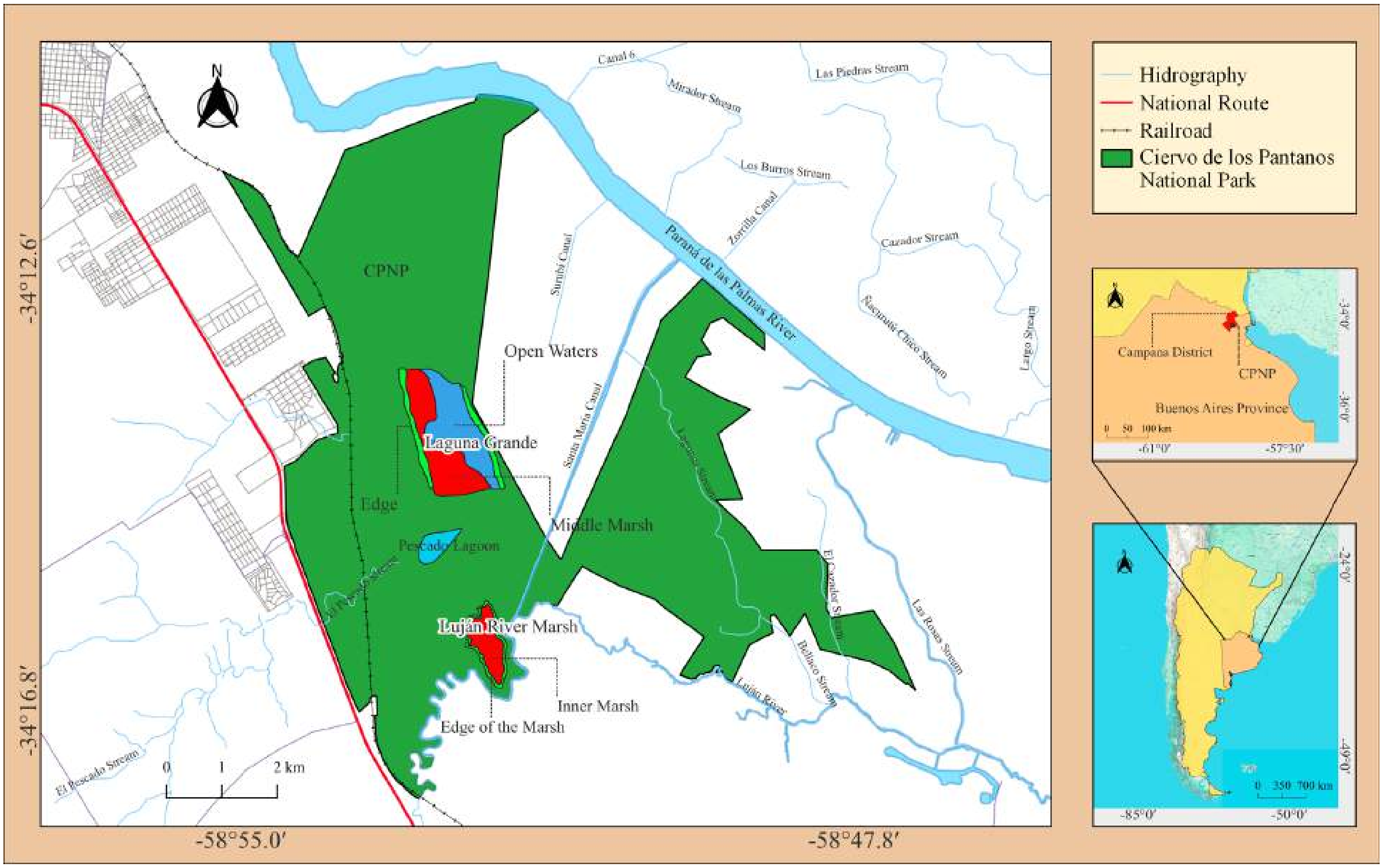

Figure 1. Map of Ciervo de los Pantanos National Park. Surveyed wetlands and its environments shown: "Laguna Grande" and "Juncal del Río Luján". Modified from Gabriela Gerardo (GIEH-FCEN-UBA).

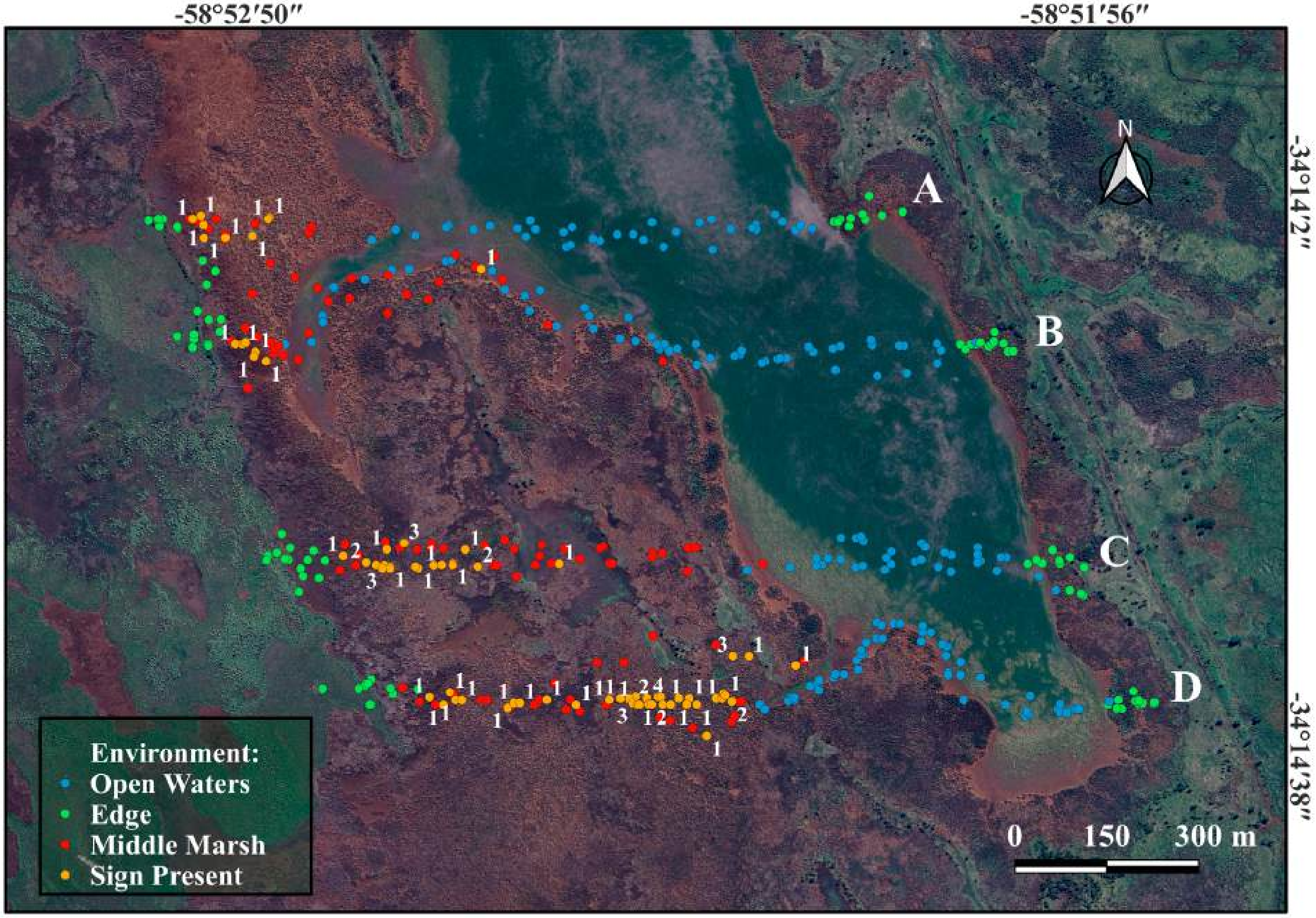

Figure 2. Accumulated point cloud of the surveyed transects during all four seasons in Laguna Grande wetland of the Ciervo de los Pantanos National Park. Transects (A, B, C and D) and points per environment are identified, highlighting in orange those where signs of presence were found, with the total number of signs found in each one written next to them.

macrohabitat. Therefore, habitat use is a multi-scale process and is a consequence of two decisions: first, where to live and establish the area of action, and second, what portion of that macrohabitat is used during daily activity (Johnson 1980; Orians and Wittenberger 1991).

The study and description of spatial patterns are central to decision-making in conservation biology (Primack 2006; Moilanen *et al.* 2009). One particular pattern, the "clustered" type, implies that individuals are grouped in clusters or patches, leaving portions of space relatively unoccupied. This pattern, so common in nature, may occur as a consequence of abiotic factors, social interactions, reproductive habits, resource availability or in response to human pressure.

Although numerous studies have been carried out along the Luján River (D'Adamo *et al.* 2000; Guichón et al. 2003a,b; Guichón and Cassini 2005, 2007), which marks the southern boundary of Ciervo de los Pantanos National Park, no studies have been conducted in two of its biggest wetlands, Laguna Grande (LG) and Juncal del Río Luján (JRL) (Figure 1). According to Guichón and Cassini (2007), who studied the habitat use by the coypu and classified the park boundaries within the "protected zone" of the Luján river, 100% of the sampled transects were positive for the presence of the coypu. Nevertheless, these transects only covered a maximum distance of 50 m perpendicular to the Luján river margin, without including the JRL or the LG.

The general objective of this work was to confirm the presence and evaluate the habitat use of the coypu in the main wetlands of the CPNP and the potential variables that determine it, as well as to generate a new source of valuable information for the park's management decisions regarding the protection of this species. The specific objectives for each studied wetland were as follows: 1) To analyze the habitat use at a macrohabitat scale; 2) To evaluate the potential association of sites with coypu presence in the CPNP to vegetation cover; 3) To describe the spatial distribution of coypu signs in the PNCP by integrating the information from points 1 and 2. Finally, the hypotheses derived from these objectives were 1) sites with and without signs of presence in the different environments will vary among seasons; 2) sites with use signs will be associated with the type of vegetation cover, and 3) the spatial arrangement of the sites with signs responds to a clustered distribution.

Materials and methods

I) Study area. The environments of the "Ciervo de los Pantanos" National Park (CPNP) are distributed in two large geomorphological units, called "High terrace" and "Low terrace", separated from each other by a ravine or paleo-cliff. The soil complex of the latter is characterized by having poor drainage, since it occupies low slopeless areas, known as marshes. It is in this area where the main wetlands are located: 1) "Laguna Grande", a large wetland covering the largest area of the park, and the reason why the CPNP is considered a "Wetland of International Importance or Ramsar Site (Secretaría de la Convención de Ramsar, 2013)", 2) The largest marsh of Luján River, located at the extreme south of the park, and 3) "Laguna del Pescado", located between both of the previously mentioned, although this last one wasn't included within the study for logistical reasons (Figure 1). These wetlands are a result of the geomorphology of the area and the base level imposed by the Río de La Plata, and are mainly maintained by groundwater from the aquifers and local precipitation that recharge the close to ground level phreatic surface. It is also regulated by the fluctuations and floods of the Paraná River, the Luján River, and by the contribution of streams such as the "Pescado" and "Otamendi" that come from the high terrace, cross the ravine, and discharge into the low terrace. Surface runoff is limited and the area remains periodically or permanently flooded (Administración de Parques Nacionales 2016).

The first site, Laguna Grande, was located on the lower terrace of the CPNP (34° 14′ 11.48″ S; 58° 52′ 26″ W), with an approximate extension of 156 ha (Rodríguez and Pizarro 2007). Based on a preliminary sampling carried out in July 2011 and the biological knowledge of the species, three main groups of environments were identified in the LG wetland at the macrohabitat scale:

Edge (E): Both edges were defined as the 100 m strip perpendicular from the limit of the waterbody. These are characterized by containing both, species typical of the saline grassland such as *Juncus acutus* (spiny rush), *Distichlis spicata* (seashore saltgrass) and *Limonium brasiliense* (caspia), and also those typical of the flooded grassland: *Schoenoplectus californicus* (California bulrush), *Typha latifolia* (common cattail) and *Scirpus giganteus* (club-rush).

Middle Marsh (MM): Dominated by *S. californicus*, with medium abundance of *T. latifolia* and lesser of *S. giganteus*, amongst others. Due to its irregular shape, this environment had variable extension in the different transects. Within this heterogeneous environment, a micro-relief was observed in which low flooded areas, with floating species such as *Azolla filiculoides* (mosquito fern), *Pistia stratiotes* (water lettuce), *Lemna minuta* (least duckweed) and *Wolffiella oblonga* (saber bogmat), were differentiated from slightly higher areas with typical flooded grassland vegetation dominated by *S. californicus*.

Open waters (OW): Characterized by lacking cover of emergent herbaceous vegetation (except for scattered patches of species such as *S. californicus*), and with high variability in terms of floating macrophytes cover.

While both, E and most of the MM, were sampled on foot, the OW and the internal flooded parts of the MM were covered using a kayak given their low accessibility. Seasonal samplings were conducted between spring 2011 and winter 2012. The transect line method (Krebs 1999) was used, identifying the presence (confirmed by the identification of at least 1 sign) or absence, counting the total number of signs and registering the GPS coordinates every 30 mts. Four transects with an approximate length of 1 km each and 100 m minimum between them were drawn, and covered with a west to east orientation, located in such a way as to proportionally cover the different types of environments. All of them were repeated throughout the four seasons, with samplings carried out within a maximum of 2 consecutive days. Signs were classified into three types: platforms (used to satisfy shelter and breeding requirements), chewed vegetation (indicators of feeding sites) and trails (signaling movement and potential feeding sites). Feces were not included due to their low detectability as a consequence of the constant fluctuation of the water level. When accessibility permitted, the longest and shortest platform diameters were measured. A Garmin® GPSmap 60CSx model was used to record the location of the points within the lagoon.

The second site, Juncal del Río Luján was located on the southernmost region of the park, adjacent to the Luján River (34° 16′ 35.10″ S; 58 ° 51′ 55.50″ W), and has been described according to the plant communities by Chichizola (1993). This section was classified by Guichón and Cassini (2007) as the "protected area" of the "deltaic portion" of the Luján River, where more than 97 % of its extension is included within three natural protected areas, one of them being the CPNP. This section, accessible only by navigation on the Luján River or an extensive hike in complex terrain, has low anthropogenic disturbance of its riparian environment (Guichón and Cassini 2007).

With regards to the vegetation that characterizes this section, it can be divided into two types of well-differentiated plant communities. On the one hand, there is the river ravine covered by a mixed grassland of *Sporobolus pyramidatus* (whorled dropseed) and *Xanthium cavanillesii* (Italian cocklebur). On the other hand, the inner marsh is a mosaic of *S. californicus*, *T. latifolia* and *S. giganteus* as the dominant species (Chichizola 1993). The edge of the marsh was considered as a transition zone between these two, containing a mixture of vegetation from the two environments. Samplings were conducted for each season of the year from summer 2013 to spring 2013. Based on a preliminary sampling conducted in this section of the park, in October 2012, it was determined that two different environments would be considered in this wetland at the macrohabitat scale: the Edge of the Marsh (EM) and the Inner Marsh (IM). Two transect lines with a maximum length of 345 m and separated by 50 m were covered at each season (A and B). However, due to the harsh conditions, a third transect (C) was covered in replacement of A, during one season. We defined EM as the transition zone corresponding to 25 % of the total length of each transect. The rest of the points correspond to the IM. At these points, separated by 15 mts, signs of coypu presence (platform, chewed vegetation, and footprints) were recorded. When accessibility permitted, the longest and shortest platform diameters were measured. The same Garmin® GPS GPSmap 60CSx model was used to record the location of the points.

II) Microhabitat scale. At each of the points surveyed within the transects, several variables were recorded in addition to the signs present:

I. The percentage cover of the dominant plant species was estimated using the Braun-Blanquet method within a 5 m radius from the point (Matteucci and Colma 1982).

II. The water depth at each of the points using the same graduated rod in all measurements in the center of the GPS point.

III) Spatial arrangement. Using QGIS Firenze 3.2.8 software, the cloud of georeferenced points was projected. Two grids containing 140 x 100 mts and 40 x 40 mts quadrats were used in LG and JRL respectively, in order to count the total number of signs observed per quadrat. These cell sizes were chosen due to the fact that the length of the transects differed between wetlands, as did the distance between points. Nevertheless, both scales represent significant areas for sign counts, and are proportional to the dimensions of the total surveyed area of each wetland. In LG wetland, only MM quadrats with at least 1 point (with or without sign presence) were included in the analysis. All quadrats fallen in OW and E were discarded given that only 1 sign was found present in the first one and none in the latter. We worked with a total of 48 quadrats where 95 signs were found to be present. In the JRL wetland, EM and MM quadrats were analyzed following the same criteria, with a total of 33 quadrats in which 17 signs were found. In both wetlands, data from all 4 seasons were analyzed together.

Statistical Analysis

1) Macrohabitat. At the macrohabitat scale, different generalized linear mixed models (GLMM) with presence/absence of signs as response variable were evaluated using the "glmmTMB" package (Brooks et al. 2017) of RStudio 4.3.2 (R Core Team 2023). Transects were identified as a random factor to declare lack of independence. Four models were tested in the two wetlands: Null (M0), Environment (M1), Environment+Season (M2) and Environment*Season (M3). In JRL wetland, spring data was not included in the analysis due the absence of signs. For the same reason, those of the E environment in LG were also excluded. A total of 369 points were evaluated in the LG and 119 in the JRL wetlands of the CPNP. Models were compared by Akaike's criterion, selecting the one with the lowest AIC. Finally, probability of presence for each environment was estimated for the final models using the "emmeans" package (Lenth 2023).

2) Microhabitat. At the microhabitat scale, multiple GLMMs with presence/absence of signs as a response variable were explored using the "glmmTMB" package (Brooks *et al*. 2017) of RStudio 4.3.2 (R Core Team 2023). Transects were identified as a random factor to declare lack of independence in the model. Models included different combinations of plant species cover (%) and water height surveyed at each point, as well as seasons as a four-level categorical variable (Summer, Fall, Winter and Spring). These were compared by Akaike's criterion, selecting the model with the lowest AIC. The assumptions of the selected model were evaluated with the DHARMa package (Hartig 2022). Once the model was selected, post-hoc contrasts were performed with the "emmeans" package (Lenth 2023) and model predictions were estimated with the "ggeffects" package (Lüdecke *et al*. 2020). In total, 448 points were evaluated in LG and 160 in JRL of the CPNP.

3) Spatial arrangement. Using "fitdistrplus" package (Delignette Muller and Dutang 2015) of RStudio 4.3.2 (R Core Team 2023) the distribution of observed signs was evaluated by goodness-of-fit tests to Poisson (random arrangement) and Negative Binomial (clustered arrangement) distributions by maximum likelihood method (Krebs 1999). Morisita's index (Krebs 1999) was also calculated for the two wetlands using the observed frequencies for each category of total sign counts per quadrat.

Results

1A) Laguna Grande Macrohabitat. A total of 448 points were surveyed, 79 corresponding to E, 193 OW and 176 MM (Table 1; Figure 2). Out of the total 448 points, 72 resulted positive for signs. Since no signs were found in environment E, points surveyed in this sector were removed from the GLMM, since the probability of presence is null and reduces the predictive capacity of the model. The 4 models (M0, M1, M2 and M3) were evaluated, and interaction between environment and season was determined to be not significant ($P > 0.05$). The additive model (M2) involving seasons and environments resulted in the lowest AIC (M0 = 366.25; M1 = 249.07; M2 = 222.12; M3 = 226.87). The final model compares the OW (193 points) and MM (176 points) environments at the 4 sampling seasons.

Table 1. Number of points with signs according to the season of the year for each environment of both studied wetlands: Laguna Grande and Juncal del Río Luján.

	Laguna Grande				**Juncal del Río Luján**		
	E[1]	**MM**[2]	**OW**[3]	**Total**	**EM**[4]	**IM**[5]	**Total**
	[79][6]	**[176]**	**[193]**	**[448]**	**[39]**	**[121]**	**[160]**
Summer	0 (0)[7]	13 (13)	0 (0)	13 (13)	0 (0)	4 (5)	4 (5)
Autumn	0 (0)	25 (35)	1 (1)	26 (36)	2 (4)	2 (2)	4 (6)
Winter	0 (0)	28 (38)	0 (0)	28 (38)	1 (3)	2 (3)	3 (6)
Spring	0 (0)	5 (9)	0 (0)	5 (9)	0 (0)	0 (0)	0 (0)
Total	0 (0)	71 (95)	1 (1)	72 (96)	3 (7)	8 (10)	11 (17)

1: Edge; 2: Middle Marsh; 3: Open Waters; 4: Edge of the Marsh; 5: Inner Marsh 6: Total points surveyed per environment between square brackets; 7: Number of points with present signs and total number of signs between regular brackets.

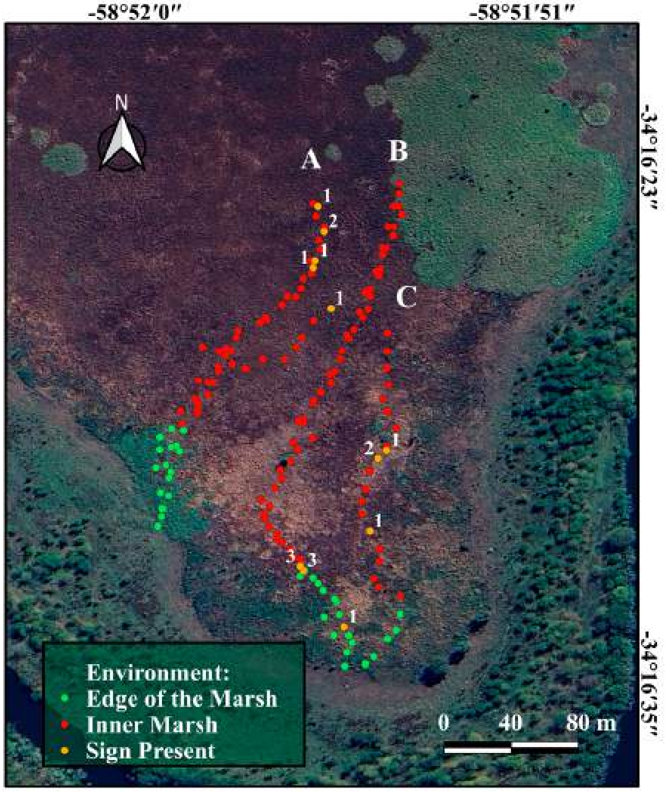

Figure 3. Accumulated point cloud of the surveyed transects during all four seasons in Juncal del Río Luján wetland of the Ciervo de los Pantanos National Park. Transects (A, B, C) and points per environment are identified, highlighting in orange those where signs of presence were found, with the total number of signs found in each one written next to them.

Significant differences were found between both environments ($P < 0.0001$) and between seasons. Given no interaction was detected, a posteriori contrasts by Tukey's method were performed only for main effects. The estimated probabilities of sign presence were estimated between [0.0004; 0.024] and [0.24; 0.49] for OW and MM, respectively. On the other hand, values between [0.03; 0.29], [0.03; 0.26], [0.002; 0.04] and [0.006; 0.07] were estimated for winter, autumn, spring and summer seasons respectively, with no significant differences between winter/autumn or spring/summer ($P = 0.58$; 0.99), but significant indeed between winter or autumn with respect to spring ($P = 0.0004$; 0.0003) or summer ($P = 0.002$), indicating that the probability of signs presence increases during cold seasons.

Platforms accessible for measurements were analyzed. During spring 2011 (n = 3) were woven solely with *S. californicus*, in an approximately elliptical shape, with an average large diameter of 40 cm and a smaller one of 30 cm. During summer low accessibility to the platforms did not allow recording their measurements. In autumn 2012, 31 platforms were analyzed, of which only one was knitted with *T. latifolia*, while the remaining ones were with *S. californicus*. These exhibited highly variable measurements, with an average long diameter of 49.68 ± 22.13 cm and a short one of 35.48 ± 20.99 cm. In winter 2012, 8 platforms were measured, all of *S. californicus* with a less elliptical shape than in the other seasons. The average large diameter resulted in 47.50 ± 19.09 cm and the smaller one 41.25 ± 16.42 cm.

1B) Juncal del Río Luján macrohabitat. A total of 160 points were surveyed, 39 corresponding to EM and 121 to IM. In JRL, signs were found in both environments (Table 1; Figure 3). Data collected during spring (41) were left out of the analysis due to the total absence of signs, so only the 119 points left from the remaining seasons were used. Assessing the M3 model no interaction between environment and season was found, and the model with the lowest AIC resulted to be M1, which only included the environments (M0 = 90.14; M1 = 89.7; M2 = 92.89; M3 = 91.84). No significant differences were found among IM and EM environments ($P = 0.7$), with estimated presence probabilities of [0.04; 0.16] and [0.03; 0.29] respectively. It should be noted that the signs found in both, EM and IM, correspond solely to platforms (17). Five of them were measured during summer, six in autumn and six in winter. Their average long diameters were [53.43 ± 10.77 cm], [47.56 ± 11.35 cm] and [47.60 ± 7.43 cm] respectively, while their short ones were of [33.44 ± 1.5 cm], [33.50 ± 4.78 cm] and [41.70 ± 2.21 cm].

It is crucial to note that the wet period during the year under study lasted from January to April, including summer and part of autumn 2013. While the driest stage was observed from May to December 2013, with a low annual accumulated rainfall of 293.8 mm. The highest flood for the Lujan River was 5.10 m, recorded in November 2012 prior to the start of the sampling period.

2A) Laguna Grande microhabitat. Two models were initially proposed: a null model containing no covariates at all, and another that included the water depth (cm) and cover of the dominant plant species: *S. californicus*, *T. latifolia*, *Chenopodium* spp. and floating macrophytes (*L. minuta*, *P. stratiotes* and *A. filiculoides*). A possible interaction between *S. californicus* cover and seasons was detected in the exploratory analysis, so not only additive models were evaluated, but also this interaction was considered. Following the selection methodology previously described, the final model presented below (in the linear predictor scale) was selected. This model includes the variables of *Chenopodium* spp. and *S. californicus* cover, seasons, and interaction between *S. californicus* cover and seasons as explanatory variables that were found significant in order to predict the probability of the sign's presence in LG.

The final model proposes that *S. californicus* cover is the main variable able to predict the probability of coypu signs presence in the LG wetland, being this relation positive. However, it is observed that when adding seasons as a categorical variable to the model, presence as a function of *S. californicus* cover is dependent on the season (Figure 4a). Due to the fact that the final model includes two vegetation cover variables (continuous), in order to evaluate differences between seasons (categorical) while accounting for the interaction, contrasts were performed by fixing cover values of *Chenopodium spp*. at 0 % and *S. californicus* at both extreme (0 and 100 %) and mean cover values of the observed data (33.2 %). Contrasts revealed that

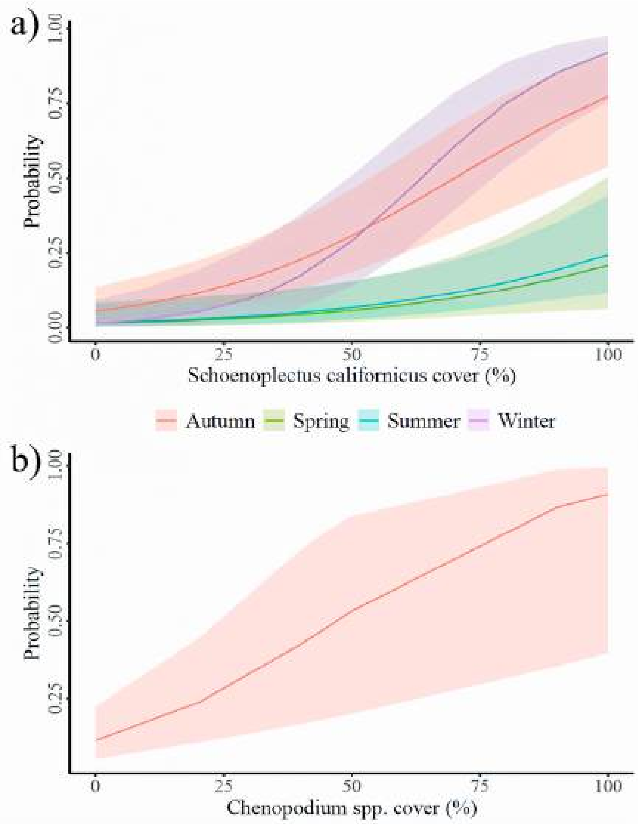

Figure 4. Predicted probabilities of coypu signs presence in Laguna Grande wetland as a function of: a) *Schoenoplectus californicus* cover (%) for each season; b) *Chenopodium spp.* cover (%), for all seasons. Each prediction is presented with its respective confidence interval (α = 0.05).

signs presence increases significantly in autumn and winter compared to summer and spring seasons (all $P < 0.0001$), when cover of *S. californicus* is maximum. Meanwhile, for a mean cover of 33.2 %, it is significantly higher solely in autumn with respect to spring ($P = 0.04$) and summer ($P = 0.04$) but no different than winter ($P = 0.85$), and no differences were detected with 0% cover (all $P > 0.99$). Therefore, a high probability of signs detection is expected for LG in winter and autumn, with predicted values of [0.76; 0.98] and [0.54; 0.91] respectively, while in spring and summer these values are lower and more variable with [0.06 %; 0.50] and [0.11; 0.44] respectively, when cover of *S. californicus* is maximum.

Chenopodium spp. cover was also significant ($P = 0.0012$), although with an imprecise confidence interval. This is due to the fact that of the total number of sites analyzed (448) only 8 registered high cover of this species. However, given that 3 of them showed clear signs of presence the model is sensitive enough to detect that there is an increasing trend in the probability of presence of coypu signs as a function of higher cover of *Chenopodium* spp. in LG (Figure 4b). Probabilities of sign presence are estimated between [0.06; 0.22.], [0.20; 0.84] and [0.40 ;0.99] for covers of 0, 50 and 100 % of *Chenopodium* spp. respectively.

A total of 96 signs were recorded, consisting of 89 nests/platforms (92.7 %), 4 chewed vegetation (4.16 %) of *S. californicus* and 3 trails (3.125 %) in *Chenopodium* sp.

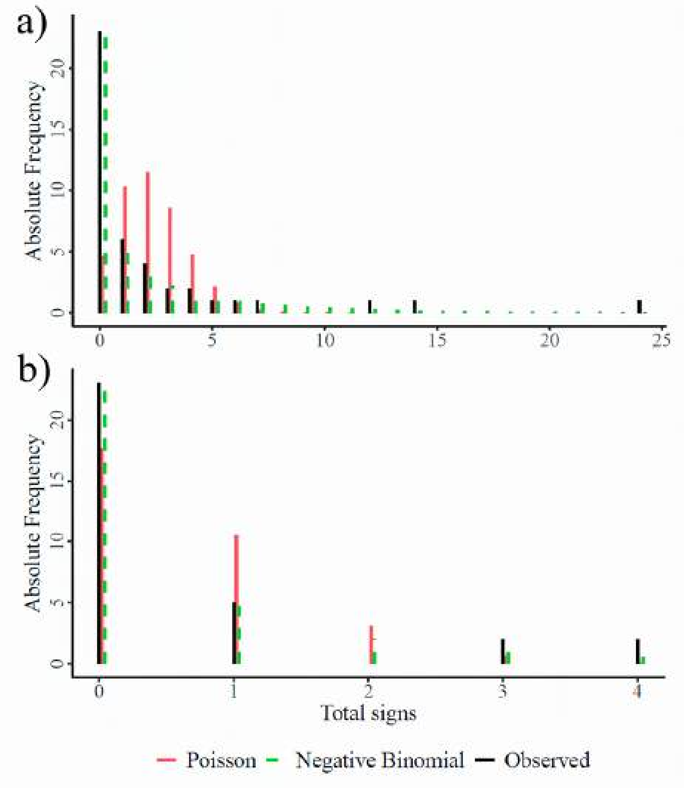

Figure 5. Comparison between the observed and theoretical Poisson and Negative binomial sign frequency distributions in: a) Laguna Grande wetland; b) Juncal del Río Luján wetland.

The OW environment presented small patches of *S. californicus* with a mean percentage cover of [15.75 ± 23.92 %]. Floating macrophytes such as *L. minuta* or *A. filiculoides* showed high seasonal variability, with percentage covers of [5.77 ± 13.02 %] and [1 ± 5.43 %] for winter and autumn respectively, while in summer and spring these values increase notably to ranges between [41.96 ± 44.90 %] and [48.57 ± 46.21 %]. In contrast, the IM presented a mean cover of [54.54 ± 45.76 %] for *S. californicus*, while floating macrophytes had were more stable amongst seasons with minimum cover in winter of [8.9 ± 20.5 %] and maximum in summer of [33.25 ± 30.84 %]. The E environment presents a *S. californicus* cover of [21.4 ± 33.7 %]. However, the cover of floating macrophytes was low or null with values of [5.6 ± 18 %].

2B) Juncal del Río Luján microhabitat. Following the same steps in the JRL wetland, 2 models, one null and another including the cover of *S. californicus*, *S. giganteus*, *T. latifolia*, *Hydrocotyle bonariensis* (largeleaf pennywort) and the seasons were initially proposed. Water height was not included due to lack of data as a consequence of the dry season. No interaction between seasons and *S. californicus* cover was observed ($P > 0.05$). In the selection process, the model with the lowest AIC was the one that only retained the *S. californicus* cover variable.

However, despite being quite a simple model due to having just one explanatory variable, SC coefficient was not significant ($P = 0.15$), indicating that no variable measured in the JRL could clearly predict coypu signs presence.

3A) Laguna Grande Spatial arrangement. The Poisson distribution hypothesis was rejected (Chi = 84.95; $P < 0.0001$), so no random arrangement of signs was detected (Figure 5). On the other hand, the negative binomial distribution hypothesis was not rejected (Chi = 0.068; $P > 0.79$). The empirical frequency distribution compared to the theoretical frequency distributions expected with the observed data show that the sign arrangement of this species in the MM of LG is clustered (Figure 5). The Morisita index was 38.13, which is also consistent with a clustered arrangement.

3B) Juncal del Río Luján Spatial arrangement. The Poisson distribution hypothesis was rejected (Chi = 4.489; $P = 0.03$), so no random arrangement of signs was detected (Figure 5). The negative binomial distribution goodness of fit p-value could not be evaluated due to lack of freedom degrees as a consequence of the low sign counts in this wetland. However, the empirical frequency distribution compared to the theoretical frequency distributions expected with the observed data suggest that the sign arrangement of this species in the JRL wetland could be clustered (Figure 5). Consistent with this, the Morisita index took a value of 10.87.

Discussion

Signs detected within the surveyed environments of the two wetlands allow us to affirm that the coypu is present in the CPNP. Returning to the particular objectives and their derived hypotheses (H), the discussion of these results will follow an orderly manner analyzing first each of the wetlands studied and finally presenting a habitat use model for the CPNP.

Laguna Grande. The macrohabitat analysis allows us to affirm that the three environments surveyed are different in terms of signs presence. No signs were found in the E environment, although some were indeed located within the MM but close to its limits with E. With the exception of 1 found in OW, all signs were detected in the MM, clearly indicating that the latter is used by coypu over the other environments. Signs found were mostly sessile platforms, built on *S. californicus*, with the exception of 3 trails detected on *Chenopodium* spp. No trails or mobile platforms built on non-rooted floating macrophytes were detected. Despite offering small patches of *S. californicus* and availability of floating macrophytes such as *L. minuta* or *A. filiculioides* as food source (Borgnia *et al.* 2000; Guichón *et al.* 2003b), the OW environment remained unused at all seasons. If we compare with the MM, not only does the latter offer higher average cover of *S. californicus*, but also food availability is relatively higher during all of the seasons. On the other hand, the E environment presents sufficient cover of *S. californicus* for coypu to weave their platforms. However, both the low cover of floating macrophytes and the mean depth of 35.7 cm could be acting as a limiting factor. Porini *et al.* (2002) proposed that coypu populations use environments with high cover of species such as *S. californicus* not only for the direct usage as nesting sites, but also that the higher water depth within these sites might be a crucial factor for protection against predators by submersion (including humans). Guichón and Cassini (1999) reported that variables associated with human disturbance were the most negatively correlated with coypu presence. Hong *et al.* (2016) carried out isotope diet studies and determined that coypu may increase the consumption of terrestrial vegetation in order to cover their daily share when availability of macrophytes is scarce. All this being considered, proximity to the park trails (anthropic) and low water level may be a more suitable explanation to why the E environment remained unused, rather than food or nesting sites availability, given that coypu could consume terrestrial vegetation. Another important result of this analysis is the distinction between seasons, with higher signs of presence during autumn and winter compared to spring and summer. This, we believe is partially correlated with the reproductive functionality of platforms, built by adults to give birth (Porini *et al.* 2019). Previous works from Courtalon *et al.* (2015) identified autumn and spring as the calving peak seasons detected in wild areas, which in contrast with these results might suggest there are other factors such as forage vegetation availability determining the higher signs presence, especially during winter. This being said, it would be of interest to carry out reproductive studies of this species within the park, in order to further understand the factors determining both peaks. Finally, evidence obtained for this wetland does not support the H1, since there is no variation between environments, only seasonal variation independent from them.

On the other hand, evidence does support H2 at the microhabitat scale, since the final model proposes that *S. californicus* cover is the main plant cover variable, able to predict the presence of coypu signs in the LG wetland, this relationship being positive. However, it is observed that when adding seasons as a categorical variable to the model, sign presence probability as a function of *S. californicus* cover is dependent on the season (Figure 4), consistent with the results obtained at the macrohabitat scale. Models shown in this study resulted in significantly higher presence for autumn and winter rather than summer and spring seasons under 100 % cover of *S. californicus*, while for a mean cover of 33.2 % it is only significantly higher in autumn. Diet studies carried by *Galende et al. (2013)* in a steppe lagoon in Argentine Patagonia demonstrated that coypu was a selective consumer, with its diet consisting primarily of *Myriophyllum* sp. (submerged macrophyte) and *S. californicus*, also with variation between seasons. Borgnia *et al.* (2000) also made microhistological feces analysis and determined that *Lemna* sp. (floating macrophyte) was the main diet component, especially during summer and spring seasons. As previously stated, winter season has the lowest availability of floating macrophytes, so *S. californicus* selection as a potential feeding component may explain the peaks observed. Also, it may be suitable for explaining why despite the presence of other emergent herbaceous vegetation species such as *T. latifolia*, coypu selected *S. californicus* over any other species in the LG wetland. Another plant species used that appears in this environment is the aforementioned *Chenopodium* sp. where coypu trails were found. Considering that during 60 to 80 % of the time spent outside their shelters, coypu actively feed on vegetation while moving (Guichón, 2003a; Salas *et al.* 2022), it is likely that this species is used for foraging, although it would be interesting to carry out diet studies to confirm this.

To test H3, a spatial analysis was carried out exclusively contemplating the MM environment, since 95 of the 96 total signs were detected there. Under the results of both goodness-of-fit tests and the calculation of Morisita's index, evidence upholds in favor of H3, indicating a clustered distribution of signs in this environment. Looking at Figure 2, there are 4 clear clusters of signs (1 per transect), the main ones being those detected in transects C and D. This spatial pattern might follow after the fact that it could allow coypu to seek refuge from multiple threats present in their environment, as well as to maximize the resting and breeding area, while minimizing the effort required at the time of feeding. This result contributes evidence that supports the behavioral tradeoff hypothesis postulated (D'Adamo *et al.* 2000; Borgnia *et al.* 2000; Gosling 1981). Domestic predators such as dogs or other natural predators such as birds of prey, ophidians (yararás) are found on the E, since it is a transitional environment between this large wetland and the saline grassland of the CPNP where these species occur. Another important problem for this species within the park is the hunting pressure exerted by poachers who enter illegally outside visiting hours (Cruz Pinzón and Courtalon 2017; Courtalon *et al.* 2019). In other words, the pressure over coypu in the CPNP is not only one of natural origin, but also anthropogenic. Therefore, in addition to what has been discussed regarding E at the macrohabitat scale, the absence of signs in this environment not only is reasonable, but also it may be one of the leading factors for coypu to seek for protection in a more isolated environment, and more suitable for their regular activities, such as the MM.

Juncal del Río Luján. At the macrohabitat scale, results show that coypu uses the 2 surveyed environments. Only platforms woven with *S. californicus*, *T. latifolia* or *S. giganteus* (species noted for their great height), were found on the Edge of the Marshand (EM) and Inner Marsh (IM) environments. As previously stated, the presence of this type of vegetation is essential for the establishment of a coypu population, since these use mainly emergent herbaceous vegetation such as the above mentioned to weave breeding and resting platforms (Borgnia *et al.* 2000; Porini et al. 2002, Bó *et al.* 2006). The presence of signs in both environments lead us to suppose that the Juncal del Río Luján is an entryway to the CPNP, and that the coypu use not only the coast of the river (D'Adamo *et al.* 2000; Guichón and Cassini, 2007), but also both, a transitional environment such as the EM, and one with a proper wetland vegetation physiognomy such as the IM. As far as the seasons are concerned, no differences were detected, except for spring, where the absence of signs was total. Given that the JRL wetland is located within the Luján River flood valley, the influence of hydrological dynamics is essential for this environment to remain saturated with water, either by direct precipitation or by the overflow of the river during floods (Comité de Cuenca del Río Luján, 2013). We believe that these results are related to the hydrological dynamics of this year, characterized by a strong drought. Such was its magnitude that, from July to December 2013, total cumulative rainfall was recorded below 60 mm and there was a single flood event of the river, reaching barely a maximum of 2.7 m, well below the 5.1 m recorded in the pre-sampling at the end of 2012 (Data from Servicio de Hidrografía Naval). Therefore, the signs present during the previous seasons (summer, autumn and winter of 2013) would be expected to belong to individuals that arrived with the floods prior to the beginning of the sampling season. In turn, their disappearance in spring, the last season surveyed, would be due to the lack of a minimum water level required to carry out their activities normally, especially reproductive ones.

With all the above mentioned, the information gathered does not support H1, since no variation was detected in the sites with signs between environments throughout the seasons, except for the particular case of spring.

Results at the microhabitat scale were inconclusive, as no vegetation or environmental variable was found to clearly predict the presence of coypu signs. For this reason, more information should be collected in order to study H2 within this wetland. It is possible that this result is due to two main reasons. First, the low number of signs detected in the total number of points surveyed in the 4 stations (17 out of 160), mainly because it was an extremely dry year, water being the limiting variable for coypu reproduction and survival (Bó *et al.* 2008; Porini et al. 2019). Secondly, most of the platforms found were woven with the dominant vegetation of this environment, *S. californicus*, so there are included in the analysis a large number of points with high cover of this species, but with no signs present, reducing the sensitivity of the model. Such is the dominance that the mean cover in EM and IM turned out to be [67.5 ± 35 %] and [72.3 ± 32.4 %] respectively. This brings us to the question of whether coypu actually selects *S. californicus* in this environment or whether its utilization is simply an availability response. To answer this and other questions, such as what happens in wetter seasons, this marsh, the most representative of the park, should be monitored seasonally.

To test H3, an implicit spatial analysis was performed to evaluate whether the arrangement of signs is random, regular or clustered. The results of the goodness-of-fit test allow us to reject a random arrangement of the signs, but the limited amount detected is not sufficient to properly calculate a p-value and evaluate whether it is indeed a clustered arrangement. However, complementing with the Morisita index whose value is 10.87 we can infer that there is an underlying clustering with two main focal zones: deep in the IM (northern end of the transects) and between both EM and IM (lower end of the transect) (Figure 3). In conclusion we assume that the signs are clustered as there is no statistical evidence to reject H3. It is important to remember that this analysis was performed with the total count of signs detected at each point, and not only with presence/absence.

Conclusions. Analyzing these results altogether, it is unmistakable that there is a resident coypu population present within both of the main CPNP wetlands, with a high probability of having active reproduction since the main signs detected consisted of platforms. In support of the behavioral tradeoff hypothesis, the clustering pattern in both wetlands could be a response of the species seeking shelter from potential threats present within the park. Also supporting this hypothesis is the fact that the Edge of LG remained unused while the Edge of the JRL was indeed used, being the first one directly connected with the park trails, while the second one is located in a more isolated area. In both wetlands, *S. californicus* was the main species used for weaving its platforms, which may be also an important food source when other resources such as *Lemna* sp. are scarce, especially during colder seasons. Nevertheless, as previously mentioned, this could be an availability response that requires further study to define whether or not it is positively selected. As for seasonal variation, clear differences were observed between the two wetlands. In LG, we found what seems to be a response mainly to reproductive peaks observed in wild environments (Courtalon *et al.* 2015), as no there were no clear limiting factors in terms of shelter or food availability observed between seasons in the MM environment. On the other hand, in the JRL wetland, differences between Spring and the rest of the seasons are highly likely to have occurred due to the influence of the hydrological regime on this wetland, fundamental in the surveyed year characterized by a strong drought.

Complementing with previous studies from D'Adamo *et al.* (2000), who detected coypu presence in the Lujan River Ravines within the park limits, this marsh located in the southernmost region of the CPNP could be an entryway for coypu to access to the other wetlands such as the LG. It would be interesting to include the Pescado Lagoon in future research, in order to further understand the connection between these three main wetlands.

Finally, we propose to carry out seasonal monitoring of the presence of this species in the park, and diet studies to help elucidate the underlying causes of these patterns of space use, observed for the coypu in the CPNP.

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Delimitation of regional management units for desert bighorn sheep in Baja California. An application of the potential species distribution model

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Models of the potential geographic distribution of species are decision-making tools for wildlife population management, especially for species with broad ranges, such as bighorn sheep. In the present study, a potential geographic distribution model was generated for managing bighorn sheep in Baja California, Mexico. The model was produced with the maximum-entropy algorithm to estimate the geographic range of the species. The variables used as predictors were climate, relief, and vegetation. Meanwhile, known sites where bighorn sheep were recorded were obtained from aerial counts in Sierra Juarez in 2012 and at the regional level in 2021 by Romero-Figueroa *et al.* (2024). Additional records of terrestrial observations used were reported by Ruiz-Mondragón *et al.* (2023) for Sierra Juarez in 2016, Sierra La Asamblea and Calamajué in 2021, and Sierra Santa Isabel and Sierra Juarez in 2022, as well as records from the Global Biodiversity Information Facility (GBIF 2021). The geographic distribution model revealed that bighorn sheep in the state of Baja California are distributed along the mountain range of the Gulf of California, covering an approximate area of 317 160 ha. The variables that contributed the most to the construction of the model were roughness, type of vegetation, and precipitation of the coldest quarter. The geographic distribution model was used to define 12 regional management units for the species. Each unit is shared between two or more agrarian communities. In Baja California, bighorn sheep should be managed through community monitoring, habitat protection, and sustainable use programs with the participation of all rural communities that own land within the distribution range of this species.

Los modelos de distribución geográfica potencial de especies son herramientas para tomar decisiones sobre el manejo de las poblaciones de vida silvestre, especialmente de especies que ocupan grandes extensiones de área, como el borrego cimarrón. En el presente estudio se generó un modelo de distribución geográfica potencial que puede ser utilizado para el manejo del borrego cimarrón en el estado de Baja California, México. El modelo se generó con el algoritmo de máxima entropía para estimar el área de distribución geográfica de la especie. Las variables utilizadas como predictoras fueron climáticas, de relieve y de vegetación. Mientras que, los sitios conocidos donde se registró al borrego cimarrón se obtuvieron de conteos aéreos realizados en Sierra Juárez en 2012, y a nivel regional en 2021, por Romero-Figueroa *et al.* (2024). Asimismo, se incluyeron registros de observaciones terrestres reportados por Ruiz-Mondragón *et al.* (2023) en Sierra Juárez en 2016; en la Sierra de la Asamblea y en Calamajué en 2021; y en Sierra Santa Isabel y Sierra Juárez en 2022. Así como, del Sistema Global de Información sobre Biodiversidad (GBIF 2021). El modelo de distribución geográfica reveló que la especie en el estado de Baja California se distribuye a lo largo del macizo montañoso del Golfo de California, en una superficie aproximada de 317,160 ha. Las variables que contribuyeron más en la construcción del modelo fueron la rugosidad, el tipo de vegetación y la precipitación del trimestre más frío. El modelo de distribución geográfica se utilizó para definir 12 unidades de manejo regional para la especie. Cada una se comparte entre dos o más comunidades agrarias. En Baja California el manejo del borrego cimarrón se debe realizar a partir de programas de monitoreo comunitario, protección del hábitat y aprovechamiento sostenible en los que, se considere la participación de todas las comunidades rurales que poseen terrenos dentro del área de distribución de esta especie.

Keywords: big game species; ecological niche model; maximum entropy; *Ovis canadensis*; wild sheep; wildlife management.

Introduction

Management units are geographic areas delimited for the conservation and sustainable management of wildlife species and their habitats (Swihart *et al.* 2020). However, their definition and delimitation consider only political boundaries without taking into account the biological aspects of the species (Bischof *et al.* 2016). An example of this issue is the management of bighorn sheep (*Ovis canadensis*) populations in Mexico. The distribution ranges of this species frequently surpass the boundaries of management units, which are delimited by property lines where there are no barriers restricting the movement of animals (Rubin *et al.* 2009; Ruiz-Mondragón *et al.* 2018). Therefore, to achieve sustainable bighorn sheep management in Mexico, it is necessary to design and delimit regional management units (RMUs) whose boundaries match the limits of the distribution area of the populations of the species (Gallina-Tessaro *et al.* 2009).

Potential geographic distribution models (PGDM) of species are useful tools for predicting the area of occurrence of species, identifying the environmental characteristics of the sites where they thrive, and projecting their presence over wider areas (Guisan and Thuiller 2005; Melo *et al.* 2020). These models are useful for designing efficient strategies for *in situ* management of populations, since they allow focusing monitoring and protection actions on the specific areas of the habitat used (Guisan and Thuiller 2005; Villero *et al.* 2017). One of the main applications of PGDMs in wildlife population management is RMU delimitation (Rodríguez-Soto *et al.* 2011; Maciel-Mata *et al.* 2015).

PGDMs are used primarily in the formulation of conservation and management plans and programs for at-risk species of hunting importance or with wide ranges (Guisan and Thuiller 2005; Refoyo *et al.* 2014; Eyre *et al.* 2022), such as wild ungulates (Ortíz-García *et al.* 2012; ENETWILD consortium *et al.* 2022). However, the reliability and functionality of the PGDM results depend on several factors: scale of the study area, accuracy and randomness of the geographic records of the species, and relevance, autocorrelation, and resolution of the environmental variables used as predictors (Austin 2007; Mateo *et al.* 2011).

Bighorn sheep are found in extensive mountain ranges with steep slopes, deep cliffs, and large canyons, where vegetation cover is scarce and temperatures are extreme, making it difficult to carry out regular and representative monitoring of population size and dispersal (Hansen 1980; Álvarez-Cárdenas *et al.* 2009; Ruiz-Mondragón *et al.* 2018). In the state of Baja California, Mexico, bighorn sheep populations inhabit 13 mountain ranges that cover an area of approximately 967 910 ha from La Rumorosa, on the border with the United States of America, to the Agua de Soda mountain range, located 50 km north of the border with Baja California Sur, Mexico (Romero-Figueroa *et al.* 2024). In this range, sheep populations are concentrated in areas where conditions are suitable for their persistence (Simmons and Hansen 1980), such as rugged terrain, open vegetation cover, presence of medium-sized shrubs (less than 1.5 m high), and water availability (Hansen 1980; Ruiz-Mondragón *et al.* 2018; Jones *et al.* 2022).

This study aimed to develop a PGDM for bighorn sheep, aiming to determine the RMUs for the species in Baja California. The following research questions were addressed: a) Where are the areas with the largest bighorn sheep populations? b) What is the extent of its potential range? c) What is the relative importance of the environmental variables that limit their distribution? d) How many bighorn sheep RMUs can be defined in the state?

Materials and methods

Description of the study area. The study was carried out in the Mexican state of Baja California, which covers an area of 71 446 km^2. This region extends from 32°43′07″ to 28° N, and from 112°17′48″ to -118°21′54″ W (Figure 1). The main relief forms include mountain ranges, hills, plateaus, descents, plains, valleys, and dunes (INEGI 2001). The predominant climates in the region are temperate dry, very warm very dry, semi-warm very dry, temperate very dry, semi-cold sub-humid, and temperate sub-humid (García and CONABIO 1998). The dominant vegetation types are chaparral, microphyllous scrub, rosetophyllous scrub, and sarcocaulescent shrub (INEGI 2021).

Baja California has 13 mountain ranges where wild bighorn sheep populations thrive (Romero-Figueroa *et al.* *2024*; Figure 1). Land tenure at these sites is mainly ejido

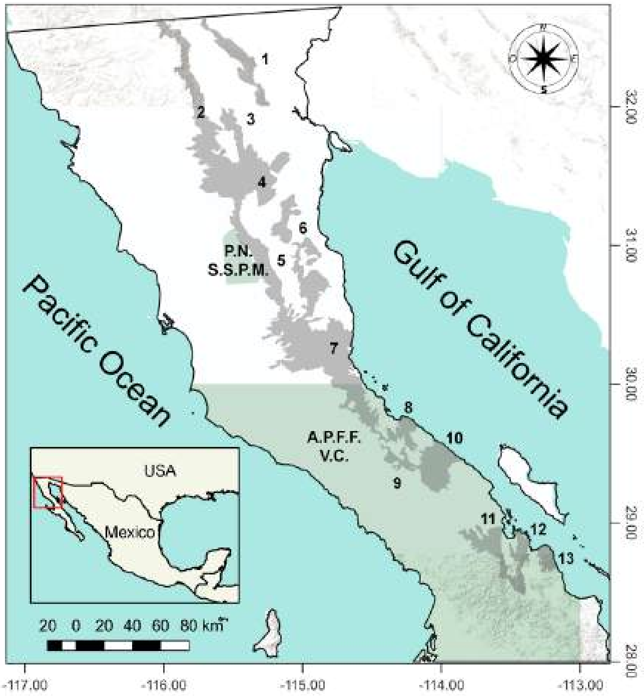

Figure 1. State of Baja California, Mexico, showing the mountain ranges (marked in grey) in which bighorn sheep occur: (1) Cucapá; (2) Sierra Juárez; (3) Las Tinajas; (4) Las Pintas; (5) San Pedro Mártir; (6) San Felipe; (7) Santa Isabel; (8) San Francisquito; (9) Calamajué; (10) La Asamblea; (11) La Libertad; (12) Las Ánimas; (13) Agua de Soda. Also shown (in green) are the NPAs that are within the distribution area of bighorn sheep. P.N. S.S.P.M.: Parque Nacional Sierra de San Pedro Mártir. A.P.F.F. V.C.: Área de Protección de Flora y Fauna Valle de los Cirios.

(RAN 2016). Similarly, the bighorn sheep distribution area is partially located within natural protected areas (NPAs): the Sierra de San Pedro Mártir National Park (PNSSPM, in Spanish) and the Valle de los Cirios Flora and Fauna Protection Area (APFFVC, in Spanish; CONANP 2024).

Generation of the database of bighorn sheep presence records. The bighorn sheep presence database was built from records of the species obtained from sampling campaigns carried out in different years. This study used data from aerial monitoring conducted by the San Diego Zoo in Sierra Juárez in 2012 (Ruiz-Mondragón *et al.* 2018) and from the surveillance flight by the Autonomous University of Baja California in 2021 that covered the 13 distribution areas recognized for bighorn sheep in Baja California (Romero-Figueroa *et al.* 2024). Additional records used regard direct sightings and indirect evidence of the presence of the species (fecal groups and footprints) obtained in terrestrial monitoring as part of a study of the distribution of bighorn sheep in the Sierra Juárez mountain range in 2016 (Ruiz-Mondragón *et al.* 2018); in terrestrial monitoring carried out within the framework of the Cimarrón Sanctuary project in the Sierra de La Asamblea and Calamajué in 2021; and in surveillance and camera-trap installation tours in Sierra Santa Isabel and Sierra Juárez in 2022 as part of a participatory community monitoring program (Ruiz-Mondragón *et al.* 2023).

The database was supplemented with records available in the Global Biodiversity Information System (GBIF; 2021), which were refined to exclude occurrence points in the sea or outside the distribution area reported in the literature. Records within 2 km of one other were excluded from our database to reduce spatial bias in the data (Merow 2013).

Predictors of environmental variables. Climate, relief, and vegetation were the environmental variables used to generate the PGDM for bighorn sheep (Rubin *et al.* 2009; Ruiz-Mondragón *et al.* 2018; Salas *et al.* 2018). Of these, 19 variables were bioclimatic, two were topographic (orientation and terrain roughness index), and two were vegetation variables (vegetation type and enhanced vegetation index). Geospatial information was handled in raster format and was processed in the QGIS 3.22.10 geographic information system (QGIS Development Team 2022).

The bioclimatic variables were generated by Cuervo-Robayo *et al.* (2013) for Mexico with a 90 m spatial resolution, available at the Idrisi Resource Center of the Autonomous University of the State of Mexico (UAEM 2021). These variables were rescaled to a 30 m spatial resolution to match the resolution of the relief and vegetation variables. Orientation and terrain roughness index (TRI) data were extracted from a digital elevation model with a 30 m resolution (INEGI 2013). The vegetation type was obtained from the Series VII of the Land Use and Vegetation Layer of Mexico (INEGI 2021) rasterized with a 30 m pixel resolution. The Enhanced Vegetation Index (EVI) was calculated from Landsat 8 OLI/TIRS satellite images captured between October and November 2022 (USGS 2022), which correspond to the months when the local vegetation greened after the passage of Hurricane Kay, thus favoring the discrimination power of the vegetation index. A mosaic was constructed with the EVI images that covered the entire study area. The generated geospatial information layers were adapted to match the extent of the study area.

The variance inflation factor ($FIV = 1*[1-r^2]^{-1}$) was used as a criterion to exclude redundant variation between variables (Akinwande *et al.* 2015). The index was obtained from multiple regressions used to estimate the correlation of the variables considered for the potential distribution model; the variables whose information was contained in any other variable were excluded ($FIV > 5$; Alvarado-Avilés *et al.* 2020). From this analysis, eight environmental variables were selected for use as predictors to estimate the distribution area of bighorn sheep in Baja California (Table 1).

Table 1. Environmental variables used as predictors to construct the potential geographic distribution model of bighorn sheep in Baja California.

Variable	Description	Units
Bio08	Mean temperature of the rainiest month	°C
Bio14	Precipitation of the dryest month	mm
Bio18	Precipitation of the warmest quarter	mm
Bio19	Precipitation of the coldest quarter	mm
Orientation	Direction of exposure of a slope	°
TRI	Degree of surface irregularity. It is calculated as changes in terrain elevation within a 3 × 3 pixel matrix and summarizes the point change in elevation in each pixel and in the 8 pixels surrounding it (Riley *et al.* 1999).	m
Vegetation type	According to the Land Use and Vegetation Layer of INEGI (2021)	
EVI	Contrast between absorption and radiation of vegetation (Liu and Huete 1995). The index is used as an indicator of vegetation cover; it takes values between 0 and 1. Values close to zero indicate areas devoid of vegetation, while values close to one (1) are typical of areas densely covered by plant species.	

Prediction of potential geographic distribution. The Maxent 3.4.4 program (Phillips *et al.* 2020) was used to predict the geographic distribution area of bighorn sheep in Baja California. The algorithm was implemented based on the criteria by Phillips *et al.* (2006) for basic niche modeling with a logistic output format, and the result indicates the probability of occurrence of the species of interest in a geographical space. The model was generated with a mean of 50 replicates with 1,000 iterations each. We used 80% of the localities of occurrence to construct the model and 20% to validate it. The predictive accuracy of the model was determined by calculating the area under the curve (AUC) of the receiving operating characteristic (ROC), and the fraction of sites classified erroneously as absences (omission errors) was determined by calculating the omission rate and the mean predicted area.

The number of replicates used for the model construction was determined based on the normality of the distribution of AUC values of the replicates (Plasencia-Vázquez *et al.*

2014) using the Shapiro-Wilk test; it was found that the 50 replicates fit a normal distribution ($W = 0.96$; $p = 0.11$). In the Maxent program interface, the *Do Jacknife to measure variable importance* and *Create response curves* options were activated. The Jacknife analysis was performed to evaluate the percentage of contribution of variables to the model generation. Response curves were created to determine the range of values for each variable within which the species is likely to occur (Phillips *et al.* 2006).

The continuous predictive model was transformed into a binary model (presence-absence). The cut-off threshold was determined from the mean of the minimum presence of training of the 50 replicates generated (Alvarado-Avilés *et al.* 2020). The binary model was projected over the Baja California mountain ranges (INEGI 2001), whose contours were used as the basis to define bighorn sheep RMUs, since these relief forms include the suitable habitat for the populations of this species (Hansen 1980; Álvarez-Cárdenas *et al.* 2009). The boundaries of RMUs were established at the points where the decrease in the concentration of binary-model pixels coincided with the boundaries of the mountain ranges, which was interpreted as an indication of the presence of barriers that limit the dispersal of organisms and, therefore, as the natural limit for a given bighorn sheep population (Epps *et al.* 2007). Similarly, the binary model was projected onto NPA polygons (CONANP 2024) to determine the fraction of the potential geographic distribution area found within an NPA in Baja California.

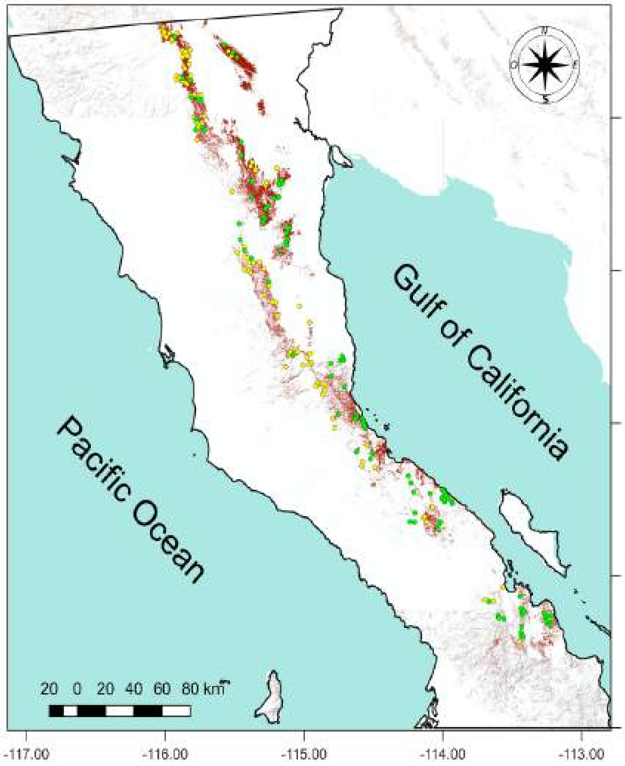

Figure 2. Potential distribution of bighorn sheep in Baja California (dark red). The image shows the presence records obtained from population monitoring (green) and the GBIF (yellow) used to generate the model.

Results

A total of 509 records of bighorn sheep were obtained for Baja California: 183 from population monitoring and 326 from the GBIF. After excluding records less than 2 km from each other, the database was reduced to 201 locations: 102 from population monitoring and 99 from the GBIF (Figure 2). The potential bighorn sheep distribution area in Baja California was calculated at 317 160 ha (4 % of the state area), stretching along the mountain massif of the Gulf of California coast from the US border to the Baja California Sur border. The AUC of the potential distribution model was 0.93, with a standard deviation of 0.02, and the omission rate was 0.40 (Figure 3).

The most important variables for the PGDM were roughness, vegetation type, and precipitation of the coldest quarter, which together contributed 78 % to the model construction. Each of the remaining variables contributed less than 10% (Table 2). According to the maximum-entropy algorithm, bighorn sheep in Baja California thrive in places where the roughness index varies between 35 m and 165 m covered by microphyllous scrub, sarcocaulescent scrub, riverbank vegetation or natural palm grove, and the precipitation of the coldest quarter ranges between 30 mm and 55 mm.

Table 2. Relative contribution (in percentage) of the environmental variables used to estimate the potential distribution area of bighorn sheep in Baja California and ranges of occurrence of the species.

Variable	Contribution (%)	Range of occurrence
TRI	38	35–165 m
Vegetation type *	23	MS, SCS, RV, NPG
Bio19	17	30–55 mm
Bio14	6	0.25–2.5 mm
Bio08	5	7–12 °C
Orientation	5	0°–100°
Bio18	4	0–65 mm
EVI	2	0–0.04

*MS = microphillous scrub; SCS = sarcocaulescent scrub; RV = riverbank vegetation; NPG = natural palm grove.

We defined 12 Regional Management Units (RMUs) for bighorn sheep in Baja California (Table 3; Figure 4). Within these RMUs, 85 % (271 044 ha) of the bighorn sheep distribution area is located on ejido land shared by 22 agrarian communities in the state. Furthermore, 23.6 % (71 520 ha) of the potential geographic distribution area of bighorn sheep is within a natural protected area: 22.3 % (70 626 ha) in Valle de los Cirios and 0.3 % (889 ha) in San Pedro Mártir.

Discussion

The most recent proposals regarding bighorn sheep distribution in Baja California were those of Lee *et al.* (2012) and Gutiérrez-Granados *et al.* (2020). Lee *et al.* (2012) indicate that bighorn sheep are distributed throughout the Gulf of California mountain range and interrupted in the

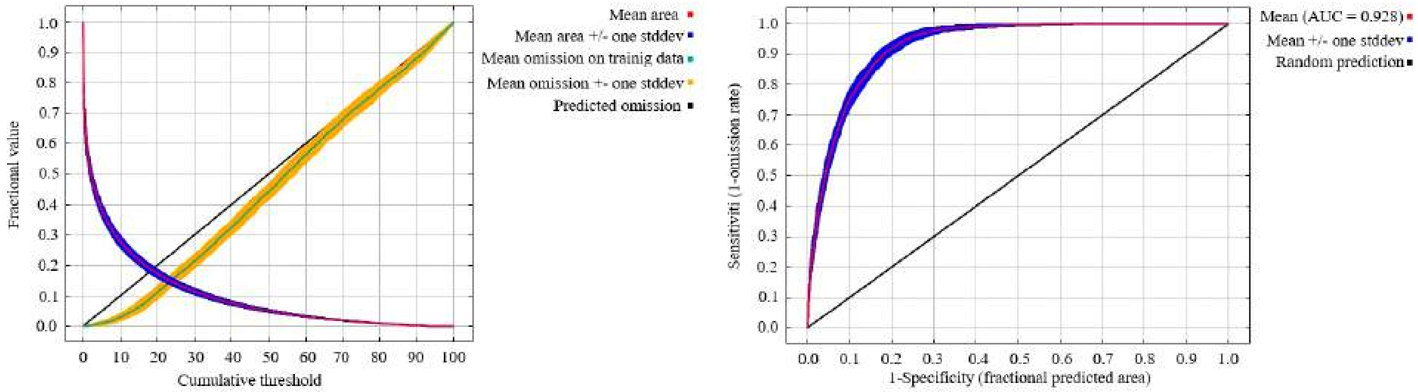

Figure 3. Area under the curve (AUC; left) and mean omission rate (right) of the 50 replicates used to estimate the potential distribution area of bighorn sheep in Baja California.

Agua de Soda mountain range, approximately 50 km north of the border with Baja California Sur (Figure 1). For their part, Gutiérrez-Granados *et al.* (2020) point out that the species is distributed continuously throughout the Gulf of California mountain range. Both proposals are inconsistent with the PGDM generated in this study, which indicates that in Baja California, bighorn sheep are distributed in patches throughout the Gulf of California mountain range (Figure 2).

Lee *et al.* (2012) defined the bighorn sheep distribution area in Baja California as the mountain ranges in which sightings were recorded in the aerial monitoring performed in the state. Therefore, the delimitation of the distribution area was conditioned by the geographical scope of the aerial surveillance. This explains why the authors point to the Agua de Soda mountain range as the distribution limit of bighorn sheep, since there were no flights south of this mountain range (Romero-Figueroa *et al.* 2024). However, despite its limitations, the distribution proposed by Lee *et al.* (2012) was hugely relevant because it represented the first effort to define the boundaries of the bighorn sheep range in Baja California, and is, therefore, one of the main references used to define the study area of the research on bighorn sheep in the state (Escobar-Flores *et al.* 2015; Ruiz-Mondragón *et al.* 2018; Ruiz-Mondragón *et al.* 2023; Romero-Figueroa *et al.* 2024).

On the other hand, Gutiérrez-Granados *et al.* (2020) constructed a PGDM to delimit the distribution area of bighorn sheep. Their work was also an important contribution to the matter because it defined the limits of the habitat available for the species in Baja California. However, it proposes a potential distribution area with an atypical pattern for any wild sheep species since they are not distributed evenly throughout the habitat but tend to concentrate around patches of habitat that provide the resources required by the species to survive, such as water, food, and escape ground (Bleich *et al.* 1990; Epps *et al.* 2007; Rubin *et al.* 2009; Salas *et al.* 2018). Another constraint of the PGDM generated by these authors is the size of the calculated distribution area, as it is too large to be used in decision-making on the management and monitoring of the species.

The lack of precision of the PGDM by Gutiérrez-Granados *et al.* (2020) is attributed to the inclusion of altitude and slope as predictor variables, the use of bioclimatic variables from WorldClim (Hijmans *et al.* 2005), and the use of a database of species occurrence made up entirely of GBIF records. In this type of analysis, altitude and slope are variables with marginal influence on the distribution of wild ungulates that inhabit mountainous areas (Keya *et al.* 2016; Khan *et al.* 2016; Ruiz-Mondragón *et al.* 2018; Salas *et al.* 2018). The low spatial resolution of WorldClim bioclimatic surfaces is a source of uncertainty for the model, as they do not reflect climate variations at the local level (Harris *et al.* 2014; Stewart *et al.* 2022). GBIF is a website with a particularly pronounced spatial bias due to uneven sampling effort, storage, and mobilization of data between the different areas in the range of a species, in addition to the lack of certainty about the quality of the data uploaded to the platform (Beck *et al.* 2014).

The PGDM presented in this study was developed from a database in which spatial bias was reduced by incorporating a similar number of records from field monitoring and the GBIF (Beck *et al.* 2014). Likewise, it was constructed using high-resolution bioclimatic variables developed especially for Mexico (Cuervo-Robayo *et al.* 2013), in addition to other predictor variables that are highly correlated with the distribution of bighorn sheep in the PGDM: terrain roughness, orientation, vegetation cover, and vegetation type (Rubin *et al.* 2009; Ruiz-Mondragón *et al.* 2018; Salas *et al.* 2018). This resulted in a PGDM showing a clustered distribution along a mountain range, consistent with the distribution pattern reported for the species (Bleich *et al.* 1990; Epps *et al.* 2007; Rubin *et al.* 2009; Ruiz-Mondragón *et al.* 2018). This suggests that this PGDM provides a more accurate representation of the distribution of bighorn sheep in Baja California than the one developed by Gutiérrez-Granados *et al.* (2020). In addition, it is a more useful tool for decision-making than the model of Gutiérrez-Granados *et al.* (2020), since it reduces by 75 % the distribution area proposed by these authors, facilitating the identification of areas of importance for the species.

The PGDM AUC assessment indicates good accuracy in discriminating between suitable and unsuitable sites for the species. However, the calculated distribution area is probably smaller than the actual range, as the omission rate of the training points did not fully match the predicted omission rate (Phillips *et al.* 2006; Figure 3).

The analysis of the contribution of the variables to the construction of the model indicated that roughness was the most relevant habitat component for bighorn sheep in Baja California (Table 3). This is an important variable for wild sheep species, as it is related to the availability of escape ground (Álvarez-Cárdenas *et al.* 2009; Salas *et al.* 2018). Furthermore, the presence of bighorn sheep was associated with sites with roughness values between 35 m and 165 m, typical of medium and high mountain ranges with canyons that provide protection to bighorn sheep in the Baja California peninsula (Álvarez-Cárdenas *et al.* 2009; Escobar-Flores *et al.* 2015; Ruiz-Mondragón *et al.* 2018).

Table 3. Fraction of the distribution area of bighorn sheep in each RMU and ejidos within its limits.

RMU	Area (ha)	Percentage	Ejidos*
Las Tinajas-Las Pintas	66 019	20.8	CM, MSC, Jam, JS, PNA
Santa Isabel	59 281	18.7	RAI, Mat, Rev
Sierra Juárez	58 736	18.5	EZ, Jac, AV, CM, MSC, Jam, DS, TK
San Pedro Mártir	30 040	9.5	Tep, PNA
Cucapá	29 474	9.3	EZ, HJ, LM
San Felipe	19 388	6.1	PNA, Del, Mat
La Asamblea	18 496	5.8	Juar, TL
La Libertad-Las Ánimas	13 567	4.3	TL, NR, CNC
Agua de Soda	12 362	3.9	TL
San Francisquito	6213	2	Mat
Calamajué	2781	0.9	Mat, Rev
La Sirena	805	0.3	Ind
Total	317 160	100	

*CM = Cordillera Molina; MSC = Misión de Santa Catarina; Jam = Jamau; JS = José Saldaña II; PNA = Plan Nacional Agrario; RAI = Reforma Agraria Integral; Mat = Matomí; Rev = Revolución; EZ = Emiliano Zapata; Jac = Jacumé; AV = Aubanel Vallejo; DS = Dieciséis de Septiembre; TK = Tribu Kiliwas; Tep = Tepi; HJ = Heriberto Jara; LM = López Mateos; Del = Delicias; Juar = Juárez; TL = Tierra y Libertad; NR = Nuevo Rosarito; CNC = Confederación Nacional Campesina; Ind = Independencia.

The vegetation type was another variable that contributed to the construction of the PGDM (Table 3) because it indicates the availability of forage and water for the species. In addition, it is related to the predator-avoidance strategy of bighorn sheep, which consists of using patches in which the vegetation foliage does not reduce visibility and, therefore, allows detection of predators from a distance (Wilson *et al.* 1980; Álvarez-Cárdenas *et al.* 2009; Escobar-Flores *et al.* 2015). As in other studies carried out in Baja California, it was determined that bighorn sheep are distributed in microphyllous scrub, sarcocaulescent scrub, riverbank vegetation, and natural palm groves (Escobar-Flores *et al.* 2015; Ruiz-Mondragón *et al.* 2018).

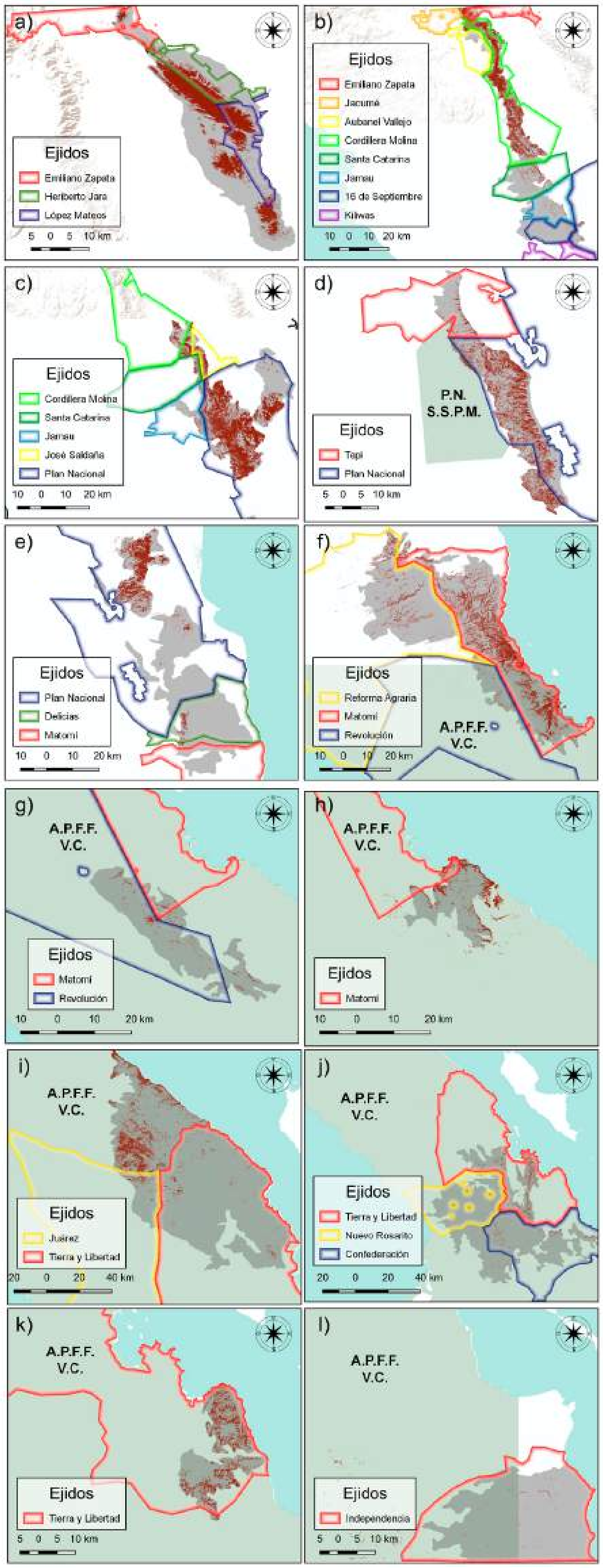

Figure 4. Regional Management Units (RMU) for bighorn sheep in Baja California (gray): a) Cucapá; b) Sierra Juárez; c) Las Tinajas - Las Pintas; d) San Pedro Mártir; e) San Felipe; f) Santa Isabel; g) Calamajué; h) San Francisquito; i) La Asamblea; j) La Libertad - Las Ánimas; k) Agua de Soda; l) La Sirena. The image shows the potential distribution of the species (dark red), NPAs (green), and ejidos that are within the limits of the RMU. P.N. S.S.P.M.: Parque Nacional Sierra de San Pedro Mártir. A.P.F.F. V.C.: Área de Protección de Flora y Fauna Valle de los Cirios.

Precipitation of the coldest quarter was the most important bioclimatic variable for bighorn sheep in Baja California (Table 3). This is attributed to the fact that the highest percentage of rainfall in the state occurs in winter (García and CONABIO 1998), and, therefore, this variable is related to water recharge in the habitat of the species. In addition, winter rains are related to the growth and flowering of quality forage for wild herbivores in Baja California (Delgadillo-Rodríguez and Macías-Rodríguez 2002).

In the state of Baja California, bighorn sheep management units are currently bounded by the boundaries of private land, ejidos, or the common use of ejidos, which have no relationship with the distribution patterns of bighorn sheep populations or their metapopulation dynamics (SEMARNAT 2022). This research outlines the first proposal for the definition of bighorn sheep management units in Baja California based on information on an ecological aspect of the species: its potential distribution. This proposal differs from the previous one by Lee *et al.* (2012), who proposed three RMUs: the northern RMU, which extends from Sierra Juárez to Sierra de San Felipe; the central RMU, from Santa Isabel to Sierra de La Asamblea; and the southern RMU, which includes the La Libertad, Las Ánimas, and Agua de Soda mountain ranges. This regional management proposal is based on the assumption that there are three metapopulations of bighorn sheep in Baja California; however, they do not provide evidence to support their existence. In this sense, there are also no studies showing that, in Baja California, a bighorn sheep metapopulation is distributed in two or more mountain ranges; on the contrary, genetic studies suggest that a mountain range can host more than one metapopulation (Buchalski *et al.* 2015).

The PGDM indicates that in Baja California the bighorn sheep shows a clustered distribution pattern, typical of wild sheep (Figure 2); that is, specimens of the species are concentrated in cores of suitable habitat connected by patches that function as biological corridors (Bleich *et al.* 1990; Epps *et al.* 2007; Rubin *et al.* 2009; Salas *et al.* 2018). The cores of suitable-habitat concentration are delimited by natural barriers that restrict the displacement of animals, which, according to the analysis of the contribution of variables, may be relatively flat areas with no escape terrain for the species (Berger 1991), a vegetation type unsuitable for the species because of a dense vegetation cover that reduces visibility (Bleich *et al.* 1997), or areas devoid of nutritious forage and water sources due to extremely aridity (Epps *et al.* 2004). This is why the boundaries of cores of suitable-habitat concentration were used to define the boundaries of RMUs for bighorn sheep, since the natural barriers between them contribute to confining the populations of the species, and, in this way, the abundance in each core of suitable-habitat concentration does not undergo significant fluctuations due to migratory processes (Epps *et al.* 2007; Creech *et al.* 2014).

The largest proportion of the bighorn sheep distribution area in Baja California is ejido land, and, in general, the land tenure of RMUs delimited in the present study corresponds to more than one ejido. This implies that each RMU should establish monitoring and protection programs for the bighorn sheep population with the participation of all landowners within the RMU (Dowsley 2009; Mandujano-Rodríguez and González-Zamora 2009). Furthermore, decision-making on the sustainable use of sheep should prioritize ejido management units and be based solely on the results of joint monitoring by all management units within an RMU (Adhikari *et al.* 2021).

The main benefit of regional management is the prevention of overexploitation of bighorn sheep populations. In Mexico, this problem is common to all game species at sites where the distribution area of local populations is shared between two or more individual management units (Gallina-Tessaro *et al.* 2009). This is due to the fact that a particular and independent exploitation quota is granted to each management unit based on the results of individual monitoring of a given local population (Mandujano-Rodríguez 2011). For this reason, to ensure the sustainable use of bighorn sheep, exploitation quotas must be established at the regional level rather than at the level of individual management units (SPA 2013; Ruiz-Mondragón 2014; 2017).

However, granting regional exploitation quotas for bighorn sheep poses a serious social challenge: the distribution of the benefits of hunting the species. The alternatives to resolve this issue could be the establishment of regional wildlife conservation management units (UMA, in Spanish) or the distribution of exploitation quotas that correspond to each RMU among the ejido management units involved, based on the fraction of the total habitat that belongs to each. The formation of regional UMAs is not considered the best option because there are considerable differences in the number of members in each ejido (Ruiz-Mondragón *et al.* 2023) and in the fraction of the bighorn sheep habitat owned by each (Figure 4). In this regard, it is worth noting that two regional UMAs were formed in Baja California whose viability could not be verified because they never started operations: the UMA named Ejidos Asociados de Baja California, comprising the ejidos Cordillera Molina, Hermenegildo Galeana, José Saldaña, and Plan Nacional Agrario; and the UMA Valle de los Cirios, made up of the ejidos Nuevo Rosarito, Revolución, and Tierra y Libertad. The distribution of hunting permits granted to each RMU among the ejido management units based on the fraction of the bighorn sheep habitat owned by each is considered the most viable alternative to solve the problem of the distribution of the economic benefits of sheep hunting, since the larger the area available, the greater the investment required for its management (Ortega-Argueta *et al.* 2016). This approach ensures that larger management units have sufficient financial resources to invest in the conservation of bighorn sheep populations and their habitat, while smaller units also participate in the economic benefits of bighorn sheep exploitation.

In Baja California, approximately 25 % of the bighorn sheep range is within an NPA, and this fraction of the habitat concentrates about 38 % of the total population of the species in the state (Romero-Figueroa *et al.* 2024). This situation has important implications for both bighorn sheep conservation and NPAs. On the one hand, Mexico's National Commission of Natural Protected Areas (CONANP, in Spanish) has the power to participate in the formulation and monitoring of the correct implementation of the work plans developed to manage one-quarter of the habitat available for the species and more than one-third of the bighorn sheep population in the state of Baja California (CONANP 2006; SEMARNAT 2013). On the other hand, bighorn sheep management units within NPAs can potentially become the main promoters of the conservation of these sites, since they can provide working groups for biodiversity surveillance and monitoring, invest in infrastructure and in the implementation of habitat improvement actions, and finance productive diversification projects around bighorn sheep (Brenner and De la Vega 2014; Sandoval *et al.* 2019).

Conclusions

The calculated distribution area for bighorn sheep in Baja California was 317 160 ha, extending throughout the state through the Gulf of California mountain range. The most influential environmental variables in the construction of the distribution model were roughness, vegetation type, and precipitation of the coldest quarter. The predictor variables were related to the presence of escape terrain, its suitability for the predator-avoidance strategy, and water and food availability. Based on the PGDM, 12 RMUs were delimited for bighorn sheep in Baja California, whose land tenure is ejido. It is recommended that the management of bighorn sheep populations within each RMU be carried out based on monitoring, protection, and sustainable use programs for bighorn sheep populations, involving the participation of all ejidos that own land within it.

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Population parameters of two Sympatric Kangaroo Rats: *Dipodomys merriami* and *Dipodomys nelsoni*

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The dynamics of climatic conditions cause diverse changes in organisms' populations. Species as the kangaroo rats (*Dipodomys*) have shown sensitivity to some climatic conditions variables such as the precipitation and temperature. For this reason, it is important to understand the relationship between these conditions and species, especially when *Dipodomys* species are considered keystone species and ecosystem engineers. The capture-recapture technique was performed monthly from 2012 to 2016. Total individual counts, age, reproductive condition, weight, and sex of *Dipodomys merriami* and *D. nelsoni* were determined and recorded. We analyzed the frequencies, age, reproductive status, sex ratio, and weight of sampled species. Daily temperature and precipitation data of the study area were analyzed with population parameters and frequency data. Further, temperature and precipitation from 2012 to 2016 in the study area were analyzed in regard with studied species frequencies. The research was conducted in two environments: shrubland and grassland in the Chihuahuan desert. The results showed that *D. merriami* was the most abundant species; however, the frequencies from the two species were higher in the shrubland. Moreover, there was no statistic relationship between species' frequencies and temperature, but there was a tendency with precipitation. Overall, the number of males and females of the two species was similar. The most found age category individuals was adults, most frequent reproductive status were non-pregnant females and males with abdominal testicles. Weight of individuals was different between species and age categories sub-adults and adults. The patterns are comparable to those found in other species of this genus. Results from this research provide information needed to take the required actions regarding the management and conservation of species with restricted distribution, such as *D. nelsoni*.

El dinamismo de las variables climáticas provoca diversos cambios en las poblaciones de los organismos. Algunas especies como las ratas canguro (*Dipodomys*), han demostrado sensibilidad ante los cambios de algunas variables climáticas. Por ello la relevancia de conocer la relación de estas variables con las especies del género *Dipodomys*, más aún cuando son consideradas como especies clave, ingenieras del sistema e indicadoras de salud de los ecosistemas. Esta investigación se planteó analizar las frecuencias, edades, estados reproductivos, proporciones de sexos y pesos de dos especies de ratas canguro (*D. merriami* y *D. nelsoni)*, así como analizar los datos de temperatura y precipitación de la zona de estudio con las frecuencias de las ratas canguro. Se utilizó la técnica de captura-recaptura durante los años 2012 al 2016 en dos tipos de ambientes matorral y pastizal del desierto Chihuahuense. Además, se obtuvieron y analizaron las temperaturas y precipitaciones de la zona de estudio durante los años estudiados con las frecuencias de las dos especies de ratas canguro. Los resultados obtenidos mostraron que *D. merriami* fue la especie con mayor abundancia; no obstante, las frecuencias de las dos especies fueron más altas en el matorral. Además, no existió relación estadística de las frecuencias de las especies con la temperatura, pero sí existió cercanía a la diferencia estadística con la precipitación. En general, el número de hembras y machos de ambas especies fue similar durante los muestreos, así como la categoría de edad más abundante fueron los adultos; mientras que los estados reproductivos más frecuentes fueron las hembras no preñadas y los machos con testículos abdominales. El peso de los organismos fue diferente entre las dos especies y entre los subadultos y adultos. Estos patrones fueron similares a los reportados para otras especies del género *Dipodomys*. Los resultados de esta investigación brindan información necesaria para tomar acciones requeridas acerca del manejo y conservación de especies con distribución restringida como *D. nelsoni*.

Keywords: Chihuahuan desert; density; desert rodents; Heteromyidae; precipitation; temperature

Introduction

The natural dynamics of climatic variables promote various changes in living beings, which can be behavioral, endocrine, metabolic, among others and which in turn lead to a series of responses that affect different trophic levels (Selwood *et al.* 2015). For example, temperature is a signal for some mammals indicating the entrance or exit of hibernation (Lane *et al.* 2012). On the other hand, precipitation is used by plant species for the production and germination of seeds, besides the emergence of seedlings. Precipitation is then used by rodents, who deploy their reproductive period during the rainy season, since they can meet the high energy demands of reproductive states such as pregnancy and lactation (Brown and Ernest 2002; Lima *et al.* 2008).

This is particularly important in semiarid environments, since precipitation pulses determine plant production, and the resource availability to organisms (Hernández *et al.* 2011; Polyakov *et al.* 2021), as well as it influences to population rates (*e.g.* density) of small mammals (Lima *et al.* 2008; Hernández *et al.* 2011). However, the exacerbated changes in climatic variables, caused by global warming, have affected ecosystems and their species (Lane *et al.* 2012; Selwood *et al.* 2015; Ward 2016). In arid systems the extension of the dry seasons has delayed the emergence and development of plant species, which in turn affects those species depending on them, either for feeding, the deployment of reproductive or hibernation behavior (Selwood *et al.* 2015).

Kangaroo rats (*Dipodomys* spp.) are usually abundant species of the North American arid systems, thus it is common for them to be the dominant species within their rodent communities (Reichman and Van De Graaff 1975; Hernández *et al.* 2005; 2011; Lightfoot *et al.* 2012). Since kangaroo rats are considered keystone species, ecosystem engineers, and indicators of the health status of their habitats (Valone *et al.*, 1995; Fernández *et al.* 2014; Hafner 2016; Longland and Dimitri 2021), several investigations have been carried out addressing their distribution, behavior, population parameters and structure (Price and Kelly 1994; Morrison *et al.* 1996; Daskalova *et al.* 2020). Furthermore, their population responses to climatic variables such as precipitation have been investigated (Hernández *et al.* 2005; Lima *et al.* 2008). Intense precipitation has caused flooding, resulting in the deaths of many kangaroo rats and, thus severe population declines (Thibault and Brown 2008). The local extinction of these species results in the loss of crucial ecological processes, including seed dispersal and soil aeration, as well as a protein source for other organisms (Valone *et al.* 1995; Goldingay *et al.* 1997; Ward 2016; Germano and Saslaw 2017).

These studies were mainly focused on species such as *Dipodomys merriami* Mearns, 1890, which has a wide distribution, ranging from the Southeastern of the U.S.A to central Mexico. Within the Chihuahuan Desert, *D. merriami*, converges with *Dipodomys nelsoni*, which is restricted only to the north of Mexico and has been studied to a lesser extent (Hafner 2016). The distributions of both species overlap with *Dipodomys ordii*, which, unlike *D. merriami* and *D. nelsoni*, specializes in sandy soils such as dunes. Therefore, these three *Dipodomys* species do not share home range (Schroder 1987). *Dipodomys merriami* and *D. nelsoni* have similar ecological and morphological characteristics, although *D. nelsoni* is larger, heavier, and has a distinctive white hair tip on the tail (Best, 1988). Both *D. merriami* and *D. nelsoni* inhabit ecosystems affected by human development activities (Lane *et al.*, 2012; Selwood *et al.*, 2015). Given their ecological importance, continuous monitoring of long-term population changes is essential. Additionally, understanding the impact of environmental variables, especially those that limit population growth, such as precipitation and temperature on their population dynamics is crucial (Hernández *et al.*, 2005; Lima *et al.*, 2008; Montero-Bagatella *et al.*, 2023). These environmental variables influence can be detected though the study of population parameters (Greenville *et al.* 2017), and provide information on their population sizes and cycles, therefore the information can be used for the management and conservation of species (Germano and Saslaw 2017). In addition, the use of biological data from organisms such as body weight, provides information on their life stage and health status (Zeng and Brown 1987; Price and Kelly 1994), along with their habitat conditions (Hernández *et al.* 2005). For these reasons, the goal of this research is to obtain the densities, population parameters and body weight of *D. nelsoni* and *D. merriami* in two environments from the Chihuahuan Desert. Further, we aim to analyze the relationship between the densities of the two species with temperature and precipitation.

Materials and methods

Study area: This research was carried out in the central region of the Chihuahuan Desert, in the Mapimí Biosphere Reserve (Reserva de la Biosfera de Mapimí), Mexico (Figure 1). The Reserve is located between the states of Durango, Chihuahua, and Coahuila (CONANP 2006). The weather is semi-warm with precipitations in summer and extreme thermal amplitude. The average annual temperature is 20.8 Celsius and mean annual precipitation is 264 mm. The rainy season is from July to January, while the dry season is from February to June. Summer precipitation (July-September) represents over 75% of annual precipitation (Cornet 1988; CONANP 2006).

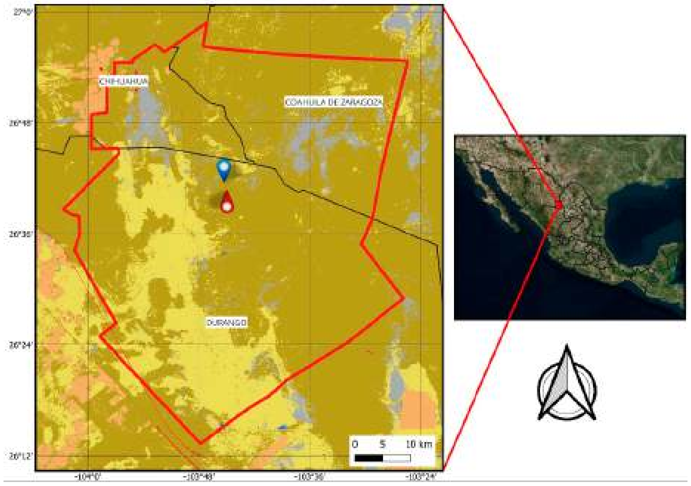

Figure 1. Map of the Mapimí Biosphere Reserve in Mexico. Blue point = shrubland site, red point = grassland site.

The main plant communities in the area are xeric shrublands (*Larrea tridentata, Fouquieria splendens* and *Opuntia rastrera*) and shrubby grasslands (*Hilaria mutica, Sporobolus airoides, Prosopis glandulosa*) (CONANP 2006; Hernández *et al.* 2011).

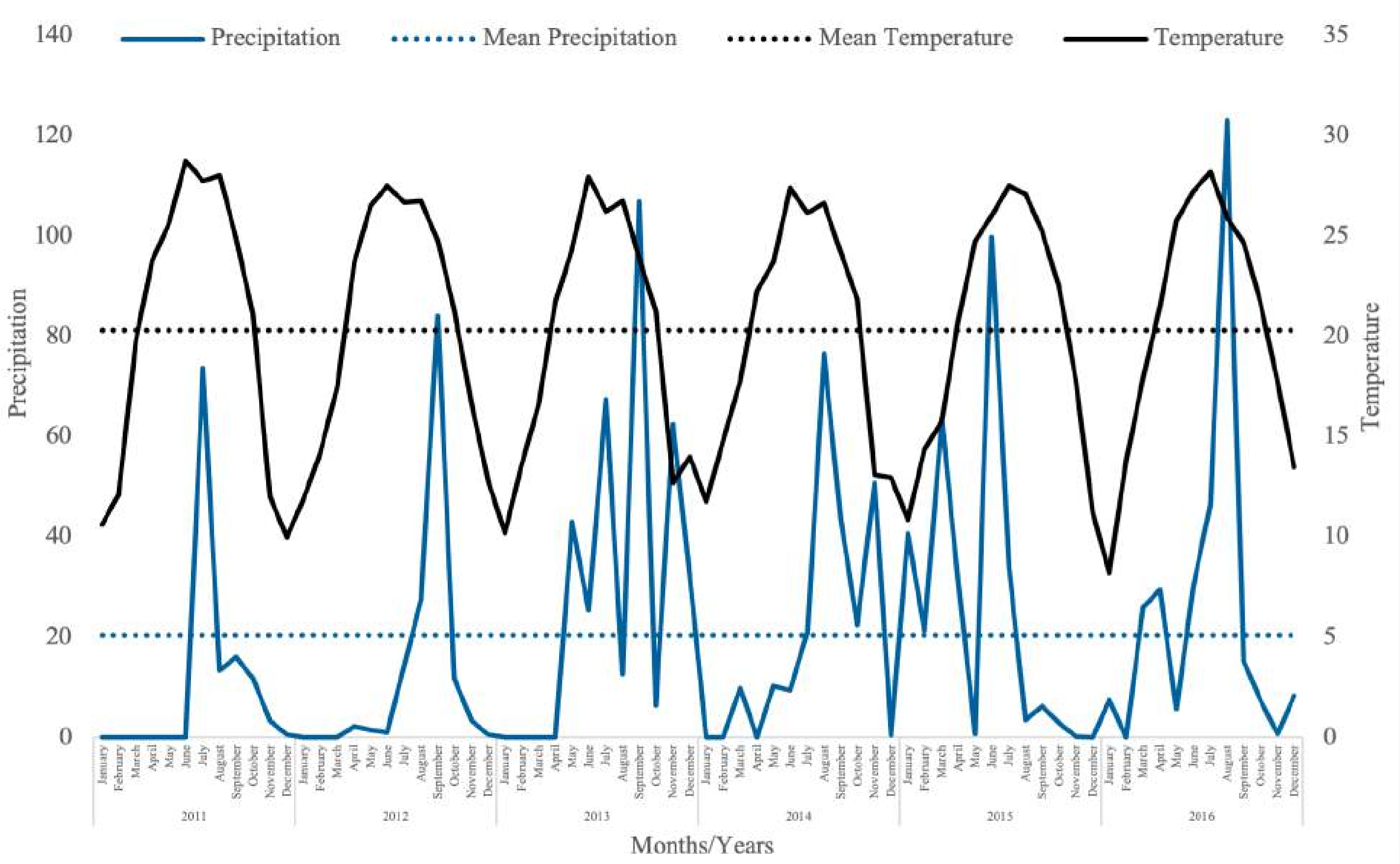

Figure 2. Mean temperature and precipitation registered during the study at Mapimí Biosphere Reserve. Black line = temperature, point black line = mean precipitation, blue line = precipitation, point blue line = mean precipitation.

Rodent trapping: We used databases from the Long-Term Ecological Research project (since 1996) from the 2012 to 2016 years to obtain the frequencies and population parameters of *D. nelsoni* and *D. merriami* in two environments from the Chihuahuan Desert. The data bases collect information from the captures of rodents in the areas of grassland and shrubland during spring and autumn (Hernández *et al.* 2005; 2011). To gather this information, at each site (3.1 ha) three established radial networks were set with 145 Sherman traps (30x10x8 cm), disposed in twelve lines (100 m each line), each radiating from a central point. 12 traps were set along each line, the first 4 at 5 m intervals and the rest at 10 m intervals, plus 1 in the center, including a 5-m peripheral buffer. Traps were baited with oat flakes and were active during three consecutive nights per season (Hernández *et al.* 2011). Traps were checked and baited each day at 7:00 and 16:00 h. From each captured individual, its species, sex, age, weight, and reproductive status (scrotal testicles in males or signs of pregnancy in females) were recorded (Zeng and Brown 1987; Hernández *et al.* 2005; Lima *et al.* 2008; Germano and Saslaw 2017). Age was determined by the size, fur coloration and reproductive status (Zeng and Brown 1987; Price and Kelly 1994; Hernández *et al.* 2005; 2011; Germano and Saslaw 2017). Captured individuals were marked in the ventral region with a semi-permanent marker and later released *in situ* (Hernández *et al.* 2011; Sikes and Gannon 2011). Due to intense precipitations in November 2013 and 2014, no sampling was carried out in those periods.

Climatic variables: Data on temperature and precipitation from the study sites and years of sampling were obtained from the Meteorological Station from the Desert Laboratory, Instituto de Ecología A.C.

Statistical analyses: To carry out the statistical analyses, based on the reported frequencies of the kangaroo rats, the total number of captures per species at each site was used. The percentages of capture with their 95% confidence intervals (CI) were obtained (O'Brien and Yi, 2016). In addition, lineal regressions between the frequencies of captures from both species with temperature, precipitation from the previous year, and from the sampling year were performed (Hernández *et al.* 2005).

Age population structure was analyzed by species with X^2 and exact Fisher tests. For the analyses of reproductive status and sex for both species, squared Chi and exact Fisher tests were carried out. Body weight for captured individuals was analyzed with ANOVAs s by species, site, sex, and age, and the respective *post hoc* Tukey tests were performed (Hernández *et al.* 2011). Analyses were carried out with the software Minitab 21.1.1.0.

Results

Temperatures at sampling sites presented marked and similar cycles in the years of study; maximum peaks were registered from June to August (summer) and descended reaching the lowest values between December and January (winter). The highest temperatures were reported in June and August, while the lowest were presented in December and January (Figure 2). The average temperature in the study site was 20.3 Celsius. There were statistical differences of temperatures among seasons (*F* = 281.38, *d. f* = 3, *P* = < 0.001); *post hoc* test indicated differences between summer and spring on the one hand and winter and autumn on the other (*P* = 0.05).

On the other hand, precipitation presented similar cycles during years 2011 and 2012, in which there were pronounced periods of drought that lasted from January to May (winter and spring). Precipitations began in June reaching its highest values in July and September (summer), decreasing in the subsequent months (autumn). From 2013 to 2015 there were constant monthly precipitations that included the winter months; the highest values of the precipitations of these years were reported from June to September. The year 2015 was the rainiest, with a total of 613.4 mm recorded annually, contrasting with 107.2 mm in 2011. There were statistically significant differences of precipitations among seasons of the years (*F* = 10.10, *d. f* = 3, *P* = < 0.001); the *post hoc* test indicated differences between summer and the other seasons (*P* = 0.05).

Total sampling effort was of 20, 880 night-traps or in other words 10,440 night-traps per site. *Dipodomys merriami* was the most abundant species, with its highest frequency recorded in the shrubland in 2015 with 110 captures (Figure 3). At the same site, there were declines in the species during March 2013 and 2014, from which they recovered in March 2015. In November 2016 the highest frequency of *D. nelsoni* was reported with 29 captures in the grassland. The percentage of captures from *D. merriami* in the grassland was 1.34 % (95 % *CI* 1.17 - 1.54), while it was of 2.80% (95% *CI* 2.54 - 3.07) in the shrubland, hence being statistically different. While the percentage of captures of *D. nelsoni* in the grassland was 0.37 % (95 % *CI* 0.29-0.49) and 0.48 % (95 % *CI* 0.38-0.61) in the shrubland, with no statistical difference.

There was no statistical relation between the densities of *D. merriami* and the precipitations from the sampling year (*F* = 0.01, *d. f.* = 1, *P* = 0.92), but there was a tendency with precipitation from the previous year (*F* = 4.9, *d. f.* = 1, *P* = 0.06). *D. nelsoni* did not present a statistical relation with precipitation from the sampling year (*F* = 0.01, *d. f.* = 1, *P* = 0.94), nor with precipitation from the previous year (*F* = 2.9, *d. f.* = 1, *P* = 0.13). Just as there was no statistical relation between temperature and the densities of *D. merriami* (*F* = 0.02, *d. f.* = 1, *P* = 0.89), nor *D. nelsoni* (*F* = 1.16, *d. f* = 1, *P* = 0.32).

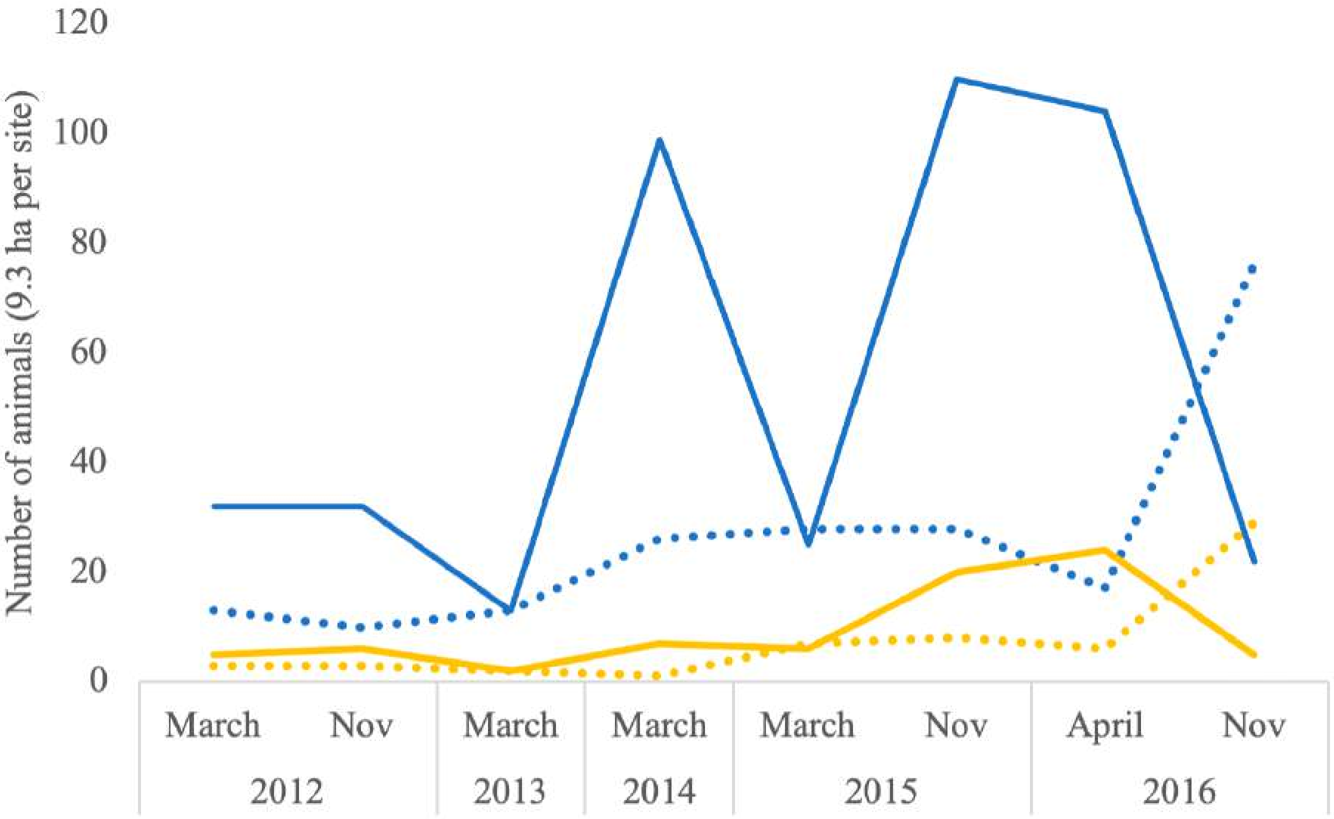

Figure 3. Number of captures of *Dipodomys merriami* and *D. nelsoni* by environment type. Blue line = *Dipodomys merriami* in shrubland, point blue line = *Dipodomys merriami* in grassland, yellow line = *D. nelsoni* in shrubland, point yellow line = *D. nelsoni* in grassland.

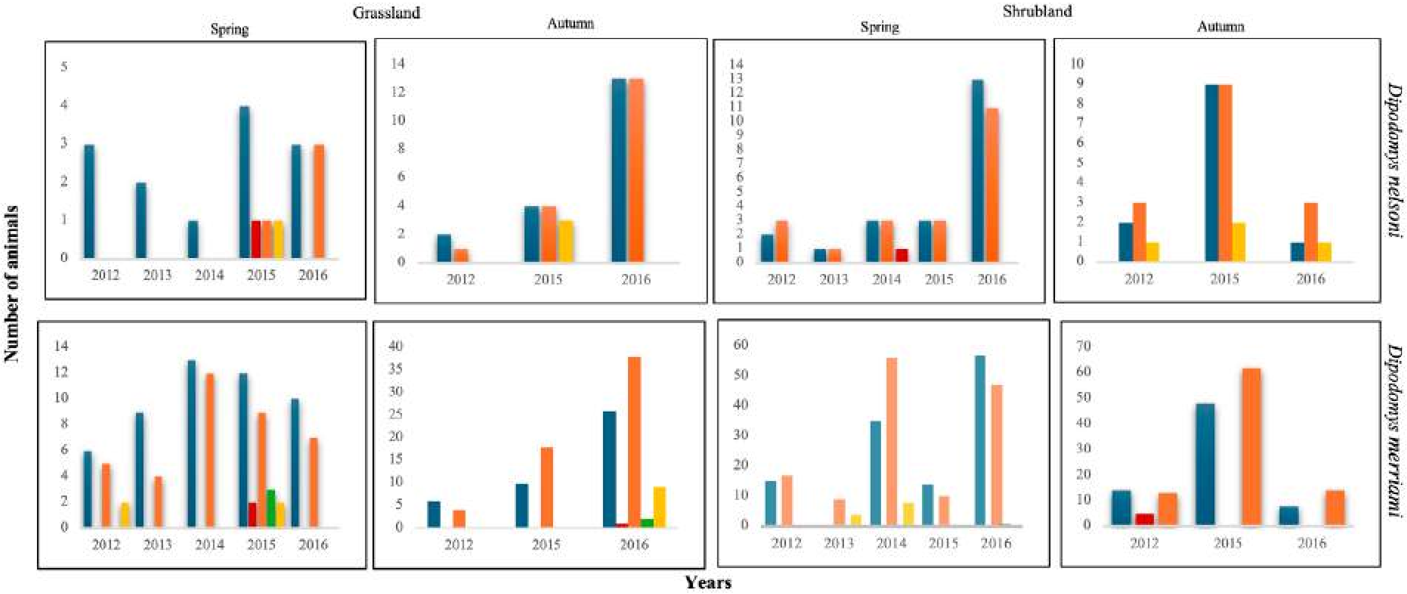

Figure 4. *Dipodomys nelsoni* and *merriami* reproductive status in the two studied environments. Blue bars = abdominal testes, yellow bars = scrotal testes orange bars = not pregnant, green bars = pregnant, red bars = lacting.

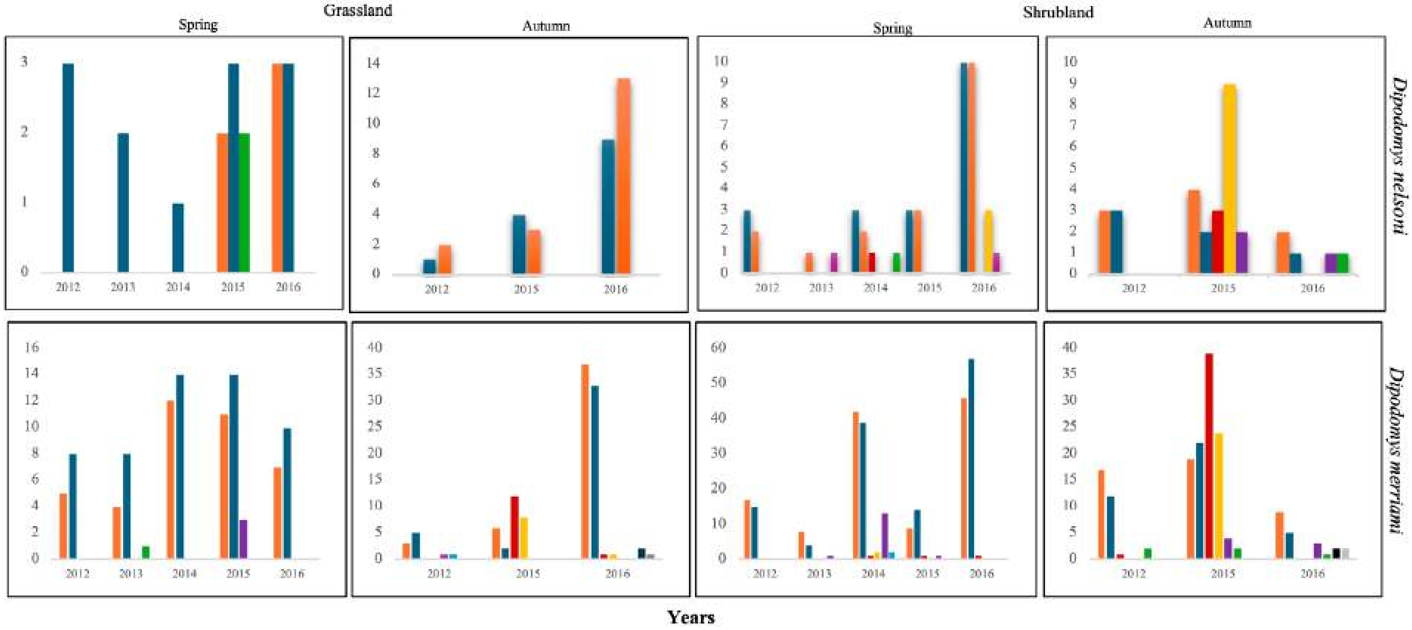

Figure 5. *Dipodomys D. nelsoni* and *D. merriami* registered by age categories and environment type. Orange bars = adult female, blue pale bars = adult male, red bars = subadult female, yellow bars = subadult male, purple bars = juvenile female, green bars = juvenile male, , black bars = young female, grey bars = young male.

In general, the number of females and males was similar during sampling. Nevertheless, the 1:1 ratio occurred only on few occasions. Females were captured slightly more often in the shrubland and males in the grassland. However, there were no statistical differences at the work sites between females and males from *D. merriami* (X^2 = 0.8, *d. f.* = 1, *P* = 0.36), though there was a tendency with *D. nelsoni* (*P* = 0.06).

Regarding the reproductive status of the two species, non-pregnant females and males with abdominal testicles were more common (Figure 4), whereas scrotal males, as well as pregnant and lactating females, were less frequently observed. In March 2015 and November 2016 all reproductively active individuals' status of *D. merriami* were registered in the grassland. There was a significant difference between reproductive status of *D. merriami* between shrubland and grassland sites (X^2 = 13.5, *d. f.* = 4, *P* = 0.009). In contrast for *D. nelsoni* presented a statistical difference at the study sites between males with abdominal testicles and scrotated males (*P* = 0.05), being males with abdominal testicles more frequent; between pregnant and non-pregnant females (*P* = 0.001), being pregnant more frequent but not between lactating and non-pregnant females (*P* = 1).

The most frequent age category for both species was adults, followed by subadults, juveniles, and finally young

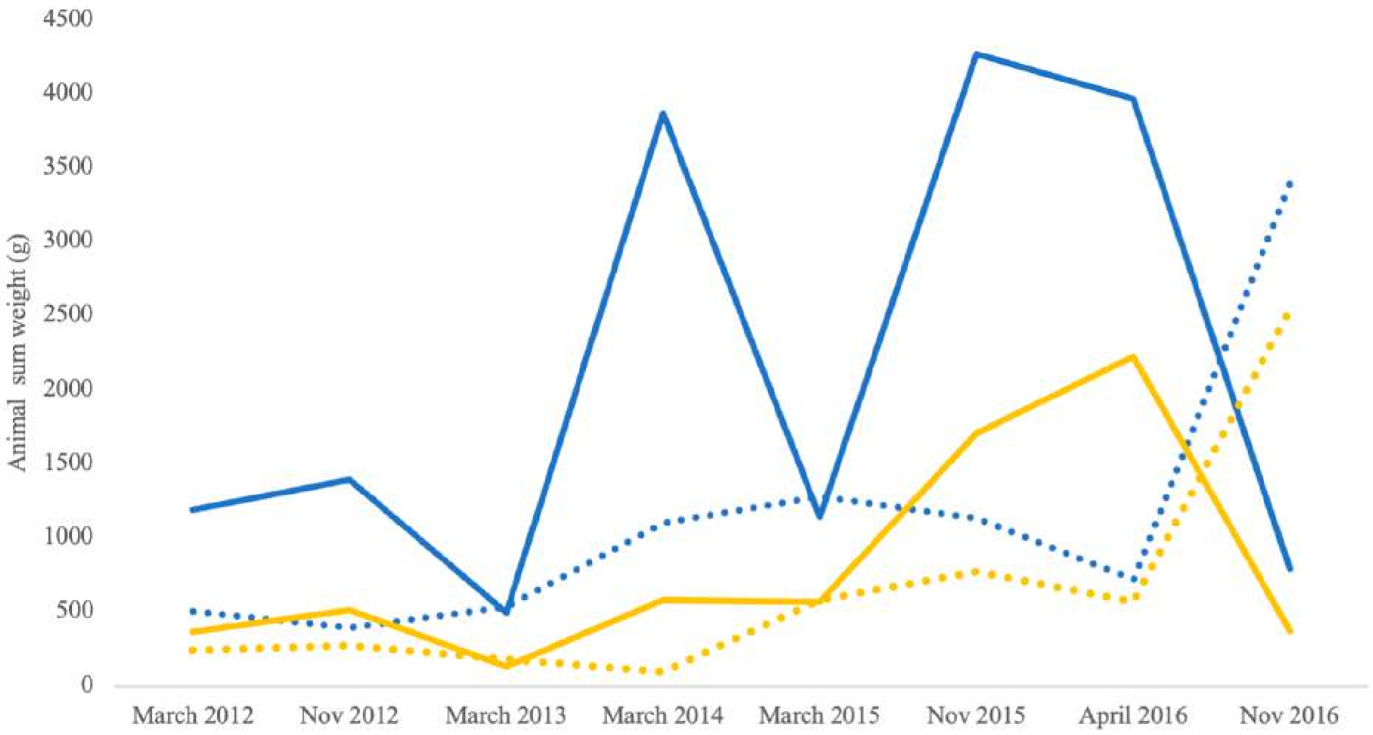

Figure 6. *Dipodomys merriami* and *D. nelsoni* total sum weights at the two studied environments. Blue line = *Dipodomys merriami* in Shrubland, point blue line = *Dipodomys merriami* in grassland, yellow line = *D. nelsoni* in Shrubland, point yellow line = *D. nelsoni* in grassland.

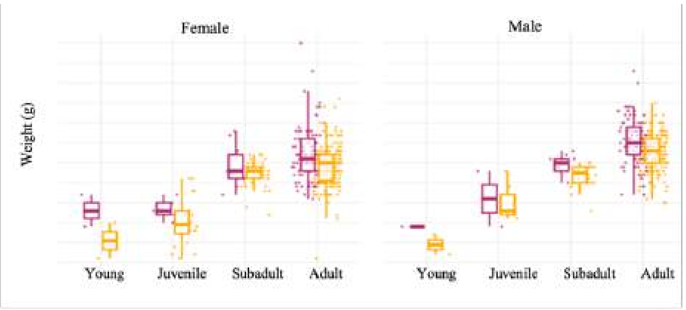

Figure 7. *Dipodomys merriami* weights by sex, age category and environment. Purple box and plots = grassland, yellow box and plots = shrubland

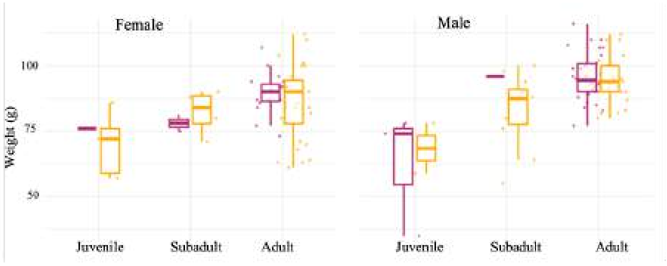

Figure 8. *Dipodomys nelsoni* weights by sex, age category and environment. Purple box and plots = grassland, yellow box and plots = shrubland

individuals which were only reported for *D. merriami* in November 2016 (Figure 5). Age categories from this species were close to a statistical difference for both study sites ($X^2 = 7.2$, *d. f.* = 3, $P = 0.06$), while there were significant differences for *D. nelsoni* at the study sites ($X^2 = 11.0$, *d. f.* = 3, $P = 0.01$).

The total summed weights of *D. merriami* were generally greater than those from *D. nelsoni* (Figure 6). The latter only exceeded the weights of *D. merriami* in March 2015 and April 2016 within the shrubland habitat. *D. merriami* exhibited sharp declines in total summed weight in March 2013 and 2015, from which it recovered by November 2016. Both species showed a decrease in total summed weight in November 2016. There were no statistically significant differences in total summed weights between study sites for *D. merriami* ($F = 2.31$, *d.f* = 1, $P = 0.15$), or for *D. nelsoni* ($F = 0.16$, *d. f.* = 1, $P = 0.69$). However, there were statistically significant differences in total summed weights between the two species ($F = 29.7$, *d. f.* = 1, $P = < 0.001$).

The heaviest individuals from *D. merriami* were registered in the shrubland, and they were females (Figure 7). There was a statistically significant difference in body weights among organisms of this species at both study sites ($F = 35.6$, *d. f.* = 1, $P = < 0.001$), sexes ($F = 35.9$, *d. f.* = 1, $P = < 0.001$), and age categories ($F = 78.2$, *d. f.* = 3, $P = < 0.001$). The *post hoc* test indicated differences in weights from subadults and adults ($P = 0.05$), being adults heavier than subadult individuals. For the case of *D. nelsoni* there was no statistic difference between sites ($F = 0.75$, *d. f.* = 1, $P = 0.38$), but there was between sexes ($F = 8.47$, *d. f.* = 1, $P = 0.004$) and age categories ($F = 21.0$, *d. f.* = 3, $P = < 0.001$). The *post hoc* test indicated differences between weights between subadults and adults ($P = 0.05$) (Figure 8). Both species shared weight ranges at different age categories.

Discussion

The temperatures recorded in the study area during sampling years were similar to previous years and did not present statistical differences between annual seasons. In addition, there was no statistical relationship between frequencies from *D. merriami* nor *D. nelsoni*. Nevertheless, studies such as Koontz *et al.* (2001) and Montero-Bagatella *et al.* (2023) reported a relationship between temperature and frequencies of *D. merriami* and *D. phillipsii* , respectively. However, these studies involved monthly samplings throughout the study years, so increasing the sampling frequency in the current study area could help detect potential changes in species abundances in relation to temperature fluctuations.

On the other hand, precipitation from the sampling years exceeded 100 mm per year that were reported by Brown and Ernest (2002) as the annual average for desert zones. Similarly, more than 60 % of the total annual rainfall occurred in summer; in 2011 and 2012, it even exceeded 90%. Nonetheless, in 2014 and 2015 there were intense and continuous rains that generated an annual total six times greater than the average. These precipitations were caused by the complex meteorological phenomenon "El Niño", that began in 2014 and ended in the summer of 2016 (IRICS 2024). The presence of this climate pattern is common in arid systems, therefore it can have an impact on the species' frequencies (Brown and Ernest 2002; Lima *et al.* 2008; Thibault and Brown 2008; Lightfoot *et al.* 2012; Polyakov *et al.* 2021). Lightfoot *et al.* (2012) registered populations growth while "El Niño" was present, thus it could also be the case in the current study, given that the highest values of captures of *D. merriami* and *D. nelsoni* were recorded while "El Niño" was developing. Nevertheless, if precipitation is so intense that it causes floods, the destruction of food resources and burrows puts populations at risk, causing the death by drowning of individuals (Valone *et al.* 1995; Valone and Thornhill 2001; Brown and Ernest 2002; Kelt *et al.* 2008; Thibault and Brown 2008; Germano and Saslaw 2017).

Despite the highest values of number of captures of *D. merriami* and *D. nelsoni* coincided with maximum precipitations, no statistical relationship was found with precipitation of the sampling year, nor with the previous year. Contrasting with the response of rodent communities in the study from Hernández *et al.* (2005), that responded to precipitation from the previous year and two years before sampling. However, these authors used the data set of several rodent species in the area, hence the handling of the information and the choice of statistical tests, could have influenced the results (Lima *et al.* 2008). On the other hand, Lightfood *et al.* (2005) and Brown and Ernest (2002) did not report a response of the rodents to precipitation, so along with Cárdenas *et al.* (2021), they suggested the substitution of this variable by direct measures of vegetation such as plant production, given that it is a direct resource for rodents and they can show a greater response to it, even 24 months after precipitation occurs (Hernández *et al.* 2005; Lightfoot *et al.* 2012).

Frequencies were different between species, being higher for *D. merriami*. Similar results have been reported by Koontz *et al.* (2001) and Hernández *et al.* (2011) where *D. merriami* was also considered as a dominant species. Whereas *D. nelsoni* was classified as subdominant due to its lower abundances (Hernández *et al.* 2011); a pattern also present in this study. Coexistence and tolerance of these species is influenced by how the species use the resources (Bowers and Brown 1982; Frye 1983; Schroder 1987; Brown 1989; Bleich and Price 1995; Perri and Randall 1999), body size (Perri and Randall 1999), activity patterns (Brown 1989), microhabitat preferences (Schroder 1987), and community structure in which dominant species exclude subordinates (Blaustein and Risser 1976; Price *et al.* 1991).

Dipodomys merriami and *D. nelsoni* exhibited the lowest frequencies in grasslands characterized by dense vegetation composed of grasses and forbs exceeding one meter in height. Such vegetation may hinder the identification of escape routes and interfere with seed distribution and collection (Schroder 1987). This type of plant composition is associated with higher productivity (Hernández *et al.* 2005) and undergoes dynamic, contrasting seasonal changes during periods of high precipitation, resulting in greater seasonal variation in vegetation abundance and diversity, which in turn may contribute to increased variability in rodent populations in grasslands, as reported by Hernández *et al.* (2005). In contrast, shrublands exhibit less seasonal fluctuation (Goldingay and Price 1997), which contributes to more stable and higher rodent densities in these systems (Hernández *et al.* 2005). Additionally, shrublands are dominated by *Larrea* spp., a plant favored by *Dipodomys* species for burrow construction (Schroder 1987; Best 1988; Hernández *et al.* 2005). Kangaroo rats prefer open habitats due to their consumption of large grass seeds and burrowing behavior, which facilitate their daily and migratory movements (Goldingay and Price 1997). Furthermore, vegetation type is closely linked to specific soil characteristics that enhance plant richness and diversity (Hernández *et al.* 2005). Soil is a critical resource for *Dipodomys* species given its role in feeding, shelter, and reproduction (Schroder 1987; Best 1988;). Moreover, species of this genus require areas with bare soil for sandbathing, a behavior performed to clean and maintain their fur. Also, through these sandbaths, they also transmit olfactory signals to conspecifics related to dominance hierarchies and reproductive status (Randall 1991). Therefore, the vegetation and soil characteristics of grasslands may act as limiting factors for the development of these two species' populations, while on the other hand, they may favor other rodents like *Sigmodon hispidus* (Elizalde-Arellano *et al.* 2014), and even lagomorphs such as *Lepus californicus* (Marin *et al.* 2003).

Captures of *D. merriami* were more frequent in the shrubland, coinciding with studies of Schroder (1987), Hernández *et al.* (2005; 2011), Lightfood *et al.* (2012), Cárdenas *et al.* (2021), and García-Feria and González-

Romero (2021). This could be due to a higher diversity and plant productivity in the area (Hernández *et al.* 2005), besides *Dipodomys* species prefer to inhabit open sites with scarce plant cover, which allows them to freely visualize possible predators (Schroder 1987; Goldingay and Price 1997), as well as escape routes (Cárdenas *et al.* 2021). In contrast, the vegetation at the shrublands was high and dense as reported by Hernández *et al.* (2005), which prevented open environment conditions (Schroder 1987). Hernández *et al.* (2005) mentioned that in grassland, vegetation growth has a rapid response to precipitation, while shrubland vegetation does not significantly change with precipitation.

The number of captures from *D. nelsoni* did not statistically change between environments, as it was also reported by Eldridge and Whitford (2014). Nevertheless, it contrasted with results obtained by Hernández *et al.* (2005), who registered the highest captures in the shrubland. Hernández *et al.* (2005) attributed the results to the use by kangaroo rats to the base of shrubs, either as support for their burrows, as shelter for feeding (Schroder 1987; Eldridge and Whitford 2014), or as protection from predators (Svejcar *et al.* 2019). Furthermore, they pointed out that due to their digging habits, the type of soil influences their establishment, so further studies considering this variable could provide more ecological information on these species and their habitats.

Despite population declines that can be caused by dispersion, migration and predation (Eisenberg 1993; Orland and Kelt 2007), the number of males and females of *D. merriami* and *D. nelsoni* was similar during the sampling years. These results coincided with those of other *Dipodomys* species, such as *D. phillipsii* (Montero-Bagatella *et al.* 2023), *D. ingens, D. heermanni, D. nitratoides* (Germano and Saslaw 2017), and *D. merriami* (Zeng and Brown 1987). On the other hand, adults were more frequently captured as it was also reported by Zeng and Brown (1987), Germano and Saslaw (2017), and Montero-Bagatella *et al.* (2023). This could be due to the exploration and search of new territories by juveniles (Price and Kelly 1994; Orland and Kelt 2007); as well as the difficulty of capturing young individuals on account of the fact that they shelter inside burrows (Price and Kelly 1994; Eisenberg 1993).

The most abundant reproductive status for both species was non-pregnant females and males with abdominal testicles, while pregnant or lactating females presented the lowest frequencies. However, this abundance pattern of reproductive status were registered during different samplings, indicating a reproductive activity from both species year round, as is the case for other species from the same genus (Orland and Kelt 2007; Germano and Saslaw 2017; Montero-Bagatella *et al.* 2023). Nevertheless, such studies had monthly sampling that provided more detailed information. Therefore, carrying out samplings covering different seasons could complement the knowledge on the frequencies and the reproductive status of these species.

Body weight from organisms was different between species because of the natural distinction of their body sizes (Best 1988). However, due to the higher number of captures of *D. merriami*, the total weight of its individuals was greater than that of *D. nelsoni*., which coincided with results from Hernández *et al.* (2011). Moreover, there were individuals from both species that had the same weight regardless of their age categories; this could have been caused by the changes in weight that individuals present during the transitions in life stages (Zeng and Brown 1987), as well as the elevated energetic demands from reproductive stages such as pregnancy and lactation (Soholt 1977).

Dipodomys species represent an important component in the diet of mesocarnivores (Hernández *et al.* 2011). These predators have shown a preference in the consumption of *Dipodomys* species rather than rodents of smaller size such as *Chaetidopus* species (Laundré *et al.* 2009; Laundré and Hernández 2003). However, the difference between the body size of *D. merriami* and *D. nelsoni* does not represent a great difference in the energetic intake of mesocarnivores. Nonetheless, this difference in the energy contribution could differ from other consumers, such as birds or snakes. Therefore, the importance of studies on the predator-prey interactions, given the influence of predators in the population dynamics of the species (Price and Kelly 1994; Laundré and Hernández 2003; Polyakov *et al.* 2021).

Results from this study suggest that *D. merriami* and *D. nelsoni* present similar patterns to the other species from the same genus. Further, their population parameters (density, sex ratio, age and reproductive status) indicate that both populations were in development and growth. It is important to note that this research was carried out in a protected and destined to conservation area, where species are not subject to the constant anthropic pressures. Nevertheless, in Mexico there remain vast extensions of natural territories that do not present any protection for their conservation (Fernández *et al.* 2014), where urban development has led fragmented environments that have favored metapopulations. Therefore, it is necessary to supplement the information about the populations subject to such pressures (Hurtado and Mabry 2017), which would allow for greater knowledge of the species in order to take the necessary actions for their management and conservation (Christensen *et al.* 2019; 2023), specially of *D. nelsoni* that has a restricted distribution and low densities.

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Biogeographic pattern and potential distribution of *Glossophaga valens* Miller, 1913 (Chiroptera: Phyllostomidae)

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Glossophaga valens is a generalist nectar-feeding bat known from the western slopes of Ecuador and Peru. Recently elevated to species level, key information about its geographic boundaries and environmental preferences remains limited. We developed a niche-based species distribution model to describe its biogeographic pattern, delimit its distribution, and evaluate its potential range. We compiled 107 occurrence records from scientific literature and museum collections, covering the complete known distribution of the species. After curating these data, 83 high-quality and spatially independent records were combined with five bioclimatic variables to build a model using the Maxent algorithm in the Wallace software. The best-performing model was selected from 50 candidates based on lower complexity criteria. Our results indicate that *G. valens* exhibits a nearly continuous distribution with high to very high suitability along the Pacific coast of Ecuador and Peru, while isolated conditions occur in northeastern Peru. Our findings update the marginal localities for the species and highlight its strong preference for arid to semi-arid conditions in western lowlands below 1500 m. The model refines the species' range, placing its northernmost boundary in Esmeraldas, Ecuador, and suggesting its absence from Colombia. It also suggests that its southern limit could extend from southern Arequipa to northern Chile, pending future surveys and specimen revisions. Additionally, we report new easternmost records in northeastern Peru, aligning with the Marañón Valley. Key geographic features such as Seasonally Dry Tropical Forests, the Andes, and the Huancabamba Depression, alongside ecological traits like generalist feeding habits and adaptability, play a crucial role in shaping its distribution. Finally, we discussed how these geographic features have influenced the distribution of *G. valens* and other western Andean bats, and remark conservation implications in their threatened habitats.

Glossophaga valens es un murciélago nectarívoro generalista distribuido en las laderas occidentales de Ecuador y Perú. Recientemente elevado al nivel de especie, presenta limitada información sobre sus límites geográficos y preferencias ambientales. Desarrollamos un modelo de distribución de especies basado en su nicho para describir su patrón biogeográfico, delimitar su distribución y evaluar su rango potencial. Compilamos 107 registros de presencia a partir de literatura científica y especímenes revisados de colecciones de museos cubriendo toda la distribución conocida de la especie. Luego de depurar los datos, utilizamos 83 registros de alta calidad e independientes espacialmente junto con cinco variables bioclimáticas para construir un modelo empleando el algoritmo Maxent en el software Wallace. El mejor modelo en base a su desempeño fue seleccionado entre 50 candidatos a partir de un criterio de menor complejidad. Nuestros resultados indican que *G. valens* presenta una distribución casi continua con una idoneidad de hábitat de alta a muy alta a lo largo de la costa del Pacífico de Ecuador y Perú, mientras que en el noreste de Perú las condiciones son más aisladas. Nuestros hallazgos actualizan las localidades marginales de la especie y resaltan su marcada preferencia por condiciones áridas a semiáridas en tierras bajas occidentales por debajo de los 1500 m. El modelo refina su rango de distribución, estableciendo su límite norte en Esmeraldas, Ecuador, y sugiriendo su ausencia en Colombia. Asimismo, sugiere que su límite sur podría extenderse desde el sur de Arequipa hasta el norte de Chile, sujeto a futuras exploraciones y revisiones de especímenes. Además, reportamos nuevos registros más orientales en el noreste de Perú, que coinciden con el Valle del Marañón. Factores geográficos clave, como los Bosques Secos Estacionales, los Andes y la depresión de Huancabamba, junto con rasgos ecológicos como su dieta generalista y adaptabilidad, juegan un papel crucial en la configuración de su distribución. Finalmente, discutimos cómo estas características geográficas han influido en la distribución de *G. valens* y otros murciélagos andinos occidentales, y destacamos las implicancias de conservación en sus hábitats amenazados.

Keywords: Arid Environments, dry ecosystems, dry forest, Huancabamba, Marañón, species distribution modelling, zoogeography

Introduction

Long-tongued and nectar-feeding bats of the genus *Glossophaga* E´. Geoffroy St.-Hilaire, 1818 are endemic to the Neotropics. Members of this genus are characterized by their small size, brown to reddish-brown coloration, and a distinct interfemoral membrane with a short tail (Griffiths and Gardner 2008). Recent taxonomic revisions using geometric morphometrics elevated four taxa previously considered subspecies of *Glossophaga soricina* to full species status (Calahorra-Oliart *et al.* 2021). Consequently, nine species are recognized within the genus: *Glossophaga antillarum* Rehn, 1902; *G. bakeri* Webster and Jones, 1987; *G. commissarisi* Gardner, 1962; *G. leachii* Gray, 1844; *G. longirostris* Miller, 1898; *G. morenoi* Martínez and Villa-R., 1938; *G. mutica* Merriam, 1898; *G. soricina* (Pallas, 1766), and *G. valens* Miller, 1913 (Calahorra-Oliart *et al.* 2021, Simmons and Cirranello 2024).

Among these species, the Robust Long-tongued Bat, *Glossophaga valens*, is known to inhabit a wide range of ecosystems, including dry forest, fog oases, wetlands, urban areas and rural landscapes (Mena and Williams 2002; Pacheco *et al.* 2020; Mena *et al.* 2021). As a generalist nectarivore, *G. valens* feeds on pollen and fruits from native plants in natural ecosystems (Arias *et al.* 2009; Novoa *et al.* 2011), but it also demonstrates adaptability to exotic, cultivated plants in urban areas (Pellón *et al.* 2020). Described initially from Balsas, Cajamarca (Miller 1913), the species' type locality was later corrected to Amazonas Department by Ortiz de la Puente (1951). Its documented distribution range includes the western (Pacific) slopes of Ecuador and Peru, with additional populations in eastern inter-Andean valleys (Miller 1913; Webster 1993; Griffiths and Gardner 2008). Although Koopman (1978) suggested these disjunct populations might be connected via Andean passes in northern Peru, this hypothesis remains untested.

While previous studies have provided valuable insights into *Glossophaga valens*, many aspects of its distribution remain unknown. Hoffman *et al.* (2019) presented the first species distribution model for the species, focusing on its genetic and taxonomic relationships. However, their model excluded the eastern distribution and geographic limits of *G. valens*. More recently, Calahorra-Oliart *et al.* (2022) developed an ecological niche to explore abiotic requirements and morphological divergence among *Glossophaga* species. They revealed that *G. valens* occupies the driest conditions (e.g. lowest precipitation values and high precipitation seasonality in their habitats) within the genus but did not account for dispersal limitations, resulting in an overestimated potential range. Consequently, critical questions regarding the species' geographic boundaries, accessible areas, and the influence of geographic features remain unresolved.

Delimiting species distributions is essential for conservation efforts, offering tools to evaluate vulnerability, understand biodiversity patterns, and anticipate threats (Lamoureux *et al.* 2006; Mota-Vargas and Rojas-Soto 2012). Niche-based species distribution models (here in SDMs) have become indispensable for predicting distributions, elucidating species-environment relationship, and approximating the Grinnellian niche (Guisan and Thuiller 2005; Peterson *et al.* 2011; Soberón and Nakamura 2009). In Neotropical bats, SDMs have been extensively applied to refine species distributions, support taxonomic decisions, identify potential populations, evaluate conservation status, and enhance natural history knowledge (Velazco *et al.* 2018; Ramírez *et al.* 2020; Zegarra *et al.* 2020; do Amaral *et al.* 2023; Avila-Bernal *et al.* 2024; Otárola-Ardila *et al.* 2024). Considering that *Glossophaga valens* was historically reported on both slopes of the Andes in Peru (Koopman 1978), we hypothesized that SDMs could help to identify environmentally suitable corridors that may connect disjunct populations, especially in northern Peru. In this study, we compiled occurrence records of *Glossophaga valens* to 1) update its distribution and describe its biogeographic patterns, and 2) infer its potential distribution and environmental requirements. Additionally, we discussed the biogeographic and non-biogeographic factors shaping its current distribution.

Materials and methods

Occurrence data and morphological identification. We collected georeferenced occurrence records for *G. valens* from two primary sources: (1) localities associated with voucher specimens housed at the Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (MUSM), Lima, Peru, and (2) records extracted from peer-reviewed articles. Voucher specimens from MUSM were morphologically reviewed to ensure accurate identification, adhering to the morphological descriptions of Miller (1913) and the morphometric criteria provided by Calahorra-Oliart *et al.* (2021, 2022). The identification of *G. valens* was based on diagnostic cranial morphology, including an inflated braincase with an oblong shape (not domed as in *G. soricina*), long nasal bones, and a relatively large and wide rostrum (versus shorter and narrow rostrum in *G. soricina*). Dentition features included four subequal, highly procumbent upper incisors; four similarly shaped lower incisors; and three molars in each tooth row. The species' greatest length of skull (GLS) ranged from 21.5 to 22.8 mm, in contrast to GLS measurements below 21 mm in *G. soricina*. External characteristics distinguishing *G. valens* included a non-elongated rostrum (respect to other glossophagines), a developed uropatagium, pale gray coloration, and a forearm length exceeding 35.5 mm (compared to <35 mm in *G. soricina*).

In addition to MUSM vouchers, we incorporated records from Males-Andocilla's (2019) thesis, as the identifications were based on voucher specimens consistent with our criteria for *G. valens*. Locality details of this last source were taken from online database of the mammal collection of the Museum of Zoology QCAZ (Pontificia Universidad Católica del Ecuador). Comparative analysis with voucher specimens of *G. soricina* and *G. bakeri*, also housed at MUSM, further supported accurate identifications (Appendix 1). Additionally, we searched the Global Biodiversity Information Facility (GBIF 2025) for occurrence records, but as of 2025, some records are mislabelled as *Glossophaga soricina*, and both species (with *G. valens*) could be externally misidentified, we opted for using records from vouchers which were available to confirm by key cranial and external characters, and for those whose identification have been already confirmed in the literature. Final dataset is provided in Appendix 1.

Cleaning occurrence records. For localities with incomplete geographic data, coordinates were assigned using a combination of label information, Google Earth Pro v. 7.3.6.10201, and gazetteers provided by Gardner (2008). This process ensured that all records were accurately georeferenced. To maintain data quality, we curated the dataset by excluding duplicate records, removing zero or erroneous coordinates, and addressing spatial biases using the *clean_coordinates* function from the *CoordinateCleaner* package (v3.0.1; Zizka *et al.* 2018) in R software (R Core Team 2022). This cleaning process ensured the reliability of the occurrence data for subsequent analyses.

To minimize sampling bias during the modeling species distribution, we employed the spThin package (Aiello-Lammens *et al.* 2015) integrated within Wallace v. 2.1.1, a platform for reproducible modelling of species niches (Kass *et al.* 2018, 2023). This tool filters occurrence records based on a user-defined minimum distance threshold to reduce spatial autocorrelation (the similarity of nearby locations in terms of a given variable, which can bias model predictions). A threshold of 5 km was selected, reflecting the dispersal capacity of a closely related species, *Glossophaga soricina*, in arid ecosystems (Aguiar *et al.* 2014). After completing these steps, the final dataset consisted of 107 georeferenced occurrence records, of which 83 spatially independent records were retained for SDM construction (see below).

Environmental data. We obtained climate data from WorldClim v. 2.0 (Fick and Hijmans 2017) at a spatial resolution of approximately 1 km^2 (0.0083 degrees). To establish the calibration area for the species distribution model, we created buffers around the species' occurrence points with a radius of 2.5°. This distance was selected to encompass a natural environmental gradient that includes suitable habitats (*e. g.* dry forests and coastal deserts based on the high number of records), transitional zones where the species is less frequent (*e. g.* inter-Andean valleys), and areas where its presence is highly unlikely (*e. g.* high lands of Puna ecosystems above 3000 m). This approach allows the model to explore a full range of environmental conditions without relying on artificial political boundaries, and without overestimating the accessible area, thus providing a reasonable extent for extrapolating the species' potential distribution. Buffers were generated using the *terra* package v. 1.7 (Hijmans 2024) in R (R Core Team 2022). Within this area, we selected independent predictors based on two criteria: (1) variables with low intercorrelation ($r < 0.7$) to avoid multicollinearity and (2) variables that effectively explain the species' climatic preferences. A Pearson correlation analysis was performed using the *corrplot* package (Wei and Simko 2021) in R, and a correlation matrix was constructed to identify and exclude highly correlated variables.

As a result, five bioclimatic variables were retained for the final dataset: BIO4 (Temperature seasonality, °C), BIO7 (Temperature Annual Range, °C), BIO8 (Mean Temperature of Wettest Quarter, °C), BIO12 (Annual Precipitation, mm), and BIO15 (Precipitation Seasonality, %). These variables were selected to capture the pronounced seasonality of dry and wet periods within the distribution range of *G. valens* and to reflect key climatic conditions relevant to their biology, especially the dry conditions the species can tolerate as temperature and precipitation fluctuations can influence their physiological performance, and foraging activity. All bioclimatic variables were managed in raster format and georeferenced using the WGS84 datum.

Niche–based species distribution modelling (SDM). We employed a non-spatial random k-fold cross-validation approach, with a partition threshold of k = 4, to evaluate model accuracy. SDM were constructed using the maximum entropy algorithm implemented in Maxent (Phillips *et al.* 2006) via Wallace v. 2.1.1 (Kass *et al.* 2018, 2023). Model calibration involved selecting 10,000 randomly sampled pixels from the study area as background points. To optimize model configuration, we tested various feature type combinations, including linear (L), quadratic (Q), linear-quadratic (LQ), hinge (H), linear-quadratic-hinge (LQH), and linear-quadratic-hinge-product (LQHP). Additionally, nine values for the regularization multiplier, ranging from 0.5 to 5.0 in 0.5 intervals, were evaluated. To enhance robustness and avoid overfitting, models were generated without extrapolation or clamping, ensuring predictions remained within the environmental values of the training data. Thus, 50 candidate models were generated.

Model performance was assessed using the *ENMeval* package (Muscarella *et al.* 2014), integrated within Wallace v. 2.1.1 (Kass *et al.* 2018, 2023). We evaluate model complexity using the corrected Akaike Information Criterion (AICc), with preference given to models of lower complexity and correspondingly lower AICc values. Additionally, we assess model accuracy by the area under the curve (AUC), with an optimal threshold set at AUC > 0.8 to ensure robust predictive performance (Peterson *et al.* 2011).

The best-performing model was projected as a continuous suitability map using the Maxent cloglog transformation. Suitability values were then classified into four categories based on the 5-percentile presence threshold (0.22): Unsuitable: 0–0.22; low suitability: 0.22–0.50; high suitability: 0.50–0.80 and very high suitability: 0.80–0.99. The best-performing model was projected as a continuous suitability map using the Maxent cloglog transformation. Suitability values were then classified into four categories based on the 5-percentile presence threshold (0.22): Unsuitable: 0–0.22; low suitability: 0.22–0.50; high suitability: 0.50–0.80; and very high suitability: 0.80–0.99. Regions identified as potentially suitable but inaccessible were excluded from the final suitability map. We followed three criteria for this step: 1) areas geographically isolated and with lack of connectivity with the rest of the species' distribution, due to major geographic barriers such as the Andes, 2) lack of confirmed records; and 3) confirmed presence of *G. soricina*, a closely related species with no known sympatry with *G. valens*.

Results

Updated distribution and biogeographic pattern. We examined 176 specimens and confirmed 107 occurrence localities for *G. valens* based on museum vouchers and published literature (Figure 1; Appendix 1). Our examination reveals that *G. valens* exhibits a nearly continuous distribution between 1°N and 17°S along the western slopes of Ecuador and Peru, with disjunct populations in north-eastern Peru. Most records (97%) occur at elevations between sea level and 1500 m (Figure 1). However, we also report new noteworthy altitudinal records: 1920 m in La Libertad Department (Locality 84: MUSM 17203) and 1733 m in Amazonas Department (Locality 49: MUSM 1345, 1346). Notably, we document significant disjunct populations on the eastern slopes of the Andes in Amazonas, Cajamarca, and La Libertad Departments. These findings extend the known eastern distribution to the intersection of the Marañón and Utcubamba rivers (Figure 1). All records from Ecuador are restricted to the western slopes of the Andes. The complete revised dataset of *G. valens* occurrence records are provided in Appendix 1.

Marginal occurrence records were identified at the northern and southern limits of the species' range. The northernmost record is in Esmeraldas Province, Ecuador (Locality 1: QCAZ 6716), while the southernmost record is in Arequipa Department, Peru (Locality 107: Webster, 1983, 1993). Most records are situated at sea level along the coast in Ecuador and Peru. In Ecuador, the easternmost records include Los Ríos Province (Locality 6: QCAZ 8909) and Bolívar Province (Locality 10: QCAZ 469, 471, 472). In Peru, the easternmost records are concentrated along the Marañón Valley, extending to the intersection of the Chinchipe, Marañón, and Utcubamba rivers in Cajamarca and Amazonas Departments (Localities 49 and 54: MUSM 1345, 1346, 56482; Appendix 1), approximately 217 km inland from the Pacific coast.

Niche-based species distribution model. The selected model, chosen from 50 candidate models, exhibited strong predictive performance with a feature class combination of LQH, a regularization multiplier of 1.5, and 14 coefficients (AUC_{train}: 0.916, $AUC_{val.avg}$: 0.895, AICc: 2098.31, ΔAICc: 0). This model demonstrated lower complexity compared to other candidates, achieving an optimal balance between accuracy and simplicity (Appendix 3).

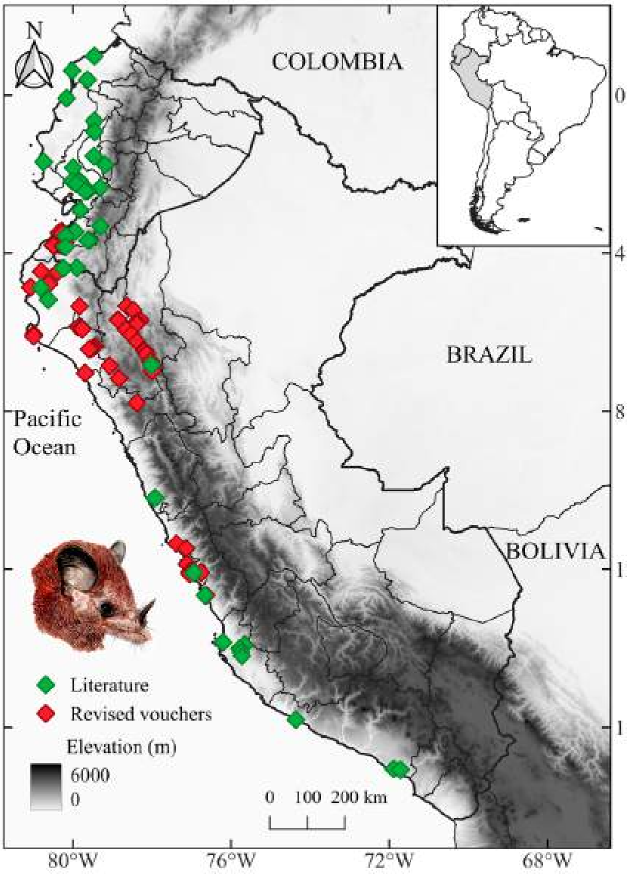

Figure 1. Occurrence records of the Robust Long-tongued Bat (*Glossophaga valens*). Red points indicate localities supported by revised voucher specimens, whereas green points correspond to localities extracted from the literature.

Predictions indicated that *G. valens* exhibits high to very high habitat suitability (suitability > 0.5) under specific environmental conditions. Important predictors selected through Wallace included temperature seasonality (BIO4) below 100 CV (%), temperature annual range (BIO7) below 15°C, and annual precipitation (BIO12) below 1200 mm. Additional factors such as mean temperatures of the wettest quarter (BIO8) above 0°C and precipitation seasonality (BIO15) over 27 CV (%) are also related to high suitability (Figure 2).

The predicted distribution of *G. valens* is predominantly continuous along the western coastline of Ecuador and Peru, with a disjunct population on the eastern slopes of the Andes between Cajamarca and Amazonas Departments (Figure 3). From north to south, the model predicts high-suitability conditions in southern Esmeraldas Province but identifies unsuitable areas northward in Colombia (Figure 3a). High to very high suitability zones are concentrated along the Pacific coastlines of southern Ecuador and northern Peru (Figure 3).

The model also identifies high-suitability areas with few or no confirmed records (n), pointing to potential under-sampled regions, including Ecuador: Manabí (n = 2), Santa Elena (n = 1), Azuay (n = 2), and Loja (n = 2) Provinces. From Peru the Inter-Andean valleys in La Libertad (n = 2), Ancash (n = 1), and Ayacucho (n = 0) (Figure 3).

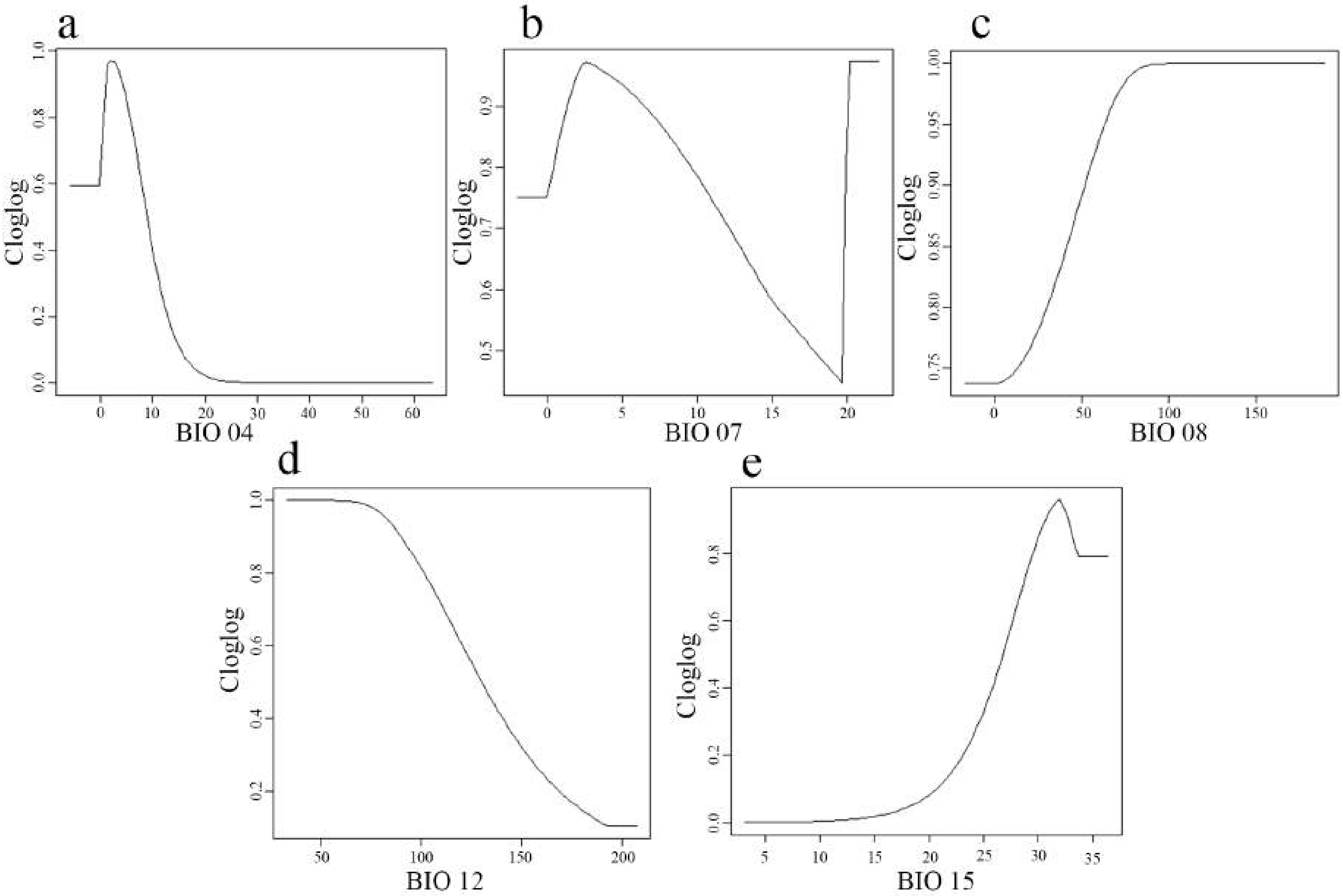

Figure 2. Response curves obtained for the selected optimal model for each selected variable: a) Temperature seasonality, °C (BIO4); b) Temperature Annual Range, °C (BIO7); c) Mean Temperature of Wettest Quarter, °C (BIO8); d) Annual Precipitation, mm (BIO2); and e) Precipitation Seasonality, % (BIO15).

In the Huancabamba Depression, located near 5–7°S, the model identifies areas of high suitability extending through the Marañón Valley. This valley is delineated by the Chamaya, Chinchipe, Marañón, and Utcubamba rivers (Figs. 3b, 4). Although the Marañón River extends northward, the predicted eastern distribution of *G. valens* terminates abruptly near the convergence of the Chinchipe, Utcubamba, and Marañón rivers (Figs. 3b, 4). A closer examination of this area reveals the truncation of its eastern range (Figure 4), with suitability values decreasing toward the east, coinciding with a shift from arid to more humid environments (Figure 4).

To the south, suitability values suggest the range of *G. valens* extends from Arequipa through Moquegua and Tacna in Peru to northern Chile (Tarapacá). However, confirmed records are lacking in these areas, requiring further field exploration (Figs. 1, 3c). Conversely, areas above 2000 m exhibit low suitability, supporting the hypothesis that the Andes act as a biogeographic barrier limiting the species' dispersal into the eastern slopes.

Discussion

Our study expands the known distribution of *Glossophaga valens*, incorporating notable museum-based records from the eastern slopes of Peru and providing a verified dataset of 107 localities for future geographic and conservation studies. Compared to previous analyses (Hoffman *et al.* 2019; Calahorra-Oliart *et al.* 2022), our approach integrated a larger dataset and accounted for dispersal limitations based on verified vouchers, producing an ecologically realistic model. The current records and SDM highlight a distribution predominantly within xeric lowlands (<1500 m) of the western slopes of Ecuador and Peru, as well as parts of the northeastern slopes characterized by low precipitation and high temperatures. These findings complement earlier geographic analyses of *G. valens* (Webster 1993; Calahorra-Oliart *et al.* 2022) and suggest that *G. valens* prefers environments characterized by low precipitation and relatively stable, high temperatures or typical dry conditions as found by Calahorra-Oliart *et al.* (2022).

Geographic limits and potential distribution. The geographic distribution of *G. valens* based on confirmed records extends from Esmeraldas Province in northern Ecuador to the Tambo Valley, Arequipa Department in southern Peru, with notable patterns at both limits. In the north, populations in Esmeraldas's match with high-suitability areas but are reduced in extent or spatially restricted to the south of this Province. Northward, unsuitable areas in Colombia delineate the northernmost limit of *G. valens* in Ecuador's Esmeraldas Province, also observed in other bats: the vesper bat, *Eptesicus innoxious* (Loaiza *et al.* 2020). Prevalent humid habitats in

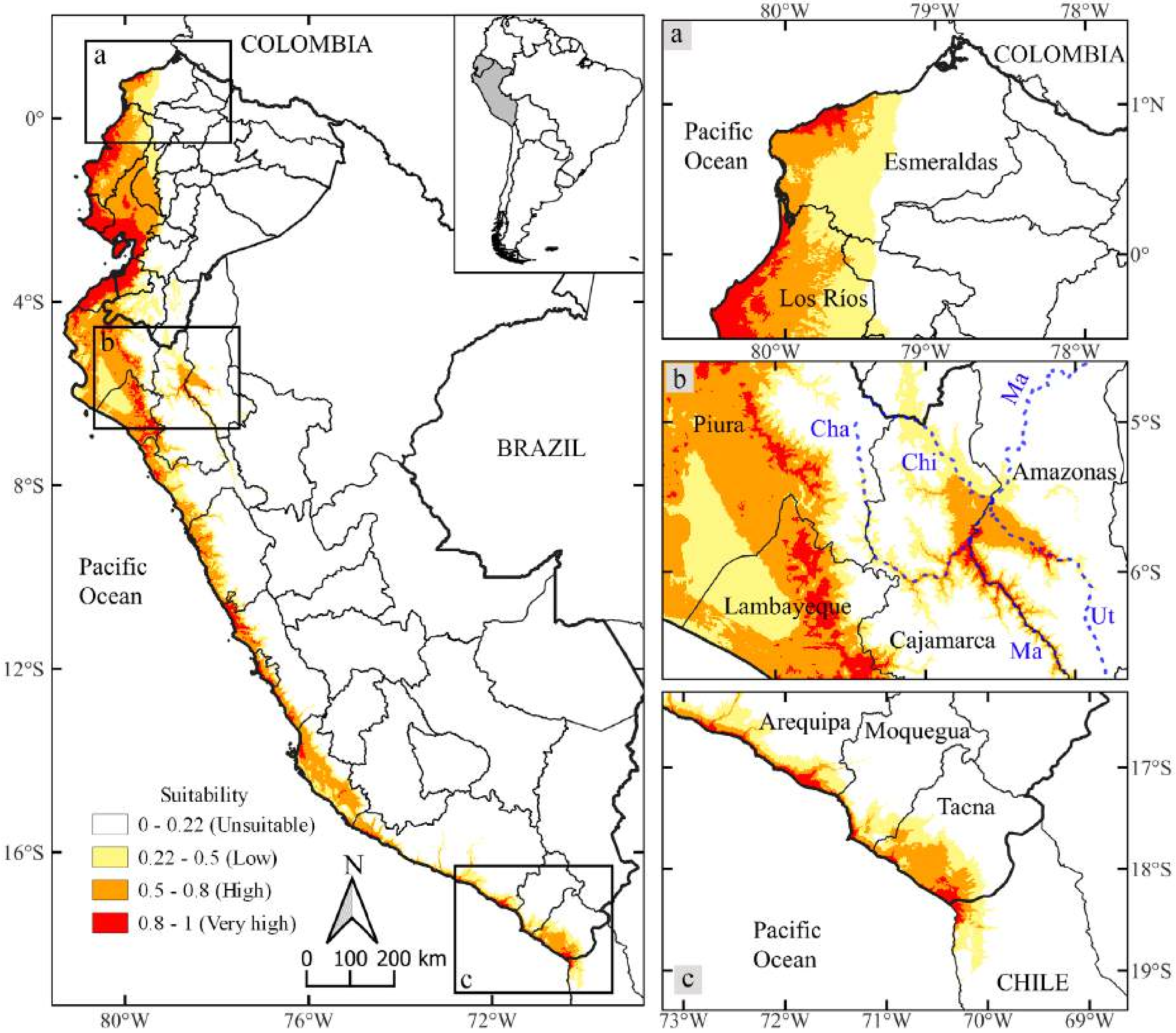

Figure 3. Niche-based species distribution model of *Glossophaga valens* displayed in four suitability categories: unsuitable, low, high, and very high. Insets highlight distribution limits and key geographic features: a) Northern limit, b) Eastern limit, and c) Southern limit. Rivers are denoted by blue dotted lines and include the Chamaya (Cha), Chinchipe (Chi), Marañón (Ma), and Utcubamba (Ut).

southwestern Colombia, part of the Western Ecuador moist forest ecoregion (Olson *et al.* 2002) limits its northward dispersion, a scenario also recorded in its easternmost distribution discussed posteriorly. While this pattern suggests that environmental conditions constrain the northern limit of *G. valens*, further studies are needed to evaluate the role of biotic interactions (*e. g.* presence of *G. mutica*; Calahorra-Oliart *et al.* 2022), resource availability, or historical factors such as orogenic events (Dias *et al.* 2017) or climatic fluctuations (as reported for other glossophagine species like *Anoura cultrata*; Molinari *et al.* 2023).

In the south, while the model predicts high suitability in Moquegua, southern Tacna, and northern Chile, bat surveys in Tacna (Aragón and Aguirre 2014; Lanchipa and Aragón-Alvarado 2018) and northern Chile (Ossa *et al.* 2016, 2018; Rodríguez-San Pedro *et al.* 2020) have not recorded *G. valens*. Given its status as a generalist nectarivore, commonly captured in both natural and urban ecosystems using mist nets (Pacheco *et al.* 2007, 2020; Pellón *et al.* 2020), its apparent absence southward may represent a sampling bias or a signal of low-density populations which evidence the need for further field surveys between southern Tacna and northern Chile. Interestingly, this apparent inability to colonize southern regions contrasts with the broader distribution of *Platalina genovensium*, a specialist nectarivore known to inhabit Tacna (Aragón and Aguirre 2014; Lanchipa and Aragón-Alvarado 2018) and northern Chile (Ossa *et al.* 2016; Ruelas and Pacheco 2018). This discrepancy raises questions about the factors restricting *G. valens*' geographical expansion.

In its eastern range, both abiotic and biotic factors seem to simultaneously act limiting the distribution of *G. valens*. While the species tolerates arid environments, its range abruptly ends in the humid lowlands of northern Amazonas.

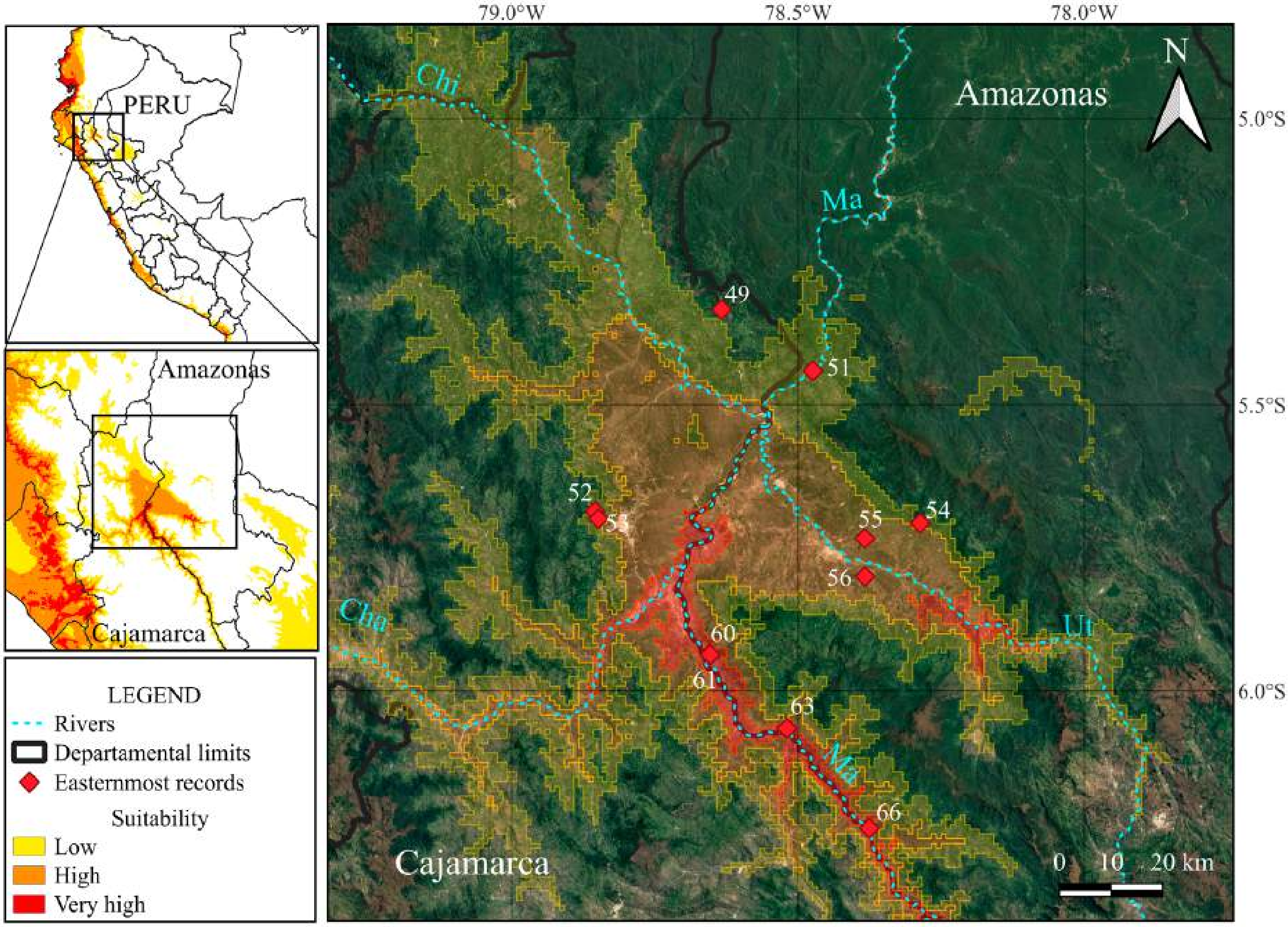

Figure 4. Eastern distribution of *Glossophaga valens* showing new easternmost records and predicted suitable areas. The map illustrates the truncation of the species' potential range across the transition from the arid Marañón valleys to the northern lowland Amazon. Key new records are numbered according to Appendix 1. Rivers are shown as blue dotted lines: Chamaya (Cha), Chinchipe (Chi), Marañón (Ma), and Utcubamba (Utc), highlighting the role of river systems and valley aridity in shaping the species' eastern range.

This truncation aligns with a shift in climatic conditions, transitioning from dry shrublands and forests to humid rainforests as shown in Figure 4. Notably, this transition occurs at the intersection of the Chinchipe, Utcubamba, and Marañón rivers, where the model shows a sharp decline in suitability. These rivers, which delineate the Marañón Valley, may also function as biogeographic boundaries or corridors influencing population connectivity. Moreover, northward in Amazonas, *G. soricina* becomes the only *Glossophaga* species (see vouchers and detailed localities in Appendix 2), suggesting potential competition and ecological exclusion. Such pattern aligns with findings indicating that non-suitable areas act as barriers to gene flow and contribute to species divergence (Calahorra-Oliart *et al.* 2022). Further studies are needed to explore and determine possible contact zones and interbreeding between *G. valens* and *G. soricina*.

In Peru, the Seasonally Dry Tropical Forest (SDTFs) of the Huallaga valley, located on the eastern slopes of the Andes, was initially recognized by the model as areas with low to high suitability for *G. valens*. However, this area was excluded from the final distribution due to its inaccessibility, lack of connectivity with the rest of *G. valens*' range, and the confirmed presence of *G. soricina* (Ruelas and Pacheco 2021). Additionally, Webster (1993) recorded a voucher (catalog number not detailed) housed in the Louisiana Museum of Natural History, collected from San José, Rio Santa Rosa, Ayacucho Department. Although the initial model predicted low suitability areas along the Apurímac Basin in Ayacucho, these regions were excluded due to their isolation, inaccessibility, and confirmed absence of *G. valens* based on recent bat surveys, which recorded only *G. soricina* (Ramos-Ascue *et al.* in prep.). As previously mentioned, the presence of *G. valens* in Ayacucho is anticipated, but restricted to the western inter-Andean valleys. Further field surveys and specimens' revisions are recommended to confirm the identity of individuals assigned to *G. soricina* in the eastern Huallaga Valley and lowlands of the Apurímac River basin as they may represent distinct populations adapted to arid conditions, in contrast to the typical humid habitats where *G. soricina* occurs.

Factors explaining current distribution of G. valens. The distribution of *G. valens* is shaped by two key geographic features: the Seasonally Dry Tropical Forests and the Andes. High suitability areas coincide with the SDTFs, particularly in coastal Ecuador and northern Peru, where the "Bosque Seco Estacional" (a Peruvian term for SDTFs, *sensu* Britto

2017) extends across both western and eastern slopes (Escribano-Avila *et al.* 2017; León *et al.* 2007; Britto 2017). This affinity for arid environments is further supported by field studies. Pacheco *et al.* (2007) reported *G. valens* (reported as *G. soricina*) exclusively in Equatorial dry forest habitats. This pattern was later confirmed by Cadenillas (2010), who, despite extensive sampling effort in Equatorial dry forests, Pacific tropical rainforest, and transitional areas between these habitats, noted a significant decline in *G. valens* abundance along the transition from dry tropical forest to Pacific tropical rainforest, with the species being dominant in the former. These findings align with our model's high suitability predictions for SDTFs and underscore the species' preference for xeric conditions.

Within this region, the Huancabamba Depression—an area of deflection in the northern Andes with its lowest point at 2150 m in Abra Porculla—is characterized by low mountain passes with arid and semi-arid conditions (Patterson *et al.* 1992) and likely connects both disjunct populations, as evidenced in the model, suggesting a corridor role for the colonization of Marañón Valley by *G. valens*. South of the Huancabamba Depression, along the western slopes of the Andes between sea level to 2500 m, the extent of the "Desierto Costero Tropical" and the "Matorral Desértico – Bosque Seco" (*sensu* Britto 2017) provides arid to semi-arid climatic conditions explaining the high to very high suitability values and high occurrence records in this area. At higher elevations, both north and south of the species' range, humid cloud forest and Altiplano highlands dominate (Weberbauer 1945; Koepcke 1954; Valencia 1992). These environments, which exhibit low to null suitability values, likely impose physiological limitations acting as a barrier to east-west dispersal and limiting the range of *G. valens*. We recommend further ecological studies to identify the abiotic and biotic factors (*e. g.* roosting sites and food availability) in the dry tropical forest and deserts inhabited by *G. valens* that strongly influence its fitness and determine the occurrence of *G. valens*.

This interplay between geographic features and physiological constraints also influences other bat species. For example, the restricted ranges of *Eptesicus innoxius, Rhogeessa velilla, Myotis moratellii, M. diminutus, Cynomops kuisha*, and *Lophostoma occidentalis* are similarly shaped by the SDTFs and the Andes (Velazco and Cadenillas 2011; Burneo and Tirira 2014; Moratelli and Wilson 2015; Loaiza *et al.* 2018; Novaes *et al.* 2021; Arenas-Viveros *et al.* 2021). Moreover, the bat assemblage distributed along the Peruvian western slopes from north to south, including *G. valens*, *Amorphochilus schnablii, Mormopterus kalinowskii, Nyctinomops aurispinosus,* and *Platalina genovensium* (Llancachahua-Tarqui *et al.,* 2023), highlights the role of geographic features (*e. g.* the Andes, the extent of dry tropical forest and western desert) in shaping species distribution. Additionally, the Huancabamba depression has also been suggested as a corridor for other bats (*e. g.* *Artibeus fraterculus, Tadarida brasiliensis*) and even birds (Chapman 1926; Patterson *et al.* 1992; Zegarra *et al.* 2020). Furthermore, this corridor role is consistent with geographic patterns observed in other bat species like *Amorphochilus schnablii, Lonchophylla hesperia,* and *Platalina genovensium,* which, are distributed on the eastern slopes despite primarily inhabiting Pacific lowlands (Tirira *et al.* 2011; Ruelas and Pacheco 2018; Guerra *et al.* 2020; Avila-Bernal *et al.* 2024).

The role of biotic factors in *G. valens* needs to be further investigated, as they may influence its occurrence and persistence, and thus play a key role in shaping its distribution. For example, the adaptability of *G. valens* to urban ecosystems appears to be relevant, as areas of high suitability overlap with cities such as Guayaquil, Lima, and Piura, where the species thrives in human-modified landscapes. Its ability to feed on exotic plants (Pellón *et al.* 2020), tolerate light pollution (Mena *et al.,* 2021), and use human structures like abandoned mines and old buildings as roosts (Ortiz de la Puente 1951; Bonifáz *et al.* 2020; Quispe-López *et al.* in prep.) highlights its ecological plasticity. Additionally, Cadenillas (2010), who conducted bat surveys in northern Peru, documented a significant increment in *G. valens* (reported as *G. soricina*) relative abundance in areas affected by cattle, reinforcing the species' capacity to inhabit disturbed habitats. As our findings reveal highly suitable habitats with several records in both natural (northern dry forest) and urban areas, comparative genetic and ecological studies are needed to assess potential differences between populations inhabiting these contrasting habitats. Such research could clarify how urbanization impacts the species' fitness and whether its capacity to exploit cultivated exotic plants (Pellón *et al.* 2020) contributes to its persistence in urban environments. It is important to emphasize that anthropogenic impacts are species-specific (Russo and Ancillotto 2014), and the observed adaptability of *G. valens* should not be extrapolated to other bats or sympatric biota.

Last remarks. The geographical distribution of *G. valens* reflects its evolutionary history shaped by abiotic conditions, biotic interactions, and geographic features such as SDTFs, the Andes, and the Huancabamba Depression (Feng *et al.* 2024). This distribution aligns with the hypothesis of a Central American origin, followed by dispersal along the western slopes of Ecuador and Peru and eventual establishment on the eastern slopes of the Peruvian Andes (Dias *et al.* 2017).

By refining the distribution boundaries of *G. valens* and identifying key areas of high environmental suitability, this research provides critical insights for guiding future survey efforts in regions where the species is likely to occur. Priority regions include areas with few to null records but with high predicted suitability, such as under-sampled inter-Andean valleys, northeastern Peru (a potential contact zone with *G. soricina*), southern Colombia (to refine boundaries with *G. mutica*), and southern Peru in Tacna and northern Chile.

Our findings have important conservation implications for *Glossophaga valens* whose distribution is closely tied to SDTFs, Pacific deserts, and inter-Andean valleys along the western slopes of Peru and Ecuador. This region represents one of the most distinctive areas of endemism for South American bats (Koopman 1982), hosting endemic species that are either known from only a few localities (*e. g. Histiotus mochica*, *Myotis bakeri*; Velazco *et al.* 2021; Llancachahua-Tarqui *et al.* 2023) or are classified under threatened categories (*e. g. Amorphochilus schnablii*, *Artibeus ravus*, *Cynomops greenhalli*, *Platalina genovensium*) (SERFOR 2018). Moreover, the Tumbesian region, (also termed SDTFs), which encompasses a great portion of western Ecuador and northwestern Peru, supports habitats for over 900 species of plants and vertebrates, 18% of which are endemic (Escribano-Avila *et al.* 2017). However, this region is also among the most threatened terrestrial biomes in the tropics, facing accelerated loss of vegetation cover due to human activities (Hoekstra *et al.* 2005; Miles *et al.* 2006).

Finally, with its elevation to the species level (Calahorra-Oliart *et al.* 2021), *G. valens* currently lacks a formal conservation status. Despite the ecological plasticity discussed earlier, its presence in fragile ecosystems such as coastal wetlands (Pacheco *et al.* 2015, 2020), fog oases (Bonifáz *et al.* 2020), and dry forests (Pacheco *et al.* 2007) underscores the urgency of assessing their populations in natural ecosystems as many of these ecosystems are undergoing rapid degradation. In this context, our revised and verified occurrence list, coupled with the identification of high-suitability areas, provides a critical foundation for guiding future research and conservation strategies. These include targeted field surveys in data-deficient regions, evaluations of habitat connectivity and gene flow, and a formal assessment of the species conservation status. This information is crucial for decision-makers aiming to implement effective conservation strategies in highly threatened habitats where *G. valens* occurs.

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Appendix

Appendix 1-3 are available at https://zenodo.org/records/15366252

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Gastrointestinal parasitism in nine-banded armadillos (*Dasypus novemcinctus*) under different hunting pressures in the Reserva Natural del Bosque Mbaracayú, Paraguay.

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The aim of this study was to characterize the gastrointestinal parasitic fauna of nine-banded armadillos (*Dasypus novemcinctus*) of the Reserva Natural del Bosque Mbaracayú (RNBM), Canindeyú, Paraguay, and to evaluate how human activities, particularly hunting, may influence parasitism patterns and zoonotic risk. We recovered 15 distinct parasites from fresh fecal samples of 73 individuals of *D. novemcinctus* including Protozoa*: Eimeria* sp., *Giardia* sp.*,* coccidia (Family Adeleidae), unidentified coccidia; Cestodes: *Hymenolepis* sp.*;* Nematodes: *Trichuris* sp., Trichostrongyloidea, *Cruzia* sp., *Ancylostoma* sp., *Uncinaria* sp., *Aspidodera* sp., *Strongyloides* sp. and Acanthocephala. We report high prevalence of potentially zoonotic parasites including *Ancylostoma, Trichuris, Uncinaria* and Coccidia. We also found an increase in parasite richness in areas where hunting was reported *vs.* areas without hunting and the same trend for Nematode probability of presence, especially during the cold dry season. These results suggest that human activities may be affecting patterns of parasitism in wild armadillos. In addition, traditional consumption of these animals in this region may present a route of human exposure to zoonotic parasites.

El objetivo de este estudio fue caracterizar la fauna parasitaria gastrointestinal de armadillos de nueve bandas (*Dasypus novemcinctus*) de la Reserva Natural del Bosque Mbaracayú (RNBM), Canindeyú, Paraguay y evaluar cómo las actividades humanas, particularmente la caza, pueden influir en los patrones de parasitismo y el riesgo de zoonosis. Recuperamos 15 parásitos distintos de muestras fecales frescas de 73 individuos de *D. novemcinctus*, incluidos protozoos: *Eimeria* sp., *Giardia* sp., coccidias (familia Adeleidae), coccidias no identificadas; Cestodos: *Hymenolepis* sp.; Nematodos: *Trichuris* sp., Trichostrongyloidea, *Cruzia* sp., *Ancylostoma* sp., *Uncinaria* sp., *Aspidodera* sp., *Strongyloides* sp. y Acanthocephala. Informamos una alta prevalencia de parásitos potencialmente zoonóticos, incluidos *Ancylostoma, Trichuris, Uncinaria* y Coccidia. También encontramos un aumento en la riqueza de parásitos en áreas donde se reportó caza *versus* áreas sin caza y la misma tendencia en la probabilidad de presencia de nematodos, especialmente durante la estación fría y seca. Estos resultados sugieren que las actividades humanas pueden estar afectando los patrones de parasitismo en los armadillos salvajes. Además, el consumo tradicional de estos animales en esta región puede presentar una vía de exposición humana a parásitos zoonóticos.

Keywords: Zoonoses, helminths, prevalence, human-wildlife interface

Introduction

Nine-banded armadillos (*Dasypus novemcinctus* Linnaeus, 1758) extend from Colombia and Venezuela to Argentina (MacDonald 1985; Redford and Eisenberg 1992; Loughry *et al.* 2015; Barthe *et al.* 2024). In Paraguay, they occur throughout the country, but are considerably rare in arid areas (Smith 2007; De la Sancha *et al.* 2017; Smith and Ríos 2018). They are nocturnal, generalist insectivores that spend their days in self-dug burrows, often shared by multiple individuals from the same family group (Taber 1945; Nowak 1991; Parera 2002; Smith 2007). In Paraguay, many human cultural groups use this species as a food resource including Aché, Mbyá, and Guaraní indigenous groups and farmers from rural settlements (McBee and Baker 1982; Esquivel 2001; Neris *et al.* 2002; Hill *et al.* 2003; Smith 2007; Morales 2009).

Although the parasites of *D. novemcinctus* are well characterized at the northern extent of their range (i.e.: Chandler 1946; Radomski *et al.* 1991; Varela-Stokes et al. 2008; Jimenez-Ruiz et al. 2013), previous research in the southern extent of their range is more limited. For example, Fujita *et al.* (1995) reported some nematodes recovered from the gastrointestinal tract of just two specimens (Dept. of San Pedro, Central Paraguay): *Ancylostoma* sp., *Aspidodera esperanzae* (possibly *A. binansata* -Jimenez-Ruiz *et al.* 2006), *Moeniggia complexus, M. pintoi* and an unidentified species of Heterakinae. Navone (1990) recorded the nematode *Aspidodera vazi* (Aspidoderidae) and the cestode *Mathevotaenia surinamensis* (Anoplocephalidae) in the Paranaense and Chaco regions of Argentina, with *Aspidodera fasciata* (Aspidoderidae) additionally recorded in the Chaco region. In Santa Cruz, Bolivia Jimenez-Ruiz *et al.* (2003) reported Aspidoderid nematodes *Aspidodera binansata* (n=173) and *Lauroia bolivari* sp. nov (n=22) from the gastrointestinal tract of one individual. Hoppe and do Nascimento (2007) reported at least 13 species of nematodes recovered from the gastrointestinal tract of nine individuals in Pantanal (Mato Grosso, Brazil). Also, in Bolivia, Deem *et al.* (2009) reported the following parasites from 22 individuals: Heterakidae, *Aspidodera* sp., *Trichuris* sp., Adelaidae (protozoan), and *Strongylus* sp.

The species can coexist with humans in rural areas because of its largely nocturnal habits and large litter size but has declined over much of its range in eastern Paraguay because of destruction of optimal habitats and hunting pressure (Smith 2007). Armadillos of the studied species may present zoonotic pathogens thus hunting and human consumption of these species or sharing edge habitats may have a significant public health impact (Stallknecht *et al.* 1987; Yaeger *et al.* 1988; Truman 2005; Deem *et al.* 2009).

The aim of the current study was twofold: 1) to begin the characterization of the gastrointestinal parasitic fauna of nine-banded armadillos (*Dasypus novemcinctus*) of the Reserva Natural del Bosque Mbaracayú (RNBM), Canindeyú, Paraguay; and 2) to explore differences in parasite diversity and prevalence between areas exposed to human activities (i.e.: traditional and illegal hunting) and more remote, undisturbed areas within the reserve. In addition to determining the parasites present and their prevalence in *Dasypus novemcinctus* within the RNBM, we also aimed to determine if the parasite communities and/or the prevalence of specific taxa differed in armadillos from hunted areas compared to those from non-hunted areas. By comparing infection patterns across zones with contrasting hunting pressure, we also aimed to evaluate the influence of these human practices on parasite in armadillos, as well as the potential zoonotic risks for individuals involved in their hunting and consumption (Medkour *et al.* 2020; Inada 2023; Mozer and Prost 2023; Salvarani *et al.* 2025).

Methods

Study site. Our study was conducted as part of a larger research program to investigate infectious diseases dynamics in the protected area Mbaracayú Forest Nature Reserve (RNBM) and its surroundings in Paraguay. Specifically, this study was done at the Reserva Natural del Bosque Mbaracayú (RNBM), (24° 00′ - 24° 15′ S, 55° 00′ - 55° 32′ W), Depto. Canindeyú, Paraguay. The RNBM represents a c. 640 km^2 protected area composed mostly of Upper Parana Atlantic Forest. Mbaracayú was established in 1991 and is privately managed by a Paraguayan non-governmental organization (Fundación Moises Bertoni). The study area is also within a UNESCO biosphere reserve, which includes the protected area Mbaracayú Forest Nature Reserve (RNBM), as a core preserved area.

The climate is warm, humid, and temperate, with an average annual temperature of 21–22 °C and annual rainfall ranging from 1,650 to 1,800 mm, peaking between October and March. Based on the classification by Owen *et al.* (2010), the year is divided into three seasons: transitional (T: February–May), dry (D: June–September), and wet (W: October–January). Frosts can occasionally occur from June to October. The landscape consists of a mosaic of natural forest and savanna ecosystems, including High Forest, Medium and Low Forests with bamboo, wetlands, cerrado, and native grasslands. Forests range from mature stands with open understories to dense, bamboo-dominated areas (*Chusquea ramosissima*, *Guadua* spp.), while savannas include Cerrado formations and grassy riparian zones, contributing to the area's ecological complexity (FMB/BM 2005).

Sampling was conducted in two types of areas within the RNBM: (1) areas under direct human influence, such as zones where hunting occurs, and (2) remote, minimally disturbed forested areas where no hunting occurs (Figure 1). One of the human-impacted sites was located near Arroyo Bandera, the closest community to the reserve. In this region, indigenous communities have legal authorization to practice traditional hunting, while illegal hunting by non-Indigenous individuals has also been reported both along the edges and within the interior of the reserve. These designations were based on reports from rangers responsible for protection and monitoring of the RNBM. In the hunting zones, the most predominant domestic species observed was the dog (*Canis lupus familiaris*), which is commonly used by hunters as a companion and a hunting aid during their excursions into the forest (Kowalewski *et al.* 2019).

Study subjects. Sample collection occurred from June 2012 to August 2013, and from September 2016 to June 2017. For subsequent analyses, samples were classified according to the three seasonal periods previously defined (transitional, dry and wet). Fresh fecal samples were collected from *D. novemcinctus*, primarily from individuals captured by hand by Aché technicians with expertise in traditional hunting practices. Additional samples were obtained opportunistically from fresh feces found in the field, attributed to non-captured individuals. Once captured, each armadillo was placed in an individual breathable bag and transported to our nearby base camp where our veterinarian performed a health assessment and obtained a fecal sample from each individual. All armadillos were weighed while in the bag and then immobilized

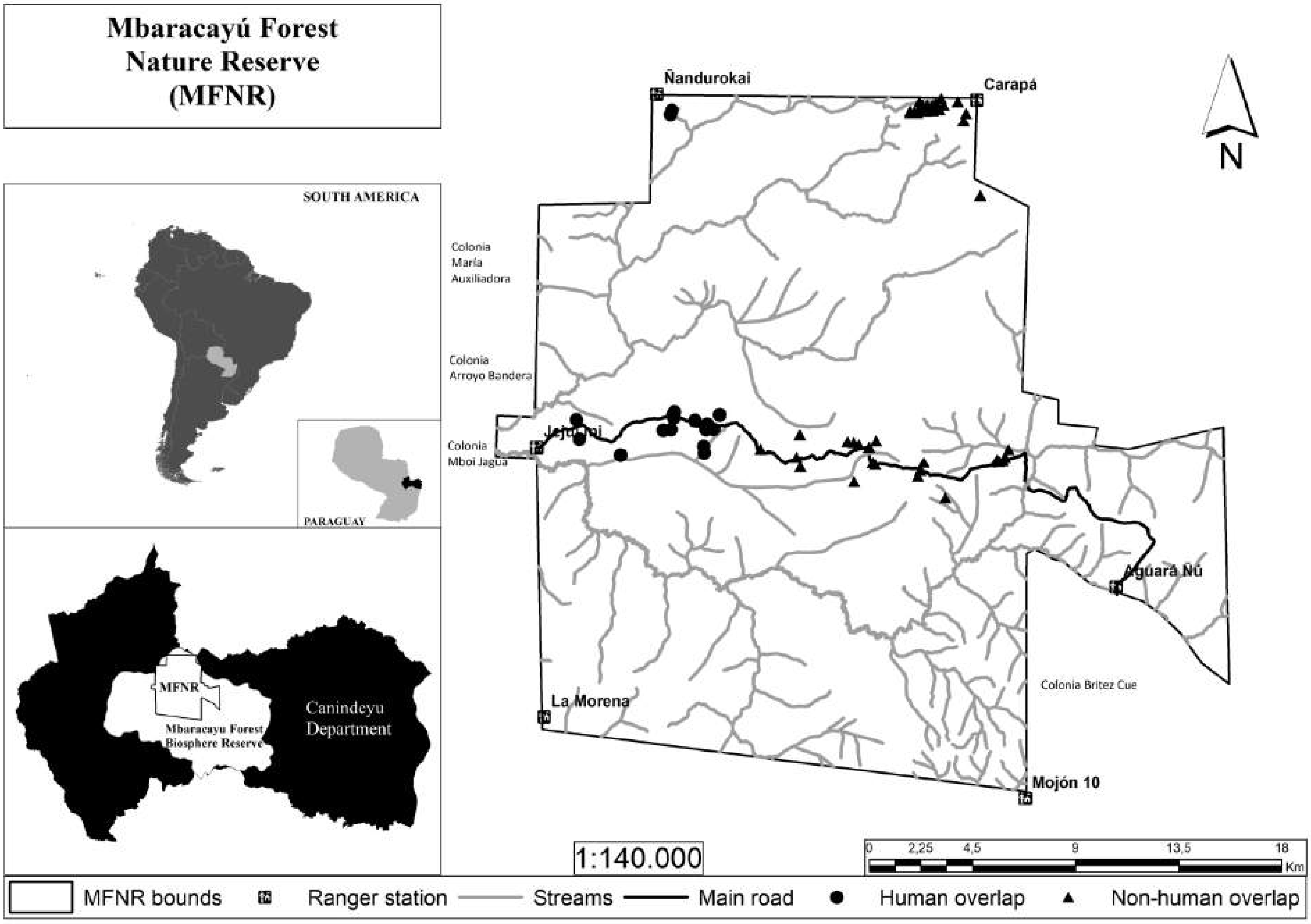


Figure 1. Location of *Dasypus novemcinctus* sampling sites with and without hunting within the Reserva Natural del Bosque Mbaracayú, Paraguay.

using ketamine-zolazepam for physical examination and collection of biologic samples. After sampling, armadillos were released at the site of capture. All fecal samples were preserved in 10% buffered formalin in 20 ml plastic tubes and labeled. We did not repeat capture sites, and armadillos were marked previous release to avoid recapture.

Parasitological Analyses. Fecal samples were examined using direct microscopic examination. Techniques to recover eggs included Willis, Sheather and zinc sulphate methods (MAAF 1986; Thienpont *et al.* 1986; Foreyt 1989) at the Parasitological Laboratory of the Centro para el Desarrollo de la Investigación Científica (CEDIC, Asunción, Paraguay). Slides (18 mm x 18 mm) prepared by each method were observed under a light microscope (LEICA DM500) after the addition of a drop of Lugol's iodine and photographed with a Leica camera (ICC50W). Parasites were identified based on egg, larvae, or cyst coloration, shape, contents, and size following Anderson *et al.* (1974), Khalil *et al.* (1994), Lainson and Shaw (1982), Mehlhorn *et al.* (1992), and Navone (1986; 1987; 1988, 1990). Each slide was scanned thoroughly under both 10x and 40x objective lenses to confirm presence or absence of helminth eggs and larvae and protozoan cysts. Representatives of each parasite species recovered per sample were measured at 400x to the nearest 0.1 µm with the Leica Application Suite software (2016) and representative parasite species were photographed. Morphological examination of eggs and oocysts allowed diagnosis to the genus level or higher levels for some parasites. Thus, the taxonomic level achieved through morphological identification of parasite eggs or oocysts depended on the parasite, as some genera had distinctive eggs enabling genus-level diagnosis, while others could only be identified at higher taxonomic levels, such as the superfamily Trichostrongyloidea

Statistical analyses. We estimated the prevalence (as the proportion of individuals positive for a given parasite divided by the total number of armadillos examined) and richness (as the number of the parasite species/group present per individual). Using Generalized Linear Models (GLM), we analyzed the effects of environmental, hunting and sex of the individual on richness and parasite occurrence (presence/absence of parasites) (Table 1). For these analyses we divided data into broad categories, presence of nematodes, presence of cestodes and presence of protozoan parasites (Model 2.a; 2.b; 2.c respectively). Full models were then restricted by removing non-significant terms in a stepwise

Table 1. Variables summary for GLM model.

Model	Variable	Definition
	Dependent variables	
1	Richness	Count (min= 1; max=9)
2. a	Presence nematodes	Binary response (absent=0; presence=1)
2. b	Presence cestodes	Binary response (absent=0; presence=1)
2. c	Presence protozoa	Binary response (absent=0; presence=1)
	Independent Variables	
Environmental	Hunting	Categorical variable, 2 levels (yes, no)
	Sex	Categorical variable, 2 levels (male, female)
	*Season	Categorical variable, 3 levels (T= transitional; D= dry; W= wet)
	Monthly total precipitation	Continuous variable (min=29; max=408, mean=166.8)

backward elimination manner, using likelihood ratio tests, until we obtained a final model that contained only significant variables. GLM were fit by maximum likelihood (Laplace Approximation), using the function "glm" from the MASS package (Venables and Ripley 2002). Poisson (log) distribution was used for the richness model and Binomial (logit) distribution for the prevalence set of models. For the first model we verified Poisson distribution of dependent variable "richness" graphically (plot distribution of cumulative frequencies and qq-plot). The assumptions of normally distributed and homogeneous residuals (Pearson residuals) were checked by visually inspecting qq-plots and the residuals plotted against the fitted values. We used R version 3.2.1 (The R Project for Statistical computing, www.r-project.org) for the analyses, and statistical significance was set at a p-value of 0.05. for all interpretations.

Compliance with ethical standards. We have followed ARRIVE guidelines and all procedures complied with relevant legislation, including the U.K. Animals (Scientific Procedures) Act (1986), EU Directive 2010/63/EU, and the NIH Guide for the Care and Use of Laboratory Animals. We used the minimum number of animals required for statistically valid results. The study adhered to Paraguayan law (N° 96/92 on wildlife), with permits for scientific collection (N°06/2012 and 270/2016) granted by Ministry of the Environment and Sustainable Development (MADES-Paraguay in Spanish).

Results

We collected a total of 78 fresh fecal samples. Of these, 66 correspond to *D. novemcinctus* captured by hand and the other 12 samples correspond to fresh fecal samples of non-captured armadillos. Of the 66 weighed armadillos, 32 were females and 34 males. There were no differences in weight between females (4.6 ±0.8 kg) and males (4 ± 1.2 kg) (p>0.05). All captured individuals showed good health status based on physical examination.

We recovered 15 distinct parasite taxa from 73 (out of 78) individuals including Protozoa: *Eimeria* sp., *Giardia* sp., coccidia (Family Adeleidae), unidentified coccidia; Cestodes: *Hymenolepis* sp.; Nematodes: *Trichuris* sp., Trichostrongyloidea, *Cruzia* sp., *Ancylostoma* sp., *Uncinaria* sp. *Aspidodera* sp., *Strongyloides* sp. and Acanthocephala (possible *Travassosia* sp.) (Table 2, Figure 2). The total

Table 2. Prevalence of parasites recovered in fresh fecal samples from 73 individual *Dasypus novemcinctus* (out of 78 individuals examined) at Reserva Natural del Bosque de Mbaracayú, Paraguay. Twenty individuals are from hunting areas and 53 individuals are from areas without hunting.

Parasite/group	**Prevalence (%)**		
Protozoa	Total	Hunting	Without Hunting
Giardia sp.*	6.9	21.4	3.4
Eimeria sp. (Coccidia)	5.5	14.3	3.4
Unidentified Coccidia	49.3	71.4	44.1
Adeleidae (Coccidia)	16.4	42.9	10.2
Cestoda			
Unknown Cestode 1	5.5	14.3	3.4
Hymenolepis sp.*	4.1	7.2	3.4
Nematoda			
Strongyloides sp.	6.9	7.2	6.8
Trichostrongyloidea	8.2	21.4	5.1
Trichuris sp.	90.4	100	88.1
Uncinaria sp.*	20.6	42.9	15.3
Ancylostoma sp.	67.1	57.1	69.5
Cruzia sp.	4.1	7.1	3.4
Aspidodera sp.	15.0	21.4	13.6
Unknown Nematode 1	1.4	7.1	0
Acanthocephala (possibly *Oligacanthorhynchus*)	1.4	7.1	0

(*) indicate new records for the species.

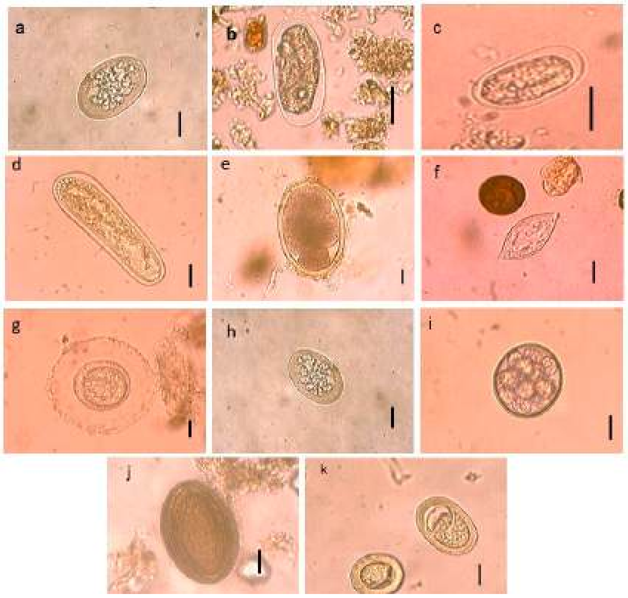
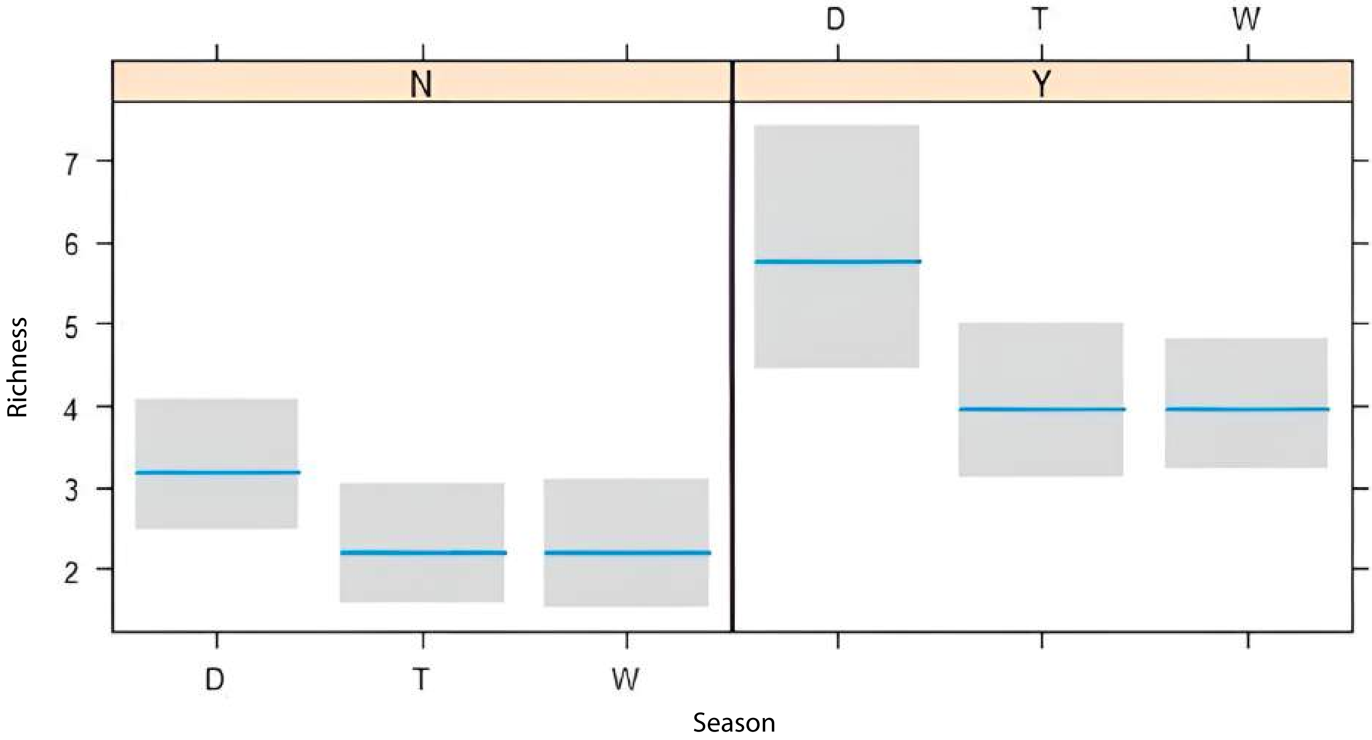


Figure 2. Parasites recovered from fecal samples of *Dasypus novemcinctus* within the Reserva Natural del Bosque Mbaracayú, Paraguay. (a) *Ancylostoma* sp. 1 (b) *Uncinaria* sp. (c) *Strongyloides* sp. (d) Trichostrongyloidea (e) *Cruzia* sp. (f) *Trichuris* sp. (g) *Hymenolepis* sp. (h) *Aspidodera* sp. (i) Coccidio, Adeleidae Family (j) Acanthocephala (possible *Travassosia* sp.) (k) Cestode 1 Scale bar is 20 µm.



N = Sites without hunting; Y = Sites with hunting.

Figure 3. Richness GLM results. Blue line represents expected richness and gray boxes are standard deviations according to GLM. Seasons are T, transitional (February–May); D, dry (June–September); W, wet (October–January) following Owen *et al.* (2010).

prevalence of gastrointestinal parasites was 93.6%. Both *Ancylostoma* sp. and *Trichuris* sp. presented the highest prevalence: 67.1% and 90.4% respectively. When coccidia parasites were pooled, the general prevalence reached 71.2% (Table 2). The number of distinct parasites per individual ranged from 1-9.

Parasite richness was higher in sites with hunting and during the dry season compared to sites without hunting and the wet season, respectively (Figure 3). Neither sex nor precipitation were significant contributors to the final model (Table 3). Presence / absence models showed a trend toward hunting pressure being associated with a higher probability of the presence of nematodes (Table 4). Null models were selected in protozoan and cestode models.

Table 3. GLM results, independent variables estimate of the model selected for richness.

Model	Estimate	Std. Error	z value	Pr(>|z|)
(intercept)	1.16	0.12	9.27	0.001***
hunting (Yes)	0.59	0.15	3.81	0.001***
season (T)	-0.37	0.16	-2.33	0.01*
season (W)	-0.37	0.16	-2.32	0.02*

Table 4. GLM results, independent variables estimate of the model selected for presence / absence of nematode.

Model	Estimate	Std. Error	z value	Pr(>|z|)
(intercept)	1.7	0.54	3.14	0.01**
hunting (Yes)	18.8	2614.2	0.007	0.99

Discussion

In this study we recovered 15 distinct parasites from 73 wild *D. novemcinctus* in Reserva Natural del Bosque Mbaracayú (RNBM) including taxa known to be prevalent in species of the Order Xenarthra, including coccidia of the Families Adeleidae and Eimeriidae (Lange and Wittenstein 2001; Ezquiaga *et al.* 2014), nematodes of the genera *Aspidodera* (Vicente 1966; Vicente *et al.* 1997; Hoppe and Nascimento 2007) and *Cruzia* (Fujita *et al.* 1995; Vicente *et al.* 1997; Hoppe and Nascimento 2007), and an acanthocephala, presumed to be *Oligacanthorhynchus* (syn, *Travassosia*) sp., (Travassos 1917; Lent and Freitas 1938; Smales 2007; Gomez-Puerta 2012). Notably, *Giardia* spp., *Hymenolepis* spp., and *Uncinaria* spp. were recorded for the first time in this host species, expanding the known parasitic diversity of *D. novemcinctus*.

The richness found in our research is similar to that recorded by Hoppe and do Nascimento (2007) in Brazil, and higher than that recorded by Fujita *et al.* (1995) in Paraguay, Navone (1990) in Argentina and Deem *et al.* (2009) in Bolivia. However, methodological differences and ecological contexts across these studies must be considered. For instance, Fujita *et al.* (1995) examined only two museum specimens, with no specific environmental information available. Navone (1990) analyzed 17 gastrointestinal tracts from individuals collected in temperate grasslands and subtropical regions of Buenos Aires and Corrientes provinces. Hoppe and do Nascimento (2007) also relied on gastrointestinal tract analysis, although detailed ecological context was not provided. In contrast, Deem et al. (2009) analyzed fecal samples from 22 individuals collected within the Gran Chaco region of Bolivia, a dry, thorny forest and savanna ecosystem. These variations among samples (feces vs. gastrointestinal tracts), sample size, and habitat likely influence parasite detection and diversity, and should be carefully considered when comparing across studies. Nevertheless, a summary of parasite records across different countries is presented in Table 5, to contextualize our findings and highlight general patterns and knowledge gaps.

Table 5. Reported parasites of *Dasypus novemcinctus* across its distribution range.

Parasite	Sample	Location	Reference
Brachylaemus virginianus, Oncicola canis, Hamanniella sp., *Aspidodera fasciata, Physocephalus* sp., *Ascarops* sp.	GI tracts (N= 8)	USA	Chandler 1946
Aspidodera vazi (Aspidoderidae) and the cestode *Mathevotaenia surinamensis* (Anoplocephalidae), *Aspidodera fasciata (Aspidoderidae)*	GI tracts (N=17)	Argentina	Navone 1990
Macracanthorhynchus ingens, Centrorhynchus sp., *Physaloptera* sp.	GI tract (N=117)	USA	Radomski *et al.* 1991
Ancylostoma sp., *Aspidodera esperanzae* (possibly *A. binansata* -Jimenez-Ruiz *et al.* 2006), *Moeniggia complexus, M. pintoi,* unidentified species of Heterakinae.	GI tract (N= 2)	Paraguay	Fujita *et al.* 1995
Aspidodera binansata, Lauroia bolivari sp. *nov*	GI tract (N=1)	Bolivia	Jimenez-Ruiz *et al.* 2003
Aspidodera sogandaresi, Aspidodera vazi, Aspidodera binansata, Lauroia trinidadensis, *L. trinidadensis,*	GI tract (N= 13)	Mexico Panama USA	Jimenez-Ruiz *et al.* 2006
Macielia macieli, M. flagellata, Moennigia moennigi, M. complexus, M. pintoi, M. littlei, Delicata variabilis, Hadrostrongylus speciosum, Strongyloides ratti, Aspidodera fasciata, A. binansata, A. vazi, Cruzia spp.	GI tract (N= 9)	Brazil	Hoppe and do Nascimento 2007
Aspidodera cf. *soganderesi, Macracanthorhynchus ingens*	GI tract (N=?)	USA	Varela-Stokes *et al.* 2008
Heterakidae, *Aspidodera* sp., *Trichuris* sp., Adelaidae, *Strongylus* sp. eggs	Fecal samples (N= 22)	Bolivia	Deem *et al.* 2009
Mathevotaenia sp., *Centrorhynchus* sp.	GI tract (N= 30)	Brazil	Gomes *et al.* 2011.
Cestoda, Trematode eggs, Trichostrongylidae and Ascarididae eggs, *Capillaria* sp.	Fecal samples (N= 4)	Brazil	Santos *et al.* 2024

GI= gastrointestinal, N= number of samples collected.

Overall parasite richness was higher in areas with hunting, with the strongest effect occurring during the dry season (from June to September). We found a trend toward the same pattern when comparing the probability of parasite presence / absence (i.e., higher probability of presence in areas with hunting and during the dry season) for nematodes, but not cestodes or protozoa. These results suggest that human activities within the reserve may alter patterns of parasitism in wild armadillos. One possible explanation for our results is that in the areas where illegal hunting is known to occur, poachers establish camps and use their dogs for hunting (Ramirez Pinto, pers. comm.). Both, dogs and humans, defecate on the ground and could contribute to a high rate of soil and grass contamination with infective parasitic stages. Studies in nearby communities have reported a high diversity of gastrointestinal parasites in domestic dogs, including *Uncinaria stenocephala*, *Strongyloides stercoralis*, *Ancylostoma caninum*, *Toxocara canis*, *Cystoisospora* sp., *Trichuris vulpis*, *Giardia duodenalis*, and *Taenia* sp. (Martínez 2019). Notably, some of these parasites—such as *Giardia*, *Trichuris*, and *Uncinaria*—were also detected in armadillos from hunting areas, suggesting potential overlap. Given their feeding habits, armadillos may be especially exposed to fecal-oral transmission routes and could act as accidental or paratenic hosts for these parasites. Furthermore, the higher parasite richness observed during the colder winter months (June to August) in comparison with the rest of the year, may be related to lower temperatures and food availability, which could compromise immune function and increase host susceptibility to infection. Additionally, some nematodes, such as *Trichuris,* produce highly resistant eggs that can remain viable in the environment under these conditions (Traversa *et al.* 2011). These survival traits, combined with seasonal nutritional stress and immune compromise, may contribute to sustained environmental infective pressure and help explain the observed increase in parasite richness during the dry season. Concerning cestodes and protozoa, there was not a significant association or trend between these groups of parasites and the independent variables. In general, cestodes have indirect cycles and use insects as intermediate hosts. Although we did not collect specific data on the diet of the captured armadillos, field observations confirm the presence of termite mounds in several areas where individuals were captured. As insectivorous mammals, armadillos are known to dig into the soil and termite nests in search of food, which could provide them access not only to termites and ants but also to insect larvae developing underground. In the case of protozoa is probably that the presence of some parasites (e.g., Adeleidae) is associates to the mechanical passage of oocysts from infected prey through the gastrointestinal tract of the nine - banded armadillos, rather than reflecting true parasitism (Lange and Wittenstein 2001; Ezquiaga *et al.* 2009).

Although we recovered some taxa with zoonotic potential, such as nematodes of the genera *Ancylostoma* and *Trichuris* (at high prevalence), *Uncinaria* (at moderate prevalence), and protozoa such as *Giardia* to a lesser extent (Table 2; Figure 2), it is important to note that the presence of these genera does not necessarily imply a direct zoonotic risk. Most species within these groups can infect both animals and humans; however, not all species possess the ability to infect humans or cause disease. Future molecular analyses are essential to identify the specific species involved and to accurately assess their zoonotic potential.

Understanding these risks is particularly important given that the traditionally consumption of these animals by legal and illegal hunters may represent a potential route of human exposure to zoonotic parasites- either through ingestion of contaminated meat or through exposure to body fluids and feces during hunting and preparation. Moreover, Ache hunters eat part of the game during their hunting expeditions (Ramirez Pinto, pers. comm.), which increases the risk of transmission when meat is consumed raw or undercook, or when handling and transportation practices do not follow food safety practices (Thompson 2013; Van Vliet *et al.* 2017).

Based on these practices, we suggest that exposure to parasites may occur not only through ingestion of infected tissue, but also via handling of raw meat or cross-contamination during food preparation. The risk may arise from indirect contact with fecal material present in the environment during traditional hunting practices. In such contexts, hunters—often accompanied by dogs—frequently dig into burrows to manually extract the animals, increasing the likelihood of exposure to soil and fecal contamination (Natalini pers. comm.) This, combined with the consumption practices described above, creates multiple potential pathways for zoonotic transmission.

Our study represents a descriptive approximation, and we do not aim to explore transmission dynamics in depth. While we acknowledge that environmental conditions such as soil moisture can influence the transmission of hookworms and other parasites, our current data are insufficient to make conclusive statements in this regard. Nonetheless, this is indeed an important aspect that warrants further investigation through targeted ecological and longitudinal studies.

Considering these findings, which highlight the possibility of zoonotic transmission, future studies employing molecular analyses and opportunistic necropsies are needed to improve our classification of the gastrointestinal parasites of armadillos. Mechanisms to reduce zoonotic risks identified may promote human health and local support for conservation.

Conclusions

We characterized the gastrointestinal parasitic fauna of nine-banded armadillos (*Dasypus novemcinctus*) recovering 15 distinct parasites from fresh fecal samples of 73 individuals at the Reserva Natural del Bosque Mbaracayú (RNBM), Canindeyú, Paraguay. We also report an increase in parasite richness in areas where hunting was reported in contrast to areas without hunting and the same trend for Nematode probability of presence. These findings suggest that human activities may be affecting patterns of parasitism in wild armadillos.

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Felid activity at artificial water troughs in a tropical forest in southeastern Mexico

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Felids play a major role in ecosystems as apex and mesopredators of numerous vertebrate species. Thus, a decline in their populations can directly impact ecosystem functioning. Recently, extreme weather events, such as prolonged droughts, have posed increasing threats to tropical forests. Implementing mitigation measures, such as providing artificial water troughs, can help alleviate water shortages for wildlife during the dry season. However, limited information is available on how species respond to such mitigation measures in tropical ecosystems, particularly concerning visitation rates and temporal patterns of use by felids. We hypothesized that water troughs would be used with similar frequency, irrespective of their spatial arrangement. Additionally, we anticipated that smaller species would exhibit lower visitation rates and reduced temporal overlap compared to larger felid species. Specifically, we evaluated the broad-scale spatial variation in visitation rates to water troughs by felid species and the degree of overlap in activity patterns to infer potential interspecific avoidance. We analyzed five years of camera-trap monitoring data collected at artificial water troughs in the Calakmul Biosphere Reserve, Campeche, Mexico. We measured visitation rates and compared frequencies among water troughs. Temporal overlap was used to evaluate similarities in diel activity patterns among felids. The ocelot (*Leopardus pardalis*) and puma (*Puma concolor*) were the most frequently observed species at water troughs, whereas the margay (*L. wiedii*) was rarely detected. Felid activity at water troughs was mainly crepuscular and nocturnal. We found greater temporal similarity between jaguars (*Panthera onca*) and pumas than with ocelots. Overall, artificial water troughs are an essential management strategy for providing water to felids. Higher visitation rates occurred during the dry years. Temporal similarity between jaguars and pumas suggested that visits by larger species to water resources occur regardless of potential competitors, whereas ocelots seem to avoid them. As prolonged droughts become extreme, management interventions that provide water sources for felid species and other wildlife will be essential for the persistence of their populations in tropical ecosystems.

Los félidos desempeñan un papel fundamental en los ecosistemas como depredadores tope y mesodepredadores de numerosas especies de vertebrados. Por lo tanto, la disminución de sus poblaciones puede afectar directamente el funcionamiento del ecosistema. Recientemente, fenómenos meteorológicos extremos, como sequías prolongadas, han aumentado las amenazas a los bosques tropicales. La implementación de medidas de mitigación, como la provisión de bebederos artificiales, puede contribuir a aliviar la escasez de agua para la fauna silvestre durante la estación seca. Sin embargo, existe información limitada sobre la respuesta de las especies a dichas medidas en los ecosistemas tropicales, en particular en lo que respecta a las tasas de visitación y los patrones temporales de uso por parte de los félidos. Planteamos la hipótesis de que los abrevaderos se utilizarían con una frecuencia similar, independientemente de su disposición espacial. Además, esperamos que las especies más pequeñas presenten tasas de visita más bajas y un traslape temporal menor en comparación con las especies de félidos más grandes. Específicamente, evaluamos la variación espacial en las tasas de visita a bebederos por parte de félidos y el grado de traslape en los patrones de actividad para inferir una posible evitación interespecífica. Analizamos cinco años de datos de monitoreo con cámaras trampa recolectados en bebederos artificiales en la Reserva de la Biosfera de Calakmul, Campeche, México. Medimos las tasas de visita y comparamos las frecuencias entre bebederos. Medimos el traslape temporal para evaluar las similitudes en los patrones de actividad entre los félidos. El ocelote (*Leopardus pardalis*) y el puma (*Puma concolor*) fueron las especies observadas con mayor frecuencia en los bebederos, mientras que el tigrillo (*L. wiedii*) fue detectada con poca frecuencia. La actividad de los félidos en los bebederos fue principalmente crepuscular y nocturna. Encontramos mayor similitud temporal entre jaguares (*Panthera onca*) y pumas que con ocelotes. En general, los bebederos artificiales son una estrategia de manejo esencial para proporcionar agua a los félidos. Las tasas de visita más altas ocurrieron durante los años más secos. La mayor similitud temporal entre jaguares y pumas sugiere que las visitas de especies grandes a los recursos hídricos ocurren independientemente de los competidores potenciales, mientras que los ocelotes parecen evitar a ambos. A medida que las sequías se vuelven extremas, las estrategias de manejo que proporcionen fuentes de agua para las especies de félidos y otros animales silvestres serán esenciales para la persistencia de sus poblaciones en los ecosistemas tropicales.

Keywords: climate change, extreme droughts, *Panthera onca*, Mayan rainforest, neotropical forests, *Leopardus pardalis*, *Puma concolor.*

Introduction

Felid species play an important role in ecosystems by preying on a wide range of small to large-sized terrestrial vertebrates, thereby influencing community structure through trophic cascade effects (Gittleman *et al.* 2001; Lucherini *et al.* 2009; Terborgh *et al.* 2010; Bogoni *et al.* 2020). The reduction or extirpation of felid populations has direct consequences for ecosystem functioning (Ripple *et al.* 2014; Wallach *et al.* 2015; Bogoni *et al.* 2020). For example, jaguars (*Panthera onca*) and pumas (*Puma concolor*), as top predators in the tropical and subtropical ecosystems of the Neotropical region, exert both direct and indirect influences on communities of medium-to-large mammal species (Gómez-Ortiz and Monroy-Vilchis 2013; Hernández-SaintMartín *et al.* 2015; Ávila-Nájera *et al.* 2018a; Galindo-Aguilar *et al.* 2022). On the other hand, medium-sized felids, such as the jaguarundi *(Herpailurus yagouaroundi*), margay (*Leopardus wiedii*), and ocelot *(Leopardus pardalis*), primarily prey on small-sized terrestrial vertebrates (Macdonald and Loveridge 2010). However, in the absence of jaguars and pumas, ocelots have been observed to shift their diet toward medium-sized terrestrial vertebrates (Moreno *et al.* 2006; Flores-Martínez *et al.* 2022).

Felid species are particularly vulnerable to habitat loss, hunting, wildlife trafficking, and prey declines, all of which may contribute to reductions in their abundance and range (Gittleman *et al.* 2001; Ceballos and Oliva 2005; McCarthy *et al.* 2017; Quigley *et al.* 2017; Goodrich *et al.* 2022; Nicholson *et al.* 2024; Stein *et al.* 2024). In Mexico, the jaguar, ocelot, and margay are categorized as "Endangered", while the jaguarundi is classified as "Threatened" (DOF 2010). Water is essential for felids for several physiological processes, including digestion, metabolism, temperature regulation, and the disposal of metabolic waste (Bothma 2005). Felids can obtain water from food or natural deposits (Delgado-Martínez *et al.* 2023), and even by consuming fruits (Bothma 2005; Kitchener *et al.* 2010). Overall, water scarcity significantly affects the abundance, spatial behavior, and interactions within the carnivore guild, posing an additional threat to their persistence under climate change scenarios (Prugh *et al.* 2018; West *et al.* 2024).

Big cats, such as jaguars and pumas, influence medium-sized felid species through interference competition and intraguild predation, modulating how these species use resources (Ritchie and Johnson 2009). In several sites across South America, pumas have been shown to negatively affect ocelots, margays, and jaguarundis (Gil-Sánchez *et al.* 2021). Ocelots exhibited temporal segregation from pumas (Finnegan *et al.* 2021), while pumas tend to avoid jaguars (Contreras-Díaz *et al.* 2021; Galindo-Aguilar *et al.* 2022). In contrast, in parts of Central America, big cats do not appear to influence the habitat use or daily activity patterns of medium-sized felid species (Santos *et al.* 2019).

The Calakmul Biosphere Reserve (CBR), southeastern Mexico, is a protected area within the Mayan Forest, which is part of the largest rainforest in Mesoamerica (Hansen *et al.* 2008). Several studies have demonstrated that this region offers favorable ecological conditions for the presence of medium-to-large felid species, including the jaguar, puma, margay, ocelot, and jaguarundi (Rodríguez-Soto *et al.* 2011; Espinosa *et al.* 2018; Jędrzejewski *et al.* 2018; Lehnen and Lombardi 2023). While deforestation represents a major threat to the Maya Forest (Ferrer-Paris *et al.* 2019), irregular rainfall has recently emerged as an additional risk factor for wildlife (Reyna-Hurtado *et al.* 2010; Pérez-Cortez *et al.* 2012; Pérez-Flores *et al.* 2021; Sandoval-Serés *et al.* 2022). Water scarcity, driven by climate change, is becoming an increasingly significant threat to tropical ecosystems (Bennett *et al.* 2023).

In recent years, ponds (locally named *aguadas*) in the Maya Forest have been observed to dry up well before replenishment during the rainy season (Garcia Gil *et al.* 2002; Dobler-Morales 2018). In response, wildlife managers have implemented artificial water sources for use by wildlife (Rosenstock *et al.* 1999) as a strategy to mitigate the consequences of dry-season water shortages. This approach has been shown to support the short-term maintenance of wildlife populations (Epaphras *et al.* 2008; Mandujano and Hernández 2019). The availability of water bodies is a critical factor influencing the habitat use by jaguars and pumas (Crawshaw *et al.* 1991; De Angelo *et al.* 2011). When water becomes scarce, some mammals change their usual behavior (Pacifici *et al.* 2015), often concentrating their activity around remaining water sources (Redfern *et al.* 2003). Although water can be obtained through food consumption, this is not always sufficient to meet metabolic and thermoregulatory demands (Simpson *et al.* 2011). In consequence, water scarcity represents a considerable challenge for felid species. Moreover, the pressure exerted by limited water availability during periods of extreme drought can facilitate intraguild predation interactions (Perera-Romero *et al.* 2021).

In the CBR, artificial water troughs have been installed and are actively managed to provide wildlife with access to water during the dry season. However, their influence on felid species remains understudied, including visitation rates to these resources, activity patterns, the impact of resource location on visitation, and the potential for temporal segregation driven by interspecific competition (Rosenstock *et al.* 1999). When ecological and morphologically similar species that are also phylogenetically related coexist in the same environment, interspecific competition is likely to arise (Begon *et al.* 1988; Hunter and Caro 2008). One common outcome of such competition is habitat segregation (Cruz *et al.* 2018). It has been found that dominant species, larger in size, exclude subordinate species from preferred areas through interference competition (Steinmetz *et al.* 2013). Consequently, smaller or subordinate species may be displaced to suboptimal habitats, which often have reduced resource availability or great anthropogenic

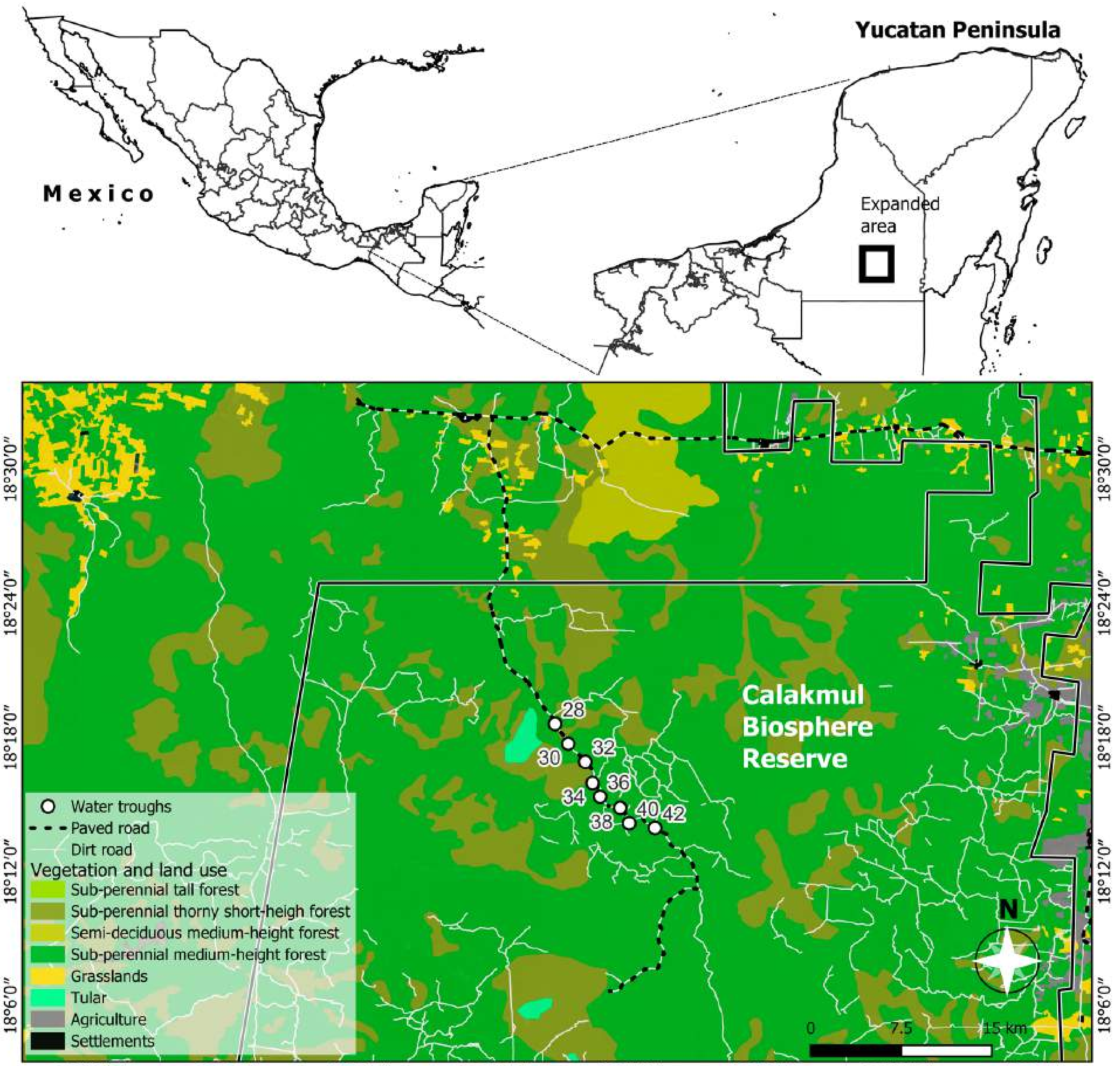

Figure 1. Location of the artificial water trough stations in the Calakmul Biosphere Reserve (CBR) in southeastern Mexico.

presence (Tannerfeldt *et al.* 2002). Therefore, competition over scarce resources, such as water, can intensify intraguild competition.

In this study, we analyzed five years of monitoring on the use of artificial water troughs by the felid species in the CBR**.** We hypothesized that felids would use water troughs with similar frequency regardless of their spatial arrangement. Also, we hypothesized that medium-sized felids (ocelot, jaguarundi, and margay) would exhibit lower temporal overlap and reduced visitation rates compared to large-sized felids (jaguar and puma), as a strategy to avoid intraguild predation (De Oliveira and Pereira 2014). In specific, we expected smaller temporal overlap between pairs of felids with the highest morphological similarity (puma-jaguar and puma-ocelot) than the more morphologically distinct jaguar and ocelot (Herrera *et al.* 2018). Our main objective was to evaluate the spatial and temporal variation in the visitation rates and the degree of temporal activity overlap among felid species to infer patterns of interspecific avoidance.

Materials and methods

Study area. The study was conducted at the CBR located in the municipality of Calakmul, Campeche, Mexico (18°40′07.7″N, 89°12′34.3″W). The area of the CBR is 7,289.08 km^2 of continuous protected tropical forest (Morales and Magaña

2001; Figure 1). The region is characterized by a diversity of plant associations, including medium sub-perennial medium-height forests, sub-perennial thorny short-height forests, short-height flooded forests, semi-deciduous medium-height forests, tular, and secondary vegetation (INEGI 2021; Figure 1). The climate is tropical, warm, humid, and warm sub-humid with rainfall in summer (García and CONABIO, 2008). The average annual temperature ranges between 24 and 28 °C (Vester *et al.* 2007), the maximum temperature is 36 °C during May and June (Hernández *et al.* 2018), but in the last years temperatures above 40 °C have been reached (Comisión Nacional del Agua; smn.conagua.gob.mx). During January occurs the minimum temperature averages 18 °C (Hernández *et al.* 2018). Average annual precipitation is highly variable, ranging from 900 mm to 1,400 mm. The dry season is from November to May, and the rainy season is from June to October (García and CONABIO 2008; Mardero *et al.* 2012; CONANP 2023).

Data collection. From 2019 to 2023, we set up eight artificial water trough stations along the primary access route of the CBR, separated by a minimum distance of 2 km (Contreras-Moreno *et al.* 2024), at an average distance of 100 m from the highway. The troughs were arranged in a latitudinal sequence reflecting a climatic gradient (Figure 1). Troughs located at higher latitudes have higher average annual temperatures and less precipitation than those located at lower latitudes; from northern to southern troughs, annual mean temperatures, calculated with interpolated data from climatic stations, are 25.3° C and 24.7° C; and annual precipitation is 1,156 mm and 1,204 mm in the same troughs (Cuervo-Robayo *et al.* 2014). The water troughs were made from plastic (Rotoplas®) with a capacity of 300 L and were supplied with water every two weeks. A simple camera-trapping station was attached to each artificial water trough. Eight Bushnell HD119876c (Trophy Cam; Outdoor Operations LLC. Los Angeles, USA), Browning Strike force 850 (Strike force; Browning Trail Cameras, Alabama, USA), or Cuddeback IR-20 (Cuddeback IR; Non-Typical Inc., Wisconsin, USA) cameras were used, which were attached to trees at approximately 50 cm above the ground, facing the troughs at approximately five meters to allow a complete view around it (Contreras-Moreno *et al.* 2024). The cameras were programmed to take three consecutive photos with a five-second delay period and remained active for 24 hours. Cameras worked during the first six months of each year, corresponding to the dry season. Revisions were conducted every two months to change batteries and replace camera memory cards. The records obtained were stored in a database using the R package of the R Studio program, version 2024.04.2+764, and a database was constructed with these data using the camtrapR interface in RStudio software 12.0 (Niedballa *et al.* 2020).

Data analysis. To evaluate the use of artificial water troughs per year, the visitation rate (*VR*) was calculated for each of the species of felids as $VR=N/SE*100$ trap-days. Where: N = number of independent records, SE = sampling effort (number of days the cameras were in operation), and 100 trap-days as a standard unit (Mandujano and Hernández 2019). A 1-hr interval was used to account for independent records (Briones-Salas *et al.* 2016). A nonparametric Kruskal-Wallis test was used to determine whether there were differences in the visitation rates between species during sampling years and between artificial water deposits. A Mann-Whitney *post hoc* test was then used to determine which cases differed.

Felid activity at the drinking troughs was described with kernel density curves (Ridout and Linkie 2009) using the times stamped on the photographs. Subsequently, the level of overlap (Δ) between species pairs and 95% confidence intervals were examined using a Bootstrap with 1,000 iterations. We compared the statistical differences between the hours of greatest activity with a Kernel density analysis. Analyses were done with packages activity (Rowcliffe 2016) and overlap (Meredith and Ridout 2014) in R 3.4.0 (R Core Team 2017).

Results

A total sampling effort of 5,465 trap-days included 588 grouped records of four felid species at artificial water troughs. Jaguar, puma, ocelot, and margay were recorded (Figure 2), whereas no records of jaguarundi were obtained. The ocelot showed the highest visitation rate throughout the five years of monitoring (4.57 records/100 trap-days), followed by the puma (3.84 records/100 trap-days) and the jaguar (2.18 records/100 trap-days). The margay was rarely observed (0.16 records/100 trap-days) (Table 1).

The visitation rate was variable over the years. Considering all the felid species, the visitation rate was higher in 2023 (*VR* = 20.10), 2022 (*VR* = 17.96), and 2019 (*VR* = 12.25). In 2021, felid species exhibited a lower rate (*VR*

Table 1. Frequency of independent records (FIR) and visitation rate (*VR*) of felid species during five sampling periods (2019-2023) in the Calakmul Biosphere Reserve (CBR) in southeastern Mexico.

	2019 FIR (*VR*)		**2020** FIR (*VR*)		**2021** FIR (*VR*)		**2022** FIR (*VR*)		**2023** FIR (*VR*)		**Total in five years** FIR (*VR*)	
Jaguar	85	3.00	18	1.32	0	0.00	4	1.07	12	2.87	119	2.18
Puma	115	4.06	24	1.77	9	1.87	34	9.11	28	6.67	210	3.84
Ocelot	146	5.15	23	1.69	10	2.08	29	7.77	42	10.05	250	4.57
Margay	1	0.03	6	0.04	0	0.00	0	0.00	2	0.48	9	0.16

Figure 2. Felid species recorded visiting artificial water troughs in the Calakmul Biosphere Reserve, Mexico. a) Jaguar (*Panthera onca*), b) Puma (*Puma concolor*), c) Ocelot (*Leopardus pardalis*), d) Margay (*Leopardus wiedii*).

= 3.96). The visitation rate of jaguars decreased between 2019 and 2020, whereas in 2021 it was not detected, and the visitation rate in 2023 was similar to 2019. Puma and ocelot also experienced a reduction in visitation rates between 2019 and 2021, but their rates increased in 2022 and 2023 (Table 1).

The jaguar exhibited significantly variable records at stations between km 32 and km 42; in contrast, the puma showed near-homogeneous records across all eight stations, and the ocelot displayed a similar trend but at stations km 36 and km 32. We found a weak significant difference between felid visitation rates at artificial water troughs (*K-W* test; $H = 6.06$, $p = 0.047$); only the ocelot had differences among water troughs ($U = 11.5$; $p = 0.035$), but we did not find differences among years (*K-W* test; $H = 6.24$, $p = 0.180$).

Felids were detected visiting the drinking troughs mainly during nocturnal hours.

Ocelots were observed mainly between 18:00-06:00 h, with a principal peak before midnight.

Jaguars and pumas exhibited a most extended activity throughout the day, with a peak around 19:00 hr and another at 06:00 hr (Figure 3).

Lastly, a high degree of use overlap was observed in felids at the artificial water trough stations over the five-year study period, with more than 70% of the recorded observations, indicating the presence of multiple species of felids at the same location. The highest overlap was observed between jaguar and puma ($\Delta = 0.89$; 95% CI = 0.79-0.93), followed by puma and ocelot ($\Delta = 0.76$; 95% CI = 0.65-0.80), and jaguar and ocelot ($\Delta = 0.72$; 95% CI = 0.62 to 0.76) (Figure 3).

Discussion

This study showed that felid species frequently used artificial water troughs in the CBR. Overall, we found that different felid species did not visit all water troughs with equal frequency, and no consistent pattern of use was observed among the species. For instance, jaguars were recorded more frequently at northern water troughs than

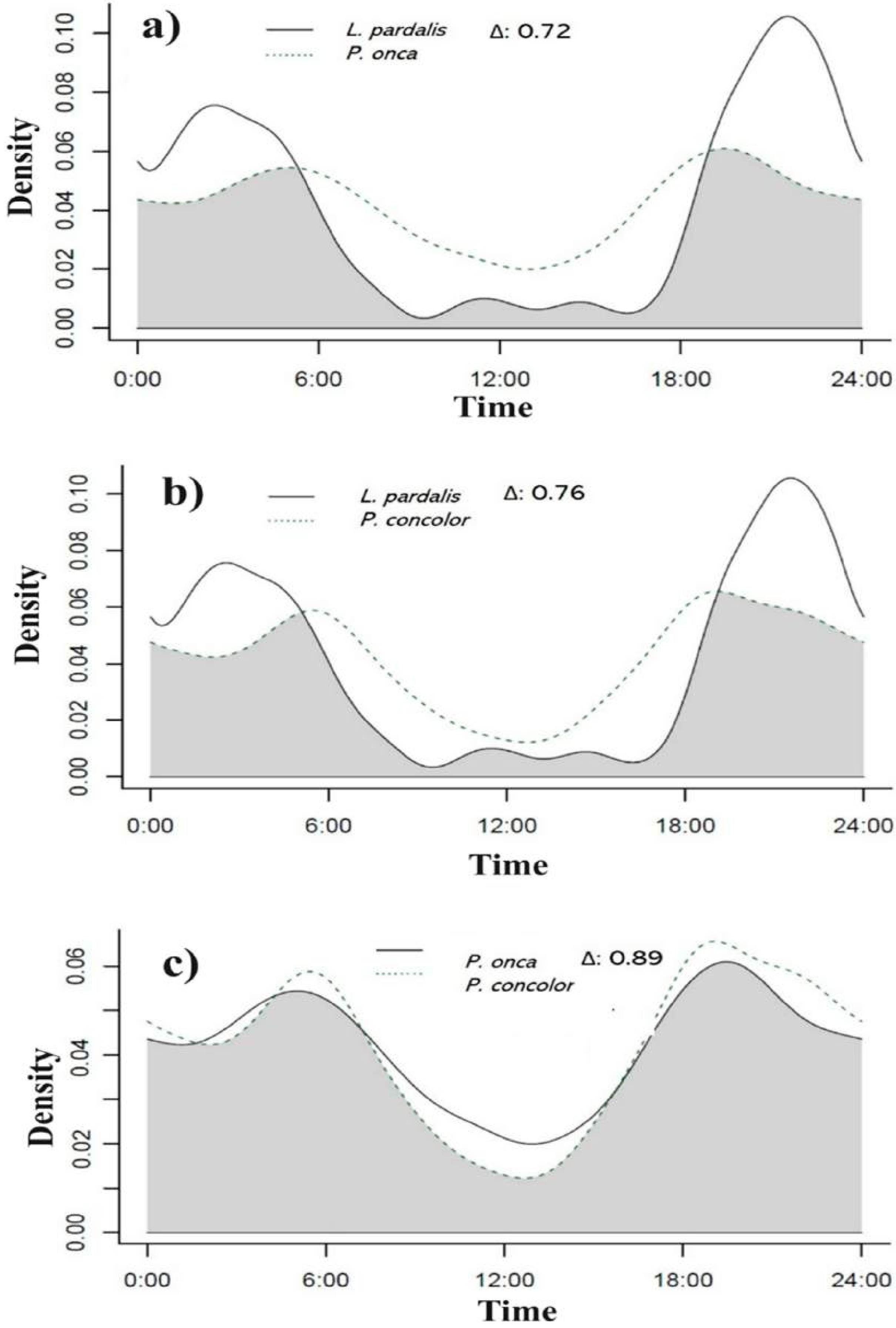

Figure 3. Temporal overlaps among felid species visiting artificial water troughs in the in the Calakmul Biosphere Reserve, Mexico. a) Ocelot and jaguar, b) ocelot and puma, and c) jaguar and puma.

pumas and ocelots. In contrast, margays were uncommon, and jaguarundis were not recorded. Compared to visitation rates at natural water bodies, our results show higher visitation rates for both jaguars and ocelots (2.18 and 4.57 grouped records/100 camera-trap days, respectively) than those reported in surrounding areas (1.31 and 2.3 grouped records/100 camera-trap days, respectively; Gaitán *et al.* 2021; Sandoval-Serés *et al.* 2022). Therefore, our results reveal the importance of water troughs as mitigation management to reduce hydric stress in felid species.

The highest visitation rate to water troughs was recorded for the ocelot, with the highest activity at night. It has been documented that ocelots are active throughout the day, with an increased activity during nocturnal hours (Sunquist 1991; Maffei *et al.* 2005; Di Bitetti *et al.* 2006). However, in certain localities, ocelot activity patterns are strongly influenced by the behavior of their prey (Murray and Gadner 1997; De Villa *et al.* 2002).

Pumas had the second-highest visitation rate at water troughs, followed by jaguars. Both species were active throughout the day but exhibited a bimodal activity pattern, with peaks at dawn and dusk. The timing of the puma visits to the water troughs was consistent with activity patterns recorded by camera traps in forested areas of the Yucatan Peninsula (Ávila-Nájera *et al.* 2018b; Argudín-Violante *et al.* 2023). Estrada (2008) and Moreira *et al.* (2009) also recorded a crepuscular and nocturnal activity pattern for puma in both the CBR and in the Maya Biosphere Reserve. Also, the activity pattern observed for jaguars in our study was similar to that found in the Maya Forest, with two peaks but higher activity after 20:00 hr (Estrada 2008). These findings align with our results, indicating that both pumas and jaguars exhibited crepuscular and nocturnal activity around water troughs. This suggests that these large-sized felids did not alter their temporal activity in response to the artificial water troughs but rather incorporated their use into their existing behavioral patterns.

The margay had the lowest visitation rate among the felid species, a result that may be attributed to several non-exclusive factors. First, the margay is primarily an arboreal species that prefers to hunt in the canopy and avoid unfavorable encounters with potential competitors (De Oliveira and Pereira 2014; De Oliveira *et al.* 2015). Second, it prefers habitats with dense shrubs and tree cover and is rarely found deep in areas with closed canopy, although it has also been recorded in open environments with some degree of disturbance (Paviolo *et al.* 2015). Third, the presence of competitors, since it has been found that margay densities increase in the absence of ocelots (Prugh *et al.* 2009; Jachowski *et al.* 2020), suggesting that ocelots may directly or indirectly suppress margay presence (Macdonald and Loveridge 2010). These factors provide explanations for the low number of observations of the margay at water troughs, as well as their comparatively low records at natural water sources compared to those of other felids (e.g., in rock pools and tree holes; Delgado-Martínez *et al.* 2023).

The overlap in activity patterns at the artificial water troughs varied among the pair of felids studied. Contrary to expectations of a smaller temporal overlap between pairs of felids with the highest morphological similarity (puma-jaguar and puma-ocelot) than the more morphologically distinct (jaguar and ocelot; Herrera *et al.* 2018), we found that jaguars and pumas exhibited high overlap in their activity patterns ($\Delta = 0.89$). This suggests that both species shared similar temporal use of artificial water troughs, a result that contrasts with expectations and with previous findings in forested areas of Calakmul ($\Delta = 0.75$; Argudín-Violante *et al.* 2023). Whereas high temporal overlap has also been observed in community-managed conservation areas in southern Mexico (Galindo-Aguilar *et al.* 2022), lower overlaps ($\Delta = 51\text{-}75$) have been reported in the northeastern part of the Yucatan peninsula (Ávila-Nájera *et al.* 2016). The necessity of water as a limiting resource likely contributes to the increased temporal overlap observed in this study.

The ocelots appear to be slightly influenced by the presence of large-sized species of felids (Macdonald and Loveridge 2010; Santos *et al.* 2019). However, the lowest overlap was observed between the jaguars and ocelots ($\Delta = 0.72$). Similarly, the temporal overlap between ocelots and pumas at water troughs was moderate ($\Delta = 0.76$). This discrepancy may be attributed to differences in peak activity times, which have been interpreted as a strategy by ocelots to avoid intraguild predation by jaguars (Fedriani *et al.* 2000; Lira-Torres and Briones-Salas 2012). Our overlap estimate between jaguar and ocelot was similar to observations at waterholes in Guatemala ($\Delta = 0.65\text{-}0.70$), where direct evidence of jaguar predation on ocelots has been recorded (Perera-Romero *et al.* 2021). It is plausible that our observed temporal overlap of ocelot with larger felids is a strategy to avoid encounters and possible predation.

The study of the mechanisms of coexistence among large-sized felids has primarily focused on three dimensions: diet, spacial use, and temporal activity (Ávila-Nájera *et al.* 2016). Among these, temporal segregation is generally considered less influential than spatial or trophic segregation in explaining coexistence (Santos *et al.* 2019; Argudín-Violante *et al.* 2023). This trend can be attributed to species-specific constraints imposed by biological rhythms and the physiological and ecological costs associated with shifting from one circadian cycle to another (Kronfeld-Schor and Dayan 2003). Factors such as density, seasonality, prey type, and prey density play a significant role in mediating the predator interaction and influence the degree of tolerance for temporal overlap (Ávila-Nájera *et al.* 2016). Predators often exhibit a higher temporal overlap and spatial association with key prey species than with competitors. In such circumstances, the coexistence of predator species may be influenced by the selection of prey with varying activity patterns (Hernández-Sánchez and Santos-Moreno 2020). The absence of strong segregation has been observed previously (Santos *et al.* 2019), but fine-scale segregation has been proposed as a spatiotemporal strategy facilitating coexistence between these felids (Galindo-Aguilar *et al.* 2022).

The use of both natural and artificial water sources by mammals highlights the value of water troughs as a management strategy in arid climates and tropical regions with pronounced seasonality, such as Calakmul (Contreras-Moreno *et al.* 2024; Sánchez-Pinzón *et al.* 2024). In general, several mammal species have shown a positive correlation between their occupancy and the presence of permanent water sources (Reyna-Hurtado *et al.* 2009; O'Farrill *et al.* 2014; Rich *et al.* 2016). Although occupancy was not evaluated because small sample size, we found high visitation use by part of felids compared to natural water sources (Gaitán *et al.* 2021; Sandoval-Serés *et al.* 2022). The importance of water is critical, as prolonged periods of low precipitation can lead to dehydration-related mortality in large-sized species such as jaguars, tapirs (*Tapirus bairdii*), and white-lipped peccaries (*Tayassu pecari*) (Gandiwa *et al.* 2016). Thus, the use of artificial water troughs represents a viable approach to ensuring water availability during the dry season for predators and other wildlife (Mandujano and Hernández 2019), while addressing the growing challenge of water scarcity (Epaphras *et al.* 2008). Accordingly, the installation of artificial water troughs emerges as a valuable strategy for mitigating the adverse effects of droughts on wildlife populations.

The high number of visits to artificial troughs by felid species in 2019, compared to the subsequent two-year period, can be attributed to the extreme drought that occurred in that year. This drought caused the widespread desiccation of natural water bodies and led to several critical incidents, including mortality events of tapirs due to dehydration, which was classified as an "environmental crisis" in the region (Contreras-Moreno 2020). The situation changed in 2020, following two major climatic events that replenished natural water bodies. A concurred decrease in the number of species of felids visiting artificial water troughs was observed, mirroring similar patterns recorded for ungulate species such as white-tailed deer (*Odocoileus virginianus*) and brockets (*Mazama temama* and *Mazama pandora*), and tapirs during the same period in the Selva Maya (Contreras-Moreno *et al.* 2024). These findings underscore the functional importance of artificial water sources in supporting wildlife during the dry season.

Our study demonstrated that felid species present in the CBR actively visited artificial water troughs. These findings are aligned with previous reports of other wildlife species using such water reservoirs, particularly during periods of extreme droughts. Although the implementation of artificial water provision has been widely recognized as a valuable management strategy, several concerns have been proposed. These include pervasive changes in abundances and diversity, risk of intoxication due to biological and non-natural contaminants, modifications in predator-prey dynamics, and the facilitation of disease transmission (Rosenstock *et al.* 1999; Simpson *et al.* 2011). However, many of these potential negative impacts are speculative (Simpson *et al.* 2011); others, such as changes in diversity and abundance, have not been empirically supported (Kluever *et al.* 2016).

The impact of climate change, particularly the severe shortage of natural water supplies, as the *aguadas* in the region, poses a significant threat to wildlife. Managing practices, such as the installation of artificial water troughs, must be implemented on a broader scale to effectively address this challenge. Further research is needed to assess variations in wildlife demand for artificial water sources across different ecosystems, as well as to evaluate any potential negative effects associated with their use in Neotropical forests.

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Molecular sexing and sex ratio of the neotropical otter (*Lontra longicaudis annectens)* using non-invasive samples from the Porce III Reservoir, Colombia

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The Neotropical otter is an important mammal in aquatic ecosystems due to its trophic position as a top predator, therefore is a valuable medium and long term bio monitor of the environment. Due to its crepuscular-nocturnal behavior, studies on this species primarily rely on non-invasive methods, such as the analysis of feces and anal-glands jellies, which allow for various ecological and population inferences. With recent advances in molecular techniques, these non-invasive samples have greatly contributed to the understanding of wild populations. Among the relevant population characteristics that might be obtained are individual identification and molecular sexing, which provide key information about demography, kinship relations, permanence, and sex-biased dispersal. This study implemented a molecular sexing methodology using DNA extracted from fresh feces and mucous of Neotropical otters, with the aim of determining the sex ratio in the otter population in a reservoir in northeastern Antioquia, Colombia. The study was conducted in the Porce III reservoir and its main tributaries. Fresh feces and anal-gland jelly from Neotropical otters were collected, DNA was extracted for genotyping. A total of 145 successfully genotyped samples were further amplified using two DNA segments: one homologous to the Y chromosome and another homologous to both sex chromosomes. The amplification products were separated on 3% agarose gels. Sex was successfully determined in 91 samples, corresponding to 40 individuals: 18 males and 22 females, with an average ratio of 2 females for every 1.4 males. A higher presence of females was found in the reservoir, while more males were detected in the tributaries. Females showed a higher recapture rate and lower mobility compared to males. The methodology used proved to be efficient for sex determination without the need to capture individuals. The results are consistent with previous studies suggesting greater female permanence. The information obtained is crucial for the management and conservation of the species, particularly in areas impacted by human activities.

La nutria neotropical es un mamífero importante en los ecosistemas acuáticos por su posición trófica como depredador tope que lo hace un buen biomonitor a mediano y largo plazo del medio ambiente. Debido a su comportamiento crepuscular-nocturno, los estudios sobre esta especie se basan principalmente en métodos no invasivos, como el análisis de heces y mucosidades, que permiten realizar diversas inferencias ecológicas y poblacionales. Con los avances recientes en las técnicas moleculares, estas muestras no invasivas han incrementado su contribución al conocimiento de las poblaciones. Entre los aspectos poblacionales relevantes que se pueden obtener se encuentran la identificación de individuos y el sexado molecular, que proporcionan información clave sobre la demografía, las relaciones de parentesco, la permanencia y la dispersión sesgada por sexo. El presente estudio implementó una metodología de sexado molecular con ADN obtenido a partir de heces y mucosidades de las glándulas anales frescas de nutria neotropical, con el objetivo de establecer la proporción de sexos en la población de nutrias de un embalse del nordeste de Antioquia, Colombia. El estudio se llevó a cabo en el embalse Porce III y sus principales afluentes. Se colectaron heces y mucosidades frescas de las glándulas anales de nutria neotropical, de las cuales se extrajo ADN para su genotipificación. Las muestras efectivas para la genotipificación fueron 145, en las cuales se amplificaron simultáneamente dos secuencias de ADN, una ligada al cromosoma Y, y otra homóloga a ambos cromosomas sexuales. Los productos de amplificación se separaron en geles de agarosa al 3%. Se logró determinar el sexo en 91 muestras, correspondientes a 40 individuos: 18 machos y 22 hembras, con una proporción promedio de 2 hembras por cada 1.4 machos. Se encontró mayor presencia de hembras en el embalse y mayor cantidad de machos en los afluentes. Las hembras presentaron una mayor frecuencia de recapturas y menor movilidad en comparación con los machos. La metodología empleada resultó eficiente para la determinación del sexo sin necesidad de capturar a los individuos. Los resultados coinciden con estudios previos que sugieren una mayor permanencia de las hembras. La información obtenida es fundamental para la gestión y conservación de la especie, especialmente en áreas afectadas por la actividad humana.

Keywords: non-invasive sampling, río Porce, sex ratios, Antioquia, SRY, ZFX/ZFY.

Introduction

Knowing the sex of individuals in wild populations allows for the evaluation of important ecological aspects such as sex ratio, type of reproductive strategy, individual movement or permanence, and home range of the different sexes within the population, which could imply territorial and resource monopolization (Griffiths *et al.* 1996; Gallo-Reynoso et al. 2013; Ferreira et al. 2018). Additionally, it provides a deeper understanding of population dynamics and structure, which is crucial for making management and conservation decisions, especially in isolated or threatened populations (Eggert and Guyétant 2003; Liu *et al.* 2014; Ancona *et al.* 2017), as is the case with some populations of the neotropical otter *Lontra longicaudis*.

This species is a semiaquatic mammal distributed from Mexico to Argentina and Uruguay (Rheingantz *et al.* 2022). Despite its wide distribution, characteristics of its populations, such as sex ratios, are poorly known. This parameter, like other ecological traits, may be especially relevant to support taxonomic differentiation, as there is current discussion regarding whether trans-Andean neotropical otters should be treated as a distinct species, *L. annectens* (de Ferran *et al.* 2024), a specific name we will use onwards.

In Colombia, *L. annectens* is distributed in the northwestern part of the Eastern Cordillera across all biogeographic regions, from lowlands to altitudes of 3,000 meters above sea level (Solari *et al.* 2013; Andrade-Ponce and Angarita-Sierra 2017). The species inhabits freshwater or brackish water bodies and feeds primarily on fish (Rheingantz *et al.* 2022). Its reproduction depends on the availability of food, shelter, and environmental conditions. Females take care of their offspring, extending up to the first year (Gallo-Reynoso 1989). In *Lontra longicaudis*, males have been reported to exhibit greater movement, in contrast to the philopatric behavior of females (Trinca et al. 2013). However, as mentioned earlier, this behavior has not yet been well characterized for trans-Andean otters.

Lontra longicaudis is classified as Near Threatened (NT) globally (Rheingantz *et al.* 2022) and as Vulnerable in Colombia (Ministerio de Medio Ambiente y Desarrollo sostenible MADS, 2024). Given its crepuscular-nocturnal activity patterns (Gallo-Reynoso et al. 2019) and its threatened conservation status, the use of non-invasive genetic methods is especially valuable for studying natural populations. These methods provide critical insights into population characteristics such as size, genetic variability, and sex determination, enabling inferences about the species' biology, behavior, and ecology (Ortega *et al.* 2012; Trinca and Eizirik 2012).

For otters (Lutrinae), sex identification using DNA extracted from feces has been approached mainly through the amplification of sex markers, such as the SRY and ZFX/ZFY genes. The SRY gene is a conserved sequence located on the Y chromosome and is key to sex determination in mammals (Graves 1998). In a PCR, positive amplification of this sequence indicates the presence of a male, while the absence of amplification would suggest a female. However, the lack of SRY amplification can also be due to the degradation or loss of the sequence in the sample, as well as procedural errors (Taberlet et al. 1996), which could lead to overestimating the proportion of females. To control for PCR sex determination failures, some authors have used the SRY marker in conjunction with other nuclear or mitochondrial sequences common to both sexes (Dallas *et al.* 2000, 2003; Huang *et al.* 2005; Hájková *et al.* 2009; Trinca and Eizirik 2012; Quaglietta *et al.* 2013; Lampa *et al.* 2015).

To reduce the risk of errors in sex assignment by tracking only one sex chromosome, several authors have implemented homologous segments from both the X and Y chromosomes in other mammals, such as the "Zinc-finger" marker (ZFX and ZFY) (Mucci and Randi 2007; Mullins *et al.* 2010; Park *et al.* 2011; Brzeski *et al.* 2013; O'Neill *et al.* 2013; Lerone *et al.* 2014; Vergara et al. 2014), a strategy that has also been applied to *Lontra longicaudis* (Ortega *et al.* 2012). This method requires subsequent digestion of the PCR products with a restriction enzyme capable of distinguishing between the amplified fragment from the X chromosome (ZFX) and the amplified fragment from the Y chromosome (ZFY) (Mucci and Randi 2007). Mowry *et al.* (2011) proposed a strategy involving the co-amplification of the ZFX/ZFY segment with the SRY marker, allowing them to identify males and females of *Lontra canadensis* without the need for restriction enzymes.

The otters in the Porce III reservoir, located in Antioquia, Colombia, form a population exposed to various environmental pressures, such as habitat loss and fragmentation, which limit their movement along the Porce River basin and may be affecting population dynamics and gene flow (Mason and Macdonald 1990; Rheingantz *et al.* 2022). This population is also exposed to multiple chemical and organic pollutants that enter the basin from industrial, domestic, and wastewater sources from 10 municipalities in the Valle de Aburrá, as well as 18 others within the Porce River basin, which together host approximately 4 million human inhabitants. Another prevalent contaminant is mercury, derived from gold mining activities around the reservoir (Gómez 2009). The high exposure to this mix of contaminants poses a long-term threat to the population's survival, as these mixtures have been shown to cause DNA mutations, hormonal disruptions, reduced fertility, and changes in sexual behavior in individuals (Storelli *et al.* 2002).

This situation makes it necessary to study the population status of the neotropical otter in the Porce River to establish effective conservation strategies. In this regard, molecular sexing can provide important information about its demographics (sex ratios), reproductive behavior, and whether there is sex-biased dispersal (Tucker et al. 2017; Hrovatin and Kunej 2018; Mengüllüoğlu *et al.* 2019). Therefore, the aim of this research is to shed light on some population aspects of the neotropical otter present in the area of influence of the Porce III reservoir (Antioquia-Colombia), through a sexing methodology using DNA obtained from feces previously individualized by genotyping with 9 microsatellites, using the SRY and ZFX/ZFY markers.

Methodology

Study Area. The Porce III reservoir is located in the northeastern part of the department of Antioquia, Colombia, approximately 147 km from the city of Medellín. It is part of a hydroelectric generation chain that includes the upstream Porce II reservoir and the Guadalupe-Troneras complex and is primarily fed by the Porce and Guadalupe rivers. This reservoir has been in operation since 2010, has a capacity of 169 million cubic meters, and floods an area of 461 hectares, with a dam height of 151 meters and a maximum flood level of 680 meters above sea level. The Porce River, the main tributary of the reservoir, receives wastewater of industrial and domestic origin from all the municipalities of the Valle de Aburrá, including rural waste from its basin (Hurtado-Alarcón *et al.*, 2007).

Fieldwork. It was conducted between 2012 and 2022, with three field trips per year between 2012 and 2014 and four trips between 2015 and 2022, each trip lasting eight days. Four sectors were selected for sampling, including both the Porce III reservoir and three adjacent lotic systems: the Porce River before and after the reservoir, and the Guadalupe River (Figure 1). Along the banks of each lotic system, two transects of 1 km in length and variable width were traversed. The reservoir was surveyed along both margins using a motorboat at an average speed of 10 km/h along the navigable perimeter, covering approximately 24 km. During each sampling campaign, two surveys per sector were conducted. Fresh otter feces and anal-glands jelly were collected in properly labeled vials with 99% ethanol. The collection sites were georeferenced using GPS, and the samples were kept at -20°C until processing.

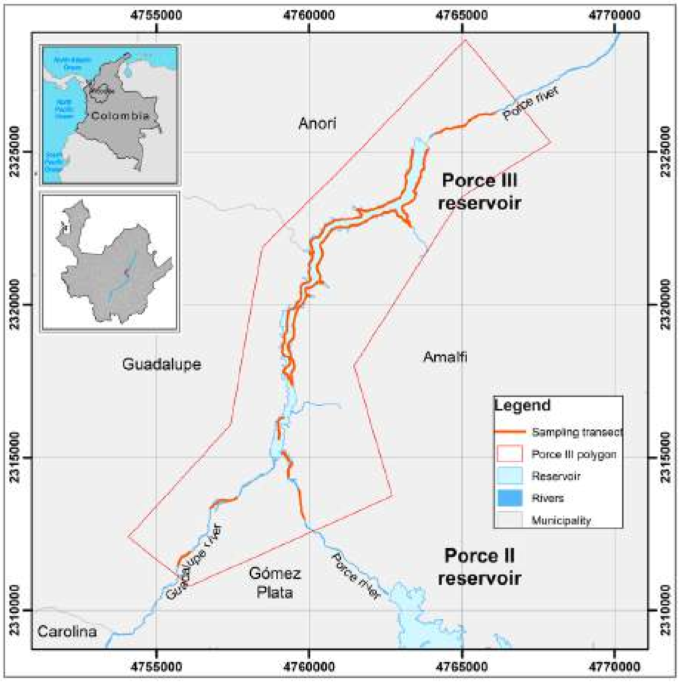

Figure 1. Study area and transects: Guadalupe River, Porce River before the Porce III reservoir and Porce River, past the reservoir.

Laboratory Work. Given that the samples were from impure sources such as feces and anal-gland jelly, a validation of the sexing method was performed using a tissue sample from a male otter and a blood sample from a female otter, whose DNA was extracted using the ZR Genomic DNA Tissue Miniprep Kit - Cat #D3051 (Zymo Research, CA, US), a specific extraction kit for tissues. Subsequently, for the extraction of total DNA from fecal samples, was used the ZymoBIOMICS DNA Miniprep Kit - Cat #D4300 (Zymo Research, CA, US), a specific extraction kit for feces. In both cases, the manufacturer's established protocol was followed.

For the total DNA extracted from otter feces, the mitochondrial DNA (mtDNA) control region was first amplified and sequenced, and polymorphisms were assigned. Then, it was determined whether the samples corresponded to the same individual through genotyping with nine microsatellite markers (Aristizábal-Duque et al. 2025, unpublished data): Lut435, Lut453, Lut715, Rio11, Lut701, 04OT17-1, RIO19, Lolo13, and Lolo29 (Dallas *et al.* 1999; Huang *et al.* 2005; Latorre-Cardenas *et al.* 2020). Both methodologies were validated with otter tissue and blood samples.

To identify the sex of the otters from fecal samples, 145 samples were selected, where the Y chromosome-specific SRY marker and the ZFX/ZFY markers present on both sex chromosomes were used. Initially, a multiplex PCR was performed where the SRY gene (Dallas *et al.* 2000) and the ZFX/ZFY gene (Mucci and Randi 2007) were simultaneously amplified (Table 1). The PCR mix included 5 µL of Phusion Flash High-Fidelity PCR Master Mix (2X) (Phusion Flash II DNA polymerase - Thermo Scientific™ CA, US), 2 µL of DNA template from fecal samples or 1 µL in the case of tissue samples, 0.5 µL of MgCl2 (0.25 mM), 0.5 µL of each primer (10 µM), 0.039 µL of BSA (20 mg/mL), and mQ H2O to adjust the final volume to 10 µL. Amplification was carried out in a MultiGene™ OptiMax thermal cycler (Labnet™) under the following conditions: one cycle at 98°C for 10 seconds, followed by 30 cycles at 98°C for 1 second, 60°C for 5 seconds, 72°C for 10 seconds, and finally, one cycle at 72°C for 1 minute for the final extension. Each amplification included a negative PCR control consisting of the reaction mix without a DNA template. PCR products were verified

Table 1. Molecular markers used for the molecular sexing of the neotropical otter.

Gene	Primer	Sequence (5'-3')
SRY	Lut-SRY (Forward)	GAATCCCCAAATGCAAAACTC
	Lut-SRY R (Reverse)	GGCTTCTGTAAGCATTTTCCAC
ZFX/ZFY	P1-5EZ (Forward)	ATAATCACATGGAGAGCCACAAGCT
	Zfxyrb (Reverse)	TTGTTCAGCTGTCTCATATTCACA

with electrophoresis on 3% agarose gels stained with Gel Red (Biotium, Hayward, CA, USA), and a DNA size marker ranging from 50 bp to 1000 bp (GeneRuler 50 bp DNA Ladder - Cat #SM0371 - Thermo Scientific™ CA, US) was used. Males were distinguished by the presence of two bands of 70 and 180 bp, corresponding to SRY and ZFX/ZFY, respectively, while females showed only a 180 bp band from the ZFX gene. Because DNA was obtained from fecal samples, three to four independent amplifications were performed (depending on the sample volume available) to validate the consistency of the PCR products (Taberlet et al. 1996). To prevent the presence of human-associated Y chromosome sequences, samples were not collected or processed by human males. Extractions, amplifications, and genotyping were performed at the laboratory GAIA at the Universidad de Antioquia.

Due to the extended sampling period, sex ratios were calculated annually using data from the years 2014, 2015, 2018, 2019, and 2022, which had the highest number of samples and individuals. The ratio was determined as the relationship between the number of individuals of each sex (females and males).

Results

The tissue and blood samples from a female and a male otter were successfully sexed using the methodology implemented in this research. As expected, since females have two X chromosomes, only a 180 bp segment corresponding to the ZFX gene was amplified. Males, having one X and one Y chromosome, produced two bands: a 70 bp band derived from the SRY gene, and a 180 bp band derived from the ZFX/ZFY segment, which is present in both the Y and X chromosomes (Figure 2). These results were consistent across all rounds of amplification evaluated.

Of the 145 samples selected for analysis, 91 were successfully sexed with consistent results in at least three independent rounds of amplification, corresponding to 40 different individuals. This means that 51 samples were recaptures, and the sex assigned to the same individual was consistent across all recaptures, further validating the effectiveness of the method.

Of the 40 individuals sexed in the study area over the 10 years of sampling, 22 were females and 18 were males. By sector, the records are as follows: at the Porce III reservoir, 14 females and 9 males were registered, with one individual of each sex recaptured in the Porce River upstream of the reservoir. In the Porce River upstream of the reservoir, 4 females and 5 males were recorded, including those shared with the reservoir. In the Porce river downstream of the reservoir, there were 4 females and 2 males, and in the Guadalupe River, there were 2 females and 2 males.

The average female-to-male ratio over the analyzed years was 2:1.4 (Table 2). However, when analyzed by system, the rivers had a lower average female-to-male ratio of 1.7:2.4, whereas the Porce III reservoir had a higher female-to-male ratio of 2.2:1. Regarding the years, the data show that while in 2014 and 2015 the proportion of males was higher compared to females, in 2018, 2019, and 2022 the proportion of females was greater. This pattern was consistent across the lotic systems, while in the reservoir, the proportion of females was always higher compared to males (Table 3).

Regarding recaptures and individual mobility, 16 individuals (10 females and 6 males) were recorded only once during the 10 years of monitoring. The remaining 24 individuals (12 females and 11 males) had between 1 and 7 recaptures during the monitoring period, with females having a higher frequency of recapture. Considering the time during which the same individual was recorded, which varied between 1 and 6 years for females and between 1 and 2 years for males, the average displacement for females in the reservoir was 3.2 km and for males, 4.4 km. In the rivers, the displacements were shorter, with an average of 0.6 km for females and 0.4 km for males, and a much lower recapture rate compared to the reservoir (Figure 3). Since the Porce River upstream of the reservoir and the Porce III reservoir are connected at their closest point, 2 individuals, 1 male and 1 female, used both sectors for more than one year, including them in their movements. Additionally, 4

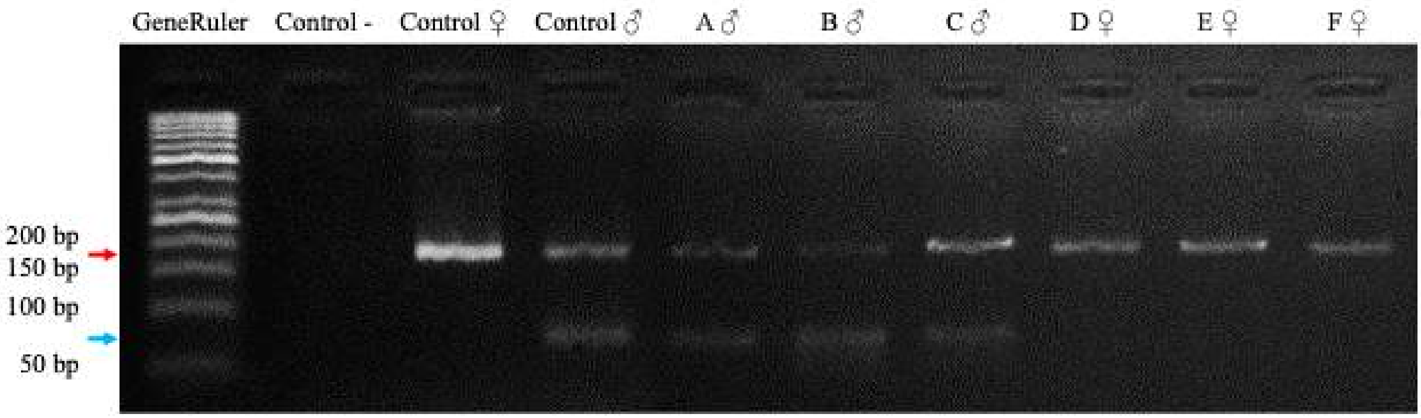

Figure 2. Verification of PCR products by 3% agarose gel electrophoresis. The bands are amplified from the co-amplification of the SRY and ZFX/ZFY genes for tissue samples (♀ and ♂ Controls) and for fecal samples (A-F) of *Lontra longicaudis* in the study area. The blue arrow indicates the size (bp) of the SRY fragment, and the red arrow indicates the size (bp) of ZFX/ZFY.

Table 2. Number of female and male otters by year

Year	Females	Males	Ratio
2014	3	7	1 : 2.3
2015	3	5	1 : 1.7
2018	13	5	2.6: 1
2019	6	3	2 : 1
2022	7	2	3.5 : 1
Average			2 : 1.4

Table 3. Number of female and male otters by year and water system

Year	Lotic (Rivers)			Lentic (Reservoir)		
	Females	Males	Ratio	Females	Males	Ratio
2014	1	6	1 : 6	2	1	2 : 1
2015	1	4	1 : 4	2	1	2 : 1
2018	5	2	2.5: 1	8	3	2.7 : 1
2019	2	0	2 : 0	5	3	1.7 : 1
2022	2	1	2 : 1	5	2	2.5 : 1
	Average		1.7 : 2.4	Average		2.2 : 1

individuals with extensive movements within the reservoir (over 7 km) were found, including 1 female and 3 males.

Discussion

The effectiveness of the sexing method in this study was 62.8%, a value higher than that obtained in similar research (Dallas *et al.* 2000, 2003; Hájková *et al.* 2009; Trinca and Eizirik 2012; Ortega *et al.* 2012; Brzeski *et al.* 2013; Lerone *et al.* 2014; Vergara *et al.* 2014; Lampa *et al.* 2015; Biffi and Williams 2017). This can be attributed to the exclusive collection of fresh samples (feces and anal gland jelly), the preservation method employed, and the use of feces-specific DNA extraction kits. However, the most determining factor of success was the selection of samples previously characterized for the control region of mtDNA, genotyped and individualized with microsatellites, which ensured high-quality DNA for sexing. Amplification was conducted using Phusion Flash II polymerase (Thermo Scientific™, CA, US), which is known for its high specificity and reduced formation of non-specific products, potentially contributing to the success rate.

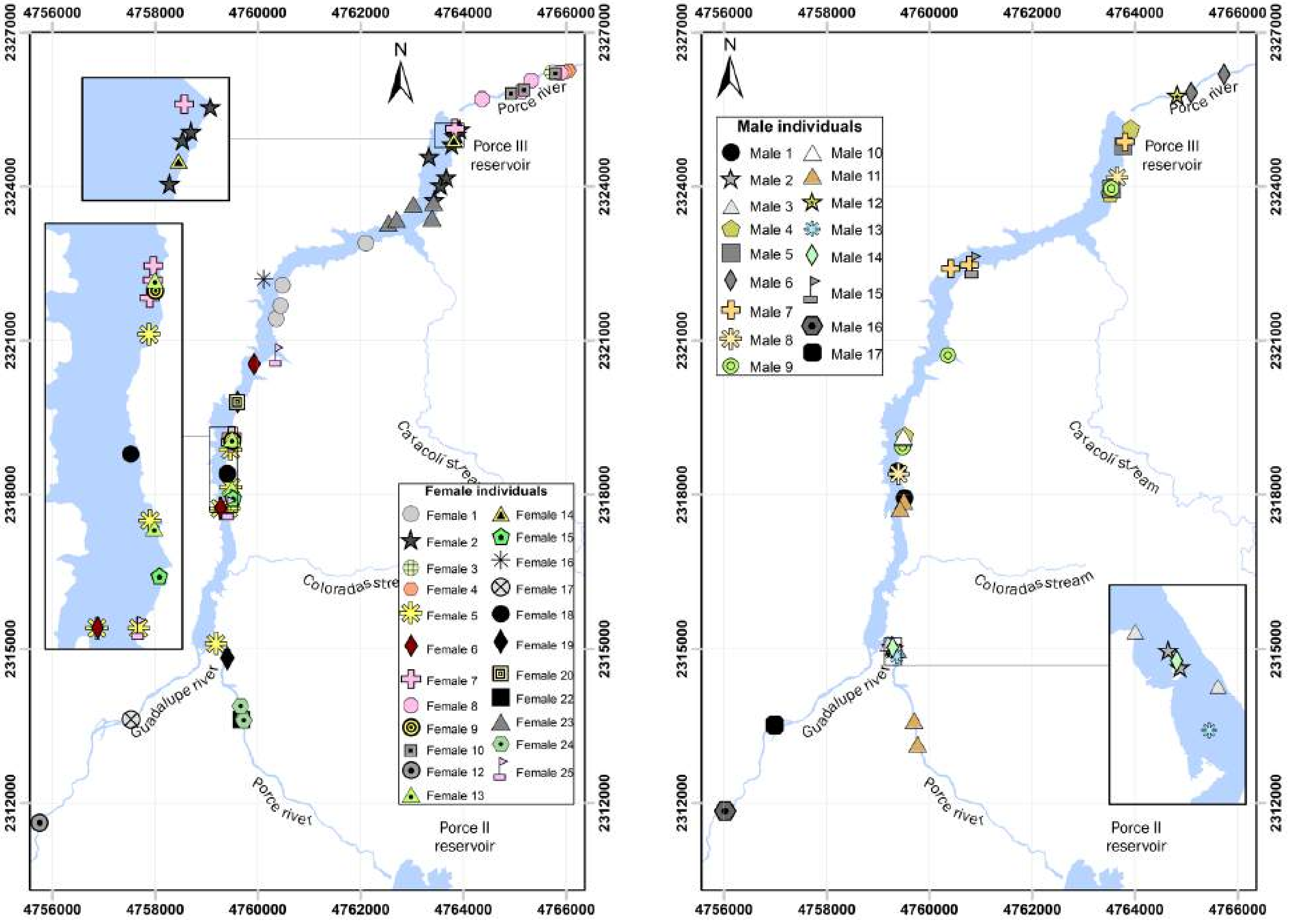

Figure 3 Distribution of individuals in Porce III reservoir and river with assigned sex. A) females B) males

The implemented methodology was effective for sexing individuals in the Porce III reservoir otter population with no need to capture and handling individuals. It is also a more economical and efficient method than others that have been used for non-invasive samples of the neotropical otter (Ortega *et al.* 2012; Trinca and Eizirik 2012). Using the ZFX/ZFY gene as a control for SRY gene amplification allowed for the identification of both males and females in a single co-amplification without the need for additional steps, such as the use of restriction enzymes. Furthermore, sex was confirmed by performing four independent PCRs for each sample, which reduced the possibility of genotyping errors due to sequence loss or amplification of non-specific products (Taberlet and Luikart 1999). In those cases where consistent results were not achieved across repetitions, this was likely due to low DNA quality, or the presence of PCR inhibitors commonly found in otter's feces.

The average sex ratios over the evaluated years in the study area, show a slight predominance of females over males. This result is strongly influenced by the otters inhabiting the reservoir area, as the proportion of males was higher in the rivers. These findings are consistent with reports in the literature for *Lontra longicaudis* (Trinca and Eizirik 2012) and *Lutra lutra* (Lampa *et al.* 2015). A greater movement of males compared to females was also observed, primarily in the reservoir, meanwhile in the rivers, movements were short and similar between sexes. These results suggest a higher dispersion of males and a philopatric tendency in females, at least in the reservoir, as reported in other studies (Ortega *et al.* 2012; Quiagletta *et al.* 2013; Trinca et al. 2013; Biffi and Williams 2017; Michalski *et al.* 2021). Considering that the availability of food and shelter are key factors influencing habitat use by otters, the movement of individuals to the lotic systems adjacent to the reservoir may reflect a search for areas with reduced competition for these resources (Zapata-Escobar et al. 2015). The higher aggregation in the reservoir area could be attributed to the sufficient availability of both food and shelter for females and their offspring. Males observed with the group of females in the reservoir area could be transient individuals or temporary residents contributing to gene flow, or juveniles remaining with their mother until the onset of the next breeding season (Michalski *et al.* 2021), a hypothesis that requires confirmation through kinship analysis.

It is important to consider that due to the high exposure to contaminants that may affect the survival and reproduction of otter individuals, it is relevant to complement this study with analyses of sex hormones to determine the levels of estrogens and testosterone in the already sexed individuals, as well as the detection of endocrine disruptors that may be affecting reproduction. Even though otters are frequently observed, the sight of neonates is low. Furthermore, it is crucial to implement effect-based methodologies to analyze whether exposure to mutagenic or endocrine-disrupting contaminants varies by sex, using fecal samples. This is essential because otters play a key role in the conservation of the aquatic and riparian ecosystems they inhabit.

In conclusion, the species *Lontra annectens* remains poorly understood compared to other otters, such as *Lutra lutra*. This lack of information hampers conservation efforts. However, this project represents a significant advancement as it is the first study in Colombia to apply molecular sexing techniques to this species. In addition to providing valuable data on sex ratios, this study also presents initial findings on individual mobility. This innovative approach not only provides valuable data for conservation but also sets a precedent for future studies on the biology and ecology of *Lontra longicaudis* and *L. annectens*.

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In Memoriam:

Dr. Miguel Ángel Armella Villalpando

(1958-2025)

El Dr. Miguel A. Armella Villapando, nació en la Ciudad de México el 18 de diciembre de 1958. Su formación educativa básica la obtuvo en la misma Ciudad de México. A lo largo de su adolescencia y juventud comenzó a interesarse por la naturaleza y particularmente por los animales. Por lo cual realizó sus estudios de Licenciatura en Biología de 1977 a 1984 en la Universidad Autónoma Metropolitana Unidad Iztapalapa (UAM-I). Posteriormente y de 1984 a 1990 cursó y obtuvo el grado de Maestría en Ciencias (Biología) por la Facultad de Ciencias de la UNAM, bajo la dirección del Dr. Rodolfo Dirzo, interesándose por la ecología de poblaciones vegetales en zonas tropicales. En 1984 obtiene su plaza como profesor asistente del Departamento de Biología en la UAM-I. En 1990 ingresa al programa de doctorado en la Universidad de Arkansas Fayetteville, EUA, bajo la dirección de Charles Amlaner Ph.D. en Ciencias Biológicas, particularmente en el área de comportamiento animal. El Dr. Armella estuvo interesado en responder preguntas centrales del comportamiento animal entre las cuales estuvieron: ¿Cómo afecta la alimentación durante el desarrollo?, así como, en estudiar los patrones de actividad animal, todo ello como temas de investigación básica del laboratorio de comportamiento animal del Dr. Amlaner.

Durante su formación académica en licenciatura, maestría y doctorado trabajó con diversos grupos de mamíferos colaborando con el grupo del Dr. José Ramírez Pulido en UAM-I. En 1996, regresa a México después de haber obtenido su grado de Doctor. En el año de 1997, ya como Profesor Titular en el Departamento de Biología de la UAM-I, inicia su abundante actividad docente al impartir numerosos cursos de Bioestadística, Ecología, Comportamiento Animal, Temas Selectos de Biología, Seminarios de Investigación, Trabajo de Investigación y Proyectos de Investigación a nivel licenciatura y posgrado, acumulando más de 120 cursos impartidos a lo largo de su labor docente entre 1984 hasta el 2025.

También en 1996 estableció su propia línea de investigación, la cual fue en ecología de la conducta y la fue consolidando al dedicar sus esfuerzos y proyectos de investigación con variados e importantes estudios con el lobo mexicano (*Canis lupus baileyi*). A lo largo de esta trayectoria amplió sus líneas de investigación hacia la fisiología de las hormonas esteroideas sexuales y su influencia en el comportamiento del lobo mexicano, así como en ecología poblacional de meso-carnívoros; colaborando con otros colegas de UAM-I y de otras universidades nacionales y extranjeras con temas de comportamiento y fisiología hormonal con otros grupos de mamíferos como roedores, murciélagos, carnívoros, aves y recientemente reptiles, como lo muestran sus diversas publicaciones en revistas científicas.

El Dr. Miguel A. Armella Villapando con una muestra del material que produjo como líder en la Vocalía de Educación Ambiental del Subcomité Técnico Consultivo de Recuperación Nacional del Lobo Mexicano.

En 1999 en colaboración con su esposa la Dra. Ma. de Lourdes Yañez-López, profesora del Departamento de Biotecnología de la UAM-I. Con la intensión de retribuir socialmente el apoyo recibido de la beca del CONACyT para obtener su doctorado fuera del país, participó en la convocatoria de proyectos interdepartamentales, iniciando estudios de cactáceas comestibles en la zona árida del centro de México en un proyecto que involucraba mejorar la producción de un fruto nativo Pitaya (*Stenocereus griseus*), con un enfoque multidisciplinario para apoyar a los productores en la región del sur de Puebla y norte de Oaxaca. Así, el Dr. Armella desarrolló exitosamente dos líneas de investigación en la UAM-I, una de investigación básica y la otra de investigación aplicada, de tal manera que su producción científica y biotecnológica fue abundante y también así cumplió con el compromiso social de apoyar a las comunidades humanas rurales en una región marginada de Puebla, México, donde también realizó conservación de la fauna silvestre.

En 1998, inició su participación en el Programa Binacional Mex-EUA de Conservación del Lobo Mexicano, destacando sus investigaciones y publicaciones sobre la fisiología de hormonas esteroides sexuales y el comportamiento reproductivo, así como la evaluación del cortisol para medir el estrés de los diferentes encierros del programa de reproducción en cautiverio, línea de trabajo que desarrolló con su colega la Dra. Ma. Asunción Soto, con ello participando activamente en la formación de recursos humanos en licenciatura y posgrado de la UAM-I. Participa como Vocal de Educación Ambiental en el Subcomité Técnico Consultivo de Recuperación Nacional de Lobo Mexicano (STCRNLM), que fue un órgano asesor para la Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT) en la toma de decisiones de la especie y particularmente para la Dirección General de Vida Silvestres (DGVS) y la Comisión Nacional de áreas Naturales Protegidas (CONANP). Con esta actividad formó parte importante en la toma de decisiones de las acciones de conservación del lobo mexicano para México. Como participante activo del STCRNLM, comienza a interactuar con colegas norteamericanos miembros del Programa Binacional de Conservación del Lobo Mexicano, siendo un asistente constante y participante activo en las reuniones binacionales anuales del grupo, lo cual lo llevó a ser muy conocido y apreciado por este selecto grupo de manejadores y conservadores del lobo tanto en México como en EUA. Como líder de la vocalía de Educación Ambiental, produjo materiales educativos como libros, folletos, trípticos, juegos para niños con el propósito de cambiar las actitudes negativas que prevalecen en los humanos hacia los lobos y siempre buscando el apoyo en la reproducción de la población cautiva, su conservación genética y su reintroducción en México.

Asistente constante de los Congresos Internacionales de Lobo, organizados por el Dr. David L. Mech, Chairman del Grupo Especialista de Lobos de la Unión Internacional de Conservación de la Naturaleza (UICN), llevando los resultados de sus actividades e investigaciones desarrolladas en México.

El repentino y sorpresivo fallecimiento del Dr. Miguel Ángel Armella Villalpando el 16 de marzo de 2025

El Dr. Miguel A. Armella en el International Wolf Symposium 2016, celebrado en Duluth, Minesotta, EUA., con el Dr. David L. Mech y un grupo de mexicanos participantes en el evento.

ha dejado un enorme vacío en la comunidad científica y académica del Departamento de Biología en la Universidad Autónoma Metropolitana (UAM), en el grupo binacional de conservación del lobo mexicano y en la mastozoología nacional en general. Fue un apasionado de la biología, un notable profesor y formador de recursos humanos en UAM-I, un incansable activista en la conservación del lobo mexicano. Su dedicación y entrega no solo quedaron reflejadas en sus investigaciones y obra publicada, sino también en el impacto que dejó en la docencia universitaria con su numeroso grupo de estudiantes, en los proyectos de biotecnología en donde colaboró con su esposa y con sus colegas de la conservación biológica tanto de EUA como en México a lo largo de su vida.

A lo largo de su trayectoria, el Dr. Miguel Ángel Armella Villalpando se distinguió por su amor a la naturaleza, su curiosidad inagotable y su inquebrantable compromiso con la ciencia, logrando un legado que perdurará en los artículos científicos que publicó, en las investigaciones que impulsó y en las generaciones de biólogos que formó.

Desde los Estados Unidos de Norteamérica hasta México, los lobos mexicanos libres y en cautiverio ululan agradeciendo la importante labor del Dr. Miguel Ángel Armella Villalpando para evitar su extinción y estar presentes en la maravillosa naturaleza de este mundo.

Jorge Servín

Reunimos estas palabras como homenaje al amigo, colega, maestro y compañero que fue el Dr. Miguel Ángel Armella Villalpando a lo largo de su vida. Con profundo respeto, estima y agradecimiento, recordamos al siempre sonriente Miguel, quien abría puertas y generaba oportunidades, convencido de que la educación ambiental era uno de los pilares fundamentales para el regreso del lobo mexicano a nuestro país. Tu huella y legado permanecen en el Laboratorio de Ecología Animal de la Universidad Autónoma Metropolitana Unidad Iztapalapa. Buen camino, colega.

Nalleli E. Lara Díaz y María Asunción Soto Álvarez

Tuve el privilegio de conocer al Dr. Miguel Ángel Armella Villalpando en 1999, cuando fue mi profesor en la Licenciatura en Biología. Desde entonces, mantuvimos una relación cercana. Con el tiempo, esa cercanía dio paso a una amistad, tanto con él como con su esposa, la Dra. Lulú Yáñez.

El Dr. Armella se distinguió por su profundo compromiso institucional y por su interés en fortalecer al Departamento de Biología. Junto a colegas como la Dra. Leticia Ponce de León García, compartimos valiosas experiencias en campo, especialmente en la Mixteca Baja Oaxaqueña. Miguel Ángel fue siempre una persona generosa y altruista con quienes colaboraban con él, dispuesto a sacar adelante los proyectos, incluso recurriendo —con humor y determinación— a lo que él llamaba el "Fondo Armella-Yáñez". Cada vivencia con él fue profundamente grata, como aquellas celebraciones de su cumpleaños, en las que compartíamos una deliciosa barbacoa preparada en horno de tierra por el Sr. Jaime Torres y su familia.

Su amistad desinteresada, su carácter paternalista, su sabiduría, su optimismo y su fortaleza ante la adversidad permanecerán en quienes tuvimos la fortuna de conocerlo.

Descansa en paz, querido Miguel Ángel. Que tu camino siga lleno de luz y bondad entre tus amados lobos.

Claudia Barbosa Martínez

Amigo Miguel, recordando cuando nos conocimos recién egresados generación 77-81, tú de UAM yo de UNAM, hace 44 años, cuando iniciamos nuestro ejercicio como biólogos profesionales en la muy querida UAM Iztapalapa, la mejor como tu decías. Después de ganar la tan añorada plaza de Profesor, fuiste tu quien sugirió la maravillosa idea que marcó nuestra vida académica y de muchos colegas más, el de trabajar en zonas áridas, dado que el 70% de nuestro país está constituido por zonas áridas y semiáridas y los estudios en ese campo, en esa época eran pocos. Fue así que iniciamos un proyecto que la SEP nos financió para trabajar en Metztitlán, Hidalgo. Así fue como se adquirió la primera computadora que el Departamento de Biología tendría, una MAC de pantalla verde, con su ruidosa impresora y un refrigerador verde para el laboratorio, iniciando nuestras primeras exploraciones en la Barranca de Metztitlán, junto con las profesoras Bety Córdoba y Martita Pérez. Y así iniciamos nuestra actividad como Profesor-Investigador, proyecto de trabajo que orgullosamente en ese entonces proponía como novedoso la UAM. La investigación en este ambiente fue en ecología de cactáceas y sus relaciones planta- animal, con la investigación enfocada al cactus columnar *Cephalocereus senilis* conocido como "viejito", con sus visitas nocturnas de murciélagos como *Leptonycteris yerbabuenae, Choeronycteris mexicana, Glossophaga soricina* que lo visitaban como herbívoros y posibles polinizadores, espectáculo que veíamos como en una galería de teatro sentados en las laderas de la Barranca en compañía de los alumnos que llevábamos para iniciarlos en el mundo de los desiertos mexicanos y sus primeros contactos como futuros biólogos con la naturaleza. Fue así como iniciamos nuestro camino como compañeros y amigos, camino que recorrimos en nuestra vida como profesionales. Nos quedó pendiente un trabajo que iniciaríamos con otro cactus columnar, *Lemnocereus hollianus*, endémico de la Reserva de la Biosfera Tehuacán-Cuicatlán, Puebla. Hasta pronto querido amigo.

María Dolores García Suárez

Conocí al Dr. Armella cuando el coordinaba estudios de sus alumnos con los lobos de los Zoológicos de la Ciudad de México. Desde el principio me pareció que sus proyectos eran de una formalidad académica notable y se diferenciaban de otros proyectos con sesgos personalistas, comunes en otros investigadores. Desde mi área de influencia, del Zoológico Aragón, siempre abrí las puertas a estos proyectos de estudiantes de la UAM y aportaron datos importantes a la conservación del lobo mexicano. Tanto Miguel Ángel como María Pía Soto, generaron investigaciones y publicaciones serias que robustecieron el proyecto.

También nos encontrábamos en las reuniones previas a la binacional y desde luego en las binacionales, donde se decidía entre otras cosas, qué ejemplares se iban a reproducir en cada temporada, un ejercicio complicado que desarrollábamos tanto lo loberos mexicanos como los norteamericanos. En estas reuniones binacionales, siempre existía la barrera del lenguaje, pues, aunque muchos de los loberos mexicanos entienden inglés, casi ningún norteamericano entiende bien el español y por ello era importante la labor de traducción que realizábamos siempre los mexicanos Frank Carlos Camacho, yo y alguno que se me olvida. Miguel Ángel se incorporó a esta difícil tarea, además de participar activamente en las presentaciones de los avances y resultados de sus investigaciones y apoyo en las discusiones técnicas.

De manera especial recuerdo la Reunión binacional en Creel Chihuahua, donde en conjunto con el Dr. Servín, nos hicieron un bonito reconocimiento a los loberos pioneros del programa.

Como miembro del subcomité técnico consultivo, Miguel Ángel participó activamente en la vocalía de educación, donde realizó publicaciones como cuadernos ilustrativos para niños, y libros como "Lobo mexicano, Iconografía".

Gerardo López Islas

Convivir con el Dr. Miguel Ángel Armella fue una experiencia que trascendió lo académico; para él, fuimos sus hijas académicas, como solía llamarnos, y verdaderamente fue un padre en nuestro crecimiento profesional. Nos cultivó el amor por la ciencia, por la biología, y nos enseñó a independizarnos: nos dio la libertad de elegir nuestro propio camino, transmitiéndonos una valiosa lección de vida: *"aprender a tomar decisiones y asumir sus consecuencias"*. Como un verdadero padre, nos permitía ser libres e independientes, pero siempre estaba atento a nuestro bienestar, cuidándonos en cada paso.

Tuvimos la fortuna de conocer al Dr. Armella desde la licenciatura y de recibir de él la oportunidad de estudiar el tema que soñábamos para nuestra maestría. Como estudiantes de biología, siempre buscamos profesores que nos impulsen a ser mejores, que nos transmitan conocimiento y nos orienten en nuestros proyectos de investigación; sin embargo, el Dr. Armella fue mucho más que un profesor: fue un amigo, un mentor, un guía. Nos ofreció no solo su saber, sino también su confianza y un apoyo incondicional que marcó nuestras vidas.

Hoy sentimos una profunda tristeza por su partida, pero cada una de nosotras llevará siempre consigo parte de su enseñanza y de su ejemplo. Siempre lo recordaremos con profundo cariño y gratitud.

Laura Morales Pimentel, Alondra Berenice, Luz Erendira Trápala Ramírez

Te conocí hace ya algunos años por no decir muchos, habías escuchado hablar del programa de conservación de lobo mexicano y estabas interesado en poder apoyar a través de la UAM, a partir de ahí, acudimos a muchas reuniones, varias de las cuales me apoyaste para poder asistir, por lo cual estaré ¡eternamente agradecida!

¡Vivimos muchas anécdotas a través de viajes y reuniones, ninguna como la de San Cayetano! Cómo olvidar nuestra decepción y tristeza, al escuchar que desaparecía el Comité Técnico Consultivo para la Conservación del Lobo Mexicano, sin decirnos ¡agua va! en un tajo dejamos de ser un grupo consultivo. Tras la decepción, vinieron los brindis y lloramos juntos, ¡qué noche pasamos todos los del extinto Comité! ¡Nunca olvidaré tu corbata amarrada a la frente!

Que tengas un buen viaje, dejas muchas cosas buenas, las malas se fueron al olvido, no me queda más que agradecer todo tu esfuerzo por la conservación del lobo mexicano, tu legado a través de trabajos de investigación en pro de la especie.

Gracias por mi último regalo en el Zoológico de Cincinnati, ¡nuestra última Reunión Binacional juntos!

Xóchitl Ramos Magaña

Hace 26 años, Miguel Ángel Armella me invitó a colaborar con él en el proyecto de recuperación del lobo mexicano, recuerdo muy bien como nos fuimos organizando y como me ayudó para aprender a analizar las hormonas esteroides sexuales y del estrés. Consiguió presupuesto para comprar el equipo necesario para hacer las cuantificaciones en el laboratorio a partir de heces que recolectábamos en nuestras visitas a las instalaciones donde alojaban lobo mexicano. Además, junto con los alumnos del laboratorio se hacían observaciones de conducta de parejas y /o grupos familiares de lobos en diversos lugares del país.

La pasión de Miguel Ángel Armella para hacer estudios de conducta y sobre todo en el lobo mexicano era admirable. Recorrimos largas distancias en la camioneta de la Universidad -que por cierto él manejaba perfectamente- y así viajamos hasta Durango, Saltillo, Ciudad Victoria, Coatepec, Monterrey, Guadalajara y Tenancingo. Durante esos viajes se dirigía a los alumnos para enriquecer sus conocimientos, como enseñarles los diferentes tipos de vegetación observados a lo largo del camino, además de poner música del gusto de todos los pasajeros de la camioneta y así hacer un viaje ligero y divertido.

En el laboratorio lamentamos mucho su fallecimiento y extrañamos las tertulias académicas que acostumbraba a organizar.

María Asunción Soto Álvarez

Recuerdo cuando me entrevisté la primera vez con el Doc Armella. ¡Fue tan estimulante y emocionante! Desde esa vez me dejaba pistas sobre la ruta a seguir. Pero claro, no me decía el camino, eso era algo que yo debía descubrir.

Creo que esa era su forma de enseñar a sus alumnos, tanto de licenciatura como de maestría. Sugiriendo, empujando amablemente, dejándonos ver que más importante que las respuestas y los resultados, eran las preguntas.

Lo recuerdo siempre con una sonrisa. Mostrándome fotos y videos de sus perros, leyendo un libro de Konrad Lorenz, escuchando música de Abba o incluso escuchando grabaciones de vocalizaciones de aves mientras viajábamos en la camioneta de la universidad rumbo a alguna zona de investigación. Creo que difícilmente se podía pedir algo más estimulante.

Las tardes que pasamos en su oficina, evaluando datos de tiempo con métodos de estadística circular fueron tan absorbentes y absolutamente enriquecedoras.

Siempre me enterneció el cariño con el que trataba su esposa. Es algo que aún me hace sonreír hoy en día.

No dudo al decir que su enseñanza y su presencia en mi vida fueron una bendición.

Siempre le estaré agradecido, Doc.

Miguel Ángel Toriz Casabal

Cuando se incorporaron al grupo de lobo mexicano, él solía decir que eran "The new kids on the block". Siempre tuvo un gran entusiasmo por hacer algo por el programa de recuperación de esta subespecie, y lo hizo: participó en reuniones de trabajo, simposios, congresos, hizo diferentes cosas en el aspecto educativo del programa, publicó algunos artículos, etc. Fue muy generoso, en incontables ocasiones puso de sus propios recursos para sacar adelante sus proyectos, a lo que llamaba "la Fundación Armella-Yáñez"; era una persona muy sociable. Sin embargo, lo que no se notaba mucho a simple vista era su gran capacidad como profesor y tutor, siempre preocupado de que sus estudiantes llevaran a buen término sus proyectos de tesis, pero además, fue buenísimo en ayudarnos a formalizar científicamente lo que al inicio eran sólo ideas dispersas, respetando nuestra opinión, al igual que su talento para que sus estudiantes aprendiéramos los conocimientos pertinentes. Recuerdo lo feliz que se veía el día que mi tesis de maestría quedó terminada y el día que aprobé mi examen de grado. Lamento aquellas conversaciones y proyectos que quedaron pendientes y espero que pueda hacerlo sentir orgulloso de haber sido parte de mi formación profesional.

Carmen Vázquez González

Mi primer contacto con Miguel Ángel fue en una fiesta en 1982. Había estado en La Paz, y me ponía al día con mi amigo Enrique Portilla (que también, epd). Otro amigo nos regañó porque hablábamos sólo entre nosotros y, decía, no buscábamos conocer gente nueva, como Miguel Ángel Armella, que empezaba a insertarse en el grupo de los "sarukhanitos", aunque él era más bien un "dirzito". Lo fui conociendo un poco más en el laboratorio de Ecología del Instituto de Biología, UNAM, cuando éramos aprendices y aspirantes a ecólogos. Ya como colegas en UAMI (desde 1986) compartimos salidas de campo tanto en la Barranca de Metztitlán como en el Valle de Zapotitlán. Tuvimos vivencias gratas y pláticas bizantinas que nos divertían mucho. Él ambicionaba liderar un grupo de investigación en zonas áridas, y de hecho, con otros colegas recreamos el Área de Ecología en nuestra Institución. Pero la vida da vueltas, y él se reformateo en etólogo y estudioso del lobo mexicano. Lo vi por última vez el 13 de marzo de 2025. Nuestro común amigo en Zapotitlán, Pedro Miranda, dijo al respecto del deceso de Miguel Ángel: "señor, se está desgranado la mazorca".

Fernando Vite González

"Deberías preguntarle al profesor Armella cómo hacerle", me dijeron algunos alumnos cuando di mi primera clase, tartamudeando por los nervios y el pavor, ante un grupo de 50 estudiantes en la UAMI.

Reconocido por su incansable pasión por la ecología y la conducta animal, Miguel Ángel fue mucho más que un académico destacado: fue un maestro cercano, siempre dispuesto a compartir con sus estudiantes su conocimiento y experiencia con una sonrisa y una historia fascinante. Especialista en el estudio de los lobos, dedicó su vida a comprender sus patrones conductuales y la manera de reintegrarlos a los ecosistemas que alguna vez habitaron; despertando en sus alumnos el mismo amor y respeto por la naturaleza que lo guiaron durante toda su carrera.

Además de su labor científica, era un amante declarado de la buena comida, un conversador entusiasta en torno a una mesa bien servida y un devoto compañero de su adorada Lulú y de sus perros, quienes lo acompañaban fielmente en sus actividades.

Su ausencia deja un vacío difícil de llenar, pero su legado vive en las aulas, en los senderos que recorrió, y en los corazones de quienes tuvimos el privilegio de considerarnos sus amigos y aprender de él.

J. Alejandro Zavala Hurtado

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Corrigendum

Matocq, M., and E. Lacey. 2025. Editorial. Keep running your traplines: Celebrating the contributions of Jim and Carol Patton to the science and people of mammalogy. Therya 16:1-4.

Editorial

Keep running your traplines: Celebrating the contributions of Jim and Carol Patton to the science and people of mammalogy

Marjorie Matocq[1], and Eileen Lacey[2]

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Change in:

Caption in Figure 3.

Figure 3. Jim and Carol Patton checking traps near Shoshone, California in December 2024. Photo: Patrick Kelly.

Caption in Figure 5.

Figure 5. Jim greeting a recently captured woodrat near Shoshone, California in December 2024. This familiarity with organisms and their natural environments is the most fundamental component of evolutionary studies. Photo: Patrick Kelly.