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La portada

Armadillo de nueve bandas (*Dasypus novemcinctus*) es posiblemente el dasipódido más ampliamente distribuido desde América del Norte hasta América del Sur, y pertenece al orden Cingulata. Se caracteriza por la presencia de escudos dérmicos que cubren todo su cuerpo y le permiten enrollarse para protegerse de sus depredadores. El patrón de escudos dérmicos se utiliza como característica de los diferentes géneros. Tiene grandes garras útiles para cavar sus madrigueras. Además de su importancia ecológica, porque su dieta es omnívora. Especialmente en México, esta especie tiene varios usos, los dos más comunes son el consumo de su carne y el uso de su caparazón, entre otras cosas para instrumentos musicales o bolsas (foto tomada por Sergio Ticul Álvarez-Castañeda).

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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REWIEWS

fascículo 39 http://www.revistas-conacyt.unam.mx/therya/index.php/THERYA/issue/view/43

DERECHOS DE AUTOR Y DERECHOS CONEXOS, año 13, No. 2, septiembre-diciembre del 2022, es una publicación cuatrimestral editada por la Asociación Mexicana de Mastozoología A. C. Hacienda Vista Hermosa 107, Colonia Villa Quietud, Coyoacan 04960. Distrito Federal, México. Telefono (612) 123-8486, www.mastozoologiamexicana.org, therya@cibnor.mx. Editor responsable: Dr. Sergio Ticul Álvarez Castañeda. Reservas de Derechos al Uso Exclusivo No. 04-2009-112812171700-102, 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. Dr. Sergio Ticul Álvarez Castañeda. Instituto Politécnico Nacional 195. La Paz, Baja California Sur, C. P. 23096. Tel. (612) 123-8486, fecha de la última modificación 30 septiembre 2022.

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 bienvenidas.

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Relative abundance, habitat selection, and diet of the coyote in northern México

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The coyote (*Canis latrans*) is a widespread predator with a high degree of adaptation to different ecosystems. The objective of this study was to estimate the relative abundance index, habitat selection, and diet of *C. latrans* through scent stations, in two types of vegetation located in northwestern Chihuahua. From April 2018 to March 2019, ten fixed scent stations (SS) were placed in pine-oak forest and other ten in semi-open grassland, with a linear separation of 500 meters between each station to achieve a five km transect in each vegetation during 12 samplings (two sampling night per month) for totaling 420 SS after discarding inactive SS. The relative abundance index of coyote showed that both types of vegetation, pine-oak forest (0.30) and semi-open grassland (0.23) were used in a similar way. The habitat selection test (Chi^2) showed that coyote abundance and type of vegetation were independent ($\chi^2 = 2.96$, $P > 0.05$), not showing statistically significant differences in annual relative abundance index of coyotes between the two vegetation types. The food items detected in thirty-four scats collected belonged to mammals (55.9 %), fruits (35.3 %) and arthropods (8.8 %). Rodents and lagomorphs were the main source of food. Throughout the sampling period, the pine-oak forest showed the greatest relative abundance index of *C. latrans*. This may be because the forest provides them with shelter from climatic situations and a greater variety of seeds, fruits and insects. In this study the two seasons with the highest relative abundance were spring and autumn in both ecosystems, coinciding with an increase in rainfall, resulting in an abundance of potential prey (rodents and lagomorphs), and other food items like fruits. Regarding annual diet the most consumed prey were mammals. We found differences in diet between seasons, that can be explained by the variation in food availability among seasons.

El coyote (*Canis latrans*) es un depredador ampliamente distribuido y con un alto grado de adaptación a diferentes ecosistemas. El objetivo de este estudio fue estimar el índice de abundancia relativa, selección de hábitat, y dieta de *C. latrans* a través de estaciones olfativas, en dos tipos de vegetación localizados en el noroeste de Chihuahua. A partir del mes de abril de 2018 hasta marzo de 2019, 10 estaciones olfativas (EO) fueron colocadas en bosque de pino-encino y otras 10 se colocaron en pastizal mediano abierto, con una separación lineal entre ellas de 500 metros entre cada estación para completar un transecto de cinco km en cada tipo de vegetación durante 12 muestreos (dos noches de muestreo por mes), totalizando 420 EO después de eliminar las EO inactivas. El índice de abundancia relativa para el coyote muestra que ambos tipos de vegetación, bosque de pino-encino (0.30) y el pastizal mediano abierto (0.23) fueron usados de forma similar. La selección de hábitat muestra (Chi^2) un valor más bajo χ^2 entre bosque y pastizal, sugiriendo que la abundancia y tipo de vegetación son independientes (2.96 $P > 0.05$), sin mostrar diferencias estadísticamente significativas en la abundancia relativa anual de coyotes entre los dos tipos de vegetación. Los alimentos detectados en 34 excretas recolectadas pertenecen a mamíferos (55.9 %), fruta (35.3 %) y artrópodos (8.8 %). Los roedores y lagomorfos fueron la principal fuente de alimento. A través del periodo de muestreo, el bosque de pino-encino mostro el índice de abundancia relativa más alto para *C. latrans*. Probablemente debido a que el bosque les provee de refugio ante situaciones climáticas y una gran diversidad de semillas, fruta e insectos. En este estudio las dos estaciones con la abundancia relativa más alta fueron primavera y otoño en ambos ecosistemas, coincidiendo con un incremento en la precipitación, resultando en una abundancia de presas potenciales (roedores y lagomorfos), y otros alimentos como fruta. Las presas más consumidas durante el año fueron mamíferos. Encontramos diferencias en dieta entre estaciones, que se puede explicar por la variación en disponibilidad de alimento entre estaciones.

Keywords: Mammals; semi-open grassland; pine-oak forest; predation; relative abundance index.

Introduction

The Coyote (*Canis latrans*) is a predator that is considered to have a high degree of ecological plasticity; consequently, it can be found in different types of ecosystems including suburban and urban human settlements. Its geographical distribution ranges from North America to northern Panamá (Bekoff and Gese 2003; Marín *et al.* 2015; Méndez-Carvajal and Moreno 2014; Hody *et al.* 2019). The coyote's reproductive success and its ability to disperse, has allowed it to have high population numbers and a wide distribution (Carreón 1998; Garrido and Arribas 2008; Hernández and Laundré 2014; Méndez-Carvajal and Moreno 2014).

The home range of the coyote may comprise 3 km^2 to 67 km^2 and its extension varies according to the characteristics of the individual and the stage of life in which it is found (Hernández 1990; Hernández *et al.* 1993; Servín and Huxley 1995; Hidalgo-Mihart *et al.* 2001). It has been hypothesized that its wide distribution and population abundance in North America is due to the elimination of the Gray wolf (*Canis lupus*), since both canids were sympatric species competing for resources. Additionally, it has been documented that *C. lupus* preyed on *C. latrans* (Hall 1981; Vaughan and Rodríguez 1986).

Although the coyote is a carnivore, the modification of its diet is an adaptation to the different environments in which it is found, adopting an omnivorous diet. All these characteristics together, have led it to be a successful predator (Bekoff 1977; Hall 1981; Vaughan and Rodríguez 1986). The coyote is considered an important regulator of populations of mammals and insects (Andelt *et al.* 1987), rodents and lagomorphs. Additionally, they consumed seeds from their herbivorous diet, fulfilling a relevant role in the regeneration of pine-oak forests. Therefore, the eradication of coyote populations would have a drastic effect on animal and plant communities and on ecosystems as a whole (Servín and Huxley 1993, 1995).

The coyote is considered an abundant and widely distributed species in Mexico, however there are only a few studies on its relative abundance, distribution, role they play in a given habitat, or diet in any ecosystem in Chihuahua, northern Mexico (Pérez *et al.* 1982; Vela 1985; Pacheco *et al.* 1999). Therefore, our objective was to estimate and compare relative abundance index, habitat selection, and diet of coyote (*C. latrans*) in two types of vegetation (semi-open grassland and pine-oak forest) in northern Mexico, through indirect methods such as scent stations and scats. The information generated might be use by local authorities and Rancho Experimental Teseachi managers to carry out conservation plans.

Materials and methods

The study was conducted at Rancho Experimental Teseachi, located in central-western Chihuahua. The ranch is owned by Universidad Autónoma de Chihuahua, and has as its main objective teaching, research, and transfer of technology in animal science and natural resources (Espinoza and Quintana 2013; Álvarez-Córdova *et al.* 2019). It encompasses approximately 12,300 ha and is located between the municipalities of Namiquipa, Bachíniva, and Guerrero (28° 53′ 44″ N, -107° 27′ 22″ W) at 2,250 masl (Figure 1). The landscape is shaped by hills and high mountain ranges displaying a mixture of arboreal pasture grasses, oak chaparral, temperate forest, semi-open grasslands, and pine-oak forest (COTECOCA 1978; Álvarez-Córdova *et al.* 2019). For this study, we selected the vegetation pine-oak forest and semi-open grassland, due to the previous information of the ranch staff and personal observations of the presence of coyotes in both areas.

From April 2018 to March 2019, we placed 10 fixed scent stations (SS) in pine-oak forest and 10 SS in semi-open grassland. A five-kilometer transect with a separation of 500 linear meters (Figure 1) between each scent stations was established in each type of vegetation. The SS consisting of 1.00 meter in diameter, where vegetation was removed and sand or soil fine were added and were baited with chicken and sardine (Linhart and Knowlton 1975). The tracks of species visited the stations at night were recorded in the SS. All scent stations were activated during the afternoon and checked the next day in the morning (two nights / month; Linhart and Knowlton 1975; Roughton and Sweeny 1982; Conner *et al.* 1983; Carreón 1998).

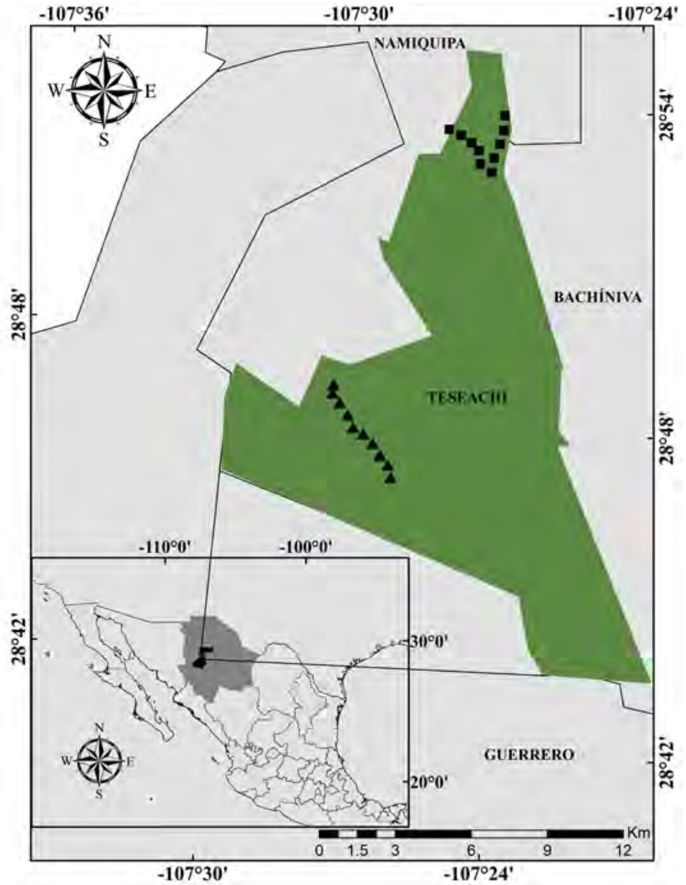

Figure 1. Geographic location of Rancho Experimental Teseachi, Chihuahua, Mexico. Black squares and black triangles indicate sampling sites in semi-open grassland and pine-oak forest respectively.

Traces and scats we found at SS and through the linear transect were identified as belonging to *C. latrans* following Aranda (2012). Each collected scat was georeferenced through a Global Positioning System (GPS; Garmin GPSMAP 66s), measurements (width and length) were taken with a measuring tape, and they were subsequently stored individually in airtight bags (Álvarez-Córdova *et al.* 2019). Once in the laboratory they were cleaned according to Arnaud (1993) and Álvarez-Córdova *et al.* (2019). Following the field guidelines of Álvarez-Castañeda *et al.* (2015), Aguirre-Segura and Barranco (2015) and Lebgue *et al.* (2015) the items within the scats were identified.

To estimate the relative abundance index, we generated a database with the records of visits of *C. latrans* at the SS, where coyote records are organized for each sampling. We defined a visit as the presence of at least one trace of coyote. To calculate the relative abundance index (RAI) we used the formula proposed by Linhart and Knowlton (1975): number of visits of the study species / number active of scent stations per night (Linhart and Knowlton 1975; Roughton and Sweeny 1982; Conner *et al.* 1983; Diefenbach *et al.* 1984; Carreón 1998). The records were analyzed on an annual and seasonal basis.

We performed a chi/square test to compare patterns of habitat selection between pine-oak forest and semi-open grassland. To achieve this, the information collected was divided between records of *C. latrans* presence and absence in scent stations in both types of vegetation. It was taken as a null hypothesis (H0) that relative abundance index of *C. latrans* was independent on the type of vegetation ($\alpha = 0.05$). A double entry contingency table was developed, in which the observed frequencies (OF) corresponded the records of scent stations that were positive during the study in the two types of vegetation and expected frequencies (EF) for forest and grassland were calculated according to chi-square formula. The results were analyzed annually and seasonally between both types of vegetation. To describe the diet of coyote, we separated the food items, and a percentage of frequency was calculated for each item (Linhart and Knowlton 1975; Roughton and Sweeny 1982; Conner *et al.* 1983; Carreón 1998; Monroy-Vilchis and Velázquez 2002).

Results

We obtained 113 coyote visits in total of 420 scent stations placed during an annual period, 67 visits correspond to pine-oak forest and 46 to semi-open grassland. The annual RAI in pine-oak forest was 0.30 versus semi-open grassland with 0.23 (Figure 2). We found differences in the RAI among seasons in both ecosystems, indicating more RAI of coyotes during Spring (0.37) and Autumn (0.40) compared to summer and winter. In semi-open grassland the highest RAI was found in Autumn (0.30). There were differences between seasons, nevertheless, regardless of the season, the RAI was always greater in pine-oak forest than in semi-open grassland (Figure 2).

For habitat selection, the double entry contingency table showed that of 420 scent stations (200 SS for grassland and 220 SS for forest) set during sampling, in 113 SS the presence of *C. latrans* were registered, corresponding to frequencies observed of 46/113 and 67/113 for semi-open grassland and pine-oak forest, respectively (Table 1). However, we did not find a significant difference between both ecosystems during the year ($\chi^2 = 2.96$, $P > 0.05$). In the same way, we did not find significant differences (all $P > 0.05$) between seasons, Spring ($\chi^2 = 3.26$), Summer ($\chi^2 = 2.63$), Autumn ($\chi^2 = 0.87$) and Winter ($\chi^2 = 0.89$). Therefore, the results suggest that the relative abundance index of coyotes and type of vegetation are independent variables across seasons.

Table 1. Double entry contingency table (χ^2; OF: observed frequencies, EF: expected frequencies) for *Canis latrans* at Rancho Experimental Teseachi, Chihuahua, Mexico.

Vegetation	Presence	Absence	Total
Semi-open grassland	OF = 46.0 EF = 53.8	OF = 154.0 EF = 146.2	200
Pine-oak forest	OF = 67.0 EF = 59.2	OF = 153.0 EF = 160.8	220
Total	113	307	420

We collected a total of 34 scats, four in semi-open grassland and 30 for pine-oak forest (2.0 to 3.1 [2.4] ± 0.33 cm width x 13.0 to 15.0 [13.3] ± 0.87 cm length). Annually, 55.8 % the food items recovered from the scats belong to mammals, 35.3 % to plants and 8.8 % to insects (Orthoptera: Acrididae). For Spring season, 16 scats were collected, in which 68.8% of the food items recovered belonged to mammals and 31.2 % to plants. During Summer, seven

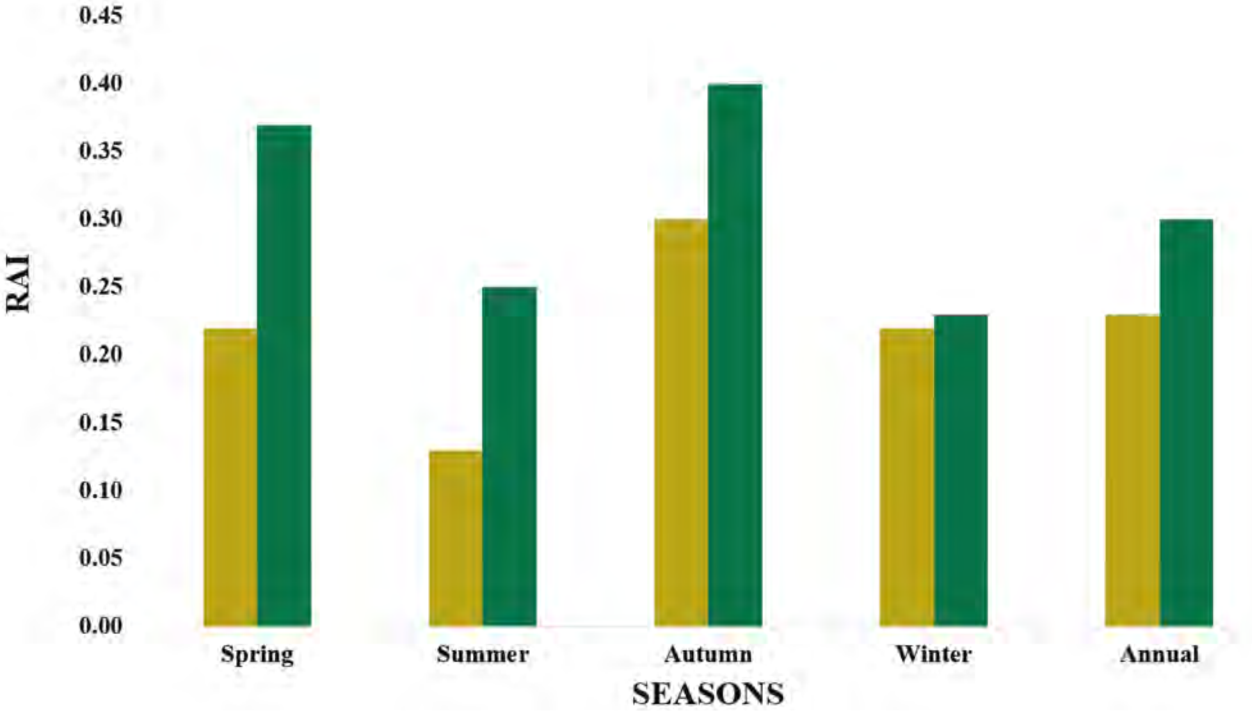

Figure 2. Relative abundance index (RAI) of *Canis latrans* seasonally and annually during 2018-2019 at Rancho Experimental Teseachi, Chihuahua, Mexico. Semi-open grassland (gray bars) and pine-oak forest (black bars).

scats were collected, with 71.4 % of the remains corresponding to mammals and 28.6 % to insects. In Autumn, only five scats were recovered where 40.0 % of the items belonged to mammals, 40.0 % to plants and 20.0 % to insects. Finally, in Winter six scats were found and 16.7 % of the items recovered were mammals and 83.3 % plants. The mammalian species identified corresponded to Cotton rats (*Sigmodon* sp.), Woodrats (*Neotoma* sp.), and Cottontail rabbits (*Sylvilagus* sp). The plants recovered corresponded to fruits of Manzanita (genus *Arctostaphylos*), and the insects were grasshoppers of the family Acrididae (Table 2). The four scats collected in semi-open grassland corresponded only to plants.

Discussion

Throughout the sampling period, the pine-oak forest showed the greatest relative abundance index of *C. latrans*. This may be because the forest provides them with shelter from climatic situations and a greater variety of seeds, fruits, and insects. Differences in the RAI between pine-oak forest and semi-open grassland were found and this result is in agreement with Monroy-Vilchis and Velázquez (2002), who found the highest RAI of *C. latrans* in pine and mixed forests. However, our results differ from those of Ponce *et al.* (2005), who found coyote lower RAI in forests, maybe because of the complexity of capturing prey, and competition with other predators (mountain lions *Puma concolor* and black bears *Ursus americanus*).

Several authors have recognized forest and grassland as important ecosystems for mammals that use them for protection and food (Servín and Huxley 1991; Hidalgo-Mihart *et al.* 2001; Randa and Yunger 2004). In this study the two seasons with the highest RAI were Spring and Autumn in both ecosystems, which are the last seasons coinciding with an increase in rainfall, resulting in a higher abundance of potential prey (rodents and lagomorphs), and other food items like fruits. According to the yearly records, Autumn seems to be a key season for coyote populations because of the dispersal of young (Randa and Yunger 2004).

Although the RAI was different between pine-oak forest and semi-open grassland, the habitat selection test did not show statistically significant differences in annual relative abundance index of coyotes between the two vegetation types. This could affirm that the coyote did not select a particular type of vegetation. Furthermore, the season that presented the greatest variation was Spring, while Autumn showed less variation. Although different authors reported that potential prey and vegetation defines *C. latrans* presence in different ecosystems, we believe that food is a key variable for the presence of coyotes in any ecosystem (Bekoff 1977; Hall 1981; Vaughan and Rodríguez 1983; Bekoff and Gese 2003; Randa and Yunger 2004; Ponce *et al.* 2005).

In the annual diet, mammals were the main food source with 55.8 %, followed by plants (35.3 %). We only know of two studies related to the coyote diet in Chihuahua. Pérez *et al.* (1982) and Vela (1985) reported that mammals (rodents and lagomorphs) are the main food item of the coyote diet, while vegetable matter was consumed occasionally in central Chihuahua. Other studies carried out in Durango reported similar food preferences, adding insects as an important food item during the dry season (Servín and Huxley 1991; Grajales-Tam and González-Romero 2014). Diet preferences coincide with the ecosystem role this canid plays as a biological control of rodent and lagomorphs populations, and seed disperser in different ecosystems in which it is distributed (Servín and Huxley 1991; Grajales-Tam *et al.* 2003; Cruz-Espinoza *et al.* 2008; Arias-Del Razo *et al.* 2011; Grajales-Tam and González-Romero 2014; Poessel *et al.* 2017).

Table 2. Annual and seasonal variation in diet of *Canis latrans* at Rancho Experimental Teseachi, Chihuahua, Mexico.

Season	Mammals	Plants	Orthoptera	Scats
Spring	68.7 %	31.2 %	0 %	16
Summer	71.4 %	0 %	28.6 %	7
Autumn	40.0 %	40.0 %	20.0 %	5
Winter	16.7 %	83.3 %	0 %	6
Annual	55.9 %	35.3 %	8.8 %	34

We found differences in diet between seasons, which can be explain by the variation in food availability among seasons (Randa and Yunger 2004; Ponce *et al.* 2005). The small rodents and lagomorphs consumed by *C. latrans* can be found throughout the four seasons (Servín and Huxley 1991); nevertheless, fruits are found in Spring, Autumn and Winter; and Orthopteran insects only appear in Summer and Autumn, corresponding to the rainy season when there is an increase in the population of Acrididae (Rivera 2006). Coyotes need the greatest energy intake for reproduction in the winter months, so that pups will be born in spring coinciding with the yearly food peak (Roughton and Sweeny 1982; Servín and Huxley 1991; Bekoff and Gese 2003; Randa and Yunger 2004; Ponce *et al.* 2005; Hernández and Laundré 2014). Even though scent stations are a very low-cost sampling technique, it has been overshadowed by other techniques such as camera traps and radiotelemetry due to the quantitative information they provide. However, the scent stations showed an efficacy in the evaluation of the relative abundance index of coyote in this study and with the data it was possible to make comparisons between ecosystems, being functional to determine population fluctuations.

In addition, in the collected scats there were specimens of a genus of nematode (*Physaloptera* sp.) not reported before for the state of Chihuahua, México (Álvarez-Córdova *et al.* 2019). The presence of this nematode in scats of the coyote agrees with the feeding habits of this canid, because this nematode is parasite of intermediate hosts like lagomorphs and rodents (Álvarez-Córdova *et al.* 2019). This study was conducted systematically in order to generate information about the ecology of *Canis latrans* in Chihuahua, specifically about the relative abundance index of this carnivore in two types of vegetation in an annual period for central-western Chihuahua populations. In addition, it is corroborated that coyote rather than a selective carnivore is an opportunistic carnivore in its diet, so it fulfills its function as a biological control of different species.

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The oldest available name for the pampas cat of the Uruguayan Savannah ecoregion is *Leopardus fasciatus* (Larrañaga 1923)

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Based on a revision of historical and taxonomic accounts, we showed that *Felis fasciatus* Larrañaga, 1923, represents the oldest available Linnean name referable to the pampas cat endemic to the Uruguayan Savannah ecoregion, currently regarded as *Leopardus munoai* (Ximénez 1961). To anchor the name *Felis fasciatus* to Uruguayan specimens, we designated a neotype for this taxon with *terra typica* in Estancia San Cristóbal, Arroyo Limetas, Conchillas, Department of Colonia, Uruguay. Since Larrañaga's *fasciatus* represents a senior synonym of *L. munoai*, it takes precedence and, in compliance with the International Code of Zoological Nomenclature, it must therefore be used to refer to the "Uruguayan" pampas cat, *L. fasciatus* (Larrañaga 1923), for which we suggested the vernacular name "Larrañaga's pampas cat".

En base a una revisión de compendios históricos y taxonómicos, demostramos que *Felis fasciatus* Larrañaga, 1923, representa el nombre Linneano disponible más antiguo referible a la especie de gato de las pampas endémico de la ecorregión Sabanas Uruguayas, considerado actualmente como *Leopardus munoai* (Ximénez 1961). Para anclar el nombre *Felis fasciatus* a los especímenes de Uruguay, designamos un neotipo para el taxón con *terra typica* en Estancia San Cristóbal, Arroyo Limetas, Conchillas, Department of Colonia, Uruguay. Dado que el *fasciatus* de Larrañaga representa un sinónimo sénior de *L. munoai*, tiene precedencia y, por tanto, en concordancia con el Código Internacional de Nomenclatura Zoológica, debe ser utilizado como el nombre para la especie, *L. fasciatus* (Larrañaga 1923), para el cual sugerimos el nombre común "gato de pajonal de Larrañaga".

Keywords: Campos grasslands; neotype; nomenclature; pampas cat; taxonomy; Uruguay.

Introduction

The term "pampas cat" has been traditionally used to refer to non-spotted small Neotropical felids of the genus *Leopardus* Gray, 1842, that, unlike their spotted congenerics that primarily use forest habitats, occur in open environments across South America (García-Perea 1994; Nascimento *et al.* 2020). The systematics of the so-called pampas cat species complex, all forms originally lumped as *Felis colocolo* Molina, 1782, has been repeatedly evaluated (*e. g.*, García-Perea 1994; Johnson *et al.* 1999; Wozencraft 2005; Barstow and Leslie 2012; Kitchener *et al.* 2017; Nascimento *et al.* 2020), yet the taxonomic status and geographical limits of several specific and subspecific designations are not yet fully resolved. Until recently, the most accepted view of the pampas cat taxonomic diversity recognized seven subspecies within a single species, *Leopardus colocola* (Molina 1782). Notwithstanding, a new taxonomic evaluation of the "*colocola*" species group based on morphological, phylogenetic, and ecological information recognized five different monotypic species of pampas cats (Nascimento *et al.* 2020); these are, *L. colocola* (Molina 1782), *L. garleppi* (Matschie 1912), *L. pajeros* (Desmarest 1816), *L. braccatus* (Cope 1889), and *L. munoai* (Ximénez 1961).

Traditionally, the pampas cat from the Campos grasslands of Southeastern South America, *i. e.*, endemic to the Uruguayan Savannah ecoregion (Tirelli *et al.* 2021; Figure 1), was regarded as a subspecies of *L. colocola* as *L. c. munoai* (Kitchener *et al.* 2017). Alternatively, it was treated as a subspecies of *L. braccatus* as *L. b. munoai*, having a much more restricted distribution compared to that of the nominotypical subspecies *L. b. braccatus* (Pantanal cat after García-Perea 1994), occurring in northeastern Argentina, eastern Paraguay, and deep into central Brazil (Barstow and Leslie 2012). Recently, Nascimento *et al.* (2020) elevated *munoai* to the species level using the binomen *Leopardus munoai* (Ximénez 1961) for the first time and referred to as Muñoa's or Uruguayan pampas cat (Nascimento *et al.* 2020).

In this contribution, we revised the taxonomic and nomenclatorial history of *L. munoai* (Ximénez 1961). We argue that *munoai* is not the oldest name that is applicable for the Uruguayan pampas cat. Whether the entity of the pampas cat inhabiting the Uruguayan Savannah is regarded as a subspecies or granted full species status, we show that there is an older available epithet (*i. e.*, a senior synonym of *munoai*), which is *Leopardus fasciatus* (Larrañaga 1923). Additionally, we selected a neotype for *L. fasciatus*.

Materials and methods

To reconstruct the nomenclature history of the Uruguayan pampas cat, we conducted an evaluation and reinterpretation of the pertinent literature and assessed the pelage coloration patterns of specimens housed at the Museo Nacional de Historia Natural (MNHN) in Montevideo, Uruguay. We detailed the history of mentions of pampas cats in Uruguay, for which some key regional references needed also to be introduced. We thus provided the necessary background to understand the specifics of the rather complex pampas cat conundrum that we seek to address, with emphasis on the milestones that build up our rationale up to Larrañaga's *fasciatus* and its applicability.

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Results

In the early 19th century, Azara (1802) described the "pajero" cat ("le chat pampa" in the French version; Azara 1801), mentioning that it occurs in the "Pampas of Buenos Ayres" and "donde al Negro" (the latter in Uruguay as explained in detail below), which constitutes the first published record of pampas cats for the La Plata River basin (García-Perea 1994). Based on Azara's "pajero", Desmarest described his *Felis pajeros* Desmarest, 1816 (currently *Leopardus pajeros*), with type locality near Santa Rosa, La Pampa, Argentina, as per the neotype designation by Nascimento *et al.* (2020).

The first reference to pampas cats in Uruguay thus corresponds to Azara (1801, 1802), whilst the first citation as a binomen for the country was by Arechavaleta (1882, 1887) under *F. pajeros*. Later, Figueira (1894) referenced *F. pajeros* for Uruguay while Aplin (1894) used *F. passerum* Sclater, 1871 instead. In a series of writings originally from the early 1800s albeit published nearly a century later, Larrañaga (1923) coined and described the species *Felis fasciatus* for Uruguayan pampas cats. This binomen remained unaccounted for in the zoological literature for almost 50 more years (*e. g.*, Devincenzi 1935; Ximénez 1970). Without reference to Larrañaga's *fasciatus*, Ximénez (1961) described a new subspecies of pampas cat under the denomination *Felis colocola muñoai* Ximénez, 1961 with type locality in Arroyo Perdido, Department of Soriano, Uruguay (Figure 1). The first mention of *fasciatus* since its formal publication in 1923 was that of Ximénez *et al.* (1972), where *Felis fasciatus* Larrañaga, 1923 was regarded as a junior synonym of *F. colocolo pajeros* Desmarest, 1816. García-Perea (1994) referred to the Uruguayan form of pampas cat as *Lynchailurus braccatus munoai* (*i. e.*, the first mention of the form *braccatus* for the country) occupying the geographical extent that is presently agreed upon for the species now recognized as *Leopardus munoai* (Nascimento *et al.* 2020; Figure 1). Klappenbach (1997) revalidated Larrañaga's *fasciatus* without a detailed justification. In turn, in our earlier work, we referred to Uruguayan populations as *Lynchailurus braccatus fasciatus* (González 2001; see also Mones *et al.* 2003), and alternatively as *Leopardus b. fasciatus* (González and Martínez-Lanfranco 2010), yet again without due argumentation (Nascimento *et al.* 2020:29).

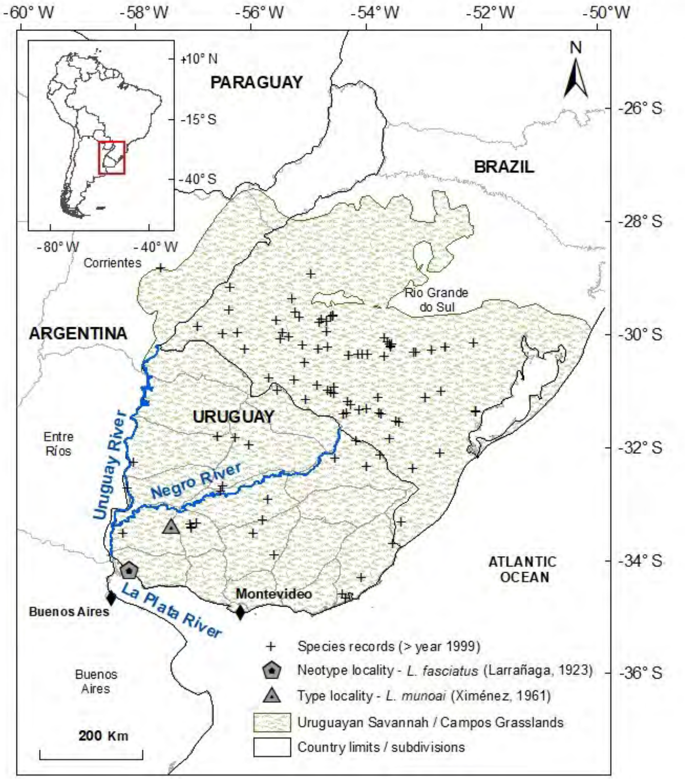

Figure 1. Map of Southeastern South America depicting the main geographical milestones referenced in the text: La Plata and Negro River; neotype locality of *Leopardus fasciatus* (Larrañaga 1923; specimen MNHN 2432 from Estancia San Cristóbal, Arroyo Limetas, Conchillas, Department of Colonia, Uruguay); the Uruguayan Savannah Ecoregion (Olson *et al.* 2001) where the Larrañaga's pampas is endemic from (Tirelli *et al.* 2021) [~ Campos sub-region of the Río de la Plata grasslands (Soriano *et al.* 1992)]; type locality of *L. munoai* (Ximénez 1961, specimen MNHN 884, Arroyo Perdido, Department of Soriano, Uruguay). Only contemporary species' records are shown (*i. e.*, 21st century; Tirelli *et al.* 2021).

Discussion

Dámaso Antonio Larrañaga (Montevideo, 1771-1848) was a transcendent figure during the Uruguayan independence process. Larrañaga was a presbyterian, architect, writer, and artist, professing a deep interest in the natural sciences; Larrañaga was, in fact, the first Uruguayan naturalist (Klappenbach 1997; Ramos 2020). His critical eye and attention to detail are well reflected in his writings and scientific illustrations (Klappenbach 1997; Duarte *et al.* 2016). Aware of the Linnean binomial system, Larrañaga described and named hundreds of plants and animals (Klappenbach 1997). Unfortunately, most of his work remained unpublished until almost 75 years after his death. Between 1922 and 1924, his writings were edited and finally published as a collection of three volumes in the "Anales del Instituto Histórico y Geográfico" of Uruguay. For this reason, several of Larrañaga's pioneer observations and taxonomic descriptions remained in the dark, for the most part, up to this day. One of such neglected taxonomic epithets is *Felis fasciatus* Larrañaga, 1923. Below, in agreement with

Klappenbach (1997) and justifying its earlier usage (*e. g.*, González and Martínez-Lanfranco 2010), we argue for the recognition of *Felis fasciatus* Larrañaga, 1923 as the oldest name applicable to the Uruguayan pampas cat.

Félix de Azara (1746–1821) was a prominent naturalist and cartographer tasked with the mapping of the territorial limits of the Spanish and Portuguese empires, with extensive explorations across the La Plata River basin in Asunción (Paraguay), Buenos Aires (Argentina), and "Banda Oriental" (Uruguay; Ballarín *et al.* 2006). In the Spanish version of his work, Azara (1802:160) wrote "*NÚM. XVIII. DEL PAJERO. Le llaman* Gato pajero, *porque habita los campos, escondiéndose en los pajonales; sin entrar en los bosques y matorrales... Yo pillé quatro en las Pampas de Buenos Ayres entre los 35 y los 36 grados y otros tres donde al Negro...*", and continued (Azara 1802:161) "*El encontrarse en ambas bandas del Río de la Plata, con identidad de formas, colores y costumbres…*"; lastly, he added (Azara 1802:161) "*La muneca hasta las unas, y lo mismo en el pie, es acanelada clara sin listas*". Our translation of Azara's fragments above is as follows: "NUM. XVIII. FROM THE PAJERO. It is called '*Gato pajero*', because it inhabits the fields, hiding in the tall grasses; without entering forests and bushes… I caught four in the Pampas of Buenos Ayres between the 35 and 36 degrees and other three where the Negro… As it is found on both sides of the La Plata River, with identity of forms, colors and costums… The wrists up until the nails, same as in the foot, is light cinnamon without bands".

Referring to Azara's XVIII "pajero" from the Spanish version [Azara 1802; the roman number "XVIII" does not appear in the French version (Azara 1801)], Larrañaga (1923:345), described *Felis fasciatus* in Latin as follows: "*Sp. 5.a.* F. fasciatus-*cauda elongata inmaculata, lanosa, corpore supra dilute fusco, infra albido cum pedibus cinnamomo-fasciato. Sp. n. HABITAT communior precedenti, nemora con ingreditur: longitude 34*$^{1/3}$, *cauda 11*$^{3/4}$. *Azara N.° XVIII Pajero*". Our translation in English is as follows: "Sp. 5.a. *F. fasciatus*-elongated tail, spotless, woolly, body pale brown above, whitish below with cinnamon-banded feet. Sp. n. HABITAT More common than the previous one, it enters forests: longitude 34$^{1/3}$, tail 11$^{3/4}$. Azara N.° XVIII Pajero".

Albeit Ximénez (1961) did not cite Larrañaga's *fasciatus* upon describing *munoai,* later Ximénez *et al.* (1972:18) referred to *fasciatus* as a junior synonym of *Felis pajeros* only noting that Larrañaga reproduced the external measurements from Azara (1802), argument followed by Nascimento *et al.* (2020). However, as we argue below, the assumption that Larrañaga's *fasciatus* merely described the "pajero" or "chat pampa" of Azara (1801, 1802) is mistaken.

Firstly, since Azara (1802) referred that the "pajero" occurred on both sides of the La Plata River, it follows that "donde al Negro" corresponds to the Negro River, with headwaters in Southern Brazil, crossing Uruguay east to west, finding its mouth in the Uruguay River (Figure 1). To strengthen the likelihood of this assertion, there is extensive evidence that Azara undertook his explorations and spent long periods in what is now Uruguay (González 1943; Mones and Klappenbach 1997; Contreras and Teta 2003; Ballarín *et al.* 2006). Thus, Azara's "pajero" encompasses populations that are now considered to belong to two different species (one in Argentina to the west of the Uruguay River, in Buenos Aires, and the other east of the Uruguay River in what is now Uruguay; Ximénez 1961; Nascimento *et al.* 2020). Larrañaga (1923), in turn, was explicit in that the taxa he was enumerating and describing, *e. g., Felis fasciatus*, were from Uruguay and not generically from the Río de la Plata (which could have also included Argentina; Larrañaga 1923:341–342). Secondly, Azara did not explicitly mention which specimens the dimensions that he reported were taken from. Hence, the argument used by Ximénez *et al.* (1972:18) regarding *fasciatus* as a synonym of *pajeros* based on those measurements is unsubstantiated, most especially detached from the whole of Larrañaga's diagnosis of *fasciatus*. Thirdly, while Azara mentioned that the "pajero" avoided forested areas, Larrañaga pointed out that *fasciatus* used wooded areas as well, in clear contrast to the former. Fourth, a key trait that Larrañaga noted in his diagnosis is represented by his chosen specific epithet, *i. e., fasciatus*. In Latin, the word "fasciate" is an adjective referring to bands or stripes (*e. g.*, broadly banded with color, see https://www.merriam-webster.com/dictionary/fasciate). Thus, "fasciatus" describes an object displaying a banded or striped pattern that, in the context of Larrañaga's diagnosis of the new species, *fasciatus* refers to the specimens as having cinnamon-colored banded feet. Azara (1802:162), in contrast, mentioned that the "pajero" had lightly cinnamon-colored limbs without bands. Fifth, while describing *munoai*, Ximénez (1961:6) noted that Uruguayan specimens displayed more vivid, rufous colorations (he used the Spanish word "anaranjado," meaning orangy in English); this difference in coloration is captured too in Larrañaga's description of *fasciatus* by using "cinnamon" versus Azara's "light cinnamon." Furthermore, whereas *munoai* specimens have

Figure 2. Ventral views of sample skins of *Leopardus fasciatus* (Larrañaga 1923) showing the variation from dark to lighter-colored talons, left to right, for Uruguayan specimens MNHN 1315, MNHN 2780, MNHN 2432 (designated neotype), and MNHN 4706, respectively.

been traditionally described as showing bicolored feet (*i. e.*, talons black versus paler dorsal side), our revision of Uruguayan specimens showed a great degree of variation in this trait (Figure 2). Notwithstanding, whereas it is worth mentioning for completeness, the fact that Larrañaga did not refer to this trait in his description is beyond the point. What stands out is that, despite Larrañaga's succinct description of *fasciatus*, the naturalist clearly distinguished between Azara's "pajero" (*i. e., F. pajeros* Desmarest, 1816) from the specimens he used for describing his *fasciatus* for Uruguay. In sum, Larrañaga made undoubtful morphologic and geographic observations that best fit the description and distribution of *L. munoai* and not that of *L. pajeros.* This is contrary to what has been supported elsewhere (*e. g.*, Ximénez *et al.* 1972; Nascimento *et al.* 2020), yet our argumentation is void of speculative claims based on partial and subjective interpretations of Larrañaga's description of *F. fasciatus.*

Figure 3. Dorsal and ventral views of the skin of the neotype specimen (MNHN 2432) of *Leopardus fasciatus* (Larrañaga 1923), female, collected in 1971 at Estancia San Cristóbal, Arroyo Limetas, Conchillas, Department of Colonia, Uruguay.

Larrañaga (1923) did not specify a type locality nor selected a holotype for the new species. This was a common practice at the time, and it was the same situation for other original descriptions within the "pampas cat" complex (García-Perea 1994). Henceforth, to anchor Larrañaga's *fasciatus* to Uruguayan pampas cats, here we designate specimen MNHN 2432 as the neotype of *L. fasciatus* (Larrañaga 1923); (Figure 3, 4). Then, the type locality of *L. fasciatus* is Estancia San Cristóbal, Arroyo Limetas, Conchillas, Department of Colonia, Uruguay (-34° 9' 54" S, -58° 5' 49 W; Figure 1). Provided this, together with the fact that there is no evidence suggesting that more than one species of pampas cat inhabits Uruguay (*e. g.*, Nascimento *et al.* 2020), we formally restrict the name *fasciatus* to pampas cat populations occurring in Uruguay, southern Brazil in the state of Rio Grande do Sul, and a portion of Corrientes province in eastern Argentina; note that this range includes the three specimens referenced by Azara from "donde al Negro" in Uruguay (Nascimento *et al.* 2020; Tirelli *et al.* 2021).

In sum, *Felis fasciatus* Larrañaga, 1923 represents the oldest available Linnean name for the Uruguayan pampas cat (see also Klappenbach 1997), an endemic species of the Uruguayan Savannah ecoregion (Tirelli *et al.* 2021). Accordingly, in compliance with the Principle of Priority of the International Code of Zoological Nomenclature (ICZN

Figure 4. Views of the skull (dorsal, ventral, and lateral including mandibles) of the neotype specimen (MNHN 2432) of *Leopardus fasciatus* (Larrañaga 1923), a female collected in 1971 at Estancia San Cristóbal, Arroyo Limetas, Conchillas, Department of Colonia, Uruguay.

1999), we formally suggest that Larrañaga's *fasciatus* is the name that applies for the "Uruguayan" pampas cat. Given the current understanding of pampas cat's taxonomic diversity (*e. g*., Nascimento *et al.* 2020), it should be used in the binomial combination *Leopardus fasciatus* (Larrañaga 1923). For an amended diagnosis of this species see Nascimento *et al.* (2020). As a corollary of our taxonomic proposition, we regard *L. munoai* (Ximénez 1961) as a junior synonym of *L. fasciatus*. Lastly, we suggest the use of the following vernacular names for *L. fasciatus* (Larrañaga 1923): Larrañaga's pampas cat (English); gato de pajonal de Larrañaga (Spanish); gato palheiro de Larrañaga (Portuguese).

There are still many unknowns about "What is a pampas cat?" (Giordano 2013). This work clarified the oldest available name for an entity readily threatened with extinction (Tirelli *et al.* 2021). Despite the clarity that we achieved about the identity of the Larrañaga's pampas cat, the likelihood of its long-term survival remains far from certain.

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Relative abundance and activity patterns of mesomammals in central Andes

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The natural history and ecology of mammals in the High Andes and Andean cloud forests are poorly known. This work aims to analyze the relative abundance and activity patterns of medium and large-sized mammals in the Andean portions of the Greater Madidi-Tambopata landscape using the camera trap methodology. Between 2012 to 2017, twelve sites were sampled covering an altitudinal gradient from 1,057 to 4,902 masl. A total of 311 digital camera trap stations were installed, and we applied a total effort of 5,144 trap nights (TN). We recorded a total of 21,346 photographs and 1,152 independent events from which we identified 28 species of mammals. The species with the highest relative abundance were *Lycalopex culpaeus* from 1 to 19.89/100TN, *Didelphis pernigra* from 0.1 to 10.71/100TN, and *Eira barbara* from 0.2 to 10.48/100TN. An analysis of activity patterns of the species that presented more than 10 independent events was carried out. The species with clearly nocturnal habits were *Conepatus chinga*, *Cuniculus paca*, *Cuniculus taczanowskii*, *Didelphis pernigra*, *Mazama chunyi*, *Mazama americana*, *Dasypus novemcinctus*, *Didelphis marsupialis*, *Lagidium viscacia, Lycalopex culpaeus* and *Leopardus tigrinus*, while *Dasyprocta variegata*, *Nasua* sp., *Eira barbara*, *Hippocamelus antisensis* and *Tremarctos ornatus* were diurnal; *Puma concolor* and *Neogale mustela* were cathemeral. The information obtained in our study responds to information gaps of a poorly studied mammal community and highlights the importance of Andean habitats for the conservation and management of montane species.

La historia natural y la ecología de los mamíferos en los hábitats andinos y los bosques nublados de los Andes son poco conocidas. Este trabajo tiene como objetivo analizar la abundancia relativa y los patrones de actividad de mamíferos medianos y grandes en áreas andinas del Gran Paisaje Madidi-Tambopata, se utilizó la metodología de registros y monitoreo con cámara trampa. Entre 2012 y 2017, se muestrearon doce sitios que cubrieron un gradiente altitudinal de 1,057 a 4,902 msnm. Se instalaron un total de 311 estaciones de trampas cámara digitales, se obtuvo un esfuerzo total de 5,144 noches trampa (TN). Se registraron un total de 21,346 fotografías y 1,152 eventos independientes, identificando 28 especies de mamíferos. Las especies con mayor abundancia fueron *Lycalopex culpaeus* de 1 a 19.89/100TN, *Didelphis pernigra* de 0.1 a 10.71/100TN y *Eira barbara* de 0.2 a 10.48/100TN. Se realizó un análisis de patrones de actividad de las especies que presentaron más de 10 eventos independientes. Las especies con hábitos claramente nocturnos fueron *Conepatus chinga, Cuniculus paca, Cuniculus taczanowskii, Didelphis pernigra, Mazama chunyi, Mazama americana, Dasypus novemcinctus, Didelphis marsupialis, Lagidium viscacia, Lycalopex culpaeus* y *Leopardus tigrinus,* mientras que *Dasyprocta variegata, Nasua* sp., *Eira barbara, Hippocamelus antisensis* y *Tremarctos ornatus* fueron diurnos. *Puma concolor* y *Neogale mustela* fueron catamerales. La información obtenida en este estudio responde al vacío de información de una comunidad de mamíferos poco estudiados y destaca la importancia de los hábitats andinos y montanos para la conservación y manejo de especies de montaña.

Keywords: Camera traps; circadian cycle; mesocarnivores; montane cloud forests; Rayleigh test.

Introduction

Research on medium and large-sized mammals in Bolivia has so far focused on lowland habitats, especially the Amazonian, Chiquitano and Chaco forests, with a lack of information from Andean montane habitats, which harbor a great diversity of poorly known species (Wallace *et al.* 2010a). Currently, Andean montane habitats are undergoing an intensification of land use, as well as the effects of climate change, which are also very noticeable and accelerated in these regions (Herzog *et al.* 2011). Andean montane forests are unique and fragile ecosystems that are the main source of water on a regional and continental scale, but they are currently experiencing accelerated deforestation rates, which are the greatest threat to their significant biological diversity (Tejedor-Garavito 2012).

Abundance and density estimates are important for wildlife management and conservation studies because they are population parameters that vary over time and space, which allow monitoring temporal variations of the population and indirectly assess the quality of habitats (Walker *et al.* 2000). Estimating the density of Neotropical mammals can be costly and difficult to obtain due to nocturnal habits, low numbers of individuals and evasiveness, and therefore the use of relative abundance indices is recommended for species without individual tags. These indices are easy to estimate and are expressed as photographic events per unit of effort (Rovero and Marshall 2009; Monroy-Vilchis *et al.* 2011).

Knowledge of the activity patterns of a species is important for understanding the biology and ecology of the species and inform management and conservation plans (Foster *et al.* 2013; Ayala *et al.* 2020). Activity patterns can be influenced by abiotic factors such as light intensity and temperature (Albanesi *et al.* 2016), as well as biotic factors such as physiology, inter- and intraspecific interactions (Porfirio *et al.* 2016), and anthropogenic disturbances (Van Dyke *et al.* 1986). In particular, the activity patterns of carnivores tend to be synchronized with the time of greatest vulnerability of their main prey (Theuerkauf *et al.* 2003).

The use of camera traps in the field of biology and particularly in wildlife research has increased exponentially in the last 25 years contributing to the knowledge of many species that are difficult to detect and study due to their low densities and elusive behavior (Karanth *et al.* 2004; Nichols *et al.* 2011). Camera traps are used as a tool for research on topics as diverse as the presence and distribution of species (Bowkett *et al.* 2007), species richness (Tobler *et al.* 2008; Ahumada *et al.* 2011), abundances and population densities (Wallace *et al.* 2003; Tobler *et al.* 2013), predator-prey relationships (Ayala *et al.* 2020), survival (Karanth *et al.* 2002), occupancy (Nichols and Karanth 2002), habitat preference (Alempijevic *et al.* 2021), activity patterns (Ayala *et al.* 2020), and behavior (Viscarra *et al.* 2019). The use of camera traps is one of the most important and versatile methods for conservation biology research (Ayala *et al.* 2020; Mena *et al.* 2020).

Our study focused on three questions: 1) What are the species of medium and large mammals present in the High Andes and montane cloud forests of the Andean portion of the Greater Madidi-Tambopata Landscape? 2) What species are the most abundant? 3) What is the pattern of activity? The information obtained will respond to information gaps in a little studied mammal community and this baseline will allow us to monitor wildlife population behavioral changes in the years to come.

Materials and methods

Study area. The research was carried out at 12 Andean sites in the Greater Madidi-Tambopata Landscape, a transboundary landscape, between northwestern Bolivia and southeastern Perú (Figure 1). This area covers approximately 142,530 km^2 (Bolivia 106,810 km^2; Perú 35,720 km^2), composed of six national protected areas, as well several indigenous communities and municipal protected areas. This landscape is characterized by an impressive altitudinal range (180 to 6,044 masl), and a varied topography and climate that have resulted in diverse plant and animal communities and a high number of endemic species. The Puina, Pampas de Apolo, Mamacona, Cargadero, Isañuj, Sarayoj, Keara and Suchez study sites are within the Madidi National Park and Natural Area of Integrated Management (PN ANMI Madidi), Pusupunku and Piwara are within the Apolobamba National Natural Area of Integrated Management (PN ANMI Apolobamba), and the Acero Marka and Camino Yungas sites are immediately adjacent to the Cototapata National Park and Natural Area of Integrated Management (PN ANMI Cotapata). Sampling sites covered Andean and montane habitats (Table 1). The High Andes region includes mountains that exceed 6,000 masl, but our study was concentrated in an altitudinal range of 4,100 to 4,902 masl characterized by undulating plateaus crossed by mountain ranges. The climate is generally cold and dry with intense solar radiation and large daily thermal amplitudes. Average annual temperatures range from below 0 °C to 9 °C (Navarro 2002). The montane cloud forests are found on the steep slopes and peaks and can be divided into the following levels: tree line forest (3,000 to 3,600 masl), upper montane forest (2,700 to 3,000 masl), mid-montane forest (1,700 to 2,600 m asl) and lower montane forest (1,100 to 1,700 masl). The climate and topography result in a relatively low and evergreen forest with leathery leaves (Ribera 1995; Identidad Madidi and SERNAP 2017, 2019). Epiphytes abound, especially mosses and lichens, which cover tree trunks, branches, and treetops, as well as a thickness soft layer that covers the ground. The average temperature is 18 °C, and the humidity is 80 % (Paniagua-Zambrana *et al.* 2003).

Camera Trap Methodology. Between 2012 to 2017, a total of 12 camera trap campaigns were implemented. A variable number of camera trap stations were placed 1 to 2 km apart from each other, each station consisted of one or two cameras placed face to face, each set at 50 to 70 cm from the ground (Noss *et al.* 2013). Three digital camera trap models were used: Reconyx® HC500, HC550® (USA) and Bushnell® HD 119477 (USA). All cameras were programmed to operate 24 hours a day, to take 1 to 10 shots, depending on the camera model (Ayala *et al.* 2020).

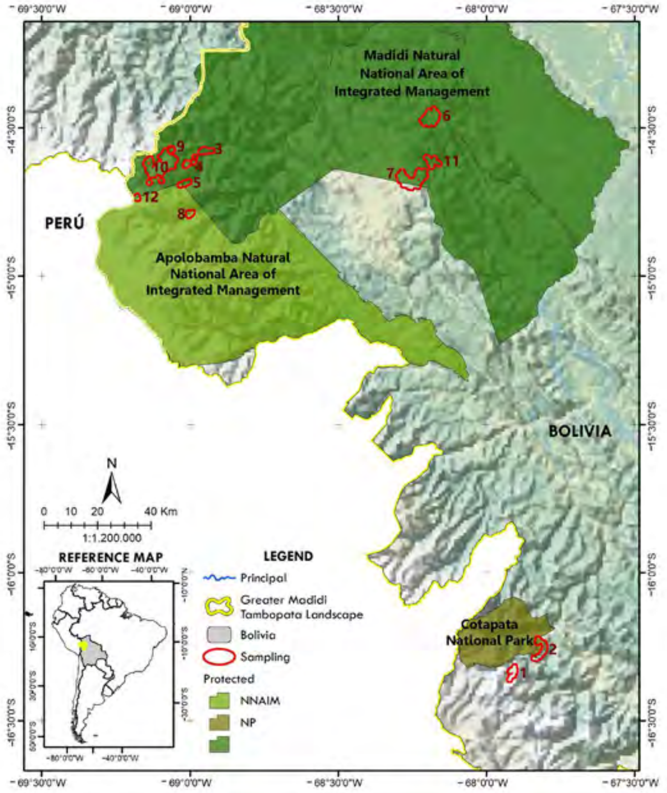

Figure 1. Map of 12 sampling sites (numbered red polygons: 1 = Acero Marka, 2 = Camino Yungas, 3 = Cargadero, 4 = Isañuj, 5 = Keara, 6 = Mamacona, 7 = Pampas de Apolo, 8 = Pasto Grande, 9 = Puina, 10 = Puina 2, 11 = Sarayoj, 12 = Suchez) in the Andean portion of the Greater Madidi-Tambopata Landscape.

The camera trap stations were placed in the predominant habitats of each study area: a) open areas along the main and secondary rivers; b) forest, often located along animal trails and small streams within the forest; c) pampa, open grassland locations, and d) rocky outcrops, this terrain is arid, sparsely vegetated, and steep. Between 7 and 71 stations were placed at each study site, sampling effective areas of between 2.1 to 92.4 km^2 (Table 1). Each camera trap campaign was conducted for a period of between 5 to 80 of effective sampling days. The location of all the camera trap stations were georeferenced.

Relative Abundance Index. To quantify photographic records, we calculated independent events for each species, where photographs of the same species at a given station separated by 30 minutes are considered as separate events (O'Brien *et al.* 2003). Relative abundance index (RAI) was calculated through the capture rate expressed as the number of independent events per total number of traps night during the study and multiplied by 100 (O'Brien *et al.* 2003). The traps night are the days that the camera traps were active during the study.

Activity Pattern Analysis. All photographs were classified according to the time automatically registered on each photograph. The following categories were defined for the analysis: a) *Sunrise*, 30 minutes before the first ray of sunlight rises above the horizon, b) *Day*, begins at the moment when the first rays of the sun appear on the horizon, c) *Sunset*, begins at the moment when the sun is lost on the horizon, d) *Night*, begins 30 minutes after the sunset on the horizon (Ayala *et al.* 2020). This classification was made based on the sunrise and sunset time of each sampling day for the location of each sampling site (www.tutiempo.net).

Statistical analyses were performed in R software version 3.3.2 (R Core Team 2011). Using the Circular package (Agostinelli and Lund 2013), a Rayleigh test was conducted to assess the distribution uniformity on the daily activity data to evaluate whether or not species exhibited a random activity pattern over the circadian cycle. The synchronization of non-random data could indicate that the animals are nocturnal, diurnal, or crepuscular, whereas if the data are evenly distributed throughout the day, the species are classified as cathemeral (Pratas-Santiago *et al.* 2016).

We used Kernel density estimation to generate the activity patterns for each species, Kernel density estimations are a non-parametric way to estimate the probability density function of a distribution of records (Linkie and Ridout 2011). The analysis of activity patterns of species with more than 10 independent events was carried out (Gerber *et al.* 2012; Monterroso *et al.* 2014).

Results

The sites sampled covered an altitudinal gradient from 1,157 to 4,902 masl. A total of 311 digital camera trap stations were installed, obtaining a sampling effort of 5,144 trap nights (TN; Table 1). A total of 21,346 photographs and 1,152 independent events were obtained, identifying 28 species of mammals. The species with the highest number of photographs were *Didelphis pernigra* (*n* = 10,305), *Lagidium viscacia* (*n* = 2,030) and *Lycalopex culpaeus* (*n* = 1,929; Table 2).

The species with the highest relative abundance were *L. culpaeus* with a range of 1 - 19.89/100 TN, *D. pernigra* with 0.1 - 10.71/100 TN, *L. viscacia* with 1.6 - 14.43/100 TN, *Eira barbara* with 0.2 - 10.48/100 TN, *Mazama chunyi* with 0.31 - 3.14/100 TN and *Nasua* sp. 0.17 - 2.9/100 TN (Table 2).

The Rayleigh test for data uniformity revealed that eleven of the 18 species with more than 10 independent events showed clear nocturnal activity patterns (Table 3). *Conepatus chinga* (92 % nocturnal) registered activity peaks between 20:30 to 21:30 hrs and 4:00 to 5:30 hrs. *Cuniculus paca* (100 %) had activity peaks between 22:30 to 24:00 hrs, whilst *C. taczanowskii* (97 %) had activity peaks between 20:30 to 22:00 hrs. Activity peaks were between 18:30 to 19:30 hrs for *Didelphis pernigra* (95 %), and between 20:30 to 22:30 hrs for *D. marsupialis* (100 %). The dwarf brocket deer (*Mazama chunyi*) is predominantly nocturnal (83 %), with very little diurnal (10 %) and crepuscular activity (7 %), and a clear activity peak between 17:30 to 19:30 hrs. The red brocket deer (*Mazama americana*) is predominantly

Table 1. Sampling sites, habitats, number of camera trap stations and camera trap nights sampled in the Andean portion of the Greater Madidi-Tambopata Landscape.

Year	Survey Site	Habitat	Altitude Range (masl)	Camera Trap Stations	Camera Trap Nights	Sampled Polygon (km^2)
2012	Acero Marka (AMK)	High Andes – Tree Line Forest	3,117 – 3,759	19	1,254.2	5.5
	Puina 1 (PNA1)	Tree Line Forest	3,157 – 3,404	7	241.1	2.2
	Pusupunku-Piwara (PP)	Tree Line Forest	2,837 – 3,514	12	373.3	2.1
2015	Pampas Apolo (PDA)	Mid-montane Cloud Forest	1,691 – 2,336	59	482.3	38.4
	Puina 2 (PNA 2)	High Andes – Tree Line Forest	3,152 – 4,761	71	974.5	92.4
2016	Mamacona (MAN)	Low-montane Cloud Forest	1,521 – 2,123	28	312.3	21.9
	Cargadero (CGR)	Mid-montane Cloud Forest	1,317 – 3,223	28	350.3	3.5
	Isañuj (ISÑ)	High Andes – Tree line Forest	3,298 – 3,611	15	298.3	2.5
	Camino Yungas (CYUN)	Mid-montane Cloud Forest	1,670 – 3,049	28	515.4	17.9
2017	Sarayoj (SRJ)	Upper Foothill Forest	1,157 – 1,448	20	135.9	8.8
	Keara (KR)	Upper Montane Cloud Forest	2,750 – 3,000	16	176.4	2.1
	Suchez (SCH)	High Andes	4,805 – 4,902	8	30.2	2.4
Total	**12**		**1,157 – 4,902**	**311**	**5,144.3**	**193.5**

nocturnal (80 %), with very little diurnal (20 %), activity peaks were between 18:30 to 24:00 hrs. The nine-banded armadillo (*Dasypus novemcinctus*) is primarily nocturnal (70 %), with little diurnal (30 %), and a clear activity peak between 18:30 to 1:30 hrs (Figures 2, 4). The Andean fox (*Lycalopex culpaeus*) is mainly nocturnal (74 %), with some diurnal (22 %) and crepuscular (4 %) activity, and peaks between 18:30 to 19:30 hrs and 22:30 to 23:30 hrs. The oncilla (*Leopardus tigrinus*) is primarily nocturnal (82 %), with some diurnal (14 %) and crepuscular (4 %) activity, and activity peaks between 17:30 to 21:30 hrs and 5:30 to 6:30 hrs. The northern vizcacha (*L. viscacia*) is nocturnal (58 %), with less diurnal (34 %) and crepuscular activity (8 %), and activity peaks between 20:30 - 01:30 hrs (Figures 3, 4).

The Rayleigh test for data uniformity revealed that the long-tailed weasel (*Neogale frenata*) is cathemeral predominantly nocturnal (43 %) and crepuscular (35 %), with less diurnal activity (21 %). The puma (*Puma concolor*) is cathemeral predominantly nocturnal (50 %), with less diurnal (40 %) and crepuscular activity (10 %) and activity peaks between 8:30 to 11:30 hrs and 18:00 to 21:30 hrs (Figures 3, 4, Table 3). Five species presented predominantly diurnal habits (Table 3), the tayra (*Eira barbara* 96 %) without showing activity peaks during the day. The Andean bear (*Tremarctos ornatus* 88 %) showed an activity peak between 13:30 to 15:30 hrs. The agouti (*Dasyprocta variegata*) is mainly diurnal (76 %) with some nocturnal and crepuscular activity (24 %) and a peak between 16:30 to 17:30 hrs. The coati (*Nasua* sp.) was predominantly diurnal (76 %), with less nocturnal activity (24 %) and an activity peak between 06:30 to 10:00 hrs. The north Andean deer (*Hippocamelus antisensis)* is predominantly diurnal (70 %) with some nocturnal and crepuscular activity (30 %) and a peak between 8:30 to 17:30 hrs (Figures 3, 4).

Table 2. Number of photographs, independent events (IE) and Relative Abundance Index (RAI) registered at 12 sample sites (AMK = Acero Marka, PNA 1 = Puina 1, PNA 2 = Puina 2, PP = Pusupunku-Piwara, PDA = Pampas de Apolo, MAM = Mamacona, CGR = Cargadero, ISÑ = Isañuj, CYUN = Camino Yungas, SRJ = Sarayoj, KR = Keara, SCH = Suchez) in the Andean portion of the Greater Madidi-Tambopata Landscape.

Species	Photos	IE	AMK	PNA1	PP	PDA	PNA2	MAM	CGR	ISÑ	CYUN	SRJ	KR	SCH
Primates														
Sapajus apella	25	5				0.86±0.40		0.35±0.33						
Lycalopex culpaeus	1,929	235	16.38±7.86				1±0.43			1.04±0.11			6.81±3.16	19.89±12.16
Leopardus colocola	27	3					0.3±0.17							
Leopardus jacobita	17	3					0.31±0.18							
Leopardus pardalis	44	4						0.36±0.34				2.14±1.55		
Leopardus tigrinus	177	22		0.41±0.12	0.54±0.11		0.31±0.17	1.72±0.66	0.3±0.29	0.63±0.44	1.51±0.59			
Puma concolor	63	11	0.08±0.08					0.35±0.33		1.96±1.11	0.39±0.36		0.57±0.57	
Puma yagouaroundi	19	2									0.39±0.39			
Conepatus chinga	608	53	3.15±2.35				1.11±1.06						1.73±0.87	3.5±3.23
Eira barbara	143	27		0.83±0.15		1.07±0.53		0.69±0.45	0.57±0.38		0.2±0.21	10.48±7.39	0.59±0.56	
Neogale frenata	76	15	0.15±0.15			0.21±0.22	0.11±0.11				0.19±0.19		0.57±0.57	
Nasua sp.	1,086	33	0.17±0.16	2.9±0.42	3.21±1.22			1.01±0.53			0.96±0.47			
Tremarctos ornatus	459	17	0.24±0.12	1.8±0.31					0.29±0.28			1±0.49		
Artiodactyla														
Hippocamelus antisensis	172	12	0.48±0.28				0.62±0.28							
Odocoileus virginianus	8	1					0.1±0.11							
Mazama chunyi	1,063	84	2.47±1.02	3.32±0.27	1.33±0.21	1.7±0.68	0.31±0.23	1.05±0.56	1.44±0.72		3.14±1.22		1.77±1.72	
Mazama americana	81	10				1.09±0.55		1.04±0.54				1.42±0.95		
Xenarthra														
Dasypus novemcinctus	92	12				0.03±0.91					1.19±1.15			
Rodentia														
Cuniculus paca	442	47						7.67±2.26	4.11±3.14		0.6±0.58	5.05±2.14		
Cuniculus taczanowskii	1,961	76	1.98±0.96	3.32±1.22	1.86±0.21	0.43±0.45			2.25±0.91	3.08±1.26	2±0.98		0.6±0.57	
Dasyprocta variegata	299	29				0.21±0.21		4.47±1.37	0.54±0.56		1.13±0.95	4.97±2.86		
Coendou bicolor	10	1				0.22±0.21								
Lagidium viscacia	2,030	170	1.66±1.12				14.43±3.67							6.74±4.13
Notosciurus pucheranii	36	8				0.63±0.47		1.02±0.53			0.19±0.19	0.76±0.69		
Hadrosciurus spadiceus	3	1										0.73±0.71		
Didelphimorphia														
Didelphis marsupialis	152	17									1.36±0.58	6.74±3.15		
Didelphis pernigra	10,305	249	6.16±3.27		10.71±2.32		0.1±0.12				0.78±0.38		2.77±1.44	
Lagomorpha														
Lepus europaeus	19	5					0.52±0.36							

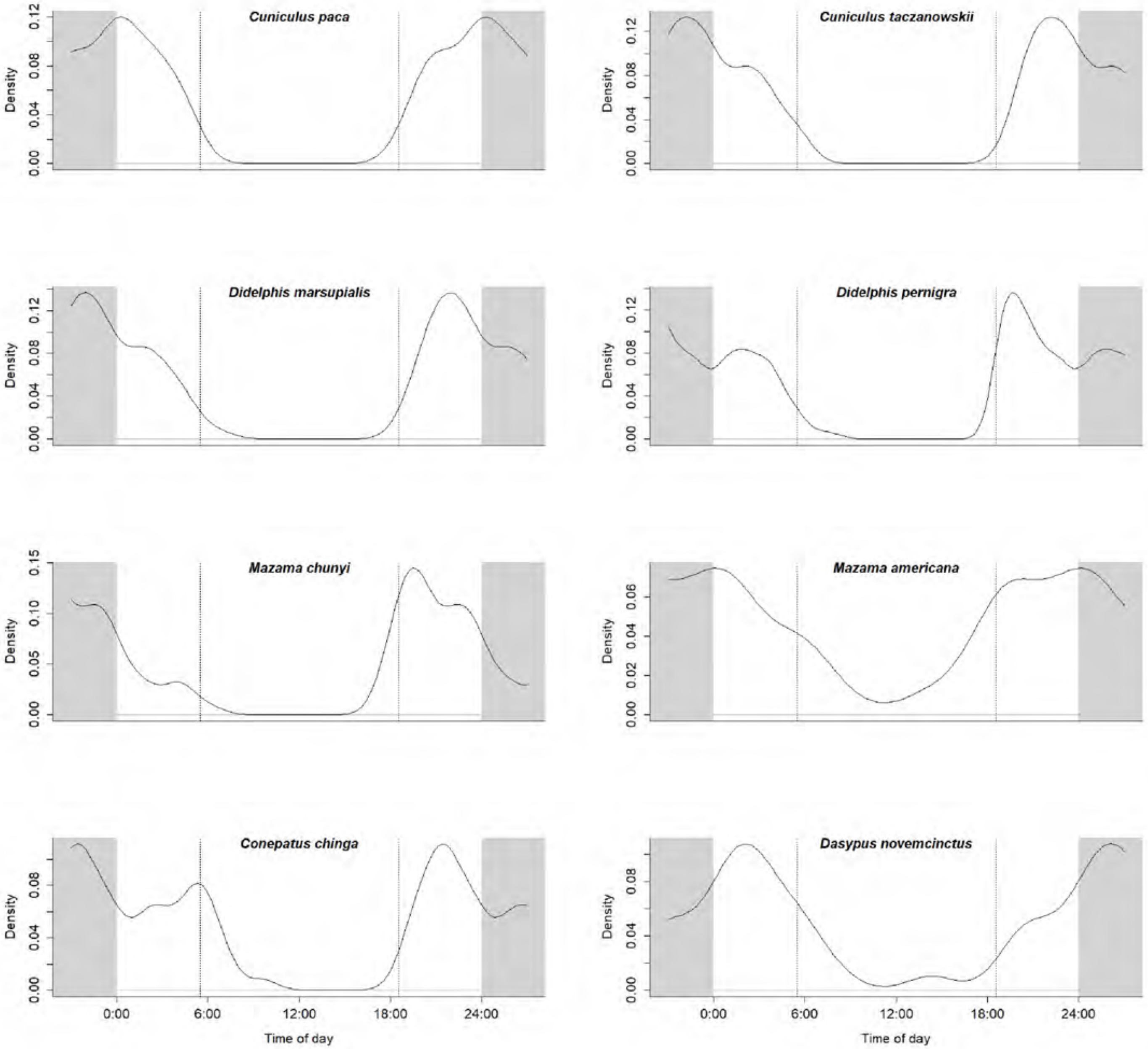

Figure 2. Activity patterns of nocturnal species in the Andean portion of the Greater Madidi-Tambopata Landscape. The two parallel dotted lines represent approximate sunrise and sunset (between 6:00 to 6:30 and 18:00 to 18:30 h, respectively, throughout the year). Shaded areas represent nighttime.

Discussion

Camera traps can record the presence of rare species, document geographic range, and reveal behaviors, as well as estimate populations, and identify habitat requirements (Karanth and Nichols 2002; O'Connel *et al.* 2011). In our study we were able to register cryptic species such as *N. frenata* and *L. tigrinus*, which previously had few records in these habitats. The camera traps also confirmed the presence of a phenotypically distinct coati (*Nasua* sp.).

Leopardus tigrinus is one of the species that has very little abundance data in montane forest and we obtained abundance data for this species from six sampled sites, where the sites with the highest abundance were Mamacona (MAM; 1.72 ± 0.66/100 TN) and Camino Yungas (CYUN; 1.51 ± 0.59/100 TN), both sites belong to the lower and mid montane forest between 1,300 to 2,700 masl where a variety of marsupials and small arboreal rodents, which are frequent preys for this feline, are present (Wang 2002). The nocturnal activity patterns found on *L. tigrinus* are similar to those reported in other studies in similar habitats (Bonilla-Sánchez *et al.* 2020).

The mountain paca (*C. taczanowskii*) is a rodent that inhabits Andean cloud forests from Venezuela to northern Bolivia and is most abundant between 2,000 to 3,350 masl (Wallace *et al.* 2010b). In this study, we registered the species between 1,943 to 3,552 masl at eight of our study sites (Table 2), with lower relative abundances than those reported in the montane forests of Peru (Jiménez *et al.* 2010: 17.0 and 1.6/100 TN). Activity patterns were predominantly nocturnal and are consistent with those reported in other studies (Jiménez *et al.* 2010). *C. taczanowskii* is considered an upland species while *Cuniculus paca* a lowland species, however, they may be sympatric in certain areas of their distribution ranges (Wallace *et al.* 2010b). In our study, both species were recorded at the Camino Yungas (CYUN) and Cargadero (CGR; Table 2), and these sites belong to the middle montane forest, which are transitional areas between upland and lowland habitats. Due to the scarce records of *C. paca*, it was not possible to carry out the analyses of the overlapping of activity patterns to observe whether there was a temporal segregation between the two species as they have similar morphology and trophic requirements. It is therefore important to continue to generate information in transitional habitats to better understand the ecology of both species.

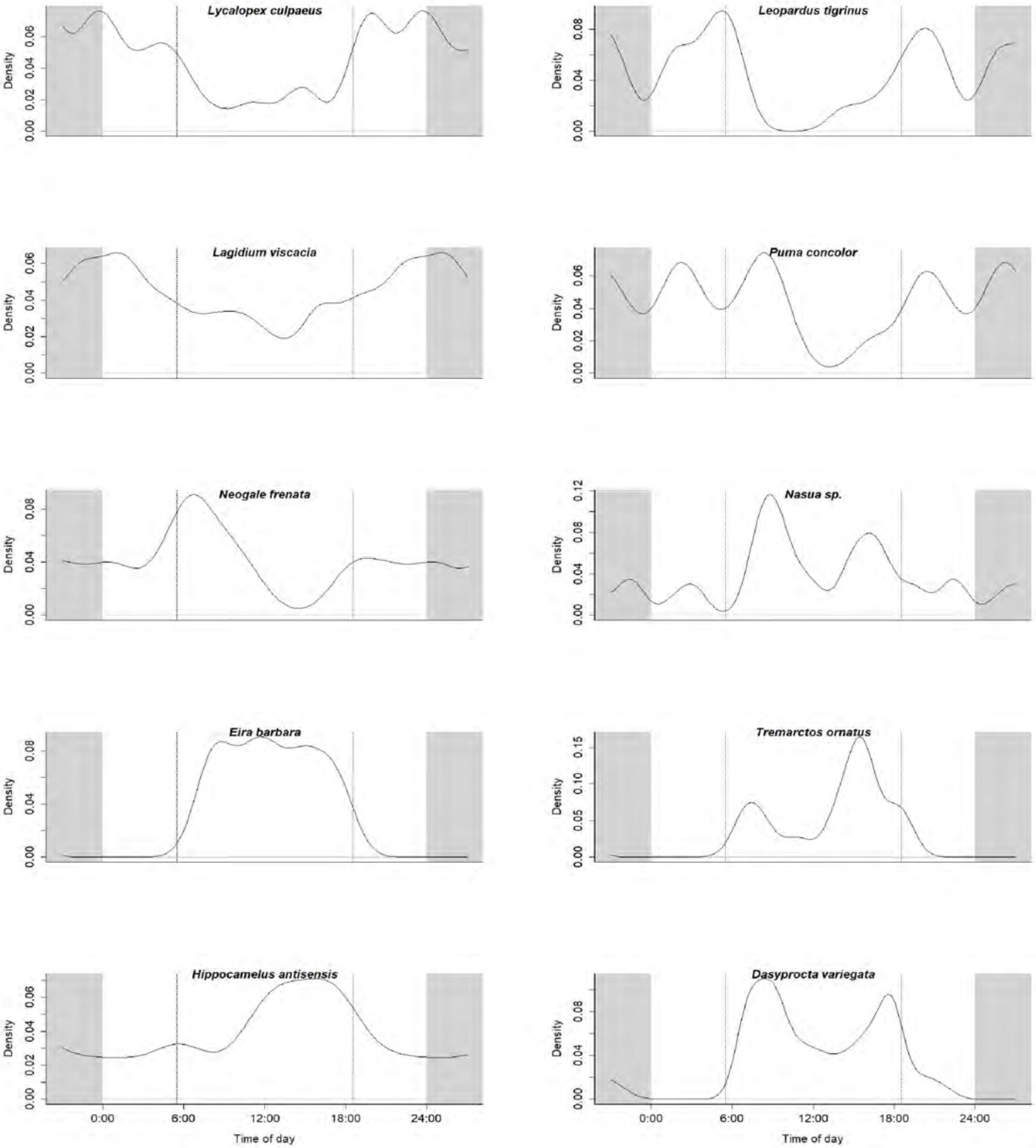

Figure 3. Activity patterns of species with nocturnal, cathemeral and diurnal habits in the Andean portion of the Greater Madidi-Tambopata Landscape. The two parallel dotted lines represent approximate sunrise and sunset (between 6:00 to 6:30 and 18:00 to 18:30 h, respectively, throughout the year). Shaded areas represent nighttime.

Table 3. Rayleigh uniformity test (Z) for the temporal activity of medium and large-sized mammals in Andean montane habitats of the Greater Madidi-Tambopata Landscape. * = for a non-random distribution ($P < 0.05$).

Species	Classification	Z	p
Leopardus tigrinus	Nocturnal	0.3709	0.046
Lycalopex culpaeus	Nocturnal	0.3533	0.01
Conepatus chinga	Nocturnal	0.5655	0.01
Mazama chunyi	Nocturnal	0.7300	0.01
Mazama americana	Nocturnal	0.4692	0.01
Dasypus novemcinctus	Nocturnal	0.6066	0.01
Cuniculus paca	Nocturnal	0.7565	0.01
Cuniculus taczanowskii	Nocturnal	0.7625	0.01
Didelphis marsupialis	Nocturnal	0.7571	0.01
Didelphis pernigra	Nocturnal	0.6527	0.01
Lagidium viscacia	Nocturnal	0.2292	0.001
Nasua sp.	Diurnal	0.2948	0.055
Hippocamelus antisensis	Diurnal	0.3262	0.028
Eira barbara	Diurnal	0.6608	0.01
Dasyprocta variegata	Diurnal	0.4517	0.01
Tremarctos ornatus	Diurnal	0.5809	0.002
Puma concolor	Cathemeral	0.2614	*0.391
Neogale frenata	Cathemeral	0.3087	*0.243

The northern vizcacha (*L. viscacia*) has been described as a diurnal-crepuscular species (Walker *et al.* 2007), but at high altitude sites nocturnal activity patterns with peaks at dawn have been reported (Lucherini *et al.* 2009; Huaranca *et al.* 2019). Our results coincide with a primarily nocturnal activity pattern, with an activity peak between 20:30 to 1:30 hrs. The sites of AceroMarka (AMK), Suchez (SCH) and Puina (PNA) where *L. viscacia* was recorded are above 3,700 masl where one of the main livelihoods of the local people is the raising of camelid cattle, *Vicugna pacos* and *Lama glama* (INE 2013). In these sites there is a trophic overlap regarding grass species (*Festuca* sp., *Stipa* sp.) between camelid livestock and vizcachas (Cortes *et al.* 2002). On the other hand, Tarifa *et al.* (2004) reported that vizcachas compete for food with cattle, therefore we consider that vizcachas are more active at night to avoid competing with camelids for food resources during the day. Dietary studies on the Andean cat (*Leopardus jacobita*) registered *L. viscacia* as the principal prey (Walker *et al.* 2007; Napolitano *et al.* 2008), and Andean cats exhibited an activity pattern similar to that of vizcachas (Lucherini *et al.* 2009; Huaranca *et al.* 2019). Our study yielded insufficient records of Andean cat to evaluate activity pattern overlap with vizcachas.

In our study, we obtained abundance estimates of *L. culpaeus* for five of the sites sampled, with the highest abundances found at the Aceromarka (AMK), Keara (KR) and Suchez (SCH) sites (Table 2). These three sites have camelid livestock, and it is possible that the high abundances of the Andean fox are related to the presence of domestic camelids, as dietary studies on *L. culpaeus* report their consumption of domestic camelids (Walker *et al.* 2007). The activity patterns of *L. culpaeus* are better studied in the region (Johnson and Franklin 1994; Salvatori *et al.* 1999; Lucherini *et al.* 2009), and in general agree with the findings in our study that *L. culpaeus* is primarily nocturnal. Dietary studies consider *L. culpeus* as a generalist and opportunistic species with *L. viscacia* as the third most important prey (Walker *et al.* 2007). Using data from our study we performed an over-

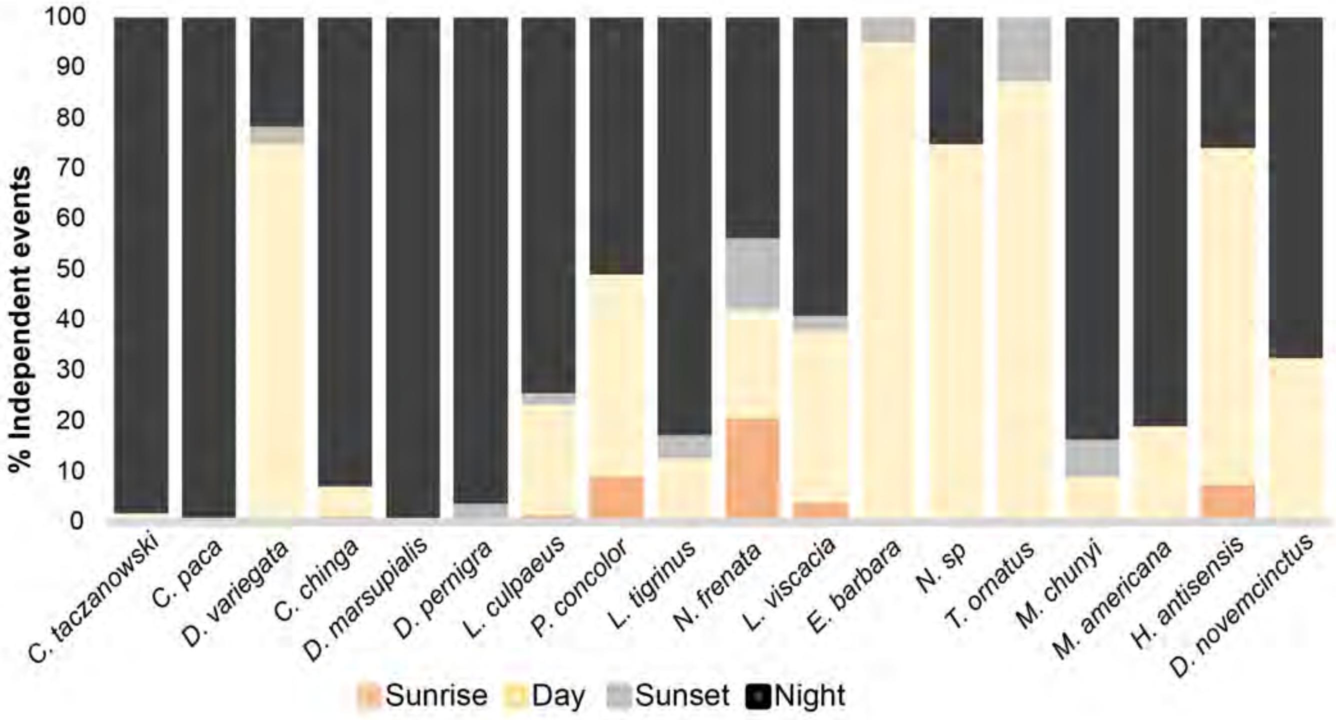

Figure 4. Percentage of activity across the day (sunrise, day, sunset, night) of fourteen species with n ≥ 10 independent events in the Andean portion of the Greater Madidi-Tambopata Landscape.

lap analysis of activity patterns between *L culpaeus* and *L. viscacia* according to standardized methodology (Ridout and Linkie 2009; Ayala *et al.* 2020), and we registered a significant overlap between both species (Δ_1 = 0.86, IC = 0.7 - 0.99, U_2 = 0.14, $P > 0.1$, Watson test of homogeneity) confirming that *L. viscacia* is one of the main preys for *L. culpaeus* (Figure 5).

The puma (*P. concolor*) despite being the largest felid in montane and high Andean habitats, has little information on abundances in these habitats. In our study it was recorded in five sites, with low abundances ranging from 0.08 to 1.96/00 TN. It is likely that this low abundance is due to conflict between local people and predators that consume domestic camelids. Studies on puma diet in the Bolivian highlands indicate that *V. pacos* and *L. glama* are the second and third most consumed food items, representing 32 % of their diet, causing pumas to be hunted or driven away (Pacheco *et al.* 2004). The cathemeral activity patterns found on *P. concolor* are similar to those reported in other studies in similar habitats (Caceres-Martinez *et al.* 2016).

The tayra (*E. barbara*), was recorded at seven sampling sites and the site with the highest abundance was Sarayoj (SRJ; Table 2). It is likely that the abundance was higher there because it was the lowest altitude site sampled and is the transition with piedmont habitats where similar abundances of 9.49/100 TN were reported with camera traps (Wallace *et al.* 2010a). Compared to previous studies in montane forests (Jiménez *et al.* 2010), relative abundance at our study sites were higher (Table 2) than in Peru (0.8 to 2/100 TN). This is a diurnal and crepuscular species with activity peaks between 13:00 to 15:00 hrs. The activity patterns we found are consistent with those reported in other studies (Delgado *et al.* 2011; Albanesi *et al.* 2016).

The Andean bear (*T. ornatus*) is considered a landscape species, principally due to its ecological role as a seed disperser (Velez-Liendo and Paisley 2010) and is also primarily diurnal (Paisley and Garshelis 2006; Jiménez *et al.* 2010; Bonilla-Sánchez *et al.* 2020). Andean bears were registered at four sites in our study, at higher relative abundances than in Peru (Jiménez *et al.* 2010).

The unidentified coati (*Nasua* sp.) was registered at five sites between 1,557 to 3,398 m a.s.l., and was primarily diurnal with a relative abundance of 0.17 to 3.2/100 TN. Conversely, the mountain coati (*Nasuella olivacea*) in the montane forests of Colombia was primarily nocturnal with activity peaks between 19:00 to 20:00 hrs, (Ramírez-Mejía and Sánchez 2016). Previous studies indicate that coatis are mainly diurnal and crepuscular (Cáceres-Martínez *et al.* 2016).

Our results clearly demonstrate that *D. pernigra* is primarily a nocturnal species and these results coincide with previous studies (Cáceres-Martínez *et al.* 2016; Ramírez-Mejía and Sánchez 2016; Albanesi *et al.* 2016). However, Pérez-Hernández *et al.* (2016) reported activity patterns in low proportions during daylight hours, indicating that this behavior is possibly due to the behavioral plasticity and generalist character of this species, which is considered tolerant to anthropic intervention and urban environments.

Molina's hog-nosed skunk (*C. chinga*) is a nocturnal species, with no data on abundance in Bolivia (Tarifa *et al.* 2010). Studies in Patagonia, Argentina, revealed a completely nocturnal activity for *C. chinga*, with activity peaks between 19:00 to 22:00 hrs and 02:00 to 05:00 hrs (Donadio *et al.* 2001), although, there are recent reports of cathemeral habits in the Velez Peninsula of Patagonia (D'Agostino and Udrizar 2021). In our study, *C. chinga* is strongly nocturnal, with very few records during the day (Figure 2).

We provide some of the first information on the relative abundance and activity patterns of mammals in Andean habitats, especially the mysterious and relatively inaccessible montane cloud forests of the Tropical Andes. Our findings reveal the presence of 28 species of mesomammals in the sampled habitats, of which 11 species are nocturnal, 5

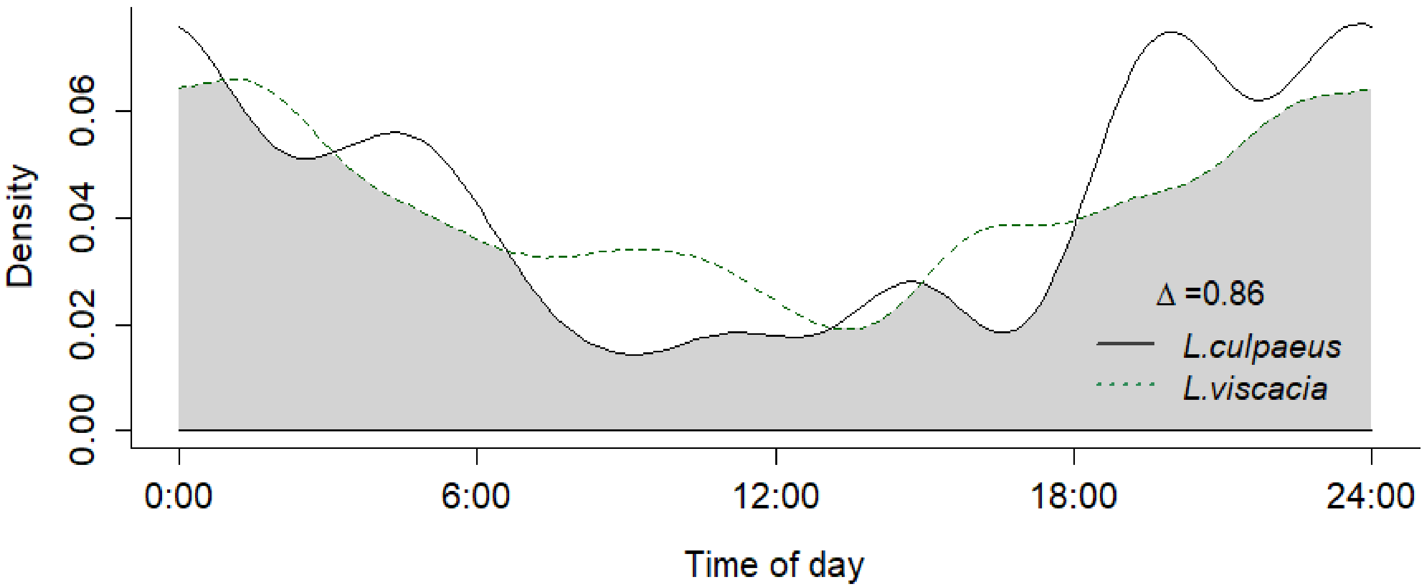

Figure 5. Overlap coefficient of daily activity patterns between *Lycalopex culpaeus* - *Lagidium viscacia*. The overlap is represented by the shaded area.

diurnals and 2 cathemeral species, where the most abundant species were *L. culpaeus* in the Andean habitats and *E. barbara* in montane habitats. The increasing threats to both habitats underscore the importance of long-term conservation research efforts to provide conservation management relevant knowledge on population and ecological dynamics, in addition to generating more support for national, municipal, and private protected areas in the Tropical Andes.

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Land use change and its implications for biodiversity and jaguar conservation

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Protected areas are frequently established to prevent declines in biodiversity, but their effectiveness in preserving biodiversity can depend on how land outside their borders is managed. We evaluated how land use changes from 1989 to 2016 in the Reserva de la Biosfera Sierra del Abra Tanchipa (RBSAT) landscape might affect the role of the RBSAT for conservation of biodiversity, with an emphasis on conservation of jaguars, a keystone species. We estimated the rate of land use change within and surrounding the RBSAT, a 215 km^2 natural reserve in San Luis Potosí, Mexico, from 1989 to 2016 using supervised classifications of satellite imagery. We also analyzed the distribution of two GPS collared male jaguars. The RBSAT and surrounding landscape became increasingly fragmented and impacted by human use over the previous *ca*. 30 years. The largest increases were seen in infrastructure and intensive agriculture, while the largest decreases were seen in pasture, tropical deciduous forest, and secondary vegetation. Jaguars were located more frequently than expected in secondary vegetation, the most common cover class, which decreased from 34.8 % of the landscape to 32.1 % by 2016. Only 23 % of jaguar locations fell within the boundaries of the RBSAT, due to increases in preferred habitat attributes of jaguars and prey outside the Reserve. Increasing fragmentation compromises the RBSAT's role as a biodiversity reserve, especially for interior-dependent species. Fragmentation and edge habitats in combination with increasing agriculture enhance suitability of the landscape surrounding the RBSAT for prey of jaguar, and only 23 % of jaguar locations were within the RBSAT itself. This increases the likelihood of jaguar-related conflicts in surrounding communities. Regional landscape planning should include policies that incentivize practices that maintain remaining larger habitat patches and minimize the likelihood of human-wildlife conflicts.

Las áreas protegidas se establecen con frecuencia para evitar la disminución de la biodiversidad, pero su eficacia para preservar la biodiversidad puede depender de cómo se gestione la tierra fuera de sus fronteras. Se evaluó cómo los cambios en el uso del suelo de 1989 a 2016 en el paisaje de la Reserva de la Biosfera Sierra del Abra Tanchipa (RBSAT) podrían afectar el papel de la RBSAT para la conservación de la biodiversidad, con énfasis en la conservación de los jaguares, una especie clave. Se estimó la tasa de cambio de uso del suelo dentro y alrededor de la RBSAT, una reserva natural de 215 km2 en San Luis Potosí, México, de 1989 a 2016 utilizando clasificaciones supervisadas de imágenes satelitales. También se analizó la distribución de dos jaguares machos con collar GPS. La RBSAT y el paisaje circundante se fragmentaron cada vez más y se vieron afectados por el uso humano durante los ca. 30 años. Los mayores aumentos se observaron en infraestructura y agricultura intensiva, mientras que las mayores disminuciones se observaron en pastos, bosque tropical caducifolio y vegetación secundaria. Los jaguares se ubicaron con mayor frecuencia de lo esperado en la vegetación secundaria, la clase de cobertura más común, que disminuyó del 34,8 % del paisaje al 32,1 % en 2016. Solo el 23 % de las ubicaciones de jaguares se ubicaron dentro de los límites de la RBSAT, debido a aumentos en la preferencia. atributos de hábitat de jaguares y presas fuera de la Reserva. La creciente fragmentación compromete el papel del RBSAT como reserva de biodiversidad, especialmente para las especies dependientes del interior. La fragmentación y los hábitats de borde en combinación con el aumento de la agricultura mejoran la idoneidad del paisaje que rodea a la RBSAT para las presas del jaguar, y solo el 23 % de las ubicaciones de los jaguares estaban dentro de la propia RBSAT. Esto aumenta la probabilidad de conflictos relacionados con jaguares en las comunidades aledañas. La planificación del paisaje regional debe incluir políticas que incentiven prácticas que mantengan los parches de hábitat más grandes restantes y minimicen la probabilidad de conflictos entre humanos y vida silvestre.

Keywords: Land classification; land use change; natural reserve; México; *Panthera onca*.

Introduction

Many areas are experiencing major declines in biodiversity (Polasky *et al.* 2007), and the establishment of protected areas is a common method used to minimize losses. However, the effectiveness of protected areas in preserving biodiversity and supporting local economies depends in part on management of lands surrounding protected areas (Brandon *et al.* 2017). Large carnivores are especially difficult to maintain through protected areas due to large home ranges and the negative attitudes they evoke from some communities (Kellert *et al.* 1996; Inskip and Zimmermann 2009; Cortina-Villar *et al.* 2012).

These issues are concerns with the Reserva de la Biosfera Sierra del Abra Tanchipa (RBSAT), in the state of San Luis Potosi, northeastern México, which is in a landscape dominated by agricultural and livestock production. This small (215 km^2) reserve was established in 1994 as a National Protected Area for the myriad of unique flora and fauna found in this environmental link among the peaks of the Sierra Madre Oriental (SMO). There are 25 species of birds and eight species of mammals considered endangered, threatened, or with restricted distributions in RBSAT (SEMARNAT 2010, 2019; Durán-Fernández *et al.* 2020), including many interior species (*i. e.*, species that require the interior of large contiguous patches of vegetation in a similar successional state ,Villard 1998, such as the elegant eagle (*Spizaetus ornatus*), red brocket deer (*Mazama temama*), and ocelot (*Leopardus pardalis*). However, the keystone species of the RBSAT and SMO is the jaguar (*Panthera onca*), and the RBSAT functions as a critical corridor in maintaining connectivity among jaguar populations in the SMO (Dueñas-López *et al.* 2015; Dueñas-López and Huerta-Rodríguez 2020). Ongoing studies of jaguar ecology (Rosas-Rosas *et al.* 2016; Silva-Caballero *et al.* 2022) further confirm the importance of the RBSAT landscape for this keystone large carnivore. Because of its role as a keystone carnivore, needs for large areas of minimally impacted habitat, and vulnerability to human-related mortality (Ripple *et al.* 2014), the jaguar is an effective management indicator species (MIS) for conservation of the RBSAT landscape, meaning that its status and trend provide insight into the overall health of the ecological system (Committee of Scientists 1999; Rosas-Rosas *et al.* 2020).

The highly productive valleys that border the Reserve are heavily impacted by human land use, including cattle ranching, cultivated crops, urban and rural developments, and mining operations (Dueñas-López *et al.* 2015; Durán-Fernández *et al.* 2020). The RBSAT is situated in the middle of two counties, Ciudad Valles and Tamuín, which have growing populations and robust local economies. Ciudad Valles had a 35 % increase in population from 1990 to 2015 (INEGI 2015) and is the number one producer of sugar cane in San Luis Potosí, with a production valued at $ 49 million USD in 2016 (SIAP 2017). The population of Tamuín increased 13 % from 1990 to 2015, and Tamuín leads the state in beef production with an economic impact of $ 269 million in 2016 (INEGI 2015; SIAP 2018). Cattle numbers more than doubled in Tamuín from 107,752 to 290,300, 2008 – 2016 (SIAP 2018), primarily related to feedlot production, but decreased in Ciudad Valles from 2,634 to 2,245.

Despite the ecological role of RBSAT as a biodiversity preserve, the types and rate of land use change remain unquantified around the RBSAT. Like many other areas in México, the landscape of the RBSAT is likely threatened by isolation, and land use surrounding the reserve has a major impact on its effectiveness in the conservation efforts of the SMO (Ortega-Huerta 2007; Chapa-Vargas and Monzalvo-Santos 2012; Rosas-Rosas *et al.* 2020). Likewise, jaguars and other sensitive wildlife move outside the boundaries of the RBSAT (Rosas-Rosas *et al.* 2016; Silva-Caballero *et al.* 2022), which highlights the need for coordinated management of the surrounding landscape to maintain viable habitat as well as decrease the likelihood of conflicts with local livestock producers and other residents of surrounding communities. Understanding the historic and current patterns of land use around RBSAT will inform strategies for future development and conservation efforts to preserve the ecological function of the Reserve and promote coexistence with its keystone species. Therefore, our goal was to document the type and rate of land use change within the RBSAT and its surrounding landscape over the last 27 years and assess how land use changes might influence efforts to conserve jaguars, one of the keystone species of the RBSAT, and thus maintain the role of the RBSAT landscape for conservation of biodiversity.

Materials and methods

Study Area. Our study area included the RBSAT and a 10 km buffer of the surrounding landscape, representing 1,080 km^2 of tropical deciduous forest, secondary vegetation, agricultural and livestock production areas, rural communities, and gravel mines (Figure 1). The RBSAT is located between 22° 04′ 38″, 22° 23′ 56″ N and -98° 53′ 07″, -99° 00′ 44″ and contains 214 km^2 of contiguous dry tropical deciduous forest, ranging in elevation from 120 to 820 masl (Arriaga *et al.* 2000; CONANP 2014). The climate is classified as subhumid with summer rains from June to September, an average annual rainfall of 965 mm, and an annual average temperature of 25.7° C (CONANP 2014). The Reserve provides habitat for 420 species of plants, 259 species of birds, 56 species of mammals, 50 species of reptiles, and 17 species of amphibians (Durán-Fernández *et al.* 2020).

Land Use Change Data. We selected satellite images used to estimate land use change based on strict criteria, including temporal availability (during the dry season) and the percentage of cloud coverage (< 10 %). These criteria emphasize the contrast between dense forest, secondary vegetation, pastures, and agriculture (most notably sugar cane, which is a crop that stays green throughout the year), facilitating the differentiation among land uses (Fichera *et al.* 2012; Mei *et al.* 2016).

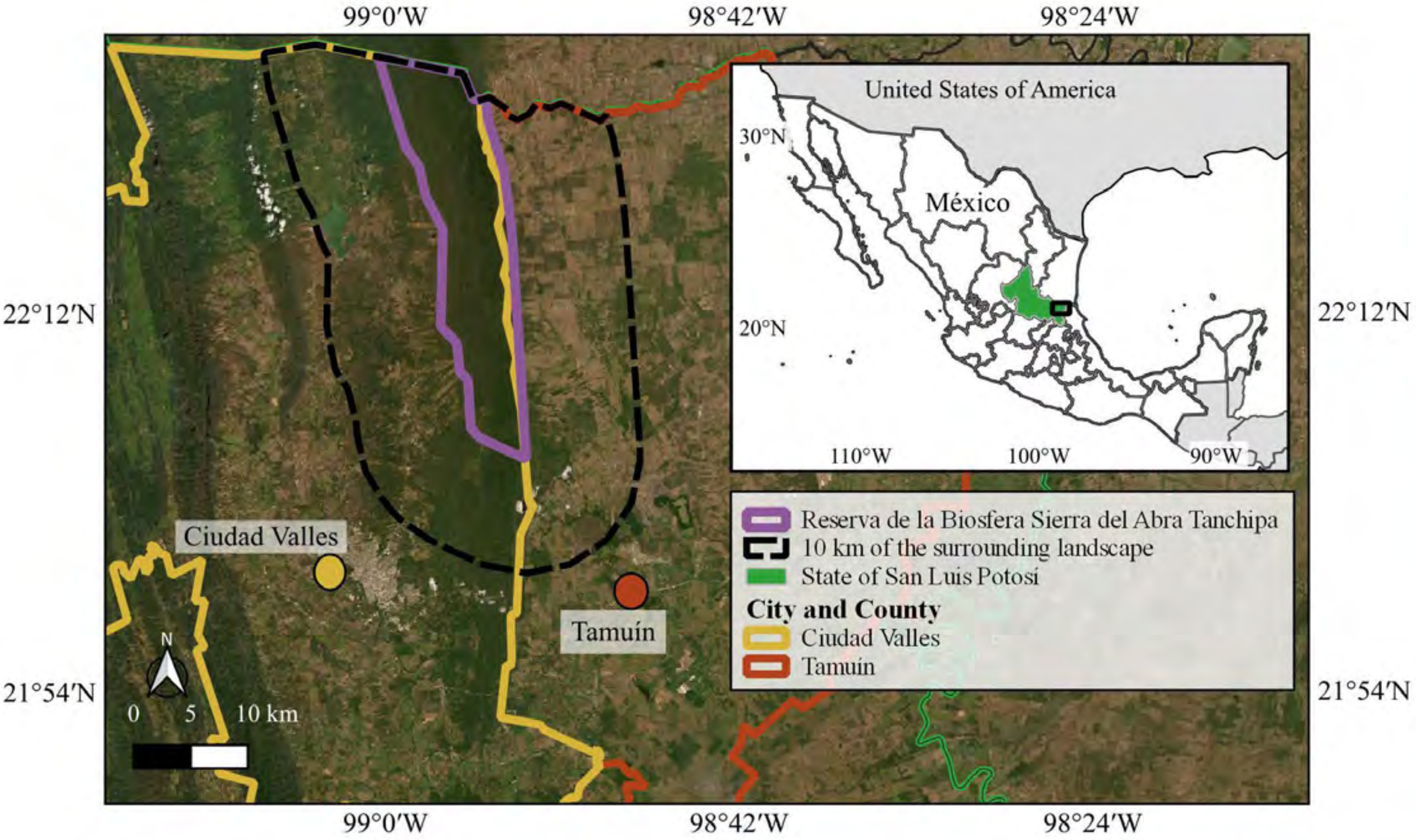


Figure 1. The study area, including the Reserva de la Biosfera Sierra del Abra Tanchipa and ten km of its surrounding landscape, and the complex matrix of land use from a satellite image captured on March 25, 2016.

We analyzed two 30-m spatial resolution satellite images, downloaded from the online platform EarthExplorer (USGS 2018). The most appropriate available images from the end of the dry season with no cloud coverage were from the Landsat 4 Thematic Mapper (TM; 7 March 1989) and Landsat 8 Operational Land Imager (OLI; 25 March 2016), which spanned a period of 27 years. We were interested in maximizing the time span as much as possible, and these images fit all criteria. We followed the methodological processing (*i. e.*, preprocessing of the satellite images, supervised classification, and accuracy assessment; see below) of data common to analyses of land use change (*e. g.*, Reyes-Hernández *et al.* 2006; Sahagún-Sánchez *et al.* 2011; Horvat 2013; Fernández-Landa *et al.* 2016; Mei *et al.* 2016). We used the software programs QGIS (QGIS Development Team 2018) and SAGA GIS (Conrad *et al.* 2015) for all analyses.

Preprocessing. Geographic, radiance, and atmospheric interference are the three components of preprocessing corrections for satellite images commonly applied before classification (Horvat 2013). Geographic corrections were not necessary for our images, as the Landsat images we used had been geo-referenced by the USGS (2015) to the Universal Transverse Mercator (UTM) projection. We corrected for radiance using the Semi-Automatic Classification Plugin for QGIS (SCP; Congedo 2018). Last, we corrected for atmospheric interference using a Dark Object Subtraction (DOS1) correction within the SCP plugin (Hadjimitsis *et al.* 2010). We then clipped the corrected images to the study area.

Supervised Classification. We included six classes in our supervised classification: agriculture, infrastructure, pasture, secondary vegetation, tropical deciduous forest, and water. We selected these classes based on two cartographic maps from the National Institute of Statistics Geography and Information Technology (INEGI 2018), Series I from 1985 and Series VI from 2016 (Table 1), which included 13 land use types (LUTs) within our study area.

We developed a training file based on the above classes using three data inputs (Foody 2002; Reyes-Hernández *et al.* 2006; Grinand *et al.* 2013). The first level of data was INEGI Series I and VI land use and vegetation maps. The second level were the satellite images projected in natural color (Landsat 8, bands 7 – 5 – 3; Landsat 4, bands 3 – 2 – 1) and false color composite images that highlight vegetation (Landsat 8, bands 6 – 5 – 4 and 4 – 3 – 2; Landsat 4, bands 5 – 4 – 3 and 4 – 3 – 2). These provided greater detail than the land use and vegetation maps. The third input was personal knowledge of the region provided by the authors. We used the classification algorithm K-nearest Neighbors Classification in SAGA GIS (Qian *et al.* 2014; Conrad *et al.* 2015) for our supervised classification, with the training file serving as the basis for the classification. We ran 15 iterations of the classification; after every iteration, we reviewed the classification results, and strengthened the training files in misclassified areas.

Accuracy Assessment. We used the final classifications from 1989 and 2016 and two verification files for accuracy assessment. The verification files included high-resolution (1-m; Planet Team 2017) satellite imagery from March 2016 and field surveys used to classify areas with a high level of certainty to serve as controls. We then ran the Confusion Matrix (Grid/Polygon) module in SAGA GIS (Conrad *et al.* 2015) for each image, which compared the classification and the verification files to assess their level of agreement. This generated the overall accuracy and kappa index values.

Land Use Trends. We ran the Confusion Matrix (Grid/Grid) module in SAGA GIS (Conrad *et al.* 2015) with the two classified images to calculate the land use change from 1989 to 2016. We calculated rate of change following Miranda-Aragón *et al.* (2013) and Sahagún-Sánchez *et al.* (2011) using RC = ,(A_2 / A_1) ^ (1 / *n*) – 1,, where RC = rate of change, A_1 = area in the first year (ha), A_2 = area in the final year (ha), and *n* = number of years in the analysis period. Positive percentages represent increases in area and negative percentages represent losses in area, and the larger the number, the greater the magnitude of change.

Jaguar and Land Use Change. We used jaguar movement data from an ongoing project in the SMO (Rosas-Rosas *et al.* 2016; Silva-Caballero *et al.* 2022) to analyze the relationship between land use change and occurrence of jaguars. Data was collected from two male jaguars in 2016 and 2017 fitted with GPS collars (VECTRONIC Aerospace GmbH; Rosas-Rosas *et al.* 2016). We used 100 randomly selected GPS records within the study area from each jaguar and assigned a land use class to each record using the plugin Add Grid Values to Points in SAGA GIS (Conrad *et al.* 2015). We then calculated the percentage of records within each class.

To address the potential effects of land use change on jaguars, we compared the location points with a transition change map that described how each pixel shifted in its land use from 1989 to 2016. We used the Chi-square goodness of fit test and Bonferroni simultaneous confidence intervals to test whether jaguar records were random or if they were associated with certain land use classes (Neu *et al*. 1974; Byers *et al*. 1984). We excluded water as a potential land use type in the analysis due to its small percentage of surface area.

Results

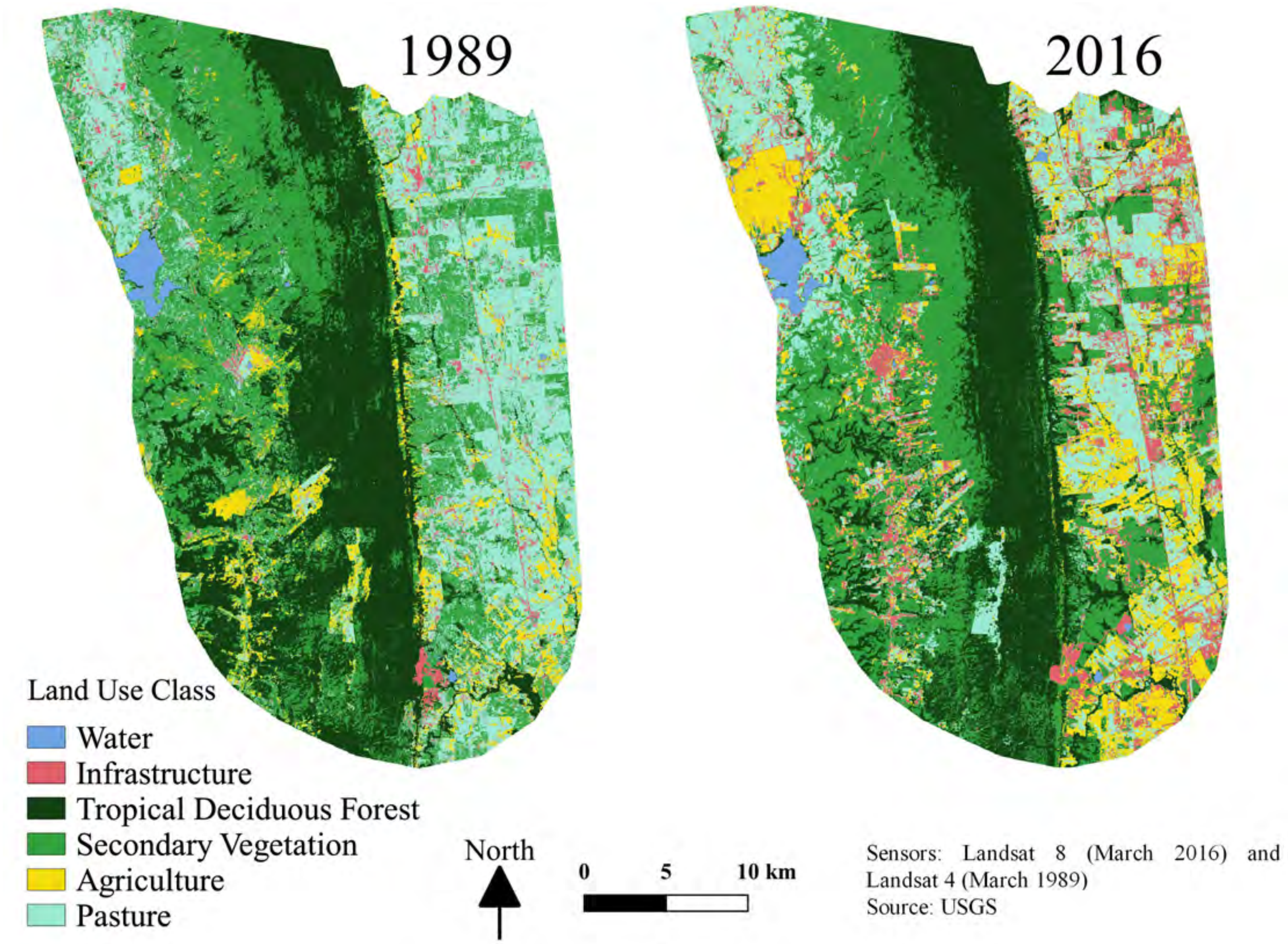
Accuracies of the classifications were 91 % for both 1989 and 2016. Kappa values were 83 % for 1989 and 84 % for 2016; kappa values of 81 % – 100 % indicate an almost perfect level of agreement (Landis and Koch 1977). From 1989 to 2016 the RBSAT landscape shifted from a more contiguous landscape towards greater heterogeneity and fragmentation (Figure 2). The largest increases were seen in infrastructure (+6.0 %) and intensive agriculture (+5.5 %), while the largest decreases were seen in pasture (–5.7 %), tropical deciduous forest (–3.0 %), and secondary vegetation (–2.7 %); the latter was the most common cover class, comprising 34.8 % of the landscape in 1989 and 32.1 % in 2016 (Table 2). The greatest rates of annual change were seen in infrastructure and agriculture (+4.5 % and +2.3 %, respectively), while pasture (–0.9 %) showed the greatest rate of annual loss.

Table 1. Original categories of land use types (LUTs) from INEGI Series I and VI, and the six classes established for use in this study.

Land Use Class/Included LUTs	I	VI
Agriculture		
Semi-permanent irrigated agriculture		X
Seasonal agriculture		X
Annual and semi-permanent seasonal agriculture		X
Semi-permanent seasonal agriculture		X
Seasonal agricultura	X	
Infrastructure		
Urban zone		X
Water		
Bodies of water	X	X
Pasture		
Permanent cultivated pasture	X	X
Managed pasture (native species)	X	X
Tropical Deciduous Forest		
Tropical deciduous forest	X	X
Secondary Vegetation		
Marsh		X
Tropical deciduous forest – secondary arboreal vegetation	X	X
Tropical deciduous forest – secondary shrub vegetation	X	X
Excluded		
Lacking vegetation		X

Notable results in the change matrix included 20,463 ha (19.1 %) of tropical deciduous forest that remained unchanged, the majority of which was located in the RBSAT, in which 21,464 ha was protected (Table 3). Similarly, 17,364 ha of secondary vegetation were conserved (16.2 %). Since 1989, pasture conversions included 5,661 ha to intensive agriculture, 5,692 ha to secondary vegetation, and 4,208 ha to infrastructure. Infrastructure, tropical deciduous forest, secondary vegetation, and pasture all transitioned to water, but only accounted for 0.2 % of the total area.

Expected distribution of jaguars based on the spatial availability of each land use class differed significantly from actual locations of jaguars ($\chi^2_4 = 121.7$, $P < 0.001$). Bonferroni CIs indicated that jaguars were found proportionately more than expected in secondary vegetation (66 % ,57 to 81 %, of locations v. 32 % expected) and were located in tropical deciduous forest (26 % ,13 to 35 %, of locations v. 27 % expected) according to its availability. Few locations were in infrastructure (3 % ,1 to 7 %, of locations v. 9 % expected), agriculture (4 % ,2 to 5 %, of locations v. 12 % expected), and pasture (2 % ,1 to 7 %, of locations v. 20 % expected). While the majority of tropical deciduous forest and other land use types were conserved from 1989 to 2016 within the RBSAT, only 23 % of jaguar locations fell within the boundaries of the RBSAT. In contrast, jaguars were comparably located in areas of stable (*i. e.*, unchanged) secondary vegetation (22 to 38 % of locations) as well as in secondary vegetation that had previously been tropical deciduous forest in 1989



Sensors: Landsat 8 (March 2016) and Landsat 4 (March 1989)
Source: USGS

Figure 2. Land use classes of the RBSAT and ten km of the surrounding landscape from 1989 and 2016 analyzed using satellite imagery.

(25 %) and in secondary vegetation that had been pasture in 1989 (38 %).

Discussion

The RBSAT and surrounding landscape changed considerably over the previous *ca*. 30 years, becoming increasingly fragmented and impacted by human use, particularly intensive agriculture and urbanization (Tables 2 and 3; Figure 2). Based on the accuracies of our supervised land use classification, these changes were real rather than artifacts of selected land use data inputs. Classifications showed high accuracy (91 % for 1989 and 2016), and kappa values (83 % for 1989 and 84 % for 2016) indicated an almost perfect level of agreement (*i. e.*, > 81 %; Landis and Koch 1977). Our values were also similar or superior to previous classifications performed in México at the state level. For example, Chapa-Vargas and Monzalvo-Santos (2012) obtained a 72 % overall accuracy and 69 % Kappa in San Luis Potosí; Porter-Bolland *et al.* (2007) obtained an 87 % overall accuracy in Campeche; and Currit (2005) obtained an 86 % overall accuracy and 85 % Kappa in Chihuahua. At the national level, Velázquez *et al.* (2010) obtained a 71 % overall accuracy of a classification of México. Thus, our mapping accurately classified the changing land use of the RBSAT landscape.

The increases in infrastructure and intensive agriculture and the decrease in pasture reflected a regional trend of shifting from less intensive cattle ranching towards intensive agriculture, specifically production of sugar cane and citrus (SIAP 2017). The increase in infrastructure reflected the development of commercial and residential infrastructure as a consequence of significant population growth in both Ciudad Valles (increase of 35 %) and Tamuín (increase of 14 %) from 1990 to 2015 (INEGI 2015). These trends follow a national and state-level trend of increasing fragmentation driven by anthropogenic modifications (Mas *et al.* 2009; Velázquez *et al.* 2010). For example, Miranda-Aragón *et al.* (2013) identified high rates of change in San Luis Potosí towards seasonal agriculture (+1.1 %) and irrigated agriculture (+4.8 %) between 1993 and 2007, similar to the annual rate of change (+2.3 %) to all forms of intensive agriculture combined that we observed from 1989 to 2016. Concurrently, less intensive agricultural uses declined, as reflected in the decrease in cover of pastures during our evaluation period. Despite the decrease in pasture, however, cattle production and associated activities are still common and

Table 2. Totals and change in area in hectares (ha) and percent (%) measured by the classification of satellite imagery from 1989 and 2016.

	1989		2016		Change 1989 to 2016		
	Ha	%	ha	%	Ha	%	% change/ year
Water	1,180	1.1	1,215	1.1	+35	0.0	0.1
Infrastructure	2,858	2.6	9,330	8.6	+6,472	6.0	4.5
TDF	31,931	29.6	28.640	26.5	−3,291	−3.0	−0.4
Secondary veg	37,599	34.8	34,631	32.1	−2,968	−2.7	−0.3
Agriculture	6,843	6.3	12,778	11.8	+5,935	5.5	2.3
Pasture	27,610	25.6	21,428	19.8	−6,182	−5.7	−0.9

economically significant in the RBSAT landscape, especially in Tamuín (Silva-Caballero *et al.* 2022).

These and other (*e. g.*, the loss of tropical deciduous forest and secondary vegetation) land use trends in the RBSAT landscape could compromise the ecological role of the reserve. Increasing fragmentation of the immediate surrounding landscape decreases habitat quantity and quality for interior-dependent species such as the elegant eagle and ocelot, thus decreasing the effectiveness of the overall RBSAT landscape as a biodiversity reserve. Loss, or decreases in the size of, large patches that support interior species can affect biodiversity by negatively impacting populations of interior species (Villard 1998), as well as increasing vulnerability to humans and other human-associated threats (*e. g.*, domestic cats and dogs; Doherty *et al.* 2016). Conversely, edge-dependent species, which include many of the key prey species of jaguars (Escamilla *et al.* 2000; Ávila-Nájera *et al.* 2011; Silva-Caballero *et al.* 2022), are benefitted by these changes in land use and increasing fragmentation of the RBSAT landscape, likely resulting in increased abundance, particularly outside of the RBSAT boundary. Consequently, most kill sites of livestock (and natural prey) by jaguars were outside the RBSAT boundary (Silva-Caballero 2019; Silva-Caballero *et al.* 2022).

These changes increase the likelihood of conflicts between jaguars and humans (Rosas-Rosas *et al.* 2015; Guerrero-Rodríguez *et al.* 2020), as only 23 % of jaguar locations were within the boundary of the RBSAT and jaguars were primarily associated with secondary vegetation, which is decreasing within the RBSAT itself. Better habitat quality for jaguars and their prey outside of the RBSAT boundary facilitates potential conflicts with humans. For example, livestock comprised 66 % of the biomass in jaguar diets in the RBSAT landscape, with all depredation occurring outside the RBSAT boundary (Silva-Caballero 2019; Silva-Caballero *et al.* 2022).

Secondary vegetation can result from abandonment of pastures or agricultural fields, and thinning or other alterations of tropical deciduous forest (Table 3; Wandelli and Fearnside 2015). Significantly, jaguars were frequently located in secondary vegetation that had transitioned from pasture and tropical deciduous forest in the RBSAT landscape (primarily outside the RBSAT border; Figure 3) in addition to their use of secondary vegetation that remained unchanged from 1989 to 2016. The apparent preference and use of secondary vegetation outside of the RBSAT boundary by jaguars in association with the preponderance of livestock in jaguar diets (Silva-Caballero *et al.* 2022) emphasizes the need to include local stakeholders in the greater RBSAT landscape in conservation efforts, including secondary vegetation.

Moreover, the expansion of intensive agriculture, increases in habitat edge due to fragmentation, and continued importance of livestock production and associated improvements (especially water) may attract prey species to, or increase their populations in, these land uses (Rosas-Rosas *et al.* 2020; Silva-Caballero *et al.* 2022). For example, Escamilla et *al.* (2000) and Ávila-Nájera *et al.* (2011) found that collared peccary (*Pecari tajacu*), white-tailed deer (*Odocoileus virginianus*), and white-nosed coati (*Nasua narica*), important prey species for jaguars in the RBSAT (Hernández-SaintMartín *et al.* 2015; Silva-Caballero 2019; Silva-Caballero *et al.* 2022, preferentially used mosaics of tropical deciduous forest, agriculture, and livestock pasture. Consequently, so did jaguars and pumas (*Puma con-*

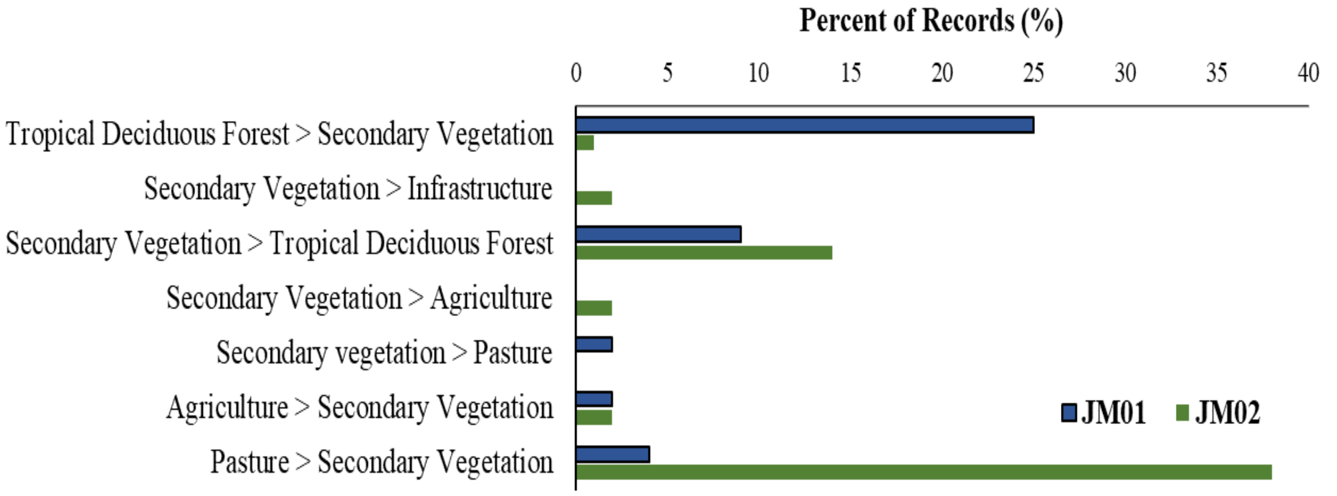


Figure 3. The percent of records associated with transitions in secondary vegetation from 1989 to 2016 from two jaguars (grey and white bars) within the RBSAT landscape. The symbol > indicates a transition between land use classes from 1989 to 2016.

Table 3. Confusion matrix of land use changes in area (%) from 1989 to 2016. Unchanged land use are in *. Retrogressive land use change is *italicized*.

	Land use classes from 2016 imagery						
Land use	**Water**	**Infrastructure**	**Tropical forest**	**Secondary veg**	**Agriculture**	**Pasture**	**Total (1989)**
Water	0.90 % *	0.04 %	0.02 %	0.07 %	0.03 %	0.05 %	1.10 %
Infrastructure	0.01 %	0.8 3% *	0.09 %	0.32 %	0.48 %	0.94 %	2.67 %
TDF	0.05 %	0.43 %	19.14 % *	8.31 %	0.84 %	0.78 %	29.54 %
Secondary Veg	0.09 %	2.81 %	5.21 %	16.24 % *	3.55 %	6.84 %	34.73 %
Agriculture	0.02 %	0.65 %	0.94 %	1.70 %	1.69 % *	1.38 %	6.38 %
Pasture	0.07 %	3.94 %	1.13 %	5.32 %	5.29 %	9.82 % *	25.57 %
Total (2016)	1.14 %	8.69 %	26.52 %	31.96 %	11.88 %	19.82 %	

color; Escamilla *et al.* 2000; Hernández-SaintMartín *et al.* 2015). Further, the RBSAT lacks permanent water sources (Hernández-SaintMartín *et al.* 2013; Silva-Caballero *et al.* 2022). Consequently, wildlife, including jaguars and their prey, seek water outside of the boundaries of the Reserve during the dry season (Hernández-SaintMartín *et al.* 2013), which increases the vulnerability of livestock and natural prey to predation (Silva-Caballero *et al.* 2022). Water developments are most common in livestock enterprises, intensive agriculture (irrigation), and human developed (*e. g.*, residential, etc.) areas, where presence of jaguars frequently results in serious conflicts (Guerrero-Rodríguez *et al.* 2020; Silva-Caballero *et al.* 2022).

The rapid increases in infrastructure, intensive agriculture, and habitat fragmentation have and will continue to dramatically change the RBSAT landscape, and thus affect the wildlife that utilize the landscape. Managers and stakeholders need to seek approaches that account for the response of resident wildlife including interior-dependent species and jaguars, mitigate conflicts, and protect the economic viability of adjacent communities (Olvera-Hernández *et al.* 2020; Silva-Caballero *et al.* 2022). Minimization of loss of tropical deciduous forest and other large habitat patches in the RBSAT itself is likely the only practical means of minimizing loss of biodiversity in the RBSAT landscape, especially for interior-dependent species given the already significant fragmentation in the surrounding landscape. Characteristics associated with human-jaguar conflict areas (especially with livestock) include proximity to a protected area and seasonal availability of water (Rosas-Rosas *et al.* 2010; Peña-Mondragón and Castillo 2013; Alfaro *et al.* 2016; Silva-Caballero *et al.* 2022). Both of these are present in the RBSAT landscape, as are associated livestock depredation conflicts (Silva-Caballero *et al.* 2022), and are likely to become more prevalent given the changes in land use away from natural vegetation towards intensive agricultural and infrastructure development. The combination of a decrease in preferred jaguar habitat within the RBSAT and likely increases in preferred prey outside of the RBSAT as a consequence of increasing fragmentation, intensive agriculture, and permanent water infrastructure, will likely lead to in increased conflicts between humans and jaguars (Rosas-Rosas *et al.* 2010; Guerrero-Rodríguez *et al.* 2020; Peña-Mondragón and Castillo 2013; Alfaro *et al.* 2016; Silva-Caballero *et al.* 2022).

Conservation of the RBSAT landscape is thus dependent upon a mutually beneficial relationship between the objectives of the Reserve and the economic viability of adjacent communities, necessitating management strategies that reflect the interests of both entities. The trends in land use in the RBSAT landscape towards increasingly fragmented intensive agricultural and infrastructure development are likely to decrease the ecological values of the Reserve (*i. e.*, biodiversity, habitat linkage, jaguar conservation because of increased conflicts and illegal killing) and increase conflicts between adjacent landowners and wildlife, including large carnivores (because even perceived conflicts with large carnivores can facilitate intolerance; Rosas-Rosas *et al.* 2015). This outcome, unless changed, results in a loss for both the RBSAT and surrounding communities. Stakeholder driven adaptable land use planning and management based upon the necessity and expectation of shared use that is advantageous for both communities and the Reserve is necessary to minimize both the future loss of ecological values and potential human-wildlife conflicts (Rosas-Rosas *et al.* 2015, 2020). However, adaptive management requires structured monitoring and a willingness to change methods and alter tactics depending on the feedback and results (Williams 2011). Consequently, working with members of the community on site-specific conservation strategies is critical to maintain or enhance the ecological role of the RBSAT and its surrounding landscape.

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Variation in population density of the Andean night monkey (*Aotus lemurinus*) in areas with different landscape characteristics

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In the Colombian Andes, most of the populations of Andean night monkeys (*Aotus lemurinus*) are found in fragmented landscapes due to the predominant changes in land use in the region. Thus, forest fragments differ in shape, size, degree of isolation, and availability of resources. These factors have had a differential effect on the ecology and permanence of their populations. In order to determine the effect of fragmentation on *A. lemurinus*, we estimated its population density in a protected area of 489 ha of sub-Andean forest - at Otún Quimbaya Wildlife Sanctuary- which is structurally connected with other protected areas; then, we compared it with another study carried out in the unprotected Sub-andean forest fragments (900 ha) of Dapa, at the Valle del Cauca department. To estimate its density, the distance sampling method was used with 31 linear transects, and the data were analyzed with the software Distance. Landscape metrics was estimate with patch analyses tools with a Corine Land Cover information 1:100,000 scale. The plant structure and diversity were measurement with transects to 50 x 4 m and taking account the trees with diameter at breast height over 10 cm. We use average comparison to evaluate the similarities between patrons of the population density and explanatory variables. A population density of 39 ind/km^2 was found in our study, which is lower than the 113 ind/km^2 found in the Dapa. This relation was similar to relations of mean shape index, mean patch size and mean diameter at breast height, and inverse with the relations of proportion of the forest in the area, weighted cover index, plant diversity and density of trees. It is likely that the protected area, by being connected and having a greater diversity of primates and trees, allows this population not to suffer from a crowding phenomenon, and that the population does not increase it carrying capacity due to the low presence of competing species, conversely to what could be occurring in the unprotected fragments. Conversely, high density in Dapa could be reflect a system depletion in diversity and ecological processes.

En los Andes colombianos, la mayoría de las poblaciones de monos nocturnos andinos (*Aotus lemurinus*) se encuentran en paisajes fragmentados debido a los cambios en el uso de suelo predominantes en la región. Los fragmentos de bosque difieren en forma, tamaño, grado de aislamiento y disponibilidad de recursos. Estos factores han tenido un efecto diferencial en la ecología y permanencia de sus poblaciones. Para determinar el efecto de la fragmentación sobre *A. lemurinus*, se estimó su densidad poblacional en un área protegida de 489 ha de bosque subandino - Santuario de Fauna y Flora Silvestre Otún Quimbaya- que está estructuralmente conectado con otras áreas protegidas; luego, lo comparamos con otro estudio realizado en fragmentos de bosque Subandino no protegidos (900 ha) de Dapa, en el departamento del Valle del Cauca. Para estimar su densidad se utilizó el método de muestreo a distancia con 31 transectos lineales, y los datos se analizaron con el programa Distance. Las métricas del paisaje se estimaron con herramientas de análisis de parche con información Corine Land Cover a escala 1:100,000. La estructura y diversidad de las plantas se midió con transectos de 50 x 4 m y teniendo en cuenta los arboles con un diámetro a la altura del pecho de más 10 cm. Usamos la comparación de medias para evaluar las similitudes entre los patrones de la densidad de población y las variables explicativas. Se encontró una densidad poblacional de 39 ind/km^2, menor a los 113 ind/km^2 encontrados en Dapa. Esta relación fue similar a las relaciones de índice medio de forma, tamaño medio de parche y diámetro medio a la altura del pecho de los árboles, e inversa a las relaciones de proporción de bosque en el área, índice de cobertura ponderado, diversidad vegetal y densidad de árboles. Es probable que el área protegida al estar conectada y tener una mayor diversidad de primates y árboles, permita que esta población no presente un fenómeno de hacinamiento, y que la población no aumente su capacidad de carga por la baja presencia de especies competidoras, al contrario de lo que podría estar ocurriendo en los fragmentos no protegidos. Por el contrario, la alta densidad en Dapa podría reflejar un agotamiento del sistema en diversidad y procesos ecológicos.

Keywords: Abundance; Landscape metrics; lemurine owl monkey; plant diversity and structure; Sub-andean Forest.

Introduction

The reduction, loss, and fragmentation of natural or semi-natural habitats are considered the main causes of the biodiversity crisis (Santos and Telleria 2006). Fragmentation increasingly modifies the environmental space accessible for species, reducing the available territory as well as the access to resources, precipitating population decline until reaching local extinctions; it also increases the amount of edge relative to the amount of interior habitat (Bender *et al.* 1998). Historical, geographical, and ecological processes have caused the largest proportion of the Colombian population is located in the Andean region and the Caribbean; therefore, these regions are the most affected in terms of transformation and fragmentation of their natural ecosystems (including mountain ranges of the Andes and its valleys; Kattan 1998; Etter and van Wyngaarden 2000).

This situation affects the Andean night monkey, *Aotus lemurinus* (Aotidae), which is categorized as Vulnerable (VU), on the IUCN red list, by a reduction in population size ≥ 30% over the last 10 years that is inferred by a decline in area of extent of occurrence and it not have ceased (Link *et al.* 2021a) and in the list of threatened species of the Colombian fauna (MADS 2018). These monkeys inhabit mature and advanced successional montane forests between 1,000 to 3,200 m of elevation, in Colombia, Ecuador, and Venezuela. They can also occupy fragments within agricultural areas, such as coffee plantations (Defler 2010). The potential distribution range of *A. lemurinus* in Colombia was 172,382 Km^2 and the remnant is 63,508 Km^2 with a loss of 63 % from 1990 to 2016 (Defler 2020).

Population density is a parameter that relates to the population and the area occupied by its individuals. This parameter is spatially and temporally dynamic, therefore, its monitoring is essential to detect trends and implement effective management strategies, as well as to compare their effectiveness (Anzures and Manson 2007; Arroyo *et al.* 2007). Thus, in this work, the population density of *A. lemurinus* in the Wildlife Sanctuary Otún Quimbaya was estimated to evaluate the differences with the results obtained in a previous study carried out in a non-protected area known as the Dapa region, Valle del Cauca (Hirche *et al.* 2017).

Materials and methods

Study area. The Wildlife Sanctuary Otún Quimbaya is located on the western slope of the Central Cordillera, Pereira municipality, Risaralda Department of Colombia (Figure 1). It comprises 489 ha and is located between 1,750 and 2,276 m of elevation. The average annual temperature is 16.8 °C, and the average rainfall is 2,638 mm/year. The Sub-Andean forest that it protects is part of a biological corridor of the Eje Cafetero Regional System of Protected Areas, which includes Los Nevados National Natural Park (58300 ha), Ucumarí Regional Natural Park (3986 ha), Barbas Bremen soil conservation district (9,651.90 ha), among others (Parques Nacionales Naturales de Colombia-PNN 2018).

Population Density. The distance sampling method with linear transects was used; this method involves measuring the perpendicular distance from the transect to the point where the animal was observed, or the geographic center of the observed group. The distribution of detection distances is then used to build a detection function that determines detection probabilities as a function of distance from the transect, assuming that there is a 100 % probability of detecting an animal on the transect and the probability decreases with perpendicular distance. The detection function allows an estimation of densities within a band extending on both sides of the transect, based on encounter rates and correcting for undetected individuals. With this method, transects can be resampled to increase the sampling effort for calculating encounter rates, and no individual recognition is necessary (Buckland *et al.* 2015). The fieldwork was carried out between July and December 2018; 31 transects were systematically located and separated in such a way that they covered as much area as possible whereas assuring the independence of the observations (Figure 1). The total length of the transects, it is mean, the sum of the distance of all transects was 11,770 m (mean length of 380 m; SD ± 34). The samplings were carried out between 18:30 and 0:30 with the appropriate conditions for data collection, mainly in the absence of rain. Each transect was traveled between 8 and 11 times, accumulating total sampling efforts of 106 km. Generally, the nocturnal monkeys were located by their vocalizations, the reflection of red light in their eyes, and the noise made by tree branches with their movements (Castaño *et al.* 2010). Once the individuals of *A. lemurinus* were visualized, the perpendicular distance, the number of individuals, time, and coordinates were taken.

The reference study, to compare the local density of this primate and infer the potential effect of some explanatory variables, was carried out in the Dapa region, between May and July 2015 with the same methodology of the current study, *i. e.*, Distance sampling (Hirche *et al.* 2017). This method estimates unbiased population density given that it estimates detectability by site and species incorporating the effect of the observer, weather, vegetation features, etc. So, the estimations between any two or more places are comparable; of course, the sampling design (location, number and sampling effort or repetitions) and number of transects (sampling units) varies in each site by area, topography, and relative abundance of the target species (Buckland *et al.* 2015). The population density is variable in space and time; however, we assume that the population density was stable given that the reproductive cycle of the *Aotus* is around three years and there were not notable changes in the any features of the site, that let us to think that the population density could be down, so, in spite of the differences of sampling period, the results of both sites are comparable. The Dapa region is located on the eastern slope of the western mountain range near the city of Cali, department of Valle del Cauca. The elevation in this region varies

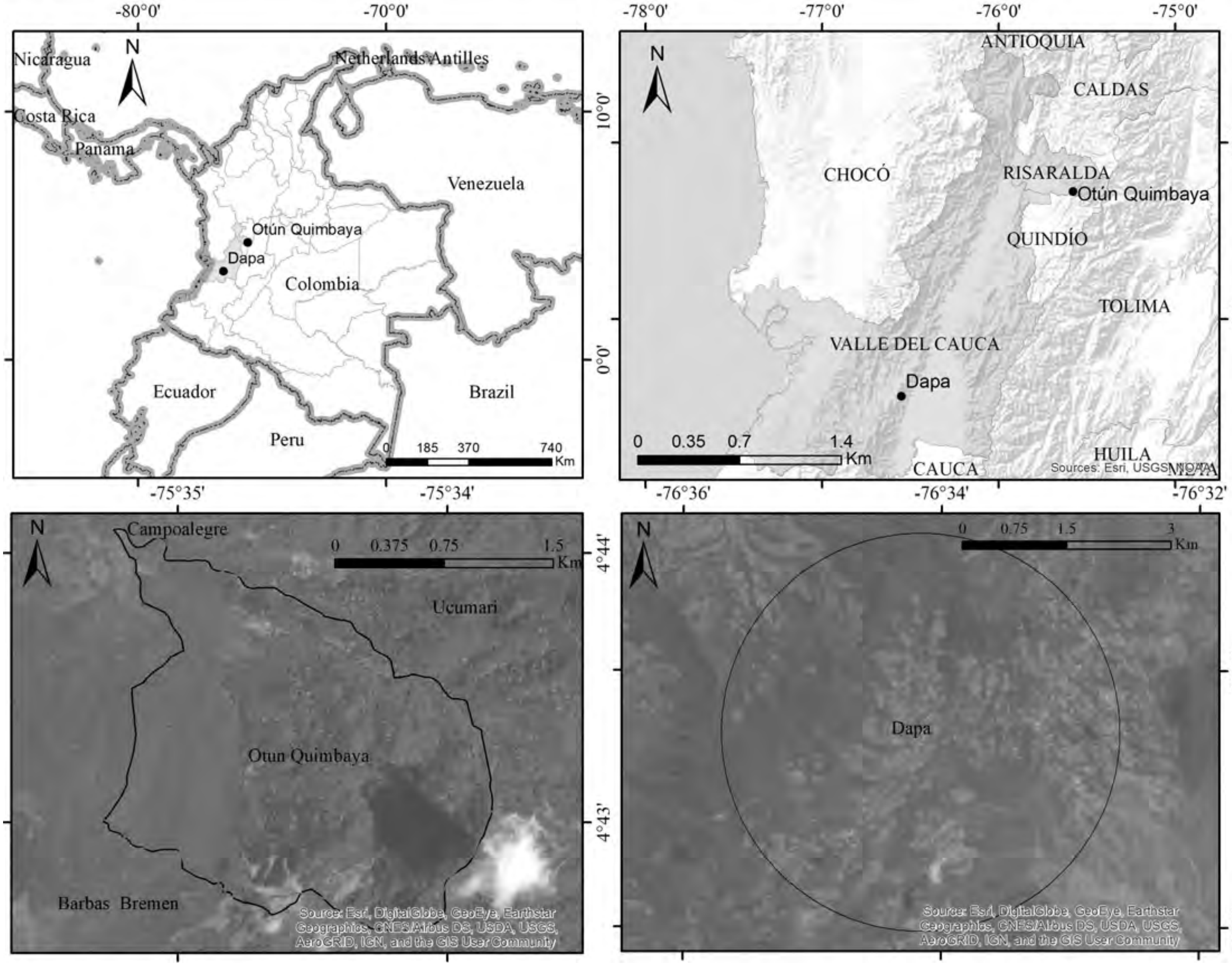

Figure 1. The map shows the location of the Otún Quimbaya SFF (4° 43' 25" N, -75° 34' 455" W) and the Dapa Reserve (comparison site; 3° 33' 46" N, -76° 33' 04"W). Dapa has not a delimited area, so, we delimited the study site with the polygon used to clip the plant cover information to estimate the cover and landscape metrics.

from 1,000 to 2,200 meters above the sea level covering the Andean and Sub-Andean humid forest (IDEAM 2021).

The population density of *A. lemurinus* was estimated with the DISTANCE 7.3 release 2 software (Thomas *et al.* 2010). The objective of the distance sampling analysis is to fit a detection function of the obtained perpendicular distances and use this function to estimate the proportion of individuals that were not detected in the sampling. In this way, the unbased value of the density and abundance of the sampled species in the total area can be obtained (Thomas *et al.* 2002; Roncancio *et al.* 2011). To find the detection function that best fits the data, the frequency distribution of the perpendicular distances was compared with six models (Key function + expansion series): 1. Half normal - Cosine, 2. Half normal - Hermite polynomial, 3. Uniform - Cosine, 4. Uniform - Simple polynomial, 5. Hazard-rate – Cosine, and 6. Hazard-rate - Simple polynomial. From the previous models, the one that presented the lowest value in the Akaike information criterion (AIC) was chosen. AIC is a quantitative method for selecting the model that best fits the data and uses the least number of parameters (Buckland *et al.* 2010, 2015).

The sample variance of population density was calculated empirically as the sum of the sample variance of the encounter rate, the sample variance of the estimated detection probability, and the sample variance of group size (Buckland *et al.* 2015). For the comparison between the population density estimated in the Wildlife Sanctuary Otún Quimbaya with the study carried out in the Dapa region, a graph of confidence intervals (CI) was made. When the confidence intervals of the sites overlapped more than 25 %, it was considered that there was no positive evidence that the population densities were different, with a 95 % confidence (Cumming *et al.* 2007).

Fragmentation statistics. A local landscape was defined for each of the study sites (Dapa and Otún Quimbaya) with the smallest perimeter circle that included all the transects used for the density sampling. This circle was overlapped on a vector file of land cover polygons (IDEAM 2021). One landscape metrics, weighted coverage index (WCI), and four class metrics were considered: mean shape index (MSI), mean patch size (MPS), class area (CA), and the number of patches (NumP) for each land cover (class) found in each site. The WCI integrates a value that allows measuring the land cover composition and structure (Type of cover and its relative area; Fahrig *et al.* 2011; Roncancio 2021); for this, the covers were classified as follows: Dense Forest = 7, Fragmented Forest = 6, Secondary or transitional vegetation = 5, Mosaic of pastures with natural spaces = 4, Mosaic of pastures, crops, and natural spaces = 3, Pastures = 2, Rivers and others = 1. Then, the value of each cover was multiplied by its size proportion in the local landscape and finally, the products were added to obtain the WCI. The MSI is the shape index average of the patches present in the landscape; it tends to 1 when the landscape fragments are circular or square, and increases without limit when the patches become irregular. The MPS is the total area of each class, divided by the number of fragments in that class; the CA is the total size of each class, and the NumP is the number of patches or fragments present by class, indicating how fragmented is that class. The MPS, MSI, CA, NumP values were estimated in the ArcGis 10.5 program, with the Patch Analysis tool (Mitchel 2005).

Plant structure and diversity. As there was not a characterization of structure and plant diversity for Dapa, we carried out the sampling in both sites. To get a representative sample of the structure and plant diversity in these sites, we stablished, as sampling units, six rectangular vegetation plots with a fixed area of 50 x 4 m (0.12 ha) in each one, these were randomly located in the study area. Trees with 10 cm or more in diameter at breast height (DBH) and 1.50 m in height were selected (Castaño *et al.* 2010). When necessary, samples of plants were taken for immediate taxonomic determination by expert personnel from the protected area. The mean DBH, tree density, and Simpson's inverse index (ISI) were estimated for each site as possible explanatory variables.

Relationship between explanatory variables and population density. To evaluate this relationship, all explanatory variables between the two sites were compared and the result was contrasted with the comparison of densities to evaluate if they had the same behavior. For the structure of the vegetation, comparisons of means were carried out. The assumptions of normality and homogeneity of variances were previously tested. The DAP frequency distribution was not normal (S-W, Otún Quimbaya: $P < 0.001 = 2.48e\text{-}13$. Dapa: $P = < 0.001$; F test: $P = < 0.001$); therefore, the nonparametric Mann-Whitney U test was used. For the tree density, we calculate the density per site by dividing the total number of trees and the area of each plot (ha) for each of the study sites (Otún Quimbaya and Dapa); then, the assumptions of normality and homogeneity of variances were tested. The density was adjusted to the normal distribution and was homoscedastic (S-W, Otún Quimbaya: $P = 0.54$, Dapa: $P = 0.80$; $F = 1.122$, $P = 0.85$), leading to a parametric T-test for two independent samples. These tests were performed in Rcmdr package of R (Fox *et al.* 2021). Additionally, Simpson's inverse index was calculated with the Vegan package of R (Oksanen *et al.* 2015). For Simpson's inverse and the fragmentation statistics, arithmetic comparisons were made between the two sites.

Results

Population density. For the Wildlife Sanctuary Otún Quimbaya, the estimated population density of *A. lemurinus* was 39 ind/km^2 (95 % CI = 24.0 to 63.2), and the estimated group density was 20 groups/km^2 (95 % CI = 12.7 to 31.6) with coefficients of variation of 24.7 % and 23.17 %, respectively. This density was estimated from 44 visual records of the species. The model that best fit according to the frequency distribution of the perpendicular distances was the Half-normal with cosine. The effective transect width (ESW) for this species in this locality was 10.39 m (95 % CI = 7.43 to 14.51, CV = 16.7) and the probability of detection was 0.45 (95 % CI = 0.32 to 0.63, CV = 16.7). The average group size was 1.9 individuals (95 % CI = 1.6 to 2.3). The coefficient of variation of the density was influenced by the detection probability 45.7 %, the encounter rate 42.3 %, and the size of the group 12 %. Significant differences were found in the estimates of the primate population densities for both sites (Figure 2), with a 95 % confidence interval.

Fragmentation, plant structure and diversity. Both local landscapes presented the same amount of vegetation covers (5), but of different types and relative area. In Dapa, 47 % of the area was a mosaic of crops, pastures, and natural spaces, while the fragmented forest and secondary or transitional vegetation represented 48 %. For Otún Quimaya, 75 % was Dense Forest and 17 % Fragmented Forest. Therefore, the WCI was higher in Otún Quimbaya. The forest cover in Otún Quimbaya presented more regular forms (Table 1). Comparatively, Dapa presented a higher DAP value and lower tree density, whereas the diversity was relatively similar among sites but bigger in Otún Quimbaya (Table 2).

Discussion

With 39 ind/km^2, the population density found for *A. lemurinus* in Otún Quimbaya was lower than in Dapa (113 ind/km^2). However, our result is similar to population density values found for the genus *Aotus* in other localities, which are mostly between 30 and 40 ind/km^2 (Maldonado-Rodriguez 2011; Roncancio *et al.* 2013; Roncancio *et al.* 2019). The population density of *A. lemurinus* was higher in Dapa, which has more elongated or irregularly shaped forests (higher MSI), with larger fragments (higher MPS; Table 1), and with bigger trees (higher DBH; Table 2). On the other hand, the population density was lower in Otún Quimbaya, which presented more mature plant cover (higher WCI), more forest (higher proportion of forest area), where trees are more abundant (Tree density) and diverse (higher ISI). In relation to altitude, it was found that the population density was lower at higher altitudes.

Table 1. Covers and landscape metrics for SFFOQ and Dapa.

	MSI	MPS	NumP	WCI	% Area	Area
Wildlife Sanctuary Otún Quimbaya						
Dense forest	1.38	270	2	5.24	0.75	540.75
Fragmented forest	1.58	120	1	1.00	0.17	119.63
Secondary or transitional vegetation	2.67	0	1	0.00	0	0.29
Mosaic of crops, pastures and natural spaces	1.97	16	2	0.13	0.04	32.06
Mosaic of pastures and natural spaces	1.73	28	1	0.16	0.04	28.15
Clouds	2.10	1	1	0.00	0.00	0.65
Rivers	1.44	1	1	0.00	0.00	0.63
				6.53		**722.16**
Dapa						
Fragmented forest	2	571	1	1.46	0.24	571.48
Secondary or transitional vegetation	1.9	184	3	1.18	0.24	550.7
Mosaic of pastures, crops and natural spaces	2.24	554	2	1.42	0.47	1107.5
Mosaic of pastures with natural spaces	1.56	31	1	0.05	0.01	31.12
Pastures	1.33	41	2	0.07	0.04	82.30
				4.18		**2343.1**

MSI = mean shape index, MPS = mean patch size, NumP = number of the patches to each plant covers, and WCI = weighted cover index.

These results suggest an apparent tolerance of *A. lemurinus* to various disturbances, which has allowed it to live, reproduce and maintain stable populations in a level of disturbance and forest fragmentation such as that of Dapa. In addition, the species seems able to increase its population density as a result of the local decrease or extinction of other primate species that could compete in some dimensions of the niche (Peres and Dolman 2000; González-Solís *et al.* 2001; Castaño *et al.* 2010). This trend has been found in other primate species, such as *Saguinus leucopus*, for which high population density values (> 100 ind/km^2) have been found in forest fragments where no other primate species were found. Whereas, in fragments where other primates (*Ateles hybridus, Alouatta seniculus,* and *Cebus versicolor*) were present, *S. leucopus* had lower density values (Roncancio *et al.* 2011; Roncancio 2021). For Dapa, it was suggested that the high population density of *A. lemurinus* could be the result of a "density compensation", where an increase in its population carrying capacity is due to the decrease in competition in niche dimensions resulting from the local extinction of other primates (*A. seniculus*, *A. fusciceps,* and *C. capucinus*) that occupied the same area but failed to adapt to the effects of fragmentation of their habitat (Hirche *et al.* 2017). The high densities found in these studies may be the product of situations resulting from, and dependent on, the population dynamics of the species in isolated conditions, the size and functional connectivity of the fragment, as well as the population's ability to adapt (Kattan and Álvarez-López 1996; Harcourt and Doherty 2005; Hirche *et al.* 2017). Also, the heavy hunting of large primates favors the densities of smaller primates that are not usually at risk of being hunted (Peres and Dolman 2000; González-Solís *et al.* 2001).

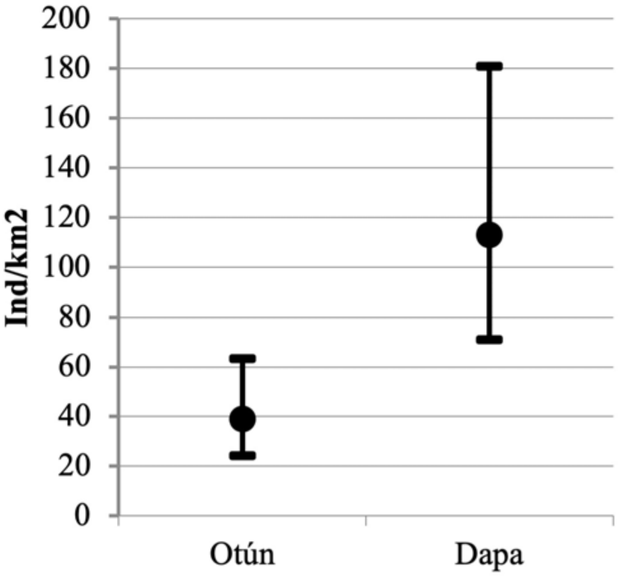

Figure 2. Comparison of confidence intervals of the estimated population density for *Aotus lemurinus* in the study sites (SFFOQ and Dapa).

The Wildlife Sanctuary Otún Quimbaya is a non-isolated area, which is part of a biological corridor for the conservation of sub-Andean and high Andean forests (PNN 2018). This connection explains, in part, the population density results, since it suggests a greater probability of movement of individuals through the corridor, and it has been documented that its distance traveled per night could be from 640 to 901 m (Montilla *et al.* 2021). On the other hand, other species of mammals such as the kinkajou (*Potos flavus*) and the red howler monkeys (*Alouatta seniculus*), as well as other medium-frugivorous such as four species of Cracids, also inhabit this area (PNN 2018), which may be a direct competition for *A. lemurinus*.

Table 2. Metrics of structure and plant diversity for both sites

	DBH (SE)	Tree density (SE)	Simpson Inverse
Otún Quimbaya	18.07 (8.83)	107 (29.09)	9.5
Dapa	99.45 (67.73)	65 (30.82)	10.2

DBH = diameter at breast height, SE = Standard error

The components that contributed the most to the variation in population density were the probability of detection and the encounter rate, which may vary for various reasons. Otún Quimbaya is a mountainous area with many slopes, cliffs, and high diversity in vegetation, therefore, some transects may be more frequented by primates than others, thus varying the quantity and frequency of detections (Roncancio *et al.* 2013; Buckland *et al.* 2015). The size of the group also influenced the variation in population density. Although it has been shown that nocturnal primates are generally in groups of three to five individuals (Castaño *et al.* 2010; Fernández-Duque *et al.* 2001), many detections of only one individual were recorded in this study; this data could come from the dispersion of young individuals by emancipation of the born group (Fernández-Duque and Huntington 2002; Castaño *et al.* 2010). In this sense, it is likely that the *Aotus* population in the Wildlife Sanctuary Otún Quimbaya is reproductively active.

The effects of forest fragmentation and reduction limit the animals to the available fragments, causing a crowding effect (Defler *et al.* 2003; Ramos-Fernández and Wallace 2008). This can generate behavioral changes due to a high level of stress in individuals such as continuous aggression, predation, low survival rates, and birth rates (Milner and Albon 1999; Begon *et al.* 2006); health conditions such as immune depression, the spread of diseases (Gómez-Posada et al. 2009); and cause an increase in the probability of overexploitation of the resources available in the habitat (Hirche *et al.* 2017).

Most of the species of the genus *Aotus* reported in Colombia are in a state of vulnerability (VU) due to the fragmentation suffered by their habitat (Carretero *et al.* 2020; Maldonado *et al.* 2020; Link *et al.* 2021a, b). In particular, for *A. lemurinus*, more of these studies are required to have information on population trends (Defler and Bueno 2010), integrated effective monitoring programs, inventories, and wildlife management and conservation plans. These require specific data such as the size and density of the populations to evaluate the state in which they are found and thus, to be able to carry out management actions at the species level (Lancia *et al.* 1994; Naranjo 2000). Knowing the specific effects of some habitat traits on local populations, and how these respond to them, should contribute to a better understanding of the differences found among protected and non-protected areas for the survival of the species of night monkeys in Colombia.

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Bison wallows effect on soil properties, vegetation composition and structure in a recently reintroduced area

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Bison are considered an ecologically keystone species of the North American grasslands because their activities influence ecosystem dynamics and interactions, particularly their wallowing behavior. In 2009, 23 bison were reintroduced within a 1,500 ha private native semi-arid grassland in Janos, Chihuahua. Our objective was to evaluate the effect of bison wallows on the composition and structure of the vegetation, on species composition of annual grasses, and soil properties. Soil and vegetation samples were taken from inside the wallows and were compared against the samples obtained outside the wallows from late August to early September. The percentage of plant cover and the height of the foliage were measured inside and outside the wallow. Soil cores were taken, and the presence of soluble salts, moisture retention capacity, percentage of clay, and concentration of nutrients was determined. The three associations preferred by bison for wallowing were toboso grassland, vine mesquite and annual grassland. Of the 27 species of grasses and forbs recorded in the three plant associations mostly used for wallowing, we found that 17 species were present inside and outside wallows. Five different species were found only inside wallows and another five, only outside of wallows. The annual grassland plants had a greater height outside the wallows. The toboso grassland association presented higher soil moisture, likely related to the higher percentage of clay, and the annual grassland presented the highest bulk density (BD) inside the wallows, which limited plant growth in this association. Soils in other associations did not show significant differences in BD among them, mainly due to their finer texture. No significant effect of the wallows on nutrient concentrations was recorded. The lack of significant differences related to bison activities could be related to the brief period since the herd was reintroduced to the site. These differences might become apparent over time with a larger herd. For this reason, we conclude that the current differences in the soil properties are mainly due to geomorphological processes. That is, at this stage after the reintroduction, the grasslands are responding to soil characteristics, and not to the activity of the bison.

Los bisontes son considerados una especie ecológicamente clave de los pastizales de Norteamérica debido a que sus actividades, particularmente el revolcarse, influyen en la dinámica del ecosistema y las interacciones. En 2009, 23 bisontes fueron reintroducidos dentro de un predio privado de pastizal semi-árido de 1,500 ha, en Janos, Chihuahua. Nuestro objetivo era evaluar el efecto de los revolcaderos de bisonte en la composición y estructura de la vegetación, en la composición de especies de pastos anuales, y en las propiedades del suelo. Se tomaron muestras de suelo y vegetación del interior de los revolcaderos, y se compararon con las muestras extraídas del exterior de los revolcaderos, de fines de agosto a principios de septiembre. Se midió el porcentaje de cobertura vegetal y la altura del follaje dentro y fuera del revolcadero. Se tomaron muestras de suelo y se determinó la presencia de sales solubles, capacidad de retención de humedad, porcentaje de arcilla y concentración de nutrientes. Tres asociaciones fueron preferidas por los bisontes para revolcarse: pastizal de toboso, pastizal de zacate guía y pastizal anual. La frecuencia en la conducta de revolcarse fue mayor en el verano y menor en invierno. De las 27 especies de pastos y hierbas registradas en las tres asociaciones de plantas seleccionadas, 17 estaban presentes dentro y fuera de los revolcaderos. Se encontraron cinco especies diferentes solo dentro de los revolcaderos y otras cinco, solo fuera de los revolcaderos. Las plantas anuales de los pastizales tenían una mayor altura fuera de los revolcaderos. La asociación pastizal de toboso presentó mayor humedad del suelo, probablemente relacionada con el mayor porcentaje de arcilla, y el pastizal anual presentó la mayor densidad aparente (BD) dentro de los revolcaderos, lo que limitó el crecimiento de las plantas en esta asociación. Los suelos en otras asociaciones no mostraron diferencias significativas en BD, principalmente debido a su textura más fina. No se registró ningún efecto significativo de los revolcaderos sobre las concentraciones de nutrientes. La falta de diferencias significativas relacionadas con las actividades de los bisontes podría estar relacionada con el breve periodo desde que la manada fue reintroducida en el sitio. Estas diferencias pueden hacerse evidentes a lo largo del tiempo con una manada más numerosa. Por esta razón, podemos concluir que las diferencias actuales en las propiedades del suelo se deben principalmente a procesos geomorfológicos. Es decir, los pastos están respondiendo a las características del suelo y no a la actividad de los bisontes. Sin embargo, este estudio representa una línea base para evaluar el efecto de los revolcaderos a mediano y largo plazo.

Keywords: Baseline study; ecological effect; ecological recovery; grassland restoration; reintroduction of vertebrates; soil compaction.

Introduction

Bison are considered an ecological keystone species as they contribute to heterogeneity and diversity in grasslands through selective grazing, seed dispersal, redistribution of nutrients via faeces, urine and carcasses, trampling of vegetation that accelerates the reintegration of nutrients into the soil, by knocking down trees and shrubs by rubbing on them, and by forming wallows (Hobbs 1996; Knapp *et al.* 1999; Reynolds *et al.* 2003; Anderson 2006; Melis *et al.* 2007). One of the most distinctive behaviors of bison is rolling over lifting its legs, and repeatedly turning from side to side on its back, creating oval depressions in the ground that alter the composition of vegetation in large areas across North American grasslands, these wallows being a distinctive feature of bison-occupied grasslands (Bracke 2011; McMillan *et al.* 2011).

Several studies have evaluated the impact of bison wallows on the diversity of North American grasslands, both in tall and mixed-grass prairie, considering soil and vegetation variables and analyzing currently used and vestigial wallows (Collins and Uno 1983; Polley and Collins 1984; Polley and Wallace 1986; Trager *et al.* 2004; McMillan *et al.* 2011). Significant differences have been reported in soil variables like texture, moisture, pH, N-NH_4, Na and Mg, as well as in the net primary production between the center, the margin and the outside of the wallows (McMillan *et al.* 2011).

In general, it has been found that wallows present less plant diversity than the adjacent grassland area, but there are plant species that only establish themselves within these, increasing the diversity of the grassland (Polley and Wallace 1986; Trager *et al.* 2004; McMillan *et al.* 2011). Wallows also have a direct effect on the establishment of other species of animals. Gerlanc and Kaufman (2003) evaluated the use of wallows by anuran species to oviposit, and complete metamorphosis, as wallows are temporary water reservoirs that remain long after the rainy season has ended.

Bison are absent from most of their former range and therefore their ecological effect is nonexistent except for the few places where they are found in significant numbers. Efforts are under way to recover the ecological role of bison at a continental scale (Sanderson *et al.* 2008). As part of this effort, a herd of bison was reintroduced in November 2009 within the Janos municipality in northern Chihuahua, México (SEMARNAT and CONANP 2009).

The objectives of this work were to determine if within the short-term after the reintroduction, ecological effects due to wallowing were evident in soil properties and vegetation features; to establish the baseline to evaluate the effect of the wallows over time; and to determine if wallowing was associated to a sex, age class, season, and a particular plant association.

We hypothesized that inside wallows the soil would be more compact, retain more moisture, and have higher nutrient contents, especially N due to bison rolling over, urinating and defecating. We further expected to find different plant species inside and outside the wallows, and expected the wallowing activity to be predominant among males.

Materials and methods

Study site. The study was conducted at *El Centro*, a 1,600 ha pasture (30° 50′ 58.58″ N, -108° 27′ 7.33″ W; 1,412 masl; Figure 1) inside the El Uno ranch, located within the Janos Biosphere Reserve, in the municipality of Janos, Chihuahua.

The pasture is located within an ancient alluvial fan formed by the direct dragging of sediments from the northern Sierra Madre Occidental. It is characterized by the presence of many shallow desert washes, with a south to north direction, which connect to a greater wash at the limit of the fan west of the site. This wash becomes evident in periods of higher rainfall and causes temporary flooding.

The dominant soils correspond to Xerosols, Regosols, Vertisols, and Phaeozems, according to the classification of INEGI and SEMARNAP 1998 (CONANP and SEMARNAT 2006), where the representative parental material is mainly alluvium. The surrounding mountain ranges are basalt and rhyolite formations formed during the Tertiary and the early Pleistocene (Nordt 2003).

The drought in the northern region of the Chihuahuan Desert, together with overgrazing, has changed perennial into annual grassland (Ceballos *et al.* 2010; Davidson *et al.* 2010), with extensive areas invaded by mesquite (*Prosopis glandulosa*; Humphrey 1987). The predominant vegetation type at the site correspond to shortgrass prairie, represented by species such as: blue grama (*Bouteloa gracilis*), black grama (*B. eriopoda*), sideoats grama (*B. curtipendula*), hairy grama (*B. hirsuta*), spreading threeawn (*Aristida divaricata*), fescue (*Festuca* sp.), and toboso (*Hilaria mutica*). There are also shrubs such as the ocotillo (*Fouquieria splendens*), prickly pear (*Opuntia* sp.), cholla (*O. imbricata*), tumbleweed (*Salsola kali*), and longleaf jointfir (*Ephedra trifurca*) in disturbed areas (Humphrey 1987; List 1997; CONANP and SEMARNAT 2006).

The climate of the region is semiarid temperate with hot summers (maximum average of 50 °C) and cold winters (minimum average of -12 °C; BSok (x'); García 1998). Average annual rainfall is 306.7 mm, of which 65 % falls between July and September (List 1997; Nordt 2003).

Study population. On November 27, 2009, a herd of bison without genetic introgression of cattle and free of diseases (brucellosis, tuberculosis) was reintroduced in El Uno. The herd came from Wind Cave National Park, in South Dakota, United States. Bison were introduced to form a reproductive herd for future reintroductions within the historical range of the species in México (SEMARNAT and CONANP 2019). During the study, the herd consisted of 16 females and 3 males between 3 and 4 years of age, a 2-year-old female, born in 2010, 9 calves born in 2011 and 7 calves born in 2012. The bison conformed a single group, they have access to permanent artificial bodies of water,

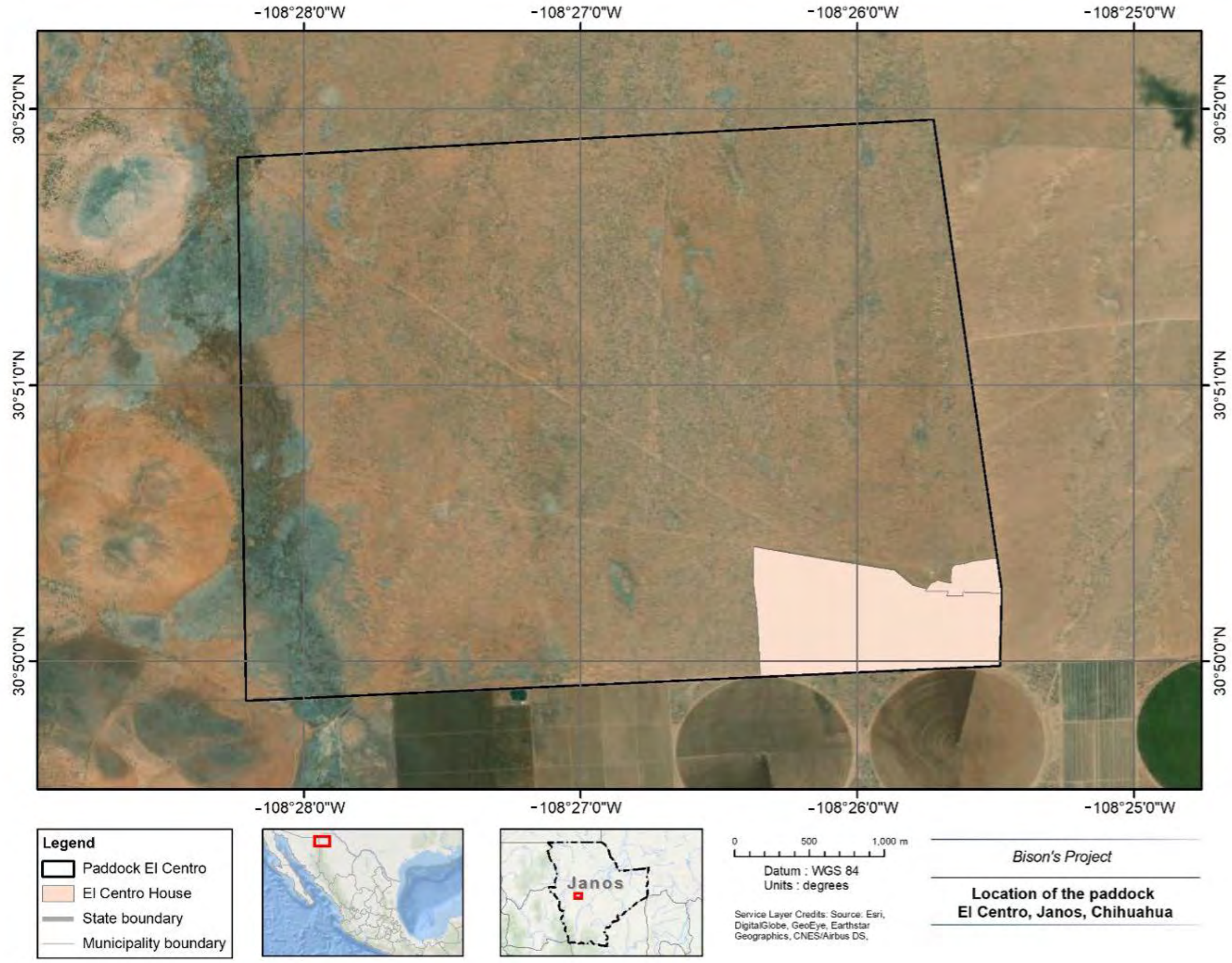

Figure 1. Location of the pasture El Centro, El Uno Ecological Reserve, Janos, Chihuahua.

the property is fenced so they do not coexist with any other ungulate species and competition and predation are not important factors for the population, since the only large carnivore present in the region is the coyote (*Canis latrans*).

Location and use of the wallows. Four periods of observation of the herd were conducted in 2012-2013: spring (April-May), summer (July-August), autumn (October-November), and winter (December-January), with a duration of 25 days each. To sample the complete daylight period, observation times were alternated: from dawn - 09:59, 10:00 -13:59, and 14:00 - to dusk. The frequency of the rolling behavior, the individual who performed it, the time of the day, and the GPS coordinates of each wallow were recorded.

We mapped the plant associations and the location of wallows using a Google Earth satellite image (2007; 1:25,000) georeferenced in UTM coordinates. For the characterization of the plant associations, the image characteristics were correlated against the vegetation characteristics. 200 reference points were obtained on field to perform a supervised classification with the Spatial Analyst extension, using the maximum likelihood statistic. Plant associations were determined based on the dominant elements (plant species). Additionally, 176 points were recorded on field to corroborate the GIS-generated map. The percentage of the total number of points that coincided was estimated to obtain an accuracy value. The area for each type of plant association (percentage) was determined with respect to the total area of the paddock using the GIS ArcMap 10.1.

The number of wallows within each of the associations was determined. The number of records expected by category (plant association) was calculated based on those observed, and the generation of wallows in the different plant associations was evaluated according to their availability using the X^2 statistic. Bonferroni confidence intervals were calculated to determine use.

The frequency of wallowing behavior was analyzed by time of year, period of the day, and sex, using the Kruskal-Wallis non-parametric statistical analysis. A multiple pair comparison test was performed following the methodology proposed by Conover (1999). Plant and soil sampling was performed during the time of maximum biomass accumulation (late August - early September).

Effect of wallows on the diversity and abundance of vegetation. Six wallows were selected in each of the three plant associations most used for this activity. These were randomly selected on the satellite map, considering that they should be separated from other wallows by a minimum distance of 10 m and a distance greater than 20 m from roads and fences, and were characterized considering the length and width of each wallow.

The percentage of plant cover, and the height of the foliage of each plant species were measured at two sampling places: inside the wallow (in five points, corresponding to the cardinal points and the center) and five meters around it (in four points, corresponding to the four cardinal points), using quadrants of 0.25 m^2 (Davidson *et al.* 2010). Vegetation samples were collected and prepared according to standard preparation methods for later identification in the laboratory (Sánchez-González and González 2007).

An analysis of variance (ANOVA) was used to establish if there were significant differences between the inside and the outside of the wallows for the variables height and vegetation cover. To estimate differences between plant species composition, a contingency table was constructed and evaluated with the X^2 statistic.

Effect of wallows on soil parameters. Soluble salts. During the same period, undisturbed soil samples with 100 ml cylindrical cores were taken. The presence of soluble salts in the soil of the study site was evaluated by measuring the electric conductivity (EC) and the potential of Hydrogen (pH) of soil-water suspensions. Ten grams of dry and sieved soil were weighed, 25 ml of distilled water were added, and the EC and pH were measured in the supernatant with a multiparameter meter (HI2020-01, Hanna Instruments, USA), equipped with a pH electrode and a conductivity probe. Additionally, the soil texture was determined with the finger probe in the field, and the moisture retention at field capacity (FC) was estimated on behalf of the soil texture, estimated organic matter content and the bulk density after Siebe *et al.* (2006). With these data, the electric conductivity at moisture contents at field capacity (EC pF1.8) was estimated at each site.

Bulk density (BD) and volumetric moisture content. To evaluate compaction and soil moisture the BD was determined. Undisturbed samples were taken at two depths (5 and 10 cm), with 100 cm^3 cylinders collected inside the wallow (12 × 2 randomly distributed samples) and around it at a distance of 5 m (12 × 2 samples distributed randomly around the circumference). The cylinders were weighed, dried in the oven at 105 °C for 48 h and reweighed (Siebe *et al.* 2006; Bélanger and Van Rees 2007).

Nutrients. To determine the concentration of nutrients, composite samples were taken with cores from 2 to 7.5 cm depth inside the wallow (12 randomly distributed samples) and five meters around it (12 randomly distributed samples around the circumference). The individual core samples were pooled and preserved in hermetically sealed plastic bags.

For the determination of nitrates and ammonium, from each sampling point a small sub-sample was kept in the refrigerator at 4 °C, until its subsequent analysis in the laboratory.

Laboratory analysis. A total of 48 composite samples were collected and extracted with 1 M Potassium Chloride (KCl) solution in duplicates. The mean of the duplicates is reported.

Nitrogen: Nitrates (NO_3) and Ammonium (NH_4). The determination of Nitrates (NO_3) and Ammonium (NH_4) was done colorimetrically following standard procedures with a nitrogen autoanalyzer (Perkin Elmer 2400). The standard solutions were prepared from Potassium Nitrate (KNO_3) and Ammonium Chloride (NH_4Cl) and readings were made at wavelengths of 410 and 660 nm, respectively.

Phosphorus. Available phosphorus was determined based on the Bray-Kurtz method (Black 1965a), since it has been considered the most suitable for acid soils. Phosphorous extractions was quantified by colorimetry and determined by spectrophotometry (Perkin Elmer 3110) at a wavelength of 720 nm.

Six of the samples were analyzed by the Olsen method (Black 1965b) because they presented a pH greater than 7. In this case, Phosphorous was extracted with a sodium bicarbonate solution, and quantified by colorimetry. Absorbance was determined by spectrophotometry at a wavelength of 660 nm.

Exchangeable Cations. Exchangeable cations were extracted with 1 N ammonium acetate at pH = 7; Calcium (Ca) and Magnesium (Mg) values were obtained in a Perkin Elmer 3110 atomic absorption spectrometer; Sodium (Na) and Potassium (K) were obtained with a Sherwood emission spectrometer (Van Reeuwijk 1992). A principal component analysis (PCA) was carried out to establish the variables that expressed the variability in the data between plant associations (field capacity (FC), BD, texture (% clay), electric conductivity (EC) and pH. We used PCA as a dimension reduction technique that estimates artificial axes for exploring dispersion graphs, with optimal properties, of the underlying variability and covariability, based on orthogonal rotations given the level of correlation of the original variables and the factors. Biplots and Minimum Path Graphs were generated from the PCA results; i) Biplot was constructed by constructing a scatter diagram of the observations from the PCA on the connections matrix (covariances) of all variables and overlapping the eigenvectors representing each variable scaled on the same space, while; ii) Minimum Path Graphs was constructed with the distances of the point rows of the data matrix in the original space, connecting variables with straight segments securing all points and linked without loops while maintaining the distance of the segments to the minimum (Gower and Ross 1969). The loads of the factors were considered when estimating all the components in order to control both for high influence of each variable and the rotation method.

To evaluate the effect of the location (inside and outside the wallows) and depth (0 to 8 cm or 8 to 13.5 cm) on BD and moisture, the non-parametric statistic Kruskal Wallis was used, since the data did not comply with the assumption of normality. Likewise, a test of multiple pairwise comparisons was carried out following the methodology proposed by Conover (1999).

Based on the concentration of nutrients, the distribution of the data was estimated using the normality test (Shapiro Wilks). A mixed effects model was generated by means of a generalized least squares analysis to evaluate the influence of the location (inside and outside the wallow) and the vegetation on the concentration of each of the analyzed nutrients.

A model with interactions between the location and the vegetation was generated and the graph of the standardized residuals against the adjusted values was obtained, since there was apparent homogeneity of variance. For this, three models with different variance structure were tested and the one with the best fit was selected according to the AKAIKE information criterion.

Results

Location and use of the wallows. A total of seven plant associations and 219 wallows were recorded within the "El Centro" pasture: longleaf jointfir scrub with grassland (41.5 %; 35 wallow records), blue grama grassland (20.1 %; 26 wallow records), annual grassland (14.2 %; 44 wallow records), toboso grassland (13.5 %; 93 wallow records), bare soil (5.4 %; 11 wallow records), vine mesquite (2.8 %; 8 wallow records), and grassland in black-tailed prairie dog colony (2.6 %; 2 wallow records). For the analysis, the three main plant associations used for this activity were selected. These corresponded to toboso grassland (mainly represented by *Hilaria mutica*), vine mesquite (*Panicum obtusum*) and annual grassland (*Aristida adscensionis*; Table 1; X^2 0.05 = 219.71, g.l. = 6 $P > 0.05$) as these three had higher observed values than expected.

Based on the frequency of the behavior of rolling on the ground, significant differences were observed between the different times of the year (H = 86.85, $P < 0.0001$); individuals wallow more frequently in summer and do less so in the winter and fall. When analyzing behavior by period of the day, significant differences were also found (F = 102.95, $P < 0.0001$). In the period between 10:00 and 14:00 the frequency of the behavior increased, being more evident during the summer. In spring, winter and autumn, the frequency decreased regardless of the period of the day (Figure 2).

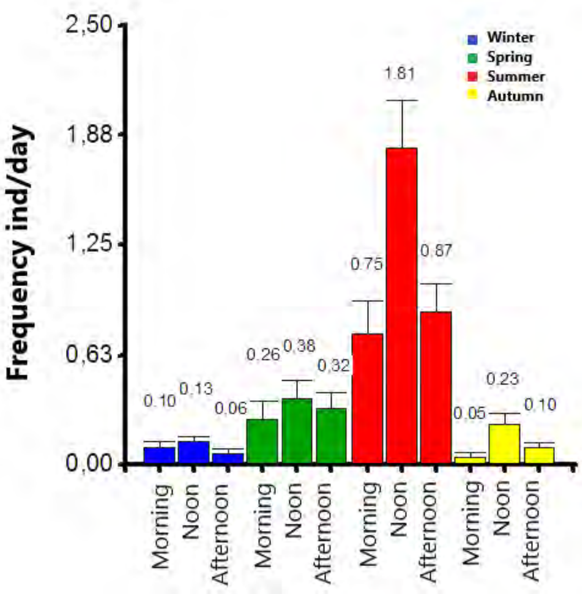

Figure 2. Frequency of wallowing by time of year and by period of the day (SE).

Significant differences were also found between the winter (H = 13.39, $P = 0.0328$), autumn (H = 15.39, $P = 0.0183$) and summer (H = 29.77, $P = 0.0002$) seasons, both between sexes and by period of the day. However, during the spring, no significant differences were found between the sexes and by period of the day (H = 7.04, $P = 0.4931$). It was determined that during the morning and at noon in winter, females, and calves wallow more than males; in autumn, the frequency of the behavior is very low in the morning and increases at noon. Although this pattern is repeated during the summer, the frequency increases markedly and the differentiation between the sexes is more evident. Males wallow more frequently than females and females in turn wallow more than calves, however, in general the behavior increases at noon. During summer, the frequency of wallowing increased regardless of the sex of the individual. In general, it was observed that calves and

Table 1. Use of the different plant associations for wallowing.

Plant Association	Number of records	Records %	Z of Bonferroni		
			Expected	Observed	
Toboso grassland	93	42.5	0.135	0.420	Major use
Vine mesquite	8	9.6	0.028	0.096	Major use
Annual grassland	44	17.4	0.142	0.174	Proportional use
Blue grama grassland	26	11.9	0.201	0.119	Less use
Longleaf Jointfir scrub with grassland	35	14.2	0.415	0.142	Less use
Bare soil	11	4.1	0.054	0.041	Proportional use
Grassland in black-tailed prairie dog colony	2	0.9	0.026	0.009	Less use
$X^2_{0.05}$ = 219.71, gl= 6 p>0.05					

females wallow less frequently during the morning and in the afternoon, except for winter, where males present the behavior to a lesser extent (Figure 3).

Effect of wallows on the diversity and abundance of vegetation. Twenty-seven species of annual grasses and herbs were recorded, of which 20 were found in the annual grassland association, 17 in the vine mesquite association, and 12 in the toboso grassland.

Wallowing did not affect species richness for any of the plant associations: annual grassland (x^2 = 26.449, *P* = 0.2329), toboso grassland (x^2 = 9.991, *P* = 0.4413) and vine mesquite (x^2 = 12.018, p = 0.8463).

Seventeen plant species were present inside and outside of wallows (*i. e.*, 67% of the total number of species). Five different species were found only inside wallows and another five, only outside of wallows.

In the annual grassland association, the plants presented a greater height outside the wallows (F = 6.50, *P* = 0.0121). However, no differences were found in the coverage for that association nor in any other plant variables measured in the associations inside and outside the wallows (Table 2).

Effect of wallows on soil parameters. Soluble salts. The results obtained from the measurement of FC, BD, % clay, EC and pH of each of the samples are shown in the Annex 1.

In general, the soils presented a medium and medium-high BD (1.23 g/cm^3 to 1.49 g/cm^3), and the soil texture varied between loam, silty clay loam and silty clay. Annual grasses grew on soils having less clay, while toboso and vine mesquite associations grew on more clayed soil. The pH corresponded mainly to acid values and the EC pF1.8 fluctuated in a range from 0.57 to 2.00 dS/m. Based on the results of X^2 of the multivariate analysis of principal components, it is shown that only the first three components present significant differences (Table 3), but with the first two 81.5 % of the variability of the observations can be explained. The first component (FC, BD, % clay) explains 57.7 % of the variability and the second (EC pF1.8 and pH) 23.8 % (Table 3; Figure 4). The differentiation of the association of annual grassland was mainly due to BD, while the associations of toboso grassland and vine mesquite were more related to the rest of the variables, mainly the percentage of clay, FC and pH (Figure 4). The minimum spanning tree shows that samples a and b from the same point do not necessarily have the greatest relationship between them.

Bulk density (BD) and volumetric moisture content. Based on the laboratory analysis, significant differences were found in soil moisture (H = 247.10, *P* = <0.0001) and soil BD (H = 157.63, *P* = <0.0001) between the different plant associations preferred for wallowing. The BD was higher in the annual grassland (1.49 g / cm^3) followed by the association of vine mesquite (1.30 g / cm^3), and the toboso grassland presented the lowest bulk density in the soil (1.23 g / cm^3). The vine mesquite association presented the highest soil moisture content (10.5 %), followed by toboso grassland (7.6 %) and annual grassland (4.8 %).

No significant differences were found in soil moisture inside and outside of the wallows in the vine mesquite association (H = 1.98, *P* = 0.5764). For the association of annual grassland, significant differences were found based

Table 2. Analysis of variance between treatments (inside and outside of the wallows) and vegetation variables (height and cover).

		df	**Sum of squares**	**Mean squares**	**F**	**p**
			Coverage			
	Treatment	1	0.014	0.014	0.02	0.887
Toboso grassland	Error	63	44.748	0.71		
	Total	64	44.762			
	Treatment	1	0.047	0.047	0.286	0.594
Annual grassland	Error	117	19.177	0.164		
	Total	118	19.224			
	Treatment	1	0.004	0.004	0.007	0.933
Vine mesquite	Error	92	51.708	0.562		
	Total	93	51.712			
			Height			
	Treatment	1	54.976	54.976	1.554	0.217
Toboso grassland	Error	63	2,228.277	35.369		
	Total	64	2,283.254			
	Treatment	1	130.028	130.028	6.503	0.012*
Annual grassland	Error	117	2,339.325	19.994		
	Total	118	2,469.353			
	Treatment	1	13.743	13.743	0.218	0.641
Vine mesquite	Error	92	5,788.396	62.917		
	Total	93	5,802.138			

*Significant differences between treatments

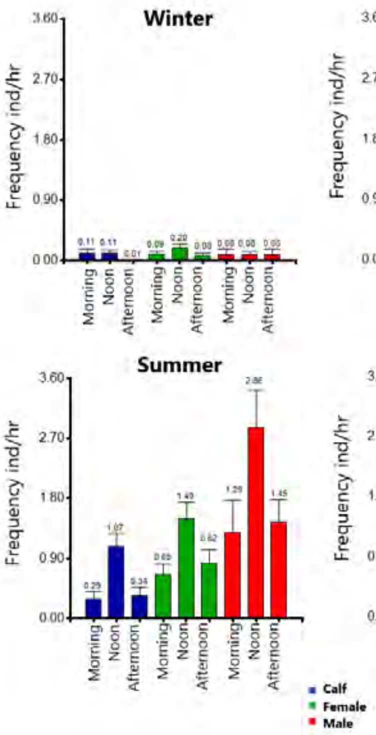

Figure 3. Frequency of wallowing by season of the year, by period of the day and by sex (SE).

on depth but regardless of the location (inside or outside of the wallow; H = 14.51, $P = 0.0023$), since higher moisture contents were measured at a depth of 8 to 13.5 cm (inside = 5.4 %; outside = 4.9 %) than in the first few centimeters of the soil (inside = 4.7 %; outside = 4.3 %). On the other hand, significant differences were found in the association of toboso grassland (H = 9.65, $P = 0.0218$). A higher soil moisture was determined inside the wallow, at both depths (surface = 8.1 %; 8-13.5 cm = 8.6 %), than outside it (surface = 6.5 %; 8-13.5 cm = 7.3 %, Figure 5).

Significantly higher values of BD were measured within the wallows (surface = 1.54 g / cm^3; 8-13.5 cm = 1.54 g / cm^3) than outside of these (surface = 1.42 g / cm^3; 8-13.5 cm = 1.46 g / cm^3) in the association of annual grassland (H = 17.33, $P = 0.0007$). No significant differences in BD were found inside and outside the wallows in the vine mesquite and toboso grassland associations (H = 6.50, $P = 0.0896$ and H = 7.58, $P = 0.0554$, respectively, Figure 5).

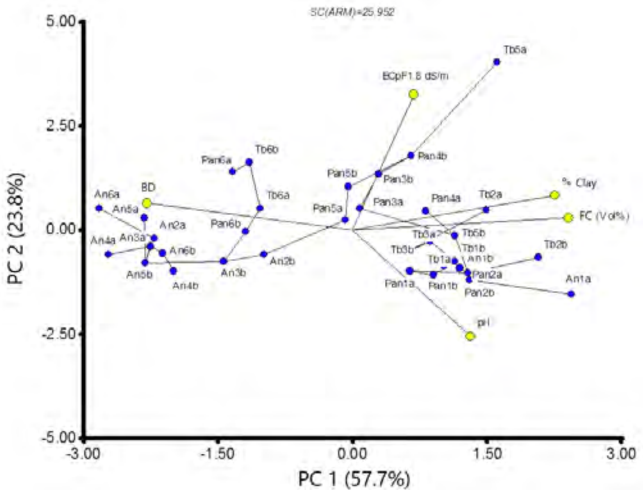

Figure 4. Minimum path tree. An (Annual grassland), Pan (Vine mesquite), Tb (Toboso grassland); a (inside), b (outside).

Table 3. Results of principal component analysis.

Number of component	Eigenvalue	%	Cumulative percentage	x^2	df	p
1	2.886	57.727	57.727	106.720	10.097	<0.0001
2	1.188	23.768	81.494	55.622	9.276	<0.0001
3	0.610	12.207	93.702	28.608	5.987	<0.0001
4	0.228	4.569	98.271	7.160	3.173	0.0759
5	0.086	1.729	100	0	0.174	1

Nutrients. Significant differences in the concentrations of nutrients in the soil were found between the different plant associations (F-value = 2.69, $P = 0.0021$), except for the concentration of NH_4. The association of toboso grassland had the highest average concentrations of Ca, Mg, P and NH_4; the vine mesquite had the highest averages in the concentrations of K and NO_3 and the association of annual grassland presented the highest average in the concentration of Na. However, the concentration of phosphorous, exchangeable cations, NO_3 and NH_4, inside and outside the wallows, did not vary significantly (F-value = 0.04, $P = 0.9998$; Table 4).

Discussion

Location and use of the wallows. As expected, wallowing behavior was more frequent among males. The three plant associations preferred for wallowing were toboso grassland, vine mesquite and annual grassland. The first two associations are found within washes, and 52 % of the total wallows generated by bison were recorded in here (*n* = 219). These associations are probably selected due to more clay and silty texture, and higher moisture content in the soil which decreases its temperature. It is important to note that the sites selected to wallow within each association generally corresponded to patches of bare soil, that is, the bison do not remove the vegetation from the surface, but rather seek that the site is devoid of vegetation cover.

Different hypotheses have been suggested that determine the origin of the rolling behavior; it can represent aggression, dominance between males during the mating season, or grooming behavior (Coppedge and Shaw 1998). McMillan *et al.* (2000), analyzed the possible causes of this behavior and determined that it can be triggered by several factors, such as hair release during molt, group cohesion (mainly to strengthen ties between females and their offspring), behavior of game, thermoregulation or to reduce the burden of ectoparasites and discomfort from insect bites. In our case, we observed a significant increase in frequency during the summer, which is directly related to an increase in the temperature and humidity of the environment. These conditions favor the increase of insect popula-

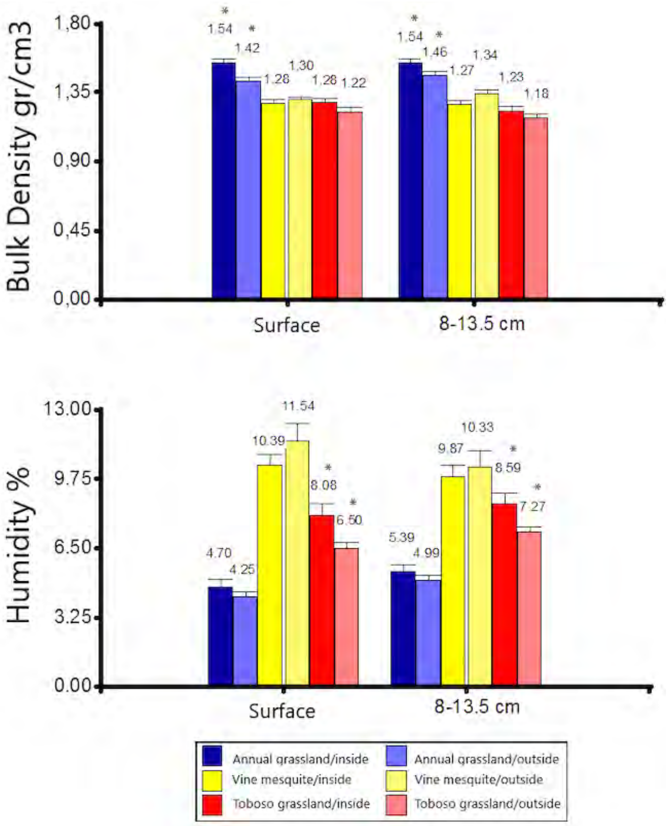

Figure 5. Bulk density (BD) and volumetric soil moisture by type of plant association and inside and outside the wallows. The bars represent the standard error and the asterisks represent significant differences between locations.

tions, particularly of the horned fly (*Haematobia irritans*), a dipteran whose bites are irritating to bison. This fly needs a high relative humidity, an air temperature around 32 °C, and a constant source of light (Morgan 1964). Minimum temperatures should not be so low as to inhibit fly activity (such as in winter), so that in summer (July-September), when the temperature remains high (both day and night), the appropriate conditions are found increasing the activity of the horned fly. Bison appeared to be disturbed by the horned flies since they were constantly shaking the head and moving the tail when the flies surrounded them. While it is not possible to discard that ticks could be related with wallowing behavior, it is during Autumn (October) when winter tick larvae are supposed to be blood feeding (Mooring and Samuel 1998, McMillan *et al.* 2000), but at this time bison wallowed much lower than during other months of the year. Based on this, our results agree with McMillan *et al.* (2000), whose results were not consistent with the idea that wallowing is a grooming behavior to remove ticks, but to relief horn flies commonly saw during summer months. The fact that the frequency of the rolling behavior increased during the period from 10:00 to 14:00 h, particularly during the summer, when ectoparasites decrease their activity, is consistent with the hypothesis of thermoregulation as when wallowing they remove the soil surface and expose soil of a cooler temperature since a temperature decrease has been found up to 70 cm depth at noon (Singh and Sharma 2017).

Although the fact that males wallow more frequently than females during the summer could also be related to dominance among males at the time of mating, however wallowing behavior was rarely accompanied by agonistic behavior; in addition to that, competition should be reduced, since there are only three sub-adult males and there is a high availability of females. It has been reported that the parasite load of flies is related to the size of the individual, the secretion of stress hormones (cortisol) and the age of the host, which in turn reflects its health (Morgan 1964). A higher parasite load on males is consistent with our observations, and that would explain the increased wallowing. It has been observed that this behavior corresponds mainly to adult individuals. However, it was observed that calves from the first month of age try to develop it, without being able to turn on the back completely but raising their legs. It was also observed that bison frequently rubbed on the burrow mounds of prairie dogs (*Cymomys ludovicianus*). Sometimes they wallowed on these, but the slope was a factor for them to turn more than 180° on their backs, which appeared to frighten them, discouraging activity. Prairie dog burrow mounds, being made up of loose material, constitute a micro-site selected by bison to rub themselves, as reported by Coppedge and Shaw (2000) for coyote and badger burrows.

Effect of wallows on the richness and abundance of vegetation. When determining the effect that the wallows currently have on the richness of the vegetation, it was observed that there are no differences in the species of plants that grew during the summer inside and outside the wallows. Even though there were some species that were only recorded inside and vice versa, none had specific characteristics that prevent them from establishing inside or outside the wallows, so the differences are possibly due to sampling size rather than an effect of the wallows.

Likewise, the height and coverage variables were not influenced by the effect of the wallows. The only association that presented a difference in the height of the plants between treatments was the association of annual grasses. Under this association the highest values of BD of the soil were observed and it was the only soil variable that was significantly higher inside the wallows than outside of them. An increase in BD of the soil, implies that the pore space diminishes, affecting the aeration capacity, limiting drainage and root penetrability, thus reducing plant growth (Bartholomew and Williams 2010). An increase in BD was related with decreases the number of leaves and the biomass of the shoots and roots (Bradshaw and Chadwick 1980; Bartholomew and Williams 2010; Wilson *et al.* 2013). Compared to perennial grasses, the growth of annual grasses at sites with high BD could be feasible even though drainage could be reduced and roots could be limited in their ability to penetrate the soil to obtain necessary resources (Singh *et al.* 2018). However, the increase in BD within the wallow limited pasture growth in this association.

Table 4. Mean values of elements by type of plant association.

Plant association/Element	Ca (cmol(+)/kg)	K (cmol(+)/kg)	Mg (cmol(+)/kg)	Na (cmol(+)/kg)	P (mg/kg)	NO_3 (mg/kg)	NH_4 (mg/kg)
Toboso grassland	18.91*	2.4	5.24*	0.07	66.31*	0.77	1.30*
Vine mesquite	12.61	2.69*	4.81	0.10	46.58	0.89*	1.27
Annual grassland	6.10	1.83	2.72	0.22*	42.55	0.48	1.05

* Indicates the maximum value of the mean per element.

Effect of wallows on BD and volumetric moisture content. Contrary to our predictions, an effect of wallows on soil properties was not detected; the soils in the toboso grassland and vine mesquite associations presented a higher percentage of clays (42.5 %), because runoff water transports the suspended clays to these sites, compared to the soil present in the annual grassland association (24.2 %), and a lower bulk density. Although fine-textured soils tend to be more susceptible to compaction (Barik *et al.* 2011; Wilson *et al.* 2013), it has been found that the aggregate size groups in the coarse textured soil has a positive effect on compaction (Barik *et al.* 2011). Likewise, higher BD values have been reported in sandy soils due to the absence of micropores, and a relationship with the amount of water in the soil has been attributed, where a decrease in the amount of water increases the contact between the particles, increasing the cohesion forces by decreasing the water pressure in the pores (Koolen and Kuipers 1983; Barik *et al.* 2011). In this way, soils with fine textures that present a good degree of structure, fluvic Cambisol (hypereutric, epilymic, chromic); protovertic Phaeozem (limic, chromic), will form larger aggregates that behave as a single particle, that present greater resistance to compaction than coarse-textured soils that present few and small aggregates, Regosol haplic (hypereutric, thaptoargic).

For this reason, the wallows formed on fine-textured soils that had greater resistance to compaction did not show significant differences when comparing the inside and outside of the wallows. These differences will probably begin to become apparent over a longer period, where the wallows are used under higher humidity regimes (the clay must be at field capacity to compact), which facilitate compaction in this type of soils with low aggregate stability.

The wallows represent a concave structure that allows the accumulation of water (Polley and Collins 1984). Sapling production has been reported to increase significantly with high water potential and moderate bulk density (Tokunaga 2006). These conditions are present within the wallows in the toboso grassland and vine mesquite associations. This type of soils are less water permeable since they have finer textures, and thus presented higher moisture contents. At the Konsa Prairie Biological Station, the differential diversity of plants was evaluated, inside and outside the wallows, and certain species were only found within them (McMillan *et al.* 2011). During the sampling period in our study site, rainfall was below the annual mean, which did not favor the growth of the vegetation cover. For this reason, it is important to note that a longer-term sampling that includes a representation of the seasonal and annual variability of the environmental parameters would allow to determine whether there are differences attributed to the effect of wallows. During the preliminary observations in 2010 (mean annual rainfall = 279.9 mm; Unifrut 2011), the growth of a marked patch of vegetation was observed within the wallows, contrary to what was observed during the sampled year.

Effect of wallows on Nutrients. The lowest concentrations of nutrients in the annual grassland association where the soils that presented a higher percentage of sand. Nutrient retention is less and leaching tends to be faster in this type of soils since they have a low cation exchange capacity, contrary to what occurs in soils with a higher percentage of clay and silt (Whitehead 2000; Schaetzl and Anderson 2005). In some grassland sites, the losses of Ca, Mg and Na due to leaching are greater than those resulting from harvested forage (Whitehead 2000).

In this way, we would expect that the effect on the accumulation of nutrients due to the biological activities of the bison would be more evident inside the wallows of the associations of toboso grassland and vine mesquite, as during the mating season, sexually mature males urinate in the wallow before rolling to assert their dominance over other males (Gates *et al.* 2010). However, no significant effect of the wallows was seen.

The leaching rate is reduced when the amount of ammonia produced in the urine is used rapidly, favoring the absorption of cations by plants. The secretion of cations is greater in the urine than in the faeces, additionally; the urine macronutrients tend to infiltrate the soil faster due to the macropores (Whitehead 2000). Each individual of domestic cattle can contribute between 0.05 and 0.1 kg/m^2 N in each urine stool (8 to 12 times/day) and between 0.02 and 0.08 kg/m^2 N in each fecal deposition (10 times/day; Bellows 2001), and N is the element more quickly recycled in the environment because it is highly mobile in the soil solution, it leaches easily and its availability in the soil is regulated mainly by biotic processes (Wedin 1996), therefore, in order to observe differences, we need a larger number of individuals and to sample in a shorter period, between the time the individuals use the wallows and the samples are taken.

The approximate quantity of cations that are recycled in the urine and excreta of cattle, at a density of 700 ind-day / ha * year, of each macronutrient has been estimated (K = 128 kg/ha; Na = 22 kg/ha; Ca = 49 kg/ha; Mg = 24 kg/

ha; Whitehead 2000). If we compare these values with the density of our study group (0.02 ind-day/ha * year), we can observe that the contribution of the recycling of macronutrients by the bison is minimal. If it is also considered that the herd was only 3 years old after being reintroduced to the site, we can conclude that indeed, we cannot expect an appreciable change in the concentration of nutrients in the soil, so we could not observe an impact of the wallows at this time. The amount of nutrients excreted is minimal and most of the cations are used by the plants or retained in the first centimeters of the soil. For this reason, we can attribute that the differences related to the type of soil are mainly due to geomorphological processes that constitute a much larger impact, both on a temporal and spatial scale, than the effect that these number of bison and for the brief period they have been in the pasture. That is, the pastures are responding to soil-geomorphology dynamics and not to the activity of the bison.

However, as the number of individuals increases in the area, it will be necessary to re-evaluate the impact that will be perceptible on a larger scale of time (10, 20, 50 years).

The limiting factor in plant growth on our site is the soil moisture, since the concentrations of nutrients are within adequate ranges to obtain maximum forage yield. In particular, K is the cation that contributes the most to maintaining the osmotic potential (opening and closing of stomata), which allows plants to adapt to drought, a very recurring factor in the study region. The values that we obtained from the K measurements far exceed the concentration value necessary to obtain the maximum yield of the forage in grasslands (> 114 mg/kg; Whitehead 2000).

Based on other studies (Polley and Collins 1984; Polley and Wallace 1986) that analyzed the effect of wallows on soil properties, differences were found inside and outside the wallows in the concentrations of NO_3, NH_4, P, Mg, pH, texture and soil moisture. However, these studies were conducted at a site with a larger herd (625 individuals), after a longer period (> 50-years) after the reintroduction of the herd, and in an area with higher rainfall.

Because our site is in the middle part of an intermediate-age alluvial fan, it can perform well in the growth of natural vegetation, integrating the impacts that bison activities can generate. For this reason, it is necessary to carry out a prolonged study that allows a greater representation of the environmental variability that influences the growth of vegetation and to obtain a follow-up of the changes generated in the properties of the soil and of the vegetation cover.

Wallowing represents a specific bison's behavior and they select certain plant associations for wallowing. Wallow formation modifies soil characteristics; however, this occurs rather in the long-term, since in the studied region no significant impact on soil properties (BD, moisture, pH, EC, texture, NO^3, NH^4, P, Na, Mg, K, Ca) or in the vegetation (cover, high, richness) could be detected after three years of bison reintroduction. At the time of the study, soil texture seems to vary in the landscape due to geomorphological processes and influences BD, moisture and nutrient contents to a greater extent than bison activity.

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Native and no-native herbivorous relationships have mutual benefit: *Lepus flavigularis* case

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Lepus flavigularis is an endemic leporid from southeastern Oaxaca, México, listed as "endangered" under Mexican law and the IUCN Red List. The main threat to the species is severe habitat fragmentation due to human activities. The jackrabbit shares the grasslands with cattle (*Bos taurus*), but the trophic interaction between these herbivores is unknown. This study aimed to determine the seasonal diet of *Lepus flavigularis* and cattle, to identify the possible dietary overlap between these mammals. The study zone covers an area around the locality of Santa María del Mar in Oaxaca, southern México. Plant species were identified using linear transects, and vegetation cover was also estimated using circular plots. Throughout the study fresh *L. flavigularis* and *B. taurus* fecal samples were collected, which were processed using the microhistological technique to determine the seasonal botanical composition of diets from the two species. Twenty-three plant species were recorded in the diet of *L. flavigularis* and 29 species in the diet of *B. taurus*. Both herbivores fed primarily on Poaceae species throughout the year (*L. flavigularis*: dry season (ds) = 79.79 %, wet season (ws) = 91.54 %; *B. taurus*: ds = 78.02 %, ws = 84.63 %) despite the significant difference in the availability of plant species between seasons. No significant differences were found in the seasonal composition of the diet between the two species. Twelve plant species were consumed by both herbivores during the two seasons. The dietary overlap between the two species was high for both seasons (ds O_{jk} = 0.7311, ws O_{jk} = 0.8459). Trophic niche breadth was low for both species, with a high dietary overlap. We recorded low seasonal use values for *L. flavigularis* and *B. taurus* when compared to forage availability. Low trophic niche breadth and high dietary overlap between the two herbivores suggest forage resources competition. However, we expect that jackrabbits benefit from cattle presence, as long as the stocking rate does not increase. Furthermore, *B. taurus* grazing reduces height, cover, and density of vegetation, which could help *L. flavigularis* to avoid predators.

Lepus flavigularis es un lepórido del sureste de Oaxaca, México, categorizado como "en peligro de extinción" de acuerdo con la legislación mexicana y con la Lista Roja de la UICN. La principal amenaza para la especie es la severa fragmentación del hábitat a causa de las actividades humanas. La liebre comparte los pastizales con ganado vacuno (*Bos taurus*), pero la interacción trófica entre estos herbívoros se desconoce. Este estudio tuvo como objetivo determinar la dieta estacional de *L. flavigularis* y el ganado, así como identificar la posible superposición alimenticia entre estos mamíferos. La zona de estudio cubre un área alrededor de la localidad de Santa María del Mar en Oaxaca, al sur de México. Las especies de plantas se identificaron utilizando transectos lineales, y se estimó la cobertura vegetal utilizando parcelas circulares. A lo largo del estudio se colectaron muestras fecales frescas de ambas especies, las cuales fueron procesadas utilizando la técnica microhistológica para determinar la composición botánica estacional de la dieta de las dos especies. Se registraron 23 especies en la dieta de *L. flavigularis* y 29 para *B. taurus*. Ambos herbívoros se alimentaron principalmente de especies de Poaceae anualmente (*L. flavigularis*: temporada seca (ts) = 79.79 %, temporada húmeda (th) = 91.54 %; *B. taurus*: ts = 78.02 %, th = 84.63 %), a pesar de la significativa diferencia en la disponibilidad de especies vegetales entre temporadas. No se encontró una diferencia significativa en la composición estacional de la dieta entre ambas especies. Doce especies vegetales fueron consumidas por ambos herbívoros en las dos temporadas. La superposición alimenticia fue alta para ambas temporadas (ts O_{jk} = 0.7311, th O_{jk} = 0.8459). La amplitud del nicho trófico fue baja para ambas especies, con un traslape alimenticio alto entre éstas. Registramos un bajo uso estacional para *L. flavigularis* y *B. taurus* cuando se le compara con la disponibilidad de alimento. La baja amplitud de nicho trófico y la alta superposición alimenticia entre los dos herbívoros sugiere una competencia por recursos alimenticios en el corto plazo. Sin embargo, de acuerdo con estudios previos sobre uso de microhábitat, sugerimos que las liebres pueden beneficiarse de la presencia del ganado, siempre que la capacidad de carga no se incremente. Además, el ramoneo de *B. taurus* reduce la altura, cobertura y densidad de la vegetación, lo cual puede ayudar a *L. flavigularis* a evitar depredadores.

Keywords: Diet competition; diet overlap; grazing; México; Tehuantepec jackrabbit; livestock.

Introduction

For small and medium mammals like rodents and lagomorphs, food availability is one of the major limitations for population density (Prevedello *et al.* 2013). Studies exploring trophic interactions between leporids and other sympatric herbivores, such as livestock, are important to design conservation plans with an adequate livestock management program. It has been widely documented that, in some cases, heavy livestock grazing, and high stocking rates have an adverse impact on the density, distribution and population dynamics of leporids (Gahr 1993; Bock *et al.* 2006) and other mammals (Cortés-Marcial *et al.* 2014). Several studies have clearly shown that trophic competition occurs between leporids and other herbivores and ruminants such as cattle (Smith *et al.* 2004; Young *et al.* 2005), producing an adverse effect on leporid populations (Hulbert and Andersen 2001). However, certain leporids and livestock can coexist and obtain mutual benefits when stocking rates do not exceed the grazing capacity of their habitat (Karmiris *et al.* 2005; du Toit 2011). Furthermore, some evidence suggests that livestock grazing, whether by goats, sheep, or cattle, is beneficial to jackrabbits due to the reduction of standing biomass, which is a key factor in habitat suitability (Kuijper *et al.* 2008; Karmiris and Nastis 2010).

The Tehuantepec jackrabbit (*Lepus flavigularis*) lives in four disjunct populations within an area of 673 km^2 in the surroundings of Laguna Inferior and Laguna Superior in the Tehuantepec Isthmus, Oaxaca, México. This jackrabbit is an endangered species, endemic to Oaxaca, and is currently considered one of the most endangered leporids in the world (SEMARNAT 2010; Lorenzo *et al.* 2015, 2018; IUCN 2022). *L. flavigularis* inhabits open grasslands and xeric shrublands with the presence of isolated tree species (Carrillo-Reyes *et al.* 2012; Lorenzo *et al.* 2015). Land-use change due to the expansion of human settlements, overgrazing by cattle, and burning of pastures for seasonal agriculture, have reduced and fragmented its habitat. Existing populations are therefore almost entirely isolated and exhibit little genetic variability (Rioja *et al.* 2011; Lorenzo *et al.* 2015). A previous study described the diet of *L. flavigularis* in one locality (Montecillo Santa Cruz) and observed that it feeds mainly on grasses (66.7 % of diet). However, the diet of this species in localities that exhibit different ecological (vegetation associations) and anthropic scenario (production activities), remains unknown. At Santa María del Mar, the jackrabbit shares the grasslands with *Bos taurus* Zebu breed (treated here as synonym of *B. indicus* or *B. t. indicus*), but the trophic interaction between these herbivores is unknown. Because there is dietary overlap between cattle and other leporids like *L. californicus, Sylvilagus audubonii* (Peña-Neira 1980; Daniel *et al.* 1993), and *Oryctolagus cuniculus* (Bonino 2006, 2011), we hypothesize trophic competition between Tehuantepec jackrabbit (*L. flavigularis*) and cattle (*B. taurus*). The study aimed not only to determine the seasonal diet of an endangered leporid (*L. flavigularis*) and cattle, but also to identify possible dietary overlap between these herbivores to better understand this relationship.

Materials and methods

The study area covers an extension of 14 km^2 around the locality of Santa María del Mar (16° 14′ 7″, 16° 12′ 46″ N and - 94° 53′ 9″, - 94° 48′ 15″ W; Figure 1), in the municipality of Juchitán de Zaragoza, in the state of Oaxaca, southern México. It is located in the region of the Tehuantepec Isthmus between a coastal lake (Mar Tileme) and the Pacific Ocean. The town is inhabited by over 800 people whose main productive activities are fishing and cattle production, and occasionally, seasonal agriculture and subsistence hunting (Carrillo-Reyes *et al.* 2010). The local climate is warm sub-humid with a pronounced dry season, summer rains, and average annual precipitation of 800 mm ($\mathrm{Aw}_{0,}$ savanna like). The driest month has less than 60 mm of precipitation and the average annual temperature is 30 °C. The wet season occurs between May and October with a short dry period in August, while the long dry season begins in November and ends in April (García and Comisión Nacional para el Conocimiento y Uso de la Biodiversidad 1998; Rioja-Paradela *et al.* 2012).

This *L. flavigularis* population is the most abundant of the four existing; Vargas (2000) recorded 12 individuals/km^2 (0.12 individuals/ha) although this value has decreased to 8 individuals/km^2 (0.08 individuals/ha; Chacón-Trinidad *et al.* 2020). The habitat of *L. flavigularis* is characterized by extensive zones of grassland, dominated by *Eragrostis prolifera*, *Jouvea pilosa* and *Whalteria preslii* (Carrillo-Reyes *et al.* 2012). Also, isolated individuals of *Opuntia tehuantepecana* and *O. decumbens* are present; these areas are grazed by cattle (Carrillo-Reyes *et al.* 2010). *B. taurus* and *L. flavigularis* are the only large and medium herbivores present in this grassland. In the scrubland surrounding the pasture occurs the Eastern cottontail (*Sylvilagus floridanus*) but does not make use of the grassland; its habitat is limited to the bush, so it does not compete for food or territory with the jackrabbit or the livestock (Rioja-Paradela 2008). A recent study showed that in the grassland association the density of cattle was eight individuals/ha (800 individuals/km^2; Chacón-Trinidad

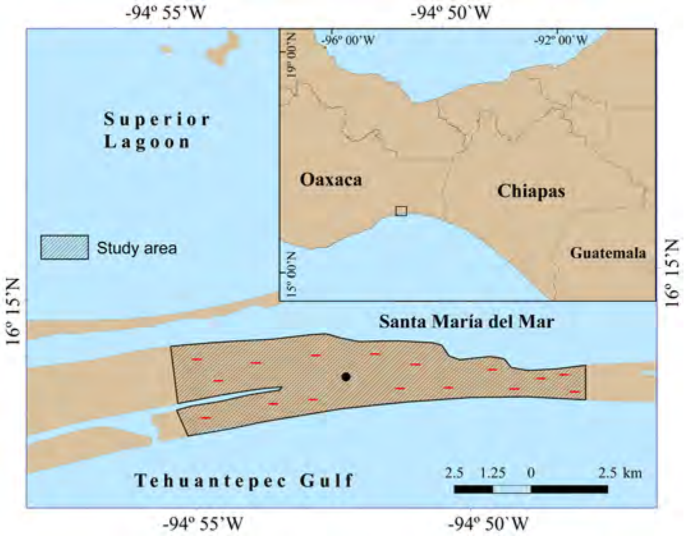

Figure 1. Study area, with vegetation transects shown in red lines.

et al. 2020), and those cattle grazed in paddocks from 8:00 to 20:00 hrs. There is no rotational grazing; cattle management depends on the availability of "paddocks to lease" and the ability of livestock owners to rent these paddocks.

We made two visits to the study area, one for dry and one for wet season, each lasting 15 days. For vegetation sampling in both seasons, 16 linear transects of 60 m were randomly established with a distance of at least 100 m between them (Carrillo-Reyes *et al.* 2010). Forage availability was estimated considering vegetation cover as an approximate measure of availability that is compatible with fecal samples at a spatial–temporal scale (Norbury and Sanson 1992; Kufner *et al.* 2008; Tirado *et al.* 2012). To record the cover of grasses and herbaceous species along each transect, five circular plots of 1 m^2 were established at 10-m intervals along each linear transect, for a total of 80 circular plots. To estimate the shrub cover, one circular plot of 12.6 m^2 was established at the center of each linear transect, for a total of 16 circular plots. Calculation of absolute coverage was based on the methodology described by Río-Olague (1999), and relative vegetation cover was calculated using the formula of Franco-López *et al.* (1989). Significant differences ($P < 0.05$) in the forage availability between dry and wet season were determined by U Mann-Whitney test (Bauer 1972). For all plant species, samples of leaves and fruits were collected and later processed by the microhistological technique to create a reference collection. This collection was later used to identify the species in the diet of the two herbivores.

During each visit to the study area, we collected *L. flavigularis* and *B. taurus* fecal samples. For each species, the freshest excreta were collected along the same transects used for vegetation (16 sampling sites, 1 per vegetation transect), to increase the probability of collecting samples from different individuals. At each transect, random *L. flavigularis* pellets and *B. taurus* dung pats were collected and placed in paper bags for transport at ambient temperature. From all fecal samples collected, a total of 20 *L. flavigularis* random pellets were chosen. Also, for *B. taurus* the sample was homogenized (the sample was dispersed and mixed homogeneously), and then a random sample of approximately 10 g of excreta was chosen. The samples were then dried at 75 °C for 24 h before subsequent analysis (Bonino 2006; Lorenzo *et al.* 2011).

The seasonal and annual botanical composition of the diet was determined by microhistological analysis of plant epidermal fragments present in the feces (Peña-Neira and Habib-de Peña 1980; Kufner *et al.* 2008; Lorenzo *et al.* 2011). This technique was used because it does not imply disturbing wildlife and no extractions are needed, which is particularly important for a critically endangered species. For each transect and every species, five temporary slides were prepared, for a total of 80 slides per species. To prepare fecal samples for analysis, the material was cleared using a commercial solution of sodium hypochlorite; samples were immersed approximately five minutes in this solution and washed in water for 20 minutes (Kufner *et al.* 2008). A plastic template with 7 mm diameter and 5 mm thick holes was used to mount the samples. The template was placed on the slides (so that each slide had the same amount of sample), and the holes were filled with the wet material after being washed. A commercial formula of corn syrup was used as mounting medium for temporary slides. After preparation, slides were sealed using transparent glaze and stored to dry for five days. Microphotographs were taken of all slides. On each slide, 20 fields were examined with an optical microscope (objective x40); five separate slides were examined for each sample, with a total of 100 fields per sampling site and a total of 1,600 per species. Slides were examined using randomly generated non-overlapping coordinates. Microscopic identification of species was performed by comparison with the reference collection of microscope slides of plants. Items whose tissue showed at least three diagnostic microhistological elements were identified to species level. Items that did not meet this criterion were not considered. Unidentified fragments were recorded as unidentified monocotyledons or dicotyledons. We recorded the plant species and accumulated frequency (number of fields in which each identified species was recorded). Botanical composition was obtained by transforming frequency to density using the Fracker and Brischle (1944) table (Lorenzo *et al.* 2011).

Species richness for each season was determined by the number of registered species (Lorenzo *et al.* 2011). Differences ($P < 0.05$) in the diet of both species between the dry and wet seasons were determined by U Mann-Whitney test (Bertolino *et al.* 2009; Desbiez *et al.* 2009) and corroborated with a Sorensen quantitative test (*QS*; Morgia and Bassano 2009). The comparison was made by contrasting the density of each plant species in the diet. Niche breadth was assessed using Levins' measure, $B_j = 1/\Sigma\ p_{ij}^2$, where p_{ij} is the proportion of diet contributed by plant species *i* on a given mammal species (Levins 1968). This was standardized to a scale of 0 - 1 following $B_j = (B - 1)/(N - 1)$ where *N* is the total number of plant species. The total number of species eaten at least once by at least one herbivore in each season was used to calculate the index (Mishra *et al.* 2004; Bertolino *et al.* 2009).

Interspecific trophic relationship was analyzed based on seasonal diet composition. Diet similarity was evaluated with U Mann-Whitney test (Bertolino *et al.* 2009) and corroborated with a Sorensen test (Morgia and Bassano 2009), contrasting the density of each plant species. Dietary overlap was estimated with the Pianka (1975) index, performing 5,000 Monte Carlo randomizations to determine the difference between the simulated data and the result of overlap (Desbiez *et al.* 2009).

To determine the relationship between the seasonal diet of each species and the forage availability in the same season the Pianka (1975) index (Kufner *et al.* 2008) was used. We use vegetation cover as an indicator of forage availability (Kufner *et al.* 2008). This analysis can indicate the degree of resource selection at a specific time and can corroborate the role of the species as generalist or specialist. All analyses were performed with the software R and RStudio (R Core Team 2020; RStudio Team 2020) and packages "EcoSimR" (Gotelli *et al.* 2015), "spaa" (Zhang 2013) and "fossil" (Vavrek 2011).

Results

During the dry season, 33 species of non-woody plants (16 grasses and 17 forbs, *i. e.*, non-grass herbaceous plants) and 16 species of woody plants were recorded. In the wet season, 68 species of non-woody plants (18 grasses and 50 forbs) and 22 species of woody plants were recorded. We found a highly significant difference in forage availability between the dry season and the wet season (U Mann-Whitney test, *W* = 357, *P* = 0.001; Figure 2).

Twenty-tree plant species were recorded in the diet of *L. flavigularis* and 29 species were in the diet of *B. taurus* (Table 1). Both herbivores fed primarily on Poaceae species throughout the year (*L. flavigularis*, dry = 79.79 %, wet = 91.54 %; *B. taurus*, dry = 78.02 %, wet = 84.63 %, Figure 3). Less consumed species were forbs (*L. flavigularis*, dry = 20.21 %, wet = 8.46 %; *B. taurus*, dry = 14.09 %, wet = 13.90 %) and shrubs (*L. flavigularis*, dry = 0.00 %, wet= 0.00 %; *B. taurus*, dry = 1.32 %, wet = 0.83 %).

The most important items in the diet of *L. flavigularis* were grasses and forbs, *Bouteloua repens* (27 %), *Stipa emi-*

Table 1. Botanical composition (%) of the diet of *L. flavigularis* and *B. taurus* by season. The percentages are presented in parentheses.

Family	Species	Life form	***L. flavigularis***		***B. taurus***	
			Dry	**Wet**	**Dry**	**Wet**
Amaranthaceae (7.69 %)	*Amaranthus scariosus*	Forb	0.56	0.00	0.00	0.00
	Gomphrena globosa	Forb	0.00	4.04	0.00	0.00
	Gomphrena decumbens	Forb	0.00	0.00	0.00	0.44
Asteraceae (5.12 %)	*Erigeron oaxacanus*	Forb	6.62	2.01	1.85	2.63
	Lactuca intybacea	Forb	0.79	1.34	0.12	2.28
Cactaceae (2.56 %)	*Opuntia tehuantepecana*	Shrub	0.00	0.00	0.49	0.00
Commelinaceae (2.56 %)	*Commelina erecta*	Forb	7.13	0.00	0.00	0.00
Convolvulaceae (2.56 %)	*Ipomoea minutiflora*	Forb	0.00	0.00	0.59	0.77
Cyperaceae (2.56 %)	*Cyperus articulatus*	Forb	4.79	0.14	0.16	0.07
Euphorbiaceae (5.12 %)	*Chamaesyce lasiocarpa*	Forb	0.00	0.00	0.40	0.12
	Dalechampia scandens	Forb	0.00	0.00	2.39	0.00
Fabaceae (15.38 %)	*Acacia* sp.	Shrub	0.00	0.00	4.79	0.00
	Centrosema pascuorum	Forb	0.00	0.00	0.00	3.56
	Chamaecrista hispidula	Forb	0.00	0.00	0.00	0.28
	Desmodium barbatum	Forb	0.00	0.57	0.00	0.00
	Desmanthus virgatus	Shrub	0.00	0.00	2.61	1.48
	Galactia argentea	Forb	0.08	0.35	0.00	0.00
Gentianaceae (5.12 %)	*Eustoma exaltatum*	Forb	0.23	0.00	0.00	0.00
Malvaceae (5.12 %)	*Melochia pyramidata*	Forb	0.00	0.00	1.95	0.55
	.	Forb	0.00	0.00	4.32	0.00
Poaceae (41.02 %)	*Sorghum halepense*	Grass	0.00	10.64	0.00	0.00
	Bouteloua aristidoides	Grass	10.11	11.38	1.81	8.42
	Bouteloua hirsuta	Grass	0.00	0.00	0.00	0.04
	Bouteloua repens	Grass	27.00	18.55	22.13	22.94
	Bouteloua sp.	Grass	0.00	0.00	0.26	0.00
	Cenchrus echinatus	Grass	2.45	4.47	1.29	4.63
	Digitaria ciliaris	Grass	10.28	6.72	8.31	5.77
	Digitaria filiformis	Grass	0.29	1.37	2.43	2.66
	Eragrostis glomerata	Grass	0.48	0.00	0.00	0.00
	Eragrostis prolifera	Grass	2.24	5.01	20.47	10.18
	Grass - Unidentified sp1	Grass	3.07	0.97	0.31	2.19
	Hilaria belangeri	Grass	1.63	1.06	1.62	2.85
	Jouvea pilosa	Grass	0.54	0.80	4.48	1.87
	Muhlenbergia sp.	Grass	2.47	0.00	0.00	0.00
	Paspalum prostatum	Grass	0.00	0.00	0.00	3.68
	Stipa eminens	Grass	16.87	29.32	14.88	19.43
	Stipa sp.	Grass	2.37	1.24	0.00	0.00
Solanaceae (5.12 %)	*Hydrolea spinosa*	Forb	0.00	0.00	2.11	3.17
Verbenaceae (5.12 %)	*Phyla nodiflora*	Forb	0.00	0.00	0.19	0.02

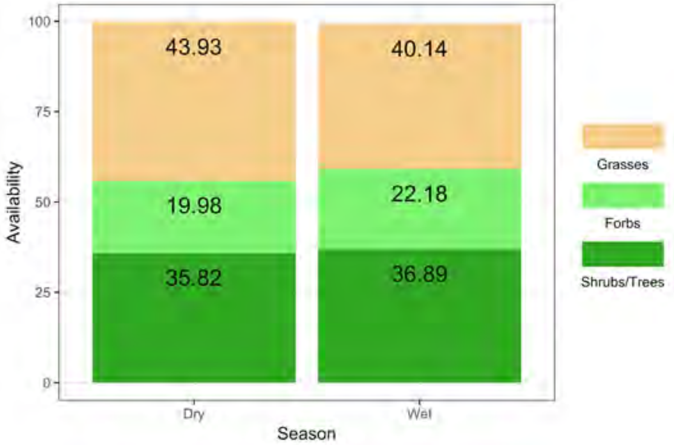

Figure 2. Availability (%) of plant species by growth form and by season in Santa María del Mar, Oaxaca, México.

nens (16.87 %) and *Digitaria ciliaris* (10.28 %), during the dry season, and *S. eminens* (29.32 %), *B. repens* (18.55 %) and *B. aristidoides* (11.38 %) during the wet season. For *B. taurus*, the most important items were grasses and forbs, *B. repens* (22.13 %), *Eragrostis prolifera* (20.43 %) and *S. eminens* (14.88 %) during the dry season, and *B. repens* (22.94 %), *S. eminens* (19.43 %) and *E. prolifera* (10.18 %) during the wet season.

No significant differences were found in the seasonal composition of the diet of *L. flavigularis* ($W = 911.5$, $P = 0.78$) or *B. taurus* ($W = 893.5$, $P = 0.91$). These results were corroborated by the Sorensen quantitative test, which showed a high similarity between the seasonal diet of *L. flavigularis* ($QS = 0.8540$) and that of *B. taurus* ($QS = 0.8957$). The trophic niche breadth was low for both species (*L. flavigularis*, dry = 0.1439, wet = 0.1250; *B. taurus*, dry = 0.1663, wet = 0.1674).

From 39 species, 12 were consumed by both species during the two seasons. Seasonally, *L. flavigularis* and *B. taurus* shared 13 species on both dry and wet seasons (Table 1). This was coincident with dietary overlap analysis; according to the Pianka (1975) index, the dietary overlap between the two species was high for both seasons (dry $O_{jk} = 0.7311$, wet $O_{jk} = 0.8459$). We recorded low seasonal use of available plant species for *L. flavigularis* (dry season $O_{jk} = 0.1788$; wet season $O_{jk} = 0.3102$) and for *B. taurus* (dry $O_{jk} = 0.3378$; wet $O_{jk} = 0.4022$).

Discussion

Results indicate that *L. flavigularis* and *B. taurus* exploited a relatively narrow variety of available species of plants, suggesting that both herbivores are selective, showing a preference for Poaceae species throughout the year despite the significant difference in the availability of plant species between the dry and wet season. The Poaceae preference of *L. flavigularis* is consistent with the findings of Lorenzo *et al.* (2011) in another isolated *L. flavigularis* population and with other leporid diet studies (López-Cortés *et al.* 2007; Karmiris and Nastis 2010; Kontsiotis *et al.* 2011; Ge *et al.* 2013; Freschi *et al.* 2014, 2015; Luna-Casanova *et al.* 2016). However, we found that some species of plants consumed by *L. flavigularis* in Santa Maria represent new records for this leporid. The Poaceae preference of *B. taurus* is also consistent with previous studies in other localities (Quinteros *et al.* 2013).

These results are also related to the plant species availability. The study area is located in a low precipitation region from the Tehuantepec Isthmus, with severe drought from November to April. Despite the fact that grasses have low protein content in comparison to other species (Codron *et al.* 2007), and contain abrasive silica (Sanson *et al.* 2007), these species are more resistant to drought than ephemeral or annual forbs (Tilman and Downing 1994) which may cause that grass availability is high year-round. Also, Poaceae species distribute in open areas (Gordon and Prins 2008), which is one of the main characteristics of grassland habitat in the Tehuantepec Isthmus (Farías and Fuller 2009; Rioja *et al.* 2011). In agreement with these findings, it has been reported that *L. flavigularis* prefer grassland over other available habitats because, besides providing food, it represents a suitable place to carry out its activities of resting, socialization and reproduction (Farías and Fuller 2009; Rioja *et al.* 2011; Carrillo-Reyes *et al.* 2012; Lorenzo *et al.* 2015; Luna-Casanova *et al.* 2016).

Studies show the clear occurrence of trophic competition between leporids and other herbivores and ruminants such as cattle (Smith *et al.* 2004, Young *et al.* 2005), producing an adverse effect on leporid populations (Hulbert and Andersen 2001). In our study, *Lepus flavigularis* and *B. taurus* had a low trophic niche breadth and high dietary overlap, which indicates a high level of resource (trophic) competition that could adversely affect the *L. flavigularis* population. Other studies state that *L. flavigularis* prefers to establish feeding, resting, and breeding sites in pastures with the presence of *B. taurus* (Carrillo-Reyes *et al.* 2010, 2012; Rioja *et al.* 2011; Luna-Casanova *et al.* 2016). It is likely that the presence of *B. taurus* influence the existence of certain plant species that could serve as a forage resource for *L. flavigularis*, through the establishment of seedlings from *B. taurus* feces (Kuijper *et al.* 2008). Furthermore, *B. taurus* grazing reduce height, cover, and density of vegetation (Smith *et al.* 2005; Karmiris

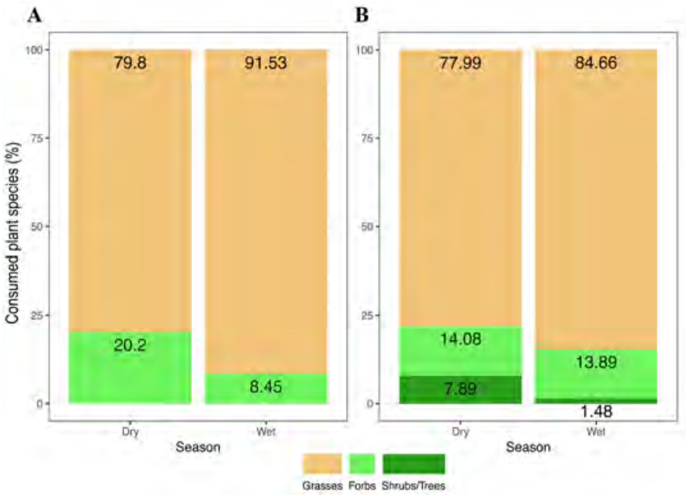

Figure 3. Percentage of consumed plant species (%) by growth form and by season in Santa María del Mar, Oaxaca, México. A: *L. flavigularis*, B: Cattle.

and Nastis 2010; Rioja *et al.* 2011). As Farías and Fuller (2009) stated, open grasslands with scattered trees and shrubs can help *L. flavigularis* to avoid predators like *Canis latrans*. Therefore, selection of areas with low vegetation cover may reduce the energy spent in monitoring its habitat, which favors the investment of more time in foraging (Karmiris and Nastis 2010; Rioja *et al.* 2011; Luna-Casanova *et al.* 2016). According to previous literature, we expect that jackrabbits benefit from cattle presence, as long as the stocking rate does not increase current levels. Research suggests that dietary partition between grazing herbivores is a common phenomenon and that leporids and cattle can coexist and obtain mutual benefits while not exceeding grazing capacity (Karmiris *et al.* 2005; Karmiris and Nastis 2010). Over long periods, livestock can even improve forage quality and foraging efficiency, facilitating food availability for leporids (Kuijper *et al.* 2008; du Toit 2011).

Low trophic niche breadth and high dietary overlap between *L. flavigularis* and *B. taurus*, combined with a low seasonal relationship between food availability and diet, suggest that forage resources competition is occurring in this system. Exotic species such as *S. eminens* and *D. ciliaris*, and a native species such as *B. repens*, were highly consumed by both species throughout the year. It is likely that these plant species remain permanently available to both herbivores, either as fresh or dry forage, due to its high silica content and their ability to survive in areas with low water content. On the other hand, certain species were consumed in a certain season by some species. For example, *C. articulatus* and *C. erecta* were consumed by *L. flavigularis* mainly during the dry season, while species *S. halepense* was consumed during the wet season. We believe that the mobility capacity of the species partly determined these differences. Cattle are restricted to using the resources available in the pastures and roads in which they are kept, while the hare can use almost the entire available area. Hence, it also has permanent or temporal access to all available plant species.

In conclusion, our results show trophic competition between native and no-native herbivorous species. However, we suggest that both species can coexist as long as the site's carrying capacity is not exceeded. On the one hand, although both species feed mainly on grasses, these plant species are the ones that persist throughout the year, even in drought conditions. Additionally, cattle have limited movement to pastures, while *L. flavigularis* can move freely throughout its range. Also, cattle grazing reduces vegetation's height, cover, and density, facilitating *L. flavigularis* to avoid potential predators. Overall, cattle management at moderate stocking densities, can be compatible with the subsistence of *L. flavigularis* populations.

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Evaluation of pocket gopher diet in a perennial productive area

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The desert pocket gopher (*Geomys arenarius*) is a fossorial herbivorous rodent of the family Geomyidae. Its distribution range is restricted to New Mexico and Texas, in the United States of America, and northern Chihuahua, in México. The Médanos de Samalayuca Flora and Fauna Protection Area (MSFFPA) is located in northern Chihuahua. Different economic activities are carried out in this region, mainly irrigated crops of pecans (*Carya illinoinensis*). Populations of *G. arenarius* have been recorded within these areas. Therefore, the objective of this work was to define the extent of the trophic niche and the changes in the physical condition of *G. arenarius* in a ranch within the MSFFPA over three contrasting seasons (dry, wet, and post-wet). Forty *G. arenarius* specimens were collected from Arantxa Ranch. Morphometric measurements and the weight of collected individuals were recorded, and the digestive tract was removed to prepare histological slides. Seven 25 m^2-quadrants were established, and the species of the vegetation cover were recorded and collected for reference. The Seasonal Fitness Index (IK) and Levin's Niche Breadth Index were calculated. Males had higher average measurements and weight than females. The IK was 2.82 ± 0.47 in males and 2.64 ± 0.61 in females. Significant differences in the IK between seasons were only found in females. The correlation between IK and plant cover was strong for males and females. The diet mainly comprised *Physalis hederifolia*, *Dimorphocarpa wislizeni*, and *Cenchrus incertus*. Levin's index showed that *G. arenarius* is a specialist rodent. Sexual dimorphism was evident, with males larger than females. The physical condition index of gophers is influenced by resource availability. In other studies, this parameter has been related to changes in food availability. It has been reported that gophers tend to feed mainly on crops; however, pecan cultivation was not a major element in the diet of the desert pocket gopher, as it feeds on the vegetation associated with crops. Gophers are considered generalists; nonetheless, the present study showed that *G. arenarius* is a specialist, although this may be a consequence of anthropogenic activities.

La tuza arenera (*Geomys arenarius*) es un roedor herbívoro fosorial perteneciente a la familia Geomyidae. Tiene una distribución restringida a Nuevo México y Texas en los Estados Unidos de América y en México al norte de Chihuahua. En el Área de Protección de Flora y Fauna Médanos de Samalayuca (APFFMS), que se ubica al norte de Chihuahua, se realizan diferentes actividades económicas destacando el cultivo por riego de nuez de pecán (*Carya illinoinensis*). Dentro de estas zonas de cultivo se han registrado poblaciones de *G. arenarius*. Por lo cual, el objetivo del presente trabajo es definir la amplitud de nicho trófico y cambios en la condición física de *G. arenarius* en un rancho dentro del APFFMS en tres temporadas (seca, húmeda y posthúmeda) con diferentes grados de humedad. Se obtuvieron un total de 40 ejemplares de *G. arenarius* del Rancho Arantxa, se registraron las medidas morfométricas y el peso, se extrajo el tracto digestivo para la elaboración de laminillas microhistológicas. Se establecieron siete cuadrantes de 25 m^2 donde se registró la cobertura de las especies vegetales y se colectó el material botánico para elaborar material de referencia. Se calculó el índice de condición física y el índice de amplitud de nicho de Levins. El promedio de las medidas y peso de los machos fue mayor al de las hembras. El IK en machos fue de 2.82 ± 0.47 y en hembras de 2.64 ± 0.61, no se detectaron diferencias estadísticamente significativas en el IK de los machos por temporada y se detectaron diferencias estadísticamente significativas en el IK por temporada en hembras. La correlación entre el IK y la cobertura para los machos y hembras fue fuerte. La dieta estuvo conformada principalmente por *Physalis hederifolia*, *Dimorphocarpa wislizeni* y *Cenchrus incertus*. El índice de Levins evidenció que *G. arenarius* es un roedor especialista. El dimorfismo sexual fue evidente al ser los machos de mayor talla que las hembras. El índice de condición física de las tuzas se ve influenciada por la disponibilidad de recursos en otros estudios se ha relacionado este parámetro con cambios en la disponibilidad de alimento. Se ha reportado que las tuzas suelen alimentarse principalmente de los cultivos, aunque para la tuza arenera, el cultivo de nogal no representó un elemento importante en la dieta y ésta se alimentó de la vegetación asociada a la parcela agrícola. Las tuzas son consideradas generalistas, sin embargo, en el presente estudio *G. arenarius* evidenció ser especialista, aunque este podría ser efecto de las actividades antropogénicas.

Keywords: Gopher pocket; NPA; Samalayuca; vegetation; walnut.

Introduction

The desert pocket gopher (*Geomys arenarius*) is an herbivorous rodent of the family Geomyidae (Williams and Baker 1974). Its distribution is restricted to the states of New Mexico and Texas in the United States and northern Chihuahua in México (Anderson 1972; Chambers *et al.* 2009). This species usually builds its burrows in sandy soils (>40 %) and avoids clay, gravel, or stone (Mauk *et al.* 1999). Gophers live near water bodies (rivers, ponds, irrigation canals) and agricultural areas (Lacher *et al.* 2019). These organisms feed mainly on leaves, roots, tubers, wood, bark, seeds, grains, nuts, fruits, and flowers of different plant species (Templeton 2006). Gophers cause significant damage to crops within their range (Monge 1999; Witmer *et al.* 1999; Engeman and Witmer 2000; Monge 2013; Baldwin *et al.* 2013), although they have been reported to feed mainly on the herbaceous plants and grass encountered while tunneling (Myers and Vaughan 1965; Foster and Stubbendieck 1980; Luce *et al.* 1980; Williams and Cameron 1986).

The Médanos de Samalayuca Flora and Fauna Protection Area (MSFFPA) is a Natural Protected Area (NPA) located in northern Chihuahua, México. It has a program that includes the management and sustainable use of wildlife, including the implementation of population ecology studies (CONANP 2013). Different economic activities are carried out within this NPA, most notably irrigated crops of pecans (*Carya illinoinensis*), where a population of *G. arenarius* is established successfully. There is a conflict between this species and local farmers, who consider it a pest. Damages to the irrigation system and crops are attributed to gophers, as they are suspected of feeding on the roots of walnut trees, thus affecting the establishment, health, and production of these plants. Consequently, pest control is conducted using traps to reduce the abundance of gophers within crop areas. It is hypothesized that gophers are generalists that feed mainly on the vegetation associated with crops and that changes in plant cover affect the physical condition of this species. Therefore, the objective of this study was to define the extent of the trophic niche, changes in the physical condition of *G. arenarius* throughout the year, and whether they are actively feeding on walnut tree roots.

Materials and methods

Study area. The Arantxa Ranch comprises 1000 ha and is located within the Médanos de Samalayuca Flora and Fauna Protection Area at coordinates 31° 12' 2.13" N, -106° 28' 11.36" W (Figure 1). The prevailing climate is very dry, with warm summer and cold winter, mean annual temperature of 15 ºC to 25 °C, and mean annual precipitation of 212 mm (Enríquez-Anchondo 2003).

The soil is sandy, originally covered by microphyllous desert shrubland (CONANP 2013). This vegetation has been replaced by irrigated crops of vines (*Vitis vinifera*), pistachios (*Pistacia vera*), and pecans (*Carya illinoinensis*); the latter is the most important crop, covering 400 ha. The vegetation associated with crops is mainly composed of species of the families Poaceae, Asteraceae, Solanaceae, and Boraginaceae.

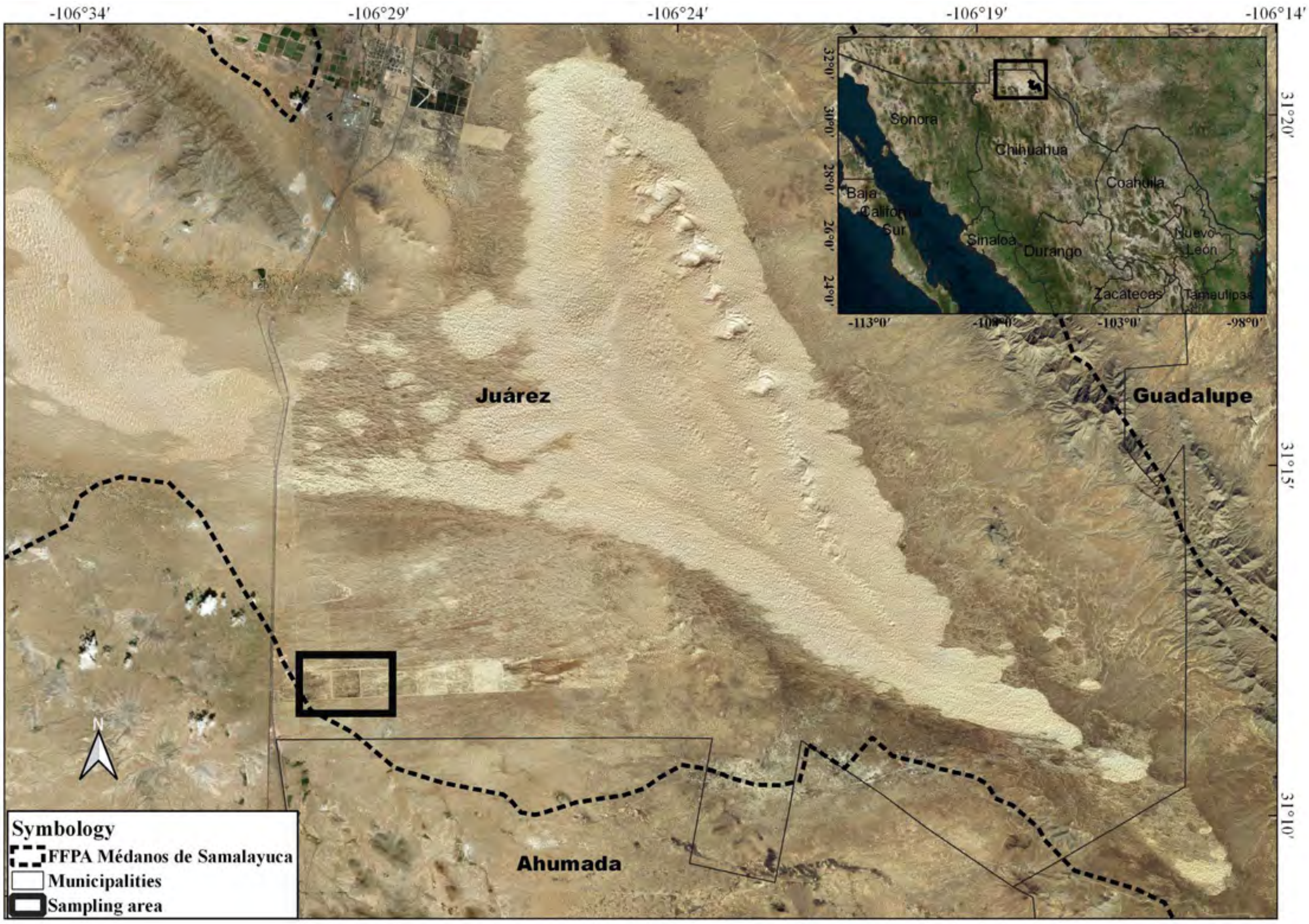

Figure 1. Location of the study area in the Arantxa Ranch within the Médanos de Samalayuca Flora and Fauna Protected Area.

Capture of Geomys arenarius specimens. Eight commercial gopher traps (Sweeney's) were installed along a 1,400 m transect within the crop area in the following seasons: dry (21 to 22 June 2020), wet (26 to 27 September 2020), and post-wet (14 to 15 November 2020). Traps were placed following the methodology by Chávez-León (2017), monitoring and relocating them every 30 minutes for six hours. Simultaneously, we collected the corpses of *G. arenarius* from the trapping conducted by the ranch pest control department.

Fitness Index. This index evaluates the differences between weights according to breed, sex, age, sexual and social status, season, climate, disease, and food, reflecting genotypic, phenotypic, and environmental interactions (Bailey, 1968). It is based on the body weight: length ratio, calculated with the formula: $IK = (W/LC^3)$ x 10^5, where W = weight (kg) and LC = body length (cm) to the base of the tail, with values ranging from 1 to 10 (Corriale *et al.* 2013). To note, the values of the morphometric and IK measurements correspond to 23 specimens (5 males and 18 females). A total of 17 specimens were excluded, 11 lacking the tail and 6 pregnant or lactating females (4 in the dry season and 2 in the wet season).

The individuals captured were euthanized by cervical dislocation following the Guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes and Gannon 2011). Conventional morphometric measurements (total length, tail length, right leg length, and ear length) were recorded using a ruler; the weight was measured with a Pesola® scale, and sex was determined based on the presence/absence of the baculum (Lorenzo *et al.* 2006). The digestive tract was removed, placed in a flask containing 10 % formol, and transported to the Animal Ecology and Biodiversity Laboratory (LEBA, for its acronym is Spanish) at Universidad Autónoma de Ciudad Juárez (UACJ). The skulls of the collected specimens were deposited in the Scientific Collection of Vertebrates at UACJ (CHI-VER 189-08-06) registered with SEMARNAT.

Vegetation sampling. Seven 25 m^2 quadrants separated by 200 m were established along the trapping transect. The plant species comprising the vegetation cover were recorded in each season following the methodology by Mostacedo and Fredericksen (2000). As reference material, we used plant samples from the PJ018 CONABIO Project deposited in the UACJ Herbarium (HERB-UACJ) and samples available in the Animal Ecology and Biodiversity Laboratory (LEBA, for its acronym in Spanish) at UACJ. Additionally, some plant samples were collected in the field following the methodology by Ricker (2019) and deposited in the LEBA reference collection as a botanical catalog (Appendix 1).

Plant material processing. Pressed plant specimens were dried at room temperature for two weeks. Once dry, the family, genus, and species were determined using the SEINet database (2020) with the assistance of the UACJ Herbarium staff. Subsequently, we performed the histological technique described by Gallina-Tessaro (2011).

We built a catalog of reference plant material with diagnostic characteristics that comprised a total of 32 species, 21 collected in the dry season, 29 in the wet season, and 9 in the post-wet season.

Processing of the digestive tracts of Geomys arenarius. The stomach contents of 40 desert pocket gophers were recovered. This material was dehydrated at 80 °C for 4 hours, grounded with a porcelain mortar with pestle, and sieved through a 1 mm-diameter mesh. Histological slides were prepared from the sieved material following the methodology by Castellaro *et al.* (2004). Five slides were prepared from each sample and examined under a light microscope at 40' including 10 fields of view. The frequency was calculated using the formula: Fr = (ai/A)*100, where ai = number of observations of a particular food element and A = number of total observations.

Levin's Niche Breadth Index. This index represents the specialization of an organism by measuring the elements that make up its diet. The standardized form has values ranging from 0 to 1, where 0 corresponds to a specialist and 1 to a generalist (Alarcón-Nieto and Palacios 2009). The Levin's niche index is expressed as $B = 1/\Sigma Pj^2$, where Σ = sum, Pj = ratio of individuals using resource j, and the standardized index as $B_a = B - 1/n+1$, where B = Levin's index and n = number of resources used by organisms, where values lower than 0.6 correspond to a specialist diet and those higher than 0.6, to a generalist diet (Krebs 1989).

Statistical analysis. The mean and standard deviation of morphometric measurements, weight, and IK of 23 specimens were obtained and sorted by season and sex. Due to the number of samples for each season, nonparametric statistics were used to determine significant differences in male IK by season using the Mood test. The Kruskal-Wallis test was used for females, and significant differences were analyzed with the Conover test. The degree of association between plant cover and IK was estimated using Spearman's correlation coefficient. Statistical analyses were performed in Excel using the SPSS Statistics Base 22.0 statistical package.

Results

A total of 40 adult specimens of *Geomys arenarius* were collected as follows: 11 females and 3 males in the dry season, 13 females and 8 males in the wet season, and 5 females in the post-wet season.

Males (n = 5) had the following mean measurements: total length (TL) 267.40 ± 9.60 mm, tail length (T) 77.6 ± 3.78 mm, hind foot (HF) 33.60 ± 3.71 mm, ear length (E) 5.0 ± 1.0 mm, and weight (w) 194.80 ± 43.75 g. Females (n = 18) had the following mean measurements: TL = 234.94 ± 19.24 mm, T = 68.05 ± 11.04 mm, HF = 32.05 ± 3.05, E = 4.05 ± 0.87, and w = 120.83 ± 26.03 g in all three seasons. The measurements and weights by season corresponded to 10 specimens (3 males and 7 females) in the dry season, 8 (2 males and 6 females) in the wet season, and 5 females in the post-wet season (See Table 1).

Table 1. Means ± standard deviation of morphometric measurements and weight of *G. arenarius* by seasons and sex.

Season	Sex	TL	T	HF	E	w
Dry	Machos (*n*=3)	269.66 ± 6.65	76.66 ± 3.05	33.66 ± 5.03	5.66 ± 0.57	223.66 ± 1.52
	Hembras (*n*=7)	229.85 ± 14.87	70.57 ± 4.46	31.00 ± 1.82	4.71 ± 0.75	134.85 ± 29.70
Wet	Machos (*n*=2)	264.00 ± 15.55	79.00 ± 5.65	33.50 ± 2.12	4.00 ± 0.0	151.50 ± 37.47
	Hembra (*n*=6)	244.83 ± 25.39	73.16 ± 8.65	34.50 ± 3.93	4.00 ± 0.63	114.00 ± 25.88
Post-wet	Hembras (*n*=5)	230.20 ± 14.68	58.4 ± 15.09	30.60 ± 1.34	3.2 ± 0.44	109.40 ± 11.90

Fitness Index (IK). The average IK of males was 2.82 ± 0.47, with 3.12 ± 0.30 in the dry season and 2.38 ± 0.22 in the wet season. For females, the average IK was 2.64 ± 0.61, with 3.28 ± 0.12 in the dry season, 2.29 ± 0.54 in the wet season, and 2.15 ± 0.13 in the post-wet season.

The Mood median test showed no statistically significant differences ($\alpha = 0.05$; d.f = 1; $p = 0.13$) in the fitness index (IK) between dry-season and wet-season males.

The Kruskal-Wallis test for the seasonal effect on the IK of females was statistically significant ($\alpha = 0.05$, d.f. = 2; $p = 0.005$). The Conover test ($\alpha = 0.05$) showed statistically significant differences between the dry season versus the wet ($p = 0.012$) and post-wet ($p = 0.0005$) seasons. On the other hand, there were no significant differences in the IK between the wet and post-wet seasons ($p = 0.57$).

A reduction in plant cover associated with the crop was observed throughout the study, mainly due to trimming and plot cleaning. A 72.88 % cover was reported for the dry season, 16.12 % for the wet season, and 4.14 % for the post-wet season. Spearman's correlation coefficient for males ($R = 0.86$) and females ($R = 0.74$) showed a strong correlation between the fitness index (IK) and plant cover (Figure 2).

Diet of Geomys arenarius. In all seasons, the diet of the 40 desert pocket gophers consisted mainly of the ivyleaf groundcherry (*Physalis hederifolia*) with 29.26 % of the consumed items, followed by the spectacle pod (*Dimorphocarpa wislizeni*; 16.29%) and the common sandbur (*Cenchrus incertus*; 14.40 %; Table 2).

In the dry season, the diet consisted of 11 species, 11 genera, and 9 families. The common sandbur (*Cenchrus incertus*) was the most abundant food item (27.97 %), followed by the spectacle pod (*Dimorphocarpa wislizeni*; 21.14 %) and the ivyleaf groundcherry (*Physalis hederifolia*; 15.78 %; Table 2).

In the wet season, 11 species, 10 genera, and 7 families were recorded in the diet. The ivyleaf groundcherry (*Physalis hederifolia*) was the dominant food item (31.24 %), followed

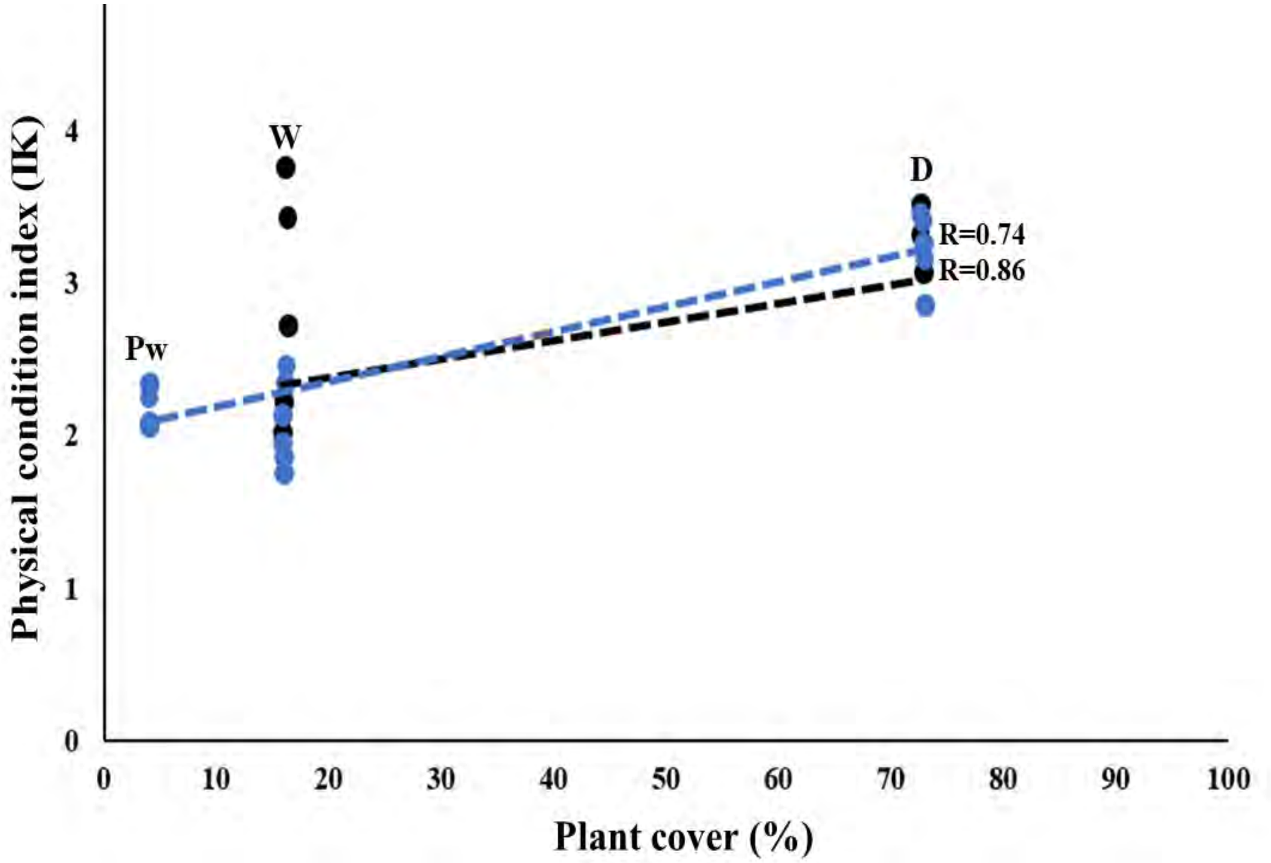

Figure 2. Correlation between plant cover and physical condition index (IK) by season and sex (males marked in black, females marked in blue).

Table 2. Overall and seasonal diet of *Geomys arenarius*

			Season		
Family	**Genus/species**	**General**	**Dry**	**Wet**	**Post-wet**
Solanaceae	*Physalis hederifolia*	29.26	15.78	31.24	58.2
Brassicaceae	*Dimorphocarpa wislizeni*	16.29	21.14	17.88	
Poaceae	*Cenchrus incertus*	14.40	27.97	5.63	2.46
Nyctaginaceae	*Tripterocalyx carneus*	10.20	6.51	17.88	
Boraginaceae	*Heliotropium curassavicum*	9.73	15.44	4.19	9.42
Poaceae	*Panicum hallii*	6.42		15.3	
Poaceae	*Setaria macrostachya*	4.94		1.61	25.82
Poaceae	*Setaria leucopila*	2.90	5.04	1.93	
Asteraceae	*Palafoxia sphacelata*	1.82	0.49	3.86	
Amaranthaceae	*Salsola kali*	1.42	3.09		0.82
Poaceae	*Sporobolus airoides*	1.01	2.43		
Onagraceae	*Oenothera pallida*	0.40	0.97		
Juglandaceae	*Carya illinoinensis*	0.54			3.28
Malvaceae	*Sphaeralcea incana*	0.54	1.14	0.16	
Poaceae	*Sporobolus contractus*	0.13		0.32	

. *All values correspond to the percentage (%) in the diet.

by the spectacle pod (*Dimorphocarpa wislizeni*; 17.88 %), and Hall's panicgrass (*Panicum hallii*; 15.29 %; Table 2).

Finally, six species, six genera, and five families were recorded in the post-wet season. The ivyleaf groundcherry (*Physalis hederifolia*) was the most abundant food item (58.05 %), followed by the plains bristle grass (*Setaria macrostachya*; 25.84 %), and the salt heliotrope (*Heliotropium curassavicum*; 13.20 %; Table 2).

Levin's Niche Breadth Index: Levin's index for *G. arenarius* was $B = 5.27$, with a standardized value of $B_a = 0.37$, indicating that the desert pocket gopher is a specialist herbivorous. Levin's values by season suggest that the gopher has a narrow trophic niche (Table 3).

Discussion

Anderson (1972) reported the morphometric measurements of male and female gopher specimens collected in the surroundings of Samalayuca; however, he did not report weight values. The present study reports the measurements of a larger number of specimens of *G. arenarius*. Males showed a larger body size than females, consistent with several studies (Hendricksen 1972; Daly and Patton 1986; Mauk *et al.* 1999; Connior 2011; Calede and Brown 2021).

Fitness Index (IK). The male IK showed no statistically significant differences between seasons (dry versus wet), probably due to the low capture rate and the lack of male specimens collected in the post-wet season. For females, the fitness index was significantly higher in the dry season, although this season had the greatest plant cover. A strong correlation between IK and plant cover was evidenced in both cases. These results are consistent with Romañach *et al.* (2007) for *G. attwateri*, *G. bursarius*, and *Thomomys bottae*, where the variation in body mass was related to changes in plant cover, with larger body size at sites with higher plant biomass. This information confirms that the physical condition of small mammals is associated with the variation in the distribution of food resources (Schulte-Hostedde *et al.* 2001). In the Arantxa Ranch, where agriculture is performed, the vegetation is influenced by anthropogenic factors such as weeding and irrigation, which has led to the reduction or expansion of plant cover, density, and species richness. These changes impact the gopher diet and are reflected in the seasonal variation of IK values.

Diet of Geomys arenarius. Of the 32 plant species recorded in the Arantxa Ranch, *G. arenarius* only consumed 15 (46.87 %) during the three seasons. Herbaceous plants were the main type, which is consistent with reports for other gopher species such as *Thomomys mazama*, *T. talpoides*, and *G. bursarius* (Tietjen *et al.* 1967; Vaughan 1967; Burton and Black 1978; Luce *et al.* 1980) where herbaceous plants make up most of the annual diet. The low density or absence of herbaceous plants is associated with a low gopher abundance. Thus, it is evident that the diet of gophers is composed of certain plant species that grow in the areas where they live, so gophers depend on the availability of these plant species. Other studies on the diet of gophers in alfalfa fields have reported that crops account for more than 90 % of the stomach contents (Ward 1960; Luce and Case 1977). Our results showed that *G. arenarius* did not actively consume walnut trees, which accounted for less than 1 % of the diet in the three seasons. This resource was only consumed during the post-wet season, reflecting the consumption of plants associated with crops.

It has been reported that gophers are associated with significant damage to agricultural crops, causing production losses (Witmer *et al.* 1999; Lacher *et al.* 2019). This is consistent with what has been mentioned by producers, who reported that gophers constantly gnaw on irrigation lines. In addition, it has been documented that gopher mounds can have other impacts on crop areas, burying plants, damaging irrigation lines, and serving as weed

Table 3. Overall and seasonal Levin's trophic niche breadth index values.

Family	Species	General	Dry	Wet	Post-wet
Solanaceae	*Physalis hederifolia*	0.085	0.024	0.097	0.338
Brassicaceae	*Dimorphocarpa wislizeni*	0.026	0.044	0.031	
Poaceae	*Cenchrus incertus*	0.020	0.078	0.003	0
Nyctaginaceae	*Tripterocalyx carneus*	0.010	0.004	0.031	
Boraginaceae	*Heliotropium curassavicum*	0.009	0.023	0.001	0.008
Poaceae	*Panicum hallii*	0.004		0.023	
Poaceae	*Setaria macrostachya*	0.002		0	0.066
Poaceae	*Setaria leucopila*	0	0.002	0	
Asteraceae	*Palafoxia sphacelata*	0	0	0.001	
Amaranthaceae	*Salsola kali*	0	0		0
Poaceae	*Sporobolus airoides*	0	0		
Onagraceae	*Oenothera pallida*	0	0		
Juglandaceae	*Carya illinoinensis*	0			0.001
Malvaceae	*Sphaeralcea incana*	0	0	0	
Poaceae	*Sporobolus contractus*	0		0	
	B=	5.21	4.54	4.20	1.40
	B_a=	0.37	0.45	0.42	0.28

seedbeds (Baldwin 2011). Likewise, tunnels can divert water, causing losses of surface irrigation water; besides, tree roots are frequently damaged from gopher tunneling (Knight 2000). Although *G. arenarius* does not actively feed on walnut trees, the presence of these rodents has adverse effects on walnut crops. The constant damage to irrigation systems restrains the establishment of new crop areas and reduces the vigor of previously established ones due to the limited water availability. This has been reported for *Thomomys* sp., where damage includes loss of vigor or mortality of crop plants due to damage in the underground drip lines and loss of water irrigation caused by the burrow system (Baldwin *et al.* 2011). This is likely the main conflict between producers and gophers in pecan production areas, as these act as artificial habitats for *G. arenarius*, which damages crops when excavating their burrows.

Levin's Niche Breadth Index. Gophers are considered general herbivores that feed on different parts (leaves, roots, and fruits) of a wide variety of plant species (Howard and Childs 1959; Williams and Cameron 1986; Hunt 1992). In the present study, Levin's index showed that *G. arenarius* is a specialist species, a finding that rejects the above hypothesis. However, Briones-Salas *et al.* (2013) described that classifying an organism as a specialist depends not only on the Levin's index value but also on a set of conditions particular to the species and the area where it lives. In the pecan orchard, the permanent change in irrigation and weeding by walnut producers forces gophers to depend on a limited range of resources in the area. Consequently, *G. arenarius* may be adopting an optimal foraging strategy that provides the greatest benefit at the lowest cost, thus maximizing the energy obtained. As Pyke (1984) reported, the dependence of organisms on a particular food type is related to abundance, search time, and energy value, which leads to selection. It has been described that in gophers, the search for food shows a relatively intense selection, balancing the energy costs and gains of food search (Vleck 1981; Andersen 1988; Reichman 1988; Jenkins and Bollinger 1989). In the case of *G. arenarius*, the narrow food niche is probably determined by the availability and energy supply of plants, as well as continued agricultural management (trimming, fertilization, irrigation, and weeding) that limits or removes food sources throughout the year. Sexual dimorphism was evident as the average morphometric and weight measurements were higher in males than in females. The fitness index (IK) of males and females is influenced by the availability of plants resulting from agronomic management in pecan orchards.

The diet of the desert pocket gopher (*Geomys arenarius*) in the Arantxa Ranch (pecan orchard) comprised 15 plant species associated with crops, mainly *Physalis hederifolia* (Solanaceae), *Dimorphocarpa wislizeni* (Brassicaceae), and *Cenchrus incertus* (Poaceae). We demonstrated that *G. arenarius* is a specialist species. The Walnut tree (*Carya illinoinensis*) was not a major food item in the gopher diet during the three seasons studied (2020).

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Appendix 1

Plant species recorded in the study area in the Arantxa Ranch in the dry (D), wet (W), and post-wet (Pw) seasons and source of the botanical reference material: PJ018 CONABIO Project (HERB-UACJ), Animal Ecology and Biodiversity Laboratory (LEBA) and material collected in the field and included in the reference collection of LEBA (collected/LEBA).

Family	Genus/species	D	W	Pw	Source
Amaranthaceae	*Amaranthus hybridus*		*		Collected/LEBA
Amaranthaceae	*Chenopodium album*		*		Collected/LEBA
Amaranthaceae	*Salsola kali*	*	*	*	LEBA
Asparagaceae	*Yucca elata*	*	*		HERB-UACJ 1992
Asteraceae	*Ambrosia acanthicarpa*		*		Collected/LEBA
Asteraceae	*Picradeniopsis absinthifolia*		*		HERB-UACJ 2233
Asteraceae	*Brickellia coulteri*		*		HERB-UACJ 2238
Asteraceae	*Dieteria canescens*		*	*	HERB-UACJ 2267
Asteraceae	*Lactuca serriola*	*	*	*	Collected/LEBA
Asteraceae	*Palafoxia sphacelata*	*			HERB-UACJ 2250
Asteraceae	*Verbesina encelioides*		*		HERB-UACJ 2236
Boraginaceae	*Heliotropium curassavicum*	*	*	*	HERB-UACJ 2307
Boraginaceae	*Euploca convolvulacea*		*		HERB-UACJ 1995
Brassicaceae	*Dimorphocarpa wislizeni*	*	*		LEBA
Convolvulaceae	*Cuscuta umbellata*	*	*		HERB-UACJ 1760
Juglandaceae	*Carya illinoinensis*	*	*	*	Collected/LEBA
Malvaceae	*Sphaeralcea incana*	*	*		Collected/LEBA
Martyniaceae	*Proboscidea louisianica*	*	*		HERB-UACJ 1904
Nyctaginaceae	*Boerhavia spicata*		*		HERB-UACJ 1987
Nyctaginaceae	*Tripterocalyx carneus*	*	*		HERB-UACJ 1938
Onagraceae	*Oenothera pallida*	*	*	*	HERB-UACJ 1771
Plantaginaceae	*Epixiphium wislizeni*	*			HERB-UACJ 2005
Poaceae	*Cenchrus incertus*	*	*	*	LEBA
Poaceae	*Chloris virgata*		*		HERB-UACJ 2046
Poaceae	*Eragrostis cilianensis*	*			HERB-UACJ 2120
Poaceae	*Panicum hallii*	*	*		LEBA
Poaceae	*Setaria leucopila*	*	*		HERB-UACJ 2080
Poaceae	*Setaria macrostachya*	*	*	*	HERB-UACJ 2184
Poaceae	*Sporobolus airoides*	*	*		HERB-UACJ 2166
Poaceae	*Sporobolus contractus*		*		HERB-UACJ 2156
Solanaceae	*Physalis hederifolia*	*	*	*	HERB-UACJ 2333
Zygophyllaceae	*Tribulus terrestris*	*	*		Collected/LEBA

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Review of Gray's beaked whale (*Mesoplodon grayi*) in the South East Pacific

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Information collected from a complete female juvenile individual of Gray's beaked whale (*Mesoplodon grayi*) stranded on the Guanaqueros coast, Coquimbo Region in Chile (30°S) is provided. Difficulties to differentiate specimens of Gray's beaked whale and Hector's beaked whale (*M. hectori*) are discussed based on the use of diagnostic phenotypic characters, such as differences in color patterns and position of teeth on the lower jaw. The identification of the studied specimen as Gray's beaked whale was supported by a detailed review of cranial characters and molecular analyses. Finally, we provide an updated list containing all known Chilean records for this species.

Se proporciona información del registro más completo de *Mesoplodon grayi* (Ziphiidae) que se conoce para Chile y que corresponde a una hembra juvenil varada en la costa de Guanaqueros, Región de Coquimbo (30°S). Se discuten los problemas de determinación de *Mesoplodon grayi* con respecto a *M. hectori* en base a la utilización de caracteres fenotipicos generales como patrones de coloración. La asignación a *Mesoplodon grayi* se apoyó en la revisión detallada de caracteres craneales y un análisis molecular. Además, se presenta un listado actualizado con todos los registros conocidos para esta especie en las costas de Chile.

Keywords: Distribution; Gray's beaked whale; Odontoceti; South America; Zhipiidae.

Introduction

Strandings represent a unique opportunity to collect information about elusive cetaceans (Canto *et al.* 1991; Leeney *et al.* 2008; Pikesley *et al.* 2011). Although these events are frequent across different coastal areas of the planet (Evans *et al.* 2005; Berta *et al.* 2015; Moore *et al.* 2018), the frequency of such stranding events differs among species (Leeney *et al.* 2008). In the case of species of the family Ziphiidae, records are scarce, and many of them are based on the stranding of specimens in an advanced state of decay or isolated bone remains (Litcher 1986; Dalebout *et al.* 1988). Therefore, only a few high-quality photographic records of ziphiids exist, given that instances of complete individuals in good condition are exceptional (Cox *et al.* 2006). Traditionally, identifying specimens of *Mesoplodon* at the species level has been a complex task (Dalebout *et al.* 2002, 2004; Van Helden *et al.* 2002; Cappozzo *et al.* 2005). For example, relying on body patterns of coloration (a trait usually used for species identification) can sometimes result in wrong taxonomic identifications (Helden *et al.* 2002; Dalebout *et al.* 2014) due to the insufficient knowledge about intra and interspecific color variation as well as a poor understanding of how coloration varies after death.

The ziphiid genus *Mesoplodon* is the most diverse, accounting for 15 of the 22 species of the family (Cappozzo *et al.* 2005; McLeod *et al.* 2006; Pitman 2009; McLeod 2009, 2017; Yamada *et al.* 2019). Ten species of *Mesoplodon* are known from the Atlantic and Pacific oceans of South America; these are *M. bowdoini* (Andrews' beaked whale), *M. densirostris* (Blainville's beaked whale), *M. europaeus* (Gervais' beaked whale), *M. ginkgodens* (Ginkgo-toothed beaked whale), *M. grayi* (Gray's beaked whale), *M. hectori* (Hector's beaked whale), *M. layardii* (Strap-toothed beaked whale), *M. mirus* (True's beaked whale), *M. peruvianus* (Pygmy beaked whale), and *M. traversii* (Spade-toothed whale ; McLeod *et al.* 2006). Six species of *Mesoplodon* have been reported in Chile: *M. densirostris*, *M. grayi*, *M. hectori*, *M. layardii*, *M. peruvianus*, and *M. traversi* (Canto and Yáñez 2009; D'Elía *et al.* 2020). Reports of these species in the Chilean coast are based on less than 20 records, a fact that highlights the importance of documenting any newly emerging record for this genus. These records are based mainly on bone remains (Sielfeld 1979, 1980, 1983; Cárdenas *et al.* 1986; Canto *et al.* 1992; Reyes *et al.* 1995; Sanino *et al.* 2007) and in only a few cases, on specimens in good condition. Among the latter, there is a male of Strap-toothed beaked whale stranded at Caleta Tortel, Aysén Region in 2019 (personal communication S.E-J.), in addition to the new specimen of *M. grayi* we are reporting in this work.

Information on Gray´s beaked whales consists of 192 records held in GBIF (2019) worldwide. A single record, not included in GBIF, is known from the South American Pacific Ocean, from the Peruvian coast (Reyes 1990). In Chile, Mann (1958) initially reported this species. Later, Venegas and Sielfeld (1978) and Sielfeld (1979) documented for the first time the presence of this species in the country, based on osteological material found at the Strait of Magellan. So far, these are the only records of this species for Chile concentrated in the southern part of the country.

Here we report a new Chilean record for Gray´s beaked whales on the basis a of a specimen initially identified as *M. hectori* by the Servicio Nacional de Pesca in 2018. This new record, from Guanaqueros beach (Figure 1), confirms the wide distribution of the species in the South-East Pacific, as suggested by MacLeod *et al.* (2006). The first DNA sequence of any Chilean specimen of this species is also provided. The analysis of skull morphology and coloration suggests that the species presents different morphs, which can be attributed to sexual dimorphism or geographic variation.

Materials and methods

On December 21, 2018, a young female odontocete stranded in the coast of Guanaqueros, Coquimbo Region, Chile, was collected by SERNAPESCA and later transferred to the Museo Nacional de Historia Natural (MNHN), Santiago. A necropsy was performed to determine the possible causes of death. External measurements were taken according to Reyes and Waerebeek (2018), while cranial measurements were taken following Ross (1984). Muscle tissue samples were fixed in 95 % ethanol. Fat samples, the respiratory system, the heart, the uterus and the ovaries were frozen at -18° C. All bone material, as well as the samples, are housed in the Mammal Collection of the MNHN under catalogue number MNHN/MAM 1972.

For the specific determination of specimen MNHN/MAM 1972, comparisons were made with the descriptions and morphometric data of adult specimens of *M. hectori*, *M. peruvianus*, *M. densirostris*, *M. bowdoini*, *M. perrini*, *M. stejnegeri*, *M.grayi*, and *M. europaeus* (Ross 1970; Venegas and Sielfeld 1978; Sielfeld 1979; 1983, Lichter 1986; Mead and Baker 1987; Nobuyuki *et al.* 1987; Mead 1989; Baker and Helden 1999; Norman and Mead 2001; Zerbini and Secchi 2001; Dalebout *et al.* 2002; Capazzo *et al.* 2005; Laporta *et al.* 2005).

In addition, genetic analyses were performed based on a fragment (801 bp) of the mitochondrial gene encoding the cytochrome b (cytb). Total genomic DNA was extracted from a sample of fresh muscle tissue using the Wizard SV Genomic DNA Purification System (Promega ©). The cytb fragment was amplified using primers MVZ 05 and MVZ 16 following the protocol mentioned in Chiquito *et al.* (2014).

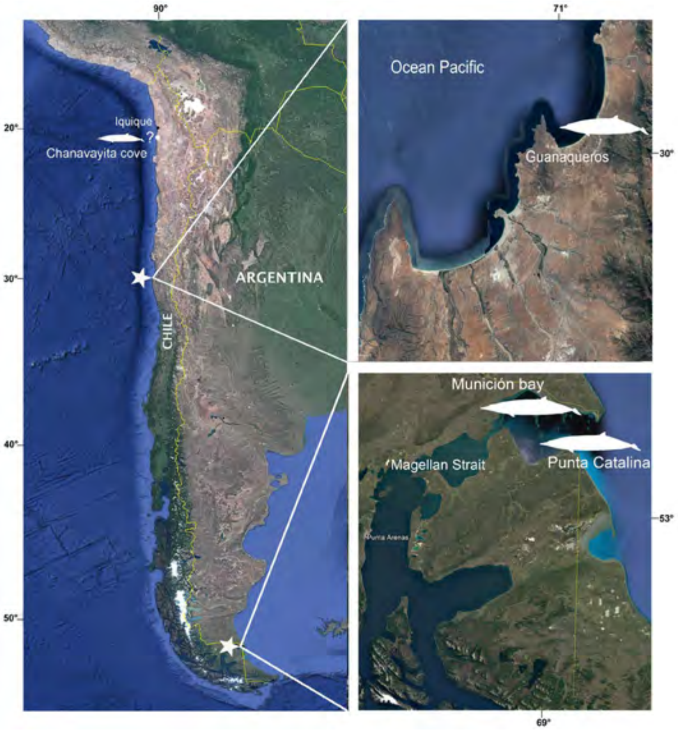

Figure 1. Map showing the three confirmed records of *Mesoplodon grayi* from the Chilean coast. The unconfirmed record from Chanavayita in northern Chile is also signalled. The record presented in this contribution corresponds to that of Guanaqueros.

The new DNA sequence was edited, including checking for the existence of internal stop codons and changes in the reading frame, using CodonCode (Codon-Code, Dedham, MA, USA). The new DNA sequences were deposited in GenBank under accession number MT813067.

Sequence alignment was done with MAFFT v.7.310 (Katoh and Standley 2013) using L-INS-i as an iterative refinement method (Katho and Toh 2008). Model TIM2+F+I+G4 was selected with IQ-TREE v.1.6.5 (Trifinopoulos *et al.* 2016). Two phylogenetic methods were used. Maximum Likelihood (ML) analysis was conducted in IQ-TREE with perturbation strength was set to 0.5 and the number of unsuccessful iterations set to 100; nodal support was evaluated by 1,000 ultrafast Bootstrap replicates (BS; Akaike 1973). A Bayesian analysis (BI) was conducted in MrBayes 3.2 (Ronquist and Huelsenbeck 2013) by means of two independent runs, each with five heated and one cold Markov chains and all model parameters estimated in MrBayes. Uniform-interval priors were assumed for all parameters except base composition and substitution model parameters, which considered a Dirichlet prior. Runs lasted 50 million generations, with trees sampled every 1,000 generations. Convergence was checked by plotting log-likelihood values against generation time. The first 25 % of the trees generated were discarded as burned, and only the remaining trees were used to compute a 50 % majority rule consensus tree and to obtain posterior probability (PP) estimates for each clade. Both analyses included sequences of all members of the family Ziphiidae, and *Physeter catodon* was employed as an outgroup; see supplementary material 1 for the accession numbers.

A list of Chilean strandings and other records involving species of *Mesoplodon* was compiled from the literature and qualified reports. References to unsupported sightings were excluded.

Results

According to SERNAPESCA, specimen MNHN/MAM 1972 would have died very shortly before stranding. This statement is based on its general good conditions, including coloration and absence of external scars or bruises (Figure 2A-B). The necropsy revealed the skull was severely damaged (see supplementary material 2).

Specimen MNHN/MAM 1972 corresponds to a juvenile female, as cranial sutures are not completely fused, especially the front-supraoccipital one. Furthermore, the skull presents a globose shape at the parietal level, which is characteristic of juvenile cetaceans (Del Castillo 2016). In the nasal region, at the vertex level, a posteroanterior projection of smaller size is observed. The mesorostral canal is cylindrical and does not present ossification like adult specimens of the same species and other species of the genus (Besharse 1971).

Body and skull-mandibular measurements of specimen MNHN/MAM1972 are presented in Table 1. The specimen measured 2.47 m and weighted 185 k. The body has a spindle shape, showing the largest circumference around its midpoint. The beak is short and narrow with the line of the mouth curved shaped (Figure 2B). There is a black patch around the eyes that projects towards the top of the head, in front of the respiratory opening, but with a less marked coloration, tending to grey. The ventral zone of the mandibular (except the area of the beak) and the gular region is greyish-white. The beak is a dark brown that fades slightly towards the melon area where it intersects with grey tones. Behind the respiratory opening, it presents a light pale cream color with a variation of shades in the form of irregular patches. The dorsal area of the body also presents a light beach color with variation in the shades that extend along the entire back. Ventrally, the coloration is greyish white and extends to the caudal peduncle. The dorsal fin is dark grey. The dorsal surface of the pectoral fins has grey color with a variation of shades to darker tones. The caudal lobes in dorsal view are grey, whilst on the ventral side, are light grey.

In the skull the anterior border of the pterygoid sinus reaches the level of the maxillary foramina (= dorsal infraorbital foramina according to Mead and Fordyce 2009). The left premaxillary ridge is not marked and is smaller than the one in the right (Figure 2C, D, E and F). A pair of teeth located in the area of the mandibular symphysis is evident. The jaw is very short compared to adult specimens of the same species (Figure 2G-H). Both the mandibular condyle and coronoid process were not preserved in both mandibular branches.

The ML and BI analyses showed that the relationship between the members of the family Ziphiidae are mostly unresolved and poorly supported; the genus *Mesoplodon* was recovered as paraphyletic with respect to *Indopacetus pacificus*. However, all the species of *Mesoplodon* were recov-

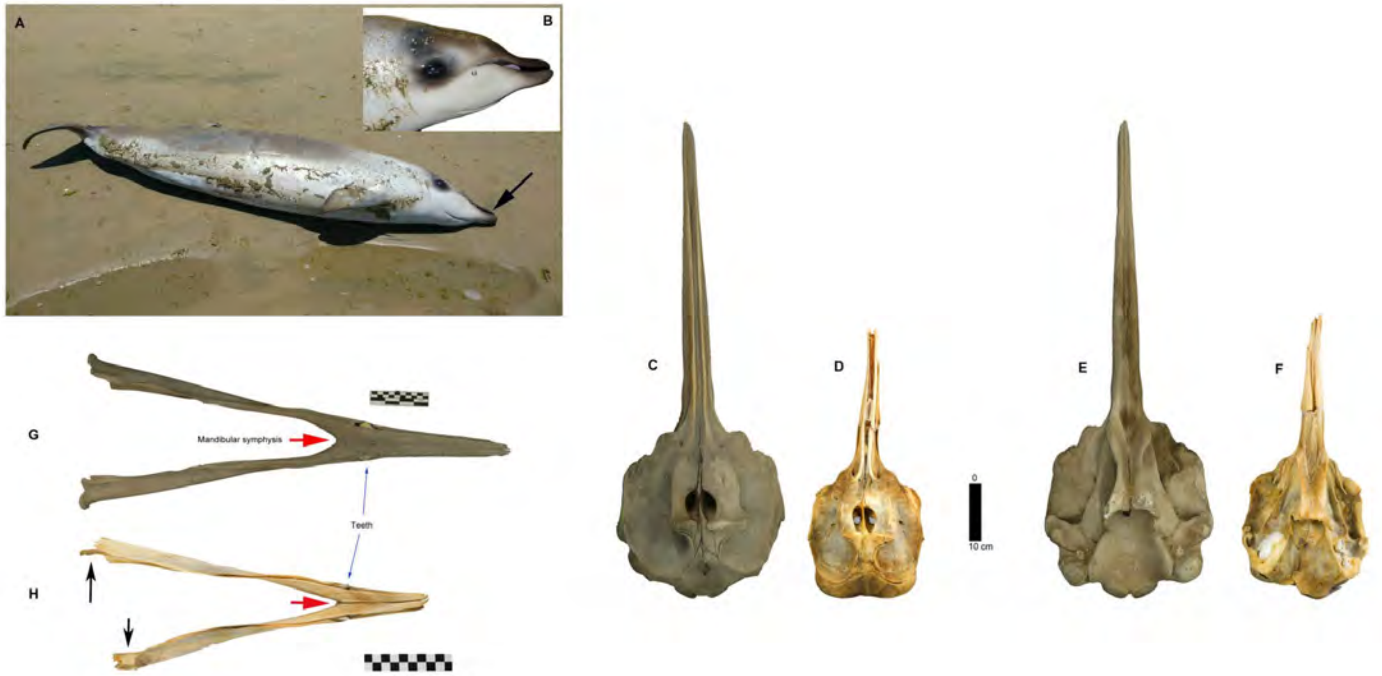

Figure 2. A) View of specimen MNHN / MAM 1972 at its site of stranding on Guanaqueros beach, Coquimbo Region, Chile. The literature mentions a dark brown, dark grey or black colouration pattern on the dorsal surface and paler on the ventral surface, with the tip of the bill being generally white, unlike the rest of the body (Wilson and Mittermeir 2014). Specimen MNHN/MAM 1972 shows a different colour pattern with a dark-coloured peak (see black arrow). The preservation of the colouration stands out because photo was taken only a few hours after the specimen stranded. B). Detail of the head of specimen MNHM / MAN 1972 showing its general colouration and the shape of the beak. C-F) Dorsal and ventral view of the skull of (C and E) an adult female specimen of *Mesoplodon grayi* (Smithsonian Museum of Natural History Mammal Collection USNM A 49880) and (D and F) specimen MNHN/MAM 1972. G-H) Dorsal view of the mandible of (G) an adult female *Mesoplodon grayi* (USNM A 49880) and (H) specimen MNHN/MAM 1972, without the mandibular condyle and coronoid process (black arrows). In both specimens, it is observed that the position of the teeth is immediately in front of the mandibular symphysis (red arrows).

ered monophyletic and with high support. The sequence obtained for MNHN/MAM 1972 falls in the clade formed by sequences of *M. grayi* (BS = 99; PP = 1; Figure 3). The sequence obtained from MNHN/MAM 1972 diverges on average from other sequences of *Gray´s beaked whales* by 1.45 %, while divergence with that of *M. perrini* is 8.97 % and *M. peruvianus* 10.71 %. In the sequence distance matrix by species, values ranging between 0.00783 and 0.0225 are observed for four *M. grayi* sequences (see supplementary material 4). *M. grayi* form a clade (BS = 95; PP=0.89) with *M. peruvianus* and *M. perrini*.

Twenty-two records of the genus *Mesoplodon* have been reported for Chile, only three of which have been identified as *M. grayi,* and additionally, a record is indicated on the coast of Iquique for the species (Figure 1, Table 1).

Discussion

Chilean records of *Mesoplodon*, based on sightings or on strandings, are scarce. Compared to other cetaceans, such *as Pseudorca, Tursiops, Lagenorhynchus, Cephalorhynchus*, and *Phocoena*, these records are considerably less frequent (Aguayo 1975). Information compiled by SERNAPESCA (2021) between 2009 and 2021 include 1.093 strandings of cetaceans in Chile, of which only two correspond to species of Ziphiidae. One of the main causes of the low frequency of occurrences is that species of *Mesoplodon*, and more generally of ziphiids, mainly inhabit the oceanic zone, reaching only occasionally the continental coasts. However, during 2018 and 2020 both sightings and strandings of various species of this family have increased throughout

Table 1. Records of species of the genus *Mesoplodon* along the Chilean coast (1968-2020).

Specie	Locality	Date	Reference	Comment
M.hectori	Windhond bay, Navarino island, Region of Magallanes and Chilean Antarctica	March, 1979	Sielfeld 1979	Skull housed in Zoological collection Instituto de la Patagonia (CE-24)
M. layardii	Windhond bay, Navarino island, Region of Magallanes and Chilean Antarctica	March, 1979	Sielfeld 1979	Skull housed in Zoological collection Instituto de la Patagonia (CE-25)
	Rio Seco, Punta Arenas, Region of Magallanes and Chilean Antarctica	1968	Venegas and Sielfeld 1978	Picture of specimen housed in files of Instituto de la Patagonia, Universidad de Magallanes
	Cabo Espiritu Santo, Tierra del Fuego	February 15, 1978	Venegas and Sielfeld 1978	Right tooth housed in Zoological collection Instituto de la Patagonia Universidad de Magallanes (CE-4)
	Tortel, Aysen Region	March 20, 2019	SERNAPESCA 2019	Tissue sample in housed in mammals collection Museo Nacional de Historia Natural (MNHN/MAM 1973)
M. grayi	Wreck Point, Posesión Strait of Magellan coast	October, 1979	Sielfeld 1979	Skull and mandible housed in Zoological collection Instituto de la Patagonia Universidad de Magallanes (CE-26)
	Wreck Point, Posesión Strait of Magellan coast	December 12, 1979	Sielfeld 1983	Skull and mandible housed in Zoological collection Instituto de la Patagonia CZIP 0510. Old number = (CE-28). The collect place is was modified (Erika Mutschke, Universidad de Magallanes)
	Catalina Point Strait of Magellan	May 28, 1978	Sielfeld 1983	Skull housed in Zoological collection Instituto de la Patagonia Universidad de Magallanes (CE-35) old number RNP 702
	Guanaqueros Coquimbo Region 30°11'S 71°24'W	December 21, 2018	This work	Specimen housed in Museo Nacional de Historia Natural MNHN/MAM 1972
M. grayi ?	Chanavayita cove, Iquique Region	April 06, 2002	SERNAPESCA 2021 Iquique	Stranded one specimen. Identification by Guillermo Guzmán UNAP
M. peruvianus	-29° S	February 1998	Sanino *et al.* 2007	Sighting, two specimens
	-20° 26' S, -70° 44' W	December 17, 1997	Sanino *et al.* 2007	Sighting, one specimen
	-17° 04' S, -71° 23' W	May, 1995	Sanino *et al.* 2007	Skull
M.densirostris	Pargua bay, Los Lagos Region -41° 45' S, -73° 29' W	March, 1980	Pastene *et al.*1990	Stranded one specimen
	Coliumo bay, Concepcion Region 36° -32' S, -73° 57' W	June 19, 1999		Stranded one specimen.
	Vaihu beach Easter island	August 31, 1994	Aguayo *et al.* 1998	Stranded one specimen. The possible cause of death was a shark attack.
Mesoplodon traversii	Juan Fernández island	June 1986	Val Helden *et al.* 2002	Specimen housed in Museo Nacional de Historia Natural MNHN/MAM 1156
Mesoplodon sp.	-32° 30' S, -88° 42' W	June, 17, 1995	Aguayo *et al.* 1998	Sighting, one specimen
	-31° 41'S, -93° 07' W	June, 18, 1995	Aguayo *et al.* 1998	Sighting, one specimen
	-33° 08' S, -74° 27' W	July, 08, 1995	Aguayo *et al.* 1998	Sighting, two specimens
	-33° 08' S, 74° 27' W	September, 15, 1995	Aguayo *et al.* 1998	Sighting, specimen

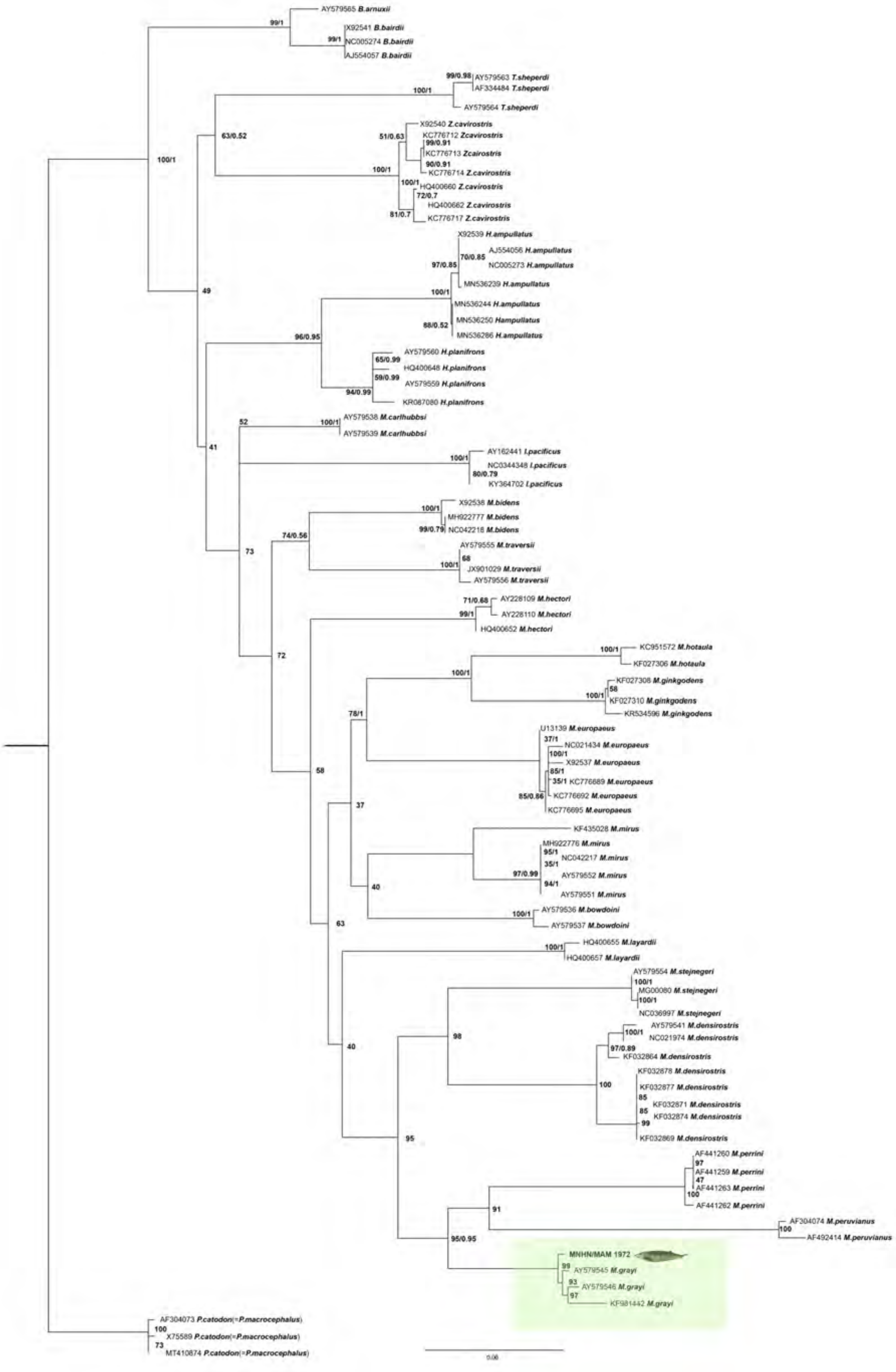

Figure 3. Bayesian phylogenetic tree resulting from the analysis of cytochrome b gene DNA sequences of specimens of *Mesoplodon*. Numbers at nodes indicate posterior probability (PP; left of the diagonal) and boostrap support (BS; right of the diagonal) values of the adjacent node. The coloured rectangle indicates the position of the specimen MNHN/MAM 1972 within the phylogenetic tree. The GenBank accession numbers of analyzed sequences are included at terminal labels. The terminal corresponding to the new record of *M. grayi* MNH/MAM 1972 is highlighted in bold.

Table 2. Measurement of specimen MNHN/MAN 1972 and of other specimens *Mesoplodon grayi* (in cm).

		MNHN/ MAM1972	**Female Mean (Thompson *et al.* 2014)**	**CE-26 Sielfeld 1979**
1	Condylobasal length	45.5	78.2-90.4	73
2	Length of rostrum, tip of beak to line connecting apices of antorbital notches	25.7	53.9-62.9	---
3	Tip of rostrum to most posterior margin of pterygoid near midline	34.0	62.4-74.5	---
4	Tip of rostrum to most posterior extension of wing of pterygoid	35.0	70.6-76.8	---
5	Tip of rostrum to most anterior extension of pterygoid	24.5	45.9-55.7	---
6	Tip of rostrum to most posterior extension of maxillaries between pterygoids on the palate	27.5	51.1-60.7	---
7	Tip of rostrum to most posterior extension of maxillary plate	41.0	73.5-83.85	---
8	Tip of rostrum to anterior margin on superior nares	30.0	59.8-69.6	---
9	Tip of rostrum to most anterior point on premaxillary crest	33.6	63.9-73.2	---
10	Tip of rostrum to most posterior extension of temporal fossae	42.8	79.1-89.5	---
11	Tip of rostrum to most posterior extension of lateral tip of premaxillary crest	33.7	---	---
12	Tip of rostrum to most anterior extension of pterygoid sinus	24.0	49.1-55.7	---
13	Length of temporal fossa	7.8	8.15-10.83	---
14	Length of orbit	7.7	8.8-10.9	8.7
15	Length of right nasal on vertex of skull	4.0	---	---
16	Length of nasal suture	3.8	2.36 - 4.3	---
17	Breadth of skull across postorbital process of frontals	19.8		---
18	Breadth of skull across zygomatic process of squamosals	19.5	28.4-31.8	---
19	Breadth of skull across centres of orbits	18.2		---
20	Least breadth of skull across posterior margins of temporal fossae	16.0		---
21	Greatest span of occipital condyles	---		---
22	Greatest width of an occipital condyle	2.6	3.43-4.53	---
23	Greatest length of an occipital condyle	5.0	1.88-3.36	---
24	Greatest breadth of foramen mangun	---		---
25	Breadht of skull across exoccipitals	16.4	26.6-31.7	---
26	Breadth of nasal vertex	4.1	---	---
27	Least distance between premaxillary crest	1.3	---	---
28	Greatest extension of right premaxillary posterior of right nasal on vertex skull	4.7	---	---
29	Greatest span of premaxillary crest	8.6	12.12-14.66	12.1
30	Least width (strictly transverse) of premaxillae where (and if) they narrow opposite superior nares	8.6	---	---
31	Greatest width of premaxillae anterior to place of measurement N°30	5.3	---	---
32	Width of premaxillae at midlenght of rostrum	1.8	---	---
33	Width of rostrum in apices of antorbital notches	12.7	---	---
34	Width of rostrum in apices of prominential notches (if any)	----	---	---
35	Greatest width of rostrum at midlength of rostrum	2.7	---	---
36	Greatest depth of rostrum at midlength of rostrum	2.7	3.1-3.9	---
37	Greatest transverse width of superior nares	---	---	---
38	Greatest inside width of inferior nares, at apices of pterygoid notches, on the pterygoids	---	---	---
39	Heigth of skull. Distance between vertex of skull and most ventral point on pterygoids	18.2	---	---
40	Greatest width of temporal fossa approximately at right angles to greatest length	5.3	---	---
41	Least distance between (main or anterior) maxillary foramina	2.0	---	---
42	Least distance between premaxillary foramina	1.4	---	---
43	Distance from posterior margin of left maxillary foramen to most anterior extension of left maxillary prominence	6.5	---	---
44	Greatest length of vomer visible at surface of palate	5.3	---	---
45	Amount added to break because of breakage	1.0	---	---
46	Length of tympanic bulla, left	--	---	---
47	Length of tympanic bulla, right	4.9	---	---

Table 2. Continuation

		MNHN/MAM1972
1	Length of mandible	38
2	Greatest length of symphysis	9,6
3	Heigth of mandible at coronoid process	7,8
4	Outside heigth of mandible at midlength of alveolus	3,3
5	Inside heigth of mandible at midlength of alveolus	---
6	Length from most posterior extension of symphysis to most posterior extension of condyle	---
7	Length from posterior margin of alveolus to condyle	---
8	Length of alveolus	3
9	Width of alveolus	0,8
10	Tip of mandible to alveolus	8,1
11	Greatest length of tooth	---
12	Greatest antero-posterior width of tooth at approximately right angles to long axis of tooth	---
13	Greatest breadth of tooth	---

the world (Whitehead *et al.* 2008; Bernaldo de Quirós et al. 2019; Grove *et al.* 2020), a pattern that was also observed for cetaceans off the coast of Chile according to records obtained by the Chilean National Fisheries Service (SERNAPESCA 2021) between 2009 and 2021. There is little information associated with the known Chilean records of the six *Mesoplodon* species that have been recorded. Most of the documented records are based on incomplete bone material, which prevents the collection of data on coloration, sex and other characteristics. As such, the record presented in this study is relevant as it belongs to Gray´s beaked whales. Mann (1958) was one of the first authors to mention the presence of *M. grayi* in Chile. However, Mann (1958) did not provide any geographic references or other information to support his mention. Therefore, we recommend this record to be ignored until further confirmation

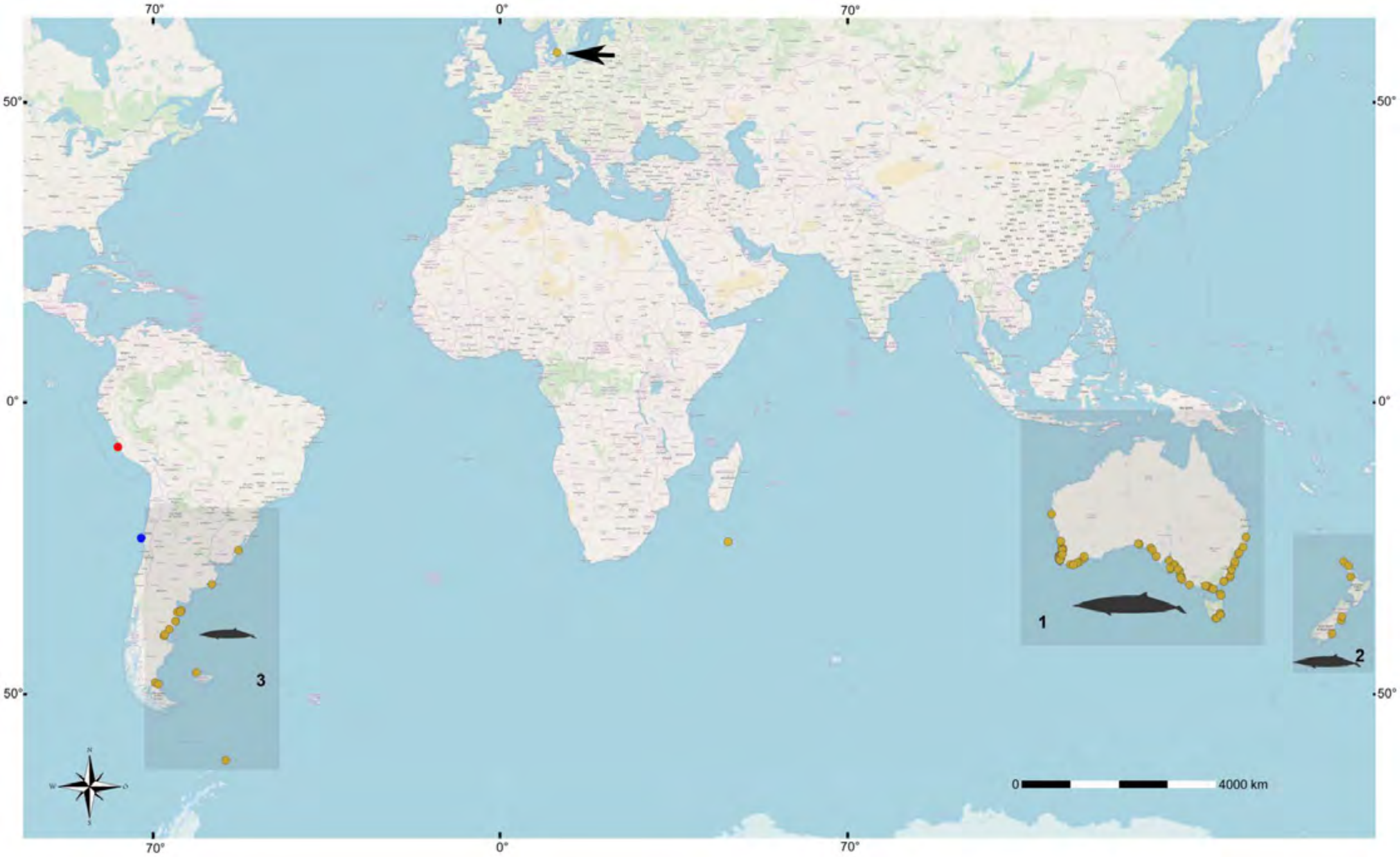

Figure 4. World map showing the distribution of *Mesoplodon grayi* records. These are concentrated in three main geographical areas: 1) Australia-Tasmania, which presents the most significant number of records, 2) New Zealand; and finally, 3) the Atlantic coast of South America. Note the records on the Pacific coast of South America are limited to two, one in Peru and the other corresponding to the one presented here. The blue circle indicates the new record of *M. grayi* in Chile (MNHN/MAM 1972); the red circle is the registry in Peru; the yellow circles are the records (strandings and sightings) in other parts of the planet. The black date that indicates the yellow circle in the northern hemisphere, indicates the existence of a specimen deposited in a collection, so it does not represent the distribution of the species

becomes available. There is also a mention for Chanavayita beach (Iquique) which is doubtful and should be taken with caution as the putative record is not associated with any preserved specimen. Therefore, only four records of this species are confirmed for Chile.

Based primarily on coloration, SERNAPESCA assigned specimen MNHN/MAM 1972 to *M. hectori*. The coloration pattern for this species is dark brown, dark grey or black and paler on the ventral surface. The tip of the beak is generally white, unlike the rest of the body (Wilson and Mittermeir 2014). However, specimen MNHN/MAM 1972 presents a different color pattern with a dark colored beak (Figure 2B). The integration of morphological and molecular information allowed us to determine that specimen MNHN/MAM 1972 belongs to *Mesoplodon grayi*. For this species, the body length varies from 474 to 564 cm in adults and 210 to 242 in newborn calf (Reidenberg and Laitman 2009). Specimen MNHN/MAM1972 presents lower values (247 cm). The beak is long and narrow with an almost straight line of the mouth (Redford and Einsenberg 1992; Wilson and Mittermeir 2014), unlike specimen MNHN/MAM 1972 which has a short beak and a curved buccal line, characteristics that could be attributed to its juvenile condition. The literature reports small teeth at the maxillary level (Von Haast 1876; Sielfeld 1979, 1983; Reyes and Molina 1997), which are also observable in the reported specimen (Figure 2). The skull shows the anterior border of the pterygoid sinus at the level of the maxillary foramen (= dorsal infraorbital foramen according to Mead and Fordyce 2009). The left premaxillary ridge is smaller and less marked than the right one. Finally, mature males have a well-developed pair of teeth in the jaw, while in females these teeth are smaller and not always visible as are covered by the gum (Mead 1989); the latter is the case of MNHN/MAM 1972. The difference in the location of the teeth in the jaw between *M. hectori* and M. *grayi* is a distinctive character. In *M. hectori*, the teeth are located at the tip of the mandible (apical), while in *M.grayi*, they are immediately anterior to the mandibular symphysis (Reyes and Molina 1997). It is important to mention that position of the teeth in *M. grayi* varies (Von Haast 1876; Robson 1975; Sielfeld 1979; Reyes 1990). The difference observed by these authors may be due to ontogenetic differences of the species. It should also be considered that there are other sources of phenotypic variation that could explain the distinction of specimen MNHN/MAM 1972. Thompson *et al.* (2014) mentioned the existence of sexual dimorphism expressed in the skull for this species in two areas of New Zealand, as well as geographic variation. The differences found in specimen MNHN/MAM 1972 may be due to its juvenile condition (Table 2).

Worldwide, 158 specimens of *Mesoplodon grayi* are currently available in collections (Figure 4). These specimens mainly come from three well-defined geographic areas: 1) Australia-Tasmania, which has the highest number of preserved and observed specimens, 2) New Zealand, and 3) the Atlantic coast of South America (GBIF 2019). Only one record is known, from the coast of Peru (Paracas), from the Pacific coast of South America; corresponds to a stranded female of *M. grayi* identified by external phenotype and skull measurements (Reyes 1990). As such, records on the Pacific coast of South America are scarcely reaching to six with this new Chilean record for *M. grayi* which is also very valuable as it is the first of a complete specimen from which molecular data are presented.

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Synthesis of knowledge of the plant diet of nectar-feeding bats of México

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The interaction between bats and plants is key to the stability of ecosystems and economically important industries, such as tequila and mezcal in México. For these reasons, it is important to determine the current state of knowledge about the plant diet of nectar-feeding bats. In this study, we conducted a systematic review of the literature on plants that have been reported as sources of food for nectar-feeding bats (subfamily Glossophaginae) in México. Based on this information, we identified bat species with the best-documented knowledge of their diet, the most consumed plant genera, and the territories with information gaps in the country. The literature search on the diet of nectar-feeding bats was carried out in the Web of Science database, Google Scholar, and digital collections of universities. We constructed rarefaction curves for bat diet richness, a heat map of the plant genera consumed by each, and a map of food localities in the different biogeographical provinces of México. This information served to explore whether knowledge of the diet of bats was related to variables such as the presence of chiropterophilous plants or the richness of nectar-feeding bats. In México, nectar-feeding bats feed mainly on plants of the genera *Agave* spp., *Pseudobombax* spp., and *Ceiba* spp., which, according to the literature, provide food to more than 75 % of nectar-feeding bats in Mexican territory. *Leptonycteris yerbabuenae* is the species with the most information on its diet in México, while *Lichonycteris obscura* is the species with the least information. None of the bat species reached a value greater than 65 % of the expected richness. Localities where the plant diet of nectar-feeding bats has been studied correspond to provinces in the Neotropical region of México. Knowledge about the diet of nectar-feeding bats in México is far from complete. The distribution of diet localities is biased over a large part of its geographic range and is positively correlated with areas with higher nectar-feeding bat species richness.

La interacción entre los murciélagos y las plantas es clave para la estabilidad de los ecosistemas y para industrias económicamente importantes, como la del tequila y mezcal en México. Por ello, es importante determinar el estado del conocimiento de la dieta vegetal de los murciélagos nectarívoros. En este estudio hicimos una revisión sistemática de literatura sobre las plantas que se han reportado como alimento de las especies de murciélagos nectarívoros glosofaginos de México. A partir de ello, identificamos las especies de murciélagos con mayor completitud en el conocimiento de su dieta, los géneros de plantas más consumidos y los territorios con vacíos de información para el país. La búsqueda de literatura sobre dieta de glosofaginos se realizó en la base de datos Web of Science, en el buscador Google Scholar y en acervos digitales de universidades. Construimos curvas de rarefacción de riqueza de la dieta de los murciélagos, un mapa de calor de los géneros consumidos por especie, así como un mapa de las localidades de dieta en las diferentes provincias biogeográficas de México, para saber si el conocimiento de la dieta vegetal de estos murciélagos estaba relacionado con variables como presencia de plantas quiropterofílicas o riqueza de especies de murciélagos. La dieta de los murciélagos se concentró en los géneros *Agave* spp., *Pseudobombax* spp. y *Ceiba* spp., los cuales proveen de alimento a más del 75 % de las especies de estudio. *Leptonycteris yerbabuenae* es la especie con mayor información sobre su dieta vegetal en México, mientras que *Lichonycteris obscura* es la especie con menos información. Ninguna especie de murciélago alcanzó un registro mayor al 65 % de la riqueza esperada. Las localidades donde se ha estudiado la dieta de estos murciélagos se concentran en provincias biogeográficas correspondientes a la región Neotropical del país. El conocimiento sobre la dieta de nectarívoros glosofaginos en México está lejos de estar completado. La distribución de localidades de dieta está sesgada en una gran parte de su distribución geográfica y parece relacionarse positivamente con áreas de mayor riqueza de especies de murciélagos.

Keywords: Biogeography; Chiroptera; conservation; plant-animal interaction; research bias.

Introduction

The food resources of animals are key to their survival and reproductive success. The diet can vary widely between species and landscapes, impacting the different consumption webs and causing cascading effects on ecosystem productivity and functioning (Cusser *et al.* 2019). An animal can be a generalist, *i. e.*, having several food sources, or specialized, feeding preferentially on a particular resource type. In the latter case, the relationship between the animal and the resource can become very close and mutually dependent (Muchhala 2006). An example is zoopollination, in which the animal feeds on the plant while contributing to its sexual reproduction (Fontaine *et al.* 2006). There is a diverse group of zoopollinators that includes bees, flies, bumblebees, beetles, butterflies, reptiles, and mammals, including bats (IPBES 2016).

The New-World nectar-feeding bats, which are specialized in nectar consumption, belong to the family Phyllostomidae, subfamily Glossophaginae (Rojas *et al.* 2016; Muchhala and Tschapka 2020). They comprise a diverse group that shows morphological, physiological, and behavioral adaptations to feeding on pollen and flower nectar (Tschapka *et al.* 2008; Ayala-Berdon *et al.* 2011; Muchhala and Tschapka 2020). These species have ecological attributes that make them particularly susceptible to extinction by anthropogenic causes (Arita and Santos del Prado 1999; Ortega-Garcia *et al.* 2020).

México is home to 12 of the 25 species of nectar-feeding bats belonging to the subfamily Glossophaginae, including two species (*Musonycteris harrisoni* and *Glossophaga morenoi*) and one genus (*Musonycteris*) endemic to the country (Ramírez-Pulido *et al.* 2014). In addition to their biological diversity and specialization, these mammals play socially and economically important roles for Mexicans by pollinating plants used in the production of alcoholic beverages such as *pulque, bacanora, mezcal*, and tequila — an industry that generated export profits of US$1.2 billion in 2015 (Trejo-Salazar *et al.* 2016). Additionally, plants that produce edible fruits such as pitaya (*Stenocereus queretaroensis*) and that are also pollinated by bats yield profits in excess of US$2,500/ha/year (Tremlett *et al.* 2020).

Given the ecological and economic importance of nectar-feeding bats, multiple studies have addressed their diet in México (Sánchez-Casas and Álvarez 2000; Sánchez and Medellín 2007; Caballero-Martínez *et al.* 2009). However, we still ignore which are the plant genera most consumed by each bat species and the degree of completeness of their dietary richness. This information is relevant for determining the key plant species in the diet of these mammals and promoting new research agendas on their ecological interactions. On the other hand, the projection of this information in the Mexican territory may contribute to evaluating whether the patterns of diet localities are related to the diversity patterns of both plants and nectar-feeding bats. This may foster conservation strategies in areas with high levels of bat-plant interactions in México.

Therefore, the objective of this study was to summarize the information on the degree of completeness of the plant diet of nectar-feeding bats and construct maps including information on the diversity of chiropterophilous plants and the distribution of nectar-feeding bats. This will promote new research agendas on the ecology of bat-plant interactions in México, especially in areas where further exploration is needed, focusing on species that are vulnerable due to the lack of knowledge of their diet.

Materials and methods

Data search. We conducted a comprehensive search of scientific literature on the Google Academic Platform (GA) and the Web of Science (WoS) database, as well as in digital thesis repositories of different universities (UNAM https://tesiunam.dgb.unam.mx; BUAP https://repositorioinstitucional.buap.mx; UV https://cdigital.uv.mx; UDG http://biblioteca.udgvirtual.udg.mx; UAEM http://ri.uaemex.mx; UANL https://cd.dgb.uanl.mx; INECOL https://inecol.repositorioinstitucional.mx). The keywords used in the search were the scientific name of each nectar-feeding bat species in the subfamily Glossophaginae distributed in México (*e. g*., *Leptonycteris nivalis*) together with the words "nectar", "diet", "pollination", "frugivory". This search was conducted in Spanish and English. Keywords were searched in the title, abstract, and keywords of the documents; the search covered from 1955 to December 2020.

Once all references were collected, any duplicate documents were removed. All documents were then evaluated according to the following inclusion criteria: 1) The study species should be nectar-feeding bats currently distributed in México, belonging to the subfamily Glossophaginae (Rojas *et al.* 2016). Documents that provided no certainty as to which bat species visited the plant were excluded. Since some of our study species, such as *Leptonycteris yerbabuenae* and *Glossophaga mutica*, had recent taxonomic changes (Simmons and Wetterer 2002; Calahorra-Oliart *et al.* 2021), the collection sites reported in these cases were reviewed and assigned to the species according to the recent taxonomic proposals. 2) The study should mention the genus of the plant used as source of nectar by the bat. 3) The document should include the collection locality. Records of nectar, pollen, seeds, fruits, and plant visits were included in the database as part of the plant diet of bats. The records used came from sources such as stomach contents, stools, hair, video records, and sightings, among others. The literature search yielded 3,200 documents. After applying the inclusion and exclusion criteria described above, 80 documents were considered for the study (Supplement 1).

A database of plant species in bat diets was constructed (see below). If studies reporting the same interaction met all criteria, the oldest was included for being the first record of the interaction. Once the database of all the plants visited by the bat species studied was gathered, it was homogenized according to the nomenclature of TROPICOS (2021) and POWO (2020).

Analysis of plant genera reported in the plant diet of nectar-feeding bats in México. Once all records of the interaction between a bat species and a plant genus in a locality were obtained, a heat map was constructed from a matrix of plant-bat interactions. This matrix contained bat species in columns and the plant genera consumed by bats in rows. The number of unique species recorded in the literature for that genus was noted in each cell. The matrix was used to construct the rarefaction curves of the species observed, to identify whether the information on the plant diet is representative for each bat species.

Species rarefaction curves were constructed with the Hill numbers corresponding to the diversity of order 0 (taxonomic richness of species). The extrapolation and rarefaction of each curve were constructed with a 95 % confidence interval, allowing extrapolating to twice the number of observations recorded. These analyses and graphs were performed using the R iNEXT V. 2.0.20 package and pheatmap (Kolde 2015; Hsieh *et al.* 2016; R Development Core Team 2021).

Since the search in GA and WoS allowed us to access information on the diet of species of nectar-feeding bats distributed in and outside the Mexican territory, we could compare the number of plant genera in the diet reported for México with the data for the rest of the geographic range of each bat species. *Musonycteris harrisoni* and *Glossophaga morenoi* were excluded from this analysis for being species endemic to México. *Choeronycteris mexicana* and *Lichonycteris obscura* were also excluded from this analysis since, according to the inclusion/exclusion criteria, records were found only within or outside México, respectively.

Spatial analysis of diet localities. We performed the following procedures to understand whether the distribution of plant diet localities reviewed and selected in this study is related to variables such as chiropterophilous plant richness or nectar-feeding bat richness. From each document selected in the search, we obtained unique geographic locations mentioned as study sites by the authors. In cases where the locality was mentioned, but not the geographic coordinates, these were estimated using a georeferencing calculator (Wieczorek and Wieczorek 2021).

To determine the areas of highest chiropterophilous plant richness, we requested the authors of a recent spatial modeling study (Ureta *et al.* 2021) to provide the localities of the plant species that are most common in the bat diet (according to our search), which corresponded to the genera *Agave*, *Pseudobombax,* and *Ceiba*. These localities represented the most complete and homogeneous source of plant presence information that could be obtained in relation to the bat species studied. The localities represented 17.8 % of the species of the genus *Agave* recorded in this study (5 species), 50 % of the species of the genus *Pseudobombax* (1 species), and 20 % of the species of the genus *Ceiba* (1 species). To obtain the areas of highest richness where these three genera of plants are found, localities were classified by sector in the Mexican territory using a grid created with ESRI ArcGIS © version 10 (Redlands, CA 1999–2010). In this sector classification, all species of a given genus were included on the same map. The resulting maps of each plant genus were combined; then, this richness map was overlapped with the plant diet localities. Since this analysis was conducted for México, plants with reported diet localities outside of the country were excluded.

The areas with the highest bat richness were determined using the distribution maps elaborated by the International Union for the Conservation of Nature (www.uicnredlist.org). The maps of the twelve bat species were combined to identify the areas with a higher richness of nectar-feeding bats. This map was overlapped on diet localities. All spatial analyses were conducted using ESRI ArcGIS © version 10 (Redlands, CA 1999–2010). Last, the biogeographical provinces of México (Morrone *et al.* 2017) were used to link and delimit the spatial distribution of diet localities and the results of both geographic overlays according to regions.

Results

The diet of nectar-feeding bats in México and the rest of their geographic range. A total of 443 plant diet records were obtained from nectar-feeding bats throughout their geographic range, corresponding to 298 plant genera (Tropicos.org. Missouri Botanical Garden; Supplement 2). Trends in records outside and within México differed between species (Figure 1), but, on average, there were a higher number of records within the country (33.1) than outside of it (8.3).

Choeroniscus godmani, Leptonycteris yerbabuenae, *L. nivalis, Glossophaga leachii*, and *G. mutica* had most of their records in México (83, 97, 90, 95, and 98 %, respectively). *G. commissarisi* had a similar number of records within and outside of México (54.8 % and 45.1 %, respectively), whereas *Anoura geoffroyi* and *Hylonycteris underwoodi* had a higher number of records outside of México (64 % and 63 % respectively).

In México, we found 370 records of plants consumed by nectar-feeding bats, corresponding to 237 genera (Tropicos.org. Missouri Botanical Garden). These plant records were attributed to 11 of the 12 species of nectar-feeding bats in México. A single record of the genus *Lonchocarpus* was obtained for the bat *Lichonycteris obscura*, but its geographic location could not be established; therefore, this bat species was excluded from the analysis. The genera *Agave* and *Pseudobombax* were recorded for the 11 bat species, followed by the genus *Ceiba* for 10 (Figure 2). The completeness of the plant diet inventory for each nectar-feeding bat species ranged from 15 % to 65 % (Figure 3). The effective number of species calculated with Hill's number did not reach an asymptote for any bat species.

Spatial analysis of diet localities. The total number of unique plant diet localities was 160. Of these, 88 % localities

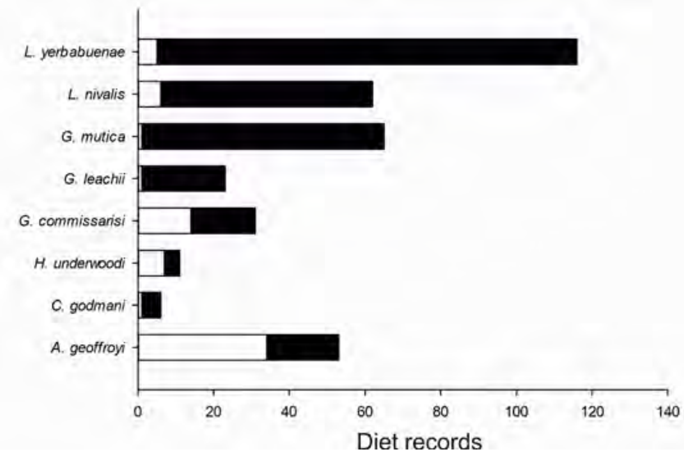

Figure 1. Comparison of the number of plant diet records in Mexico (in black) with the rest of the geographic distribution (in white) for eight nectar-feeding bat species. The rationale for the exclusion of the other study species in this chart is detailed in the Material and Methods section.

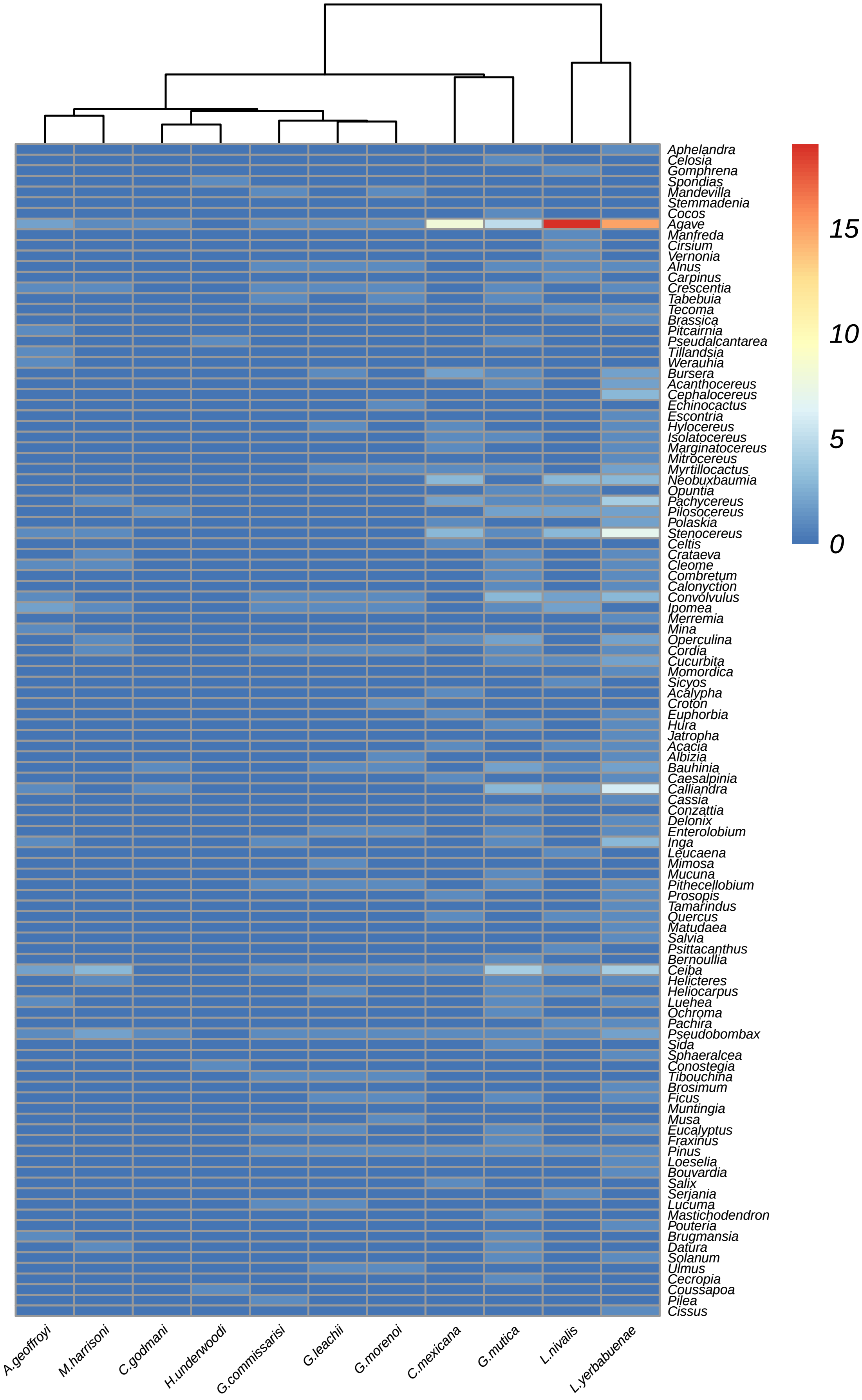

Figure 2. Dendrogram based on similarities of plants consumed by nectar-feeding bats of Mexicoand heat map of plant diet records: Matrix cells show the plant-bat pairs (rows and columns corresponding to each bat species and the plant genus with which it interacts) for which an increase (red) or decrease (blue) occurs in the number of interactions. Plant genera are ranked according to the family to which they belong. For details of each species by genus and family, refer to Supplement 2.

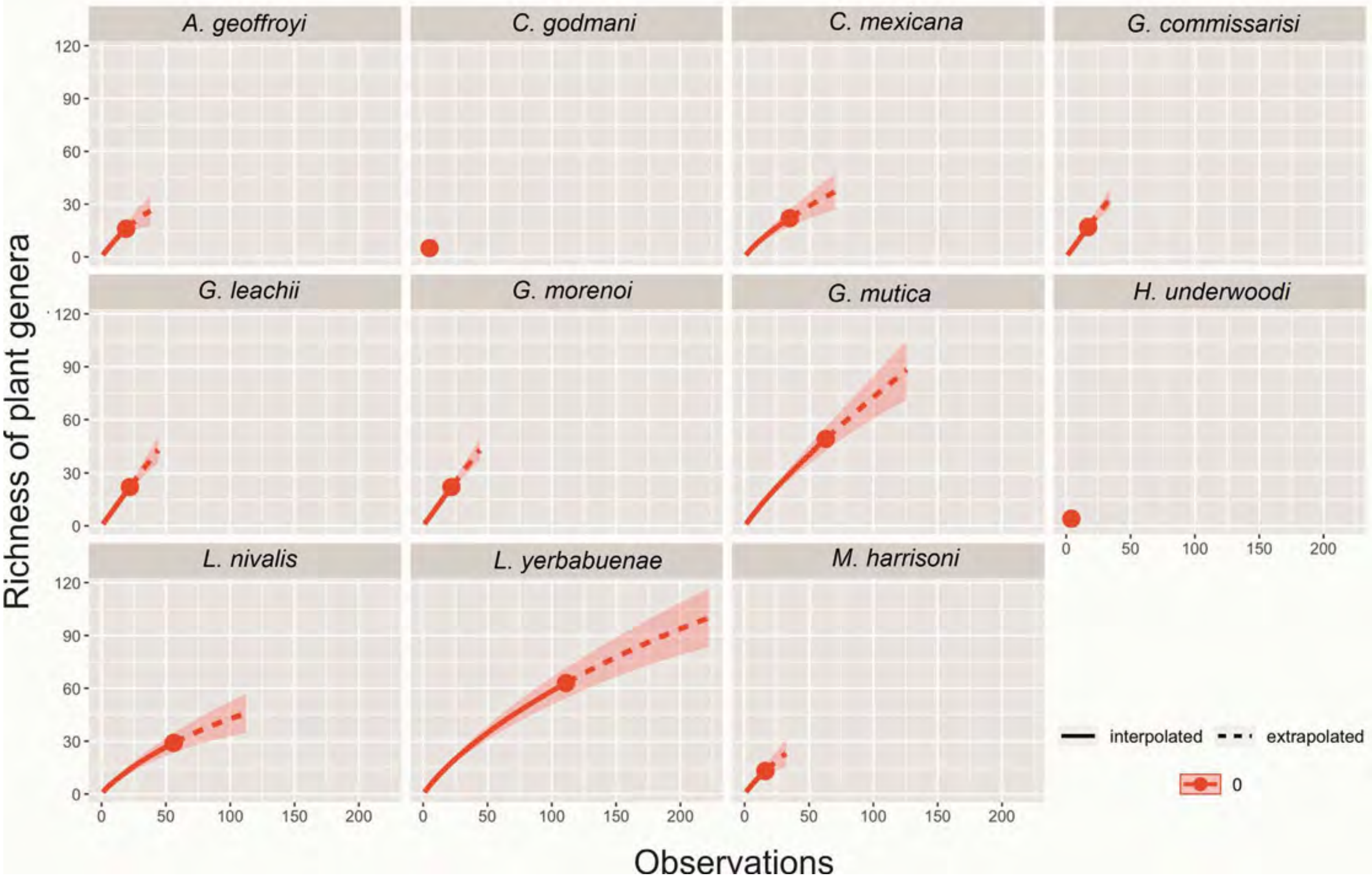

Figure 3. Rarefaction curves for eleven species of nectar-feeding bats in Mexico in relation to the number of plant genera visited. To note, the extrapolation curve does not reach the asymptote for any of the bat species. The rationale for the exclusion of *L. obscura* in this chart is detailed in the Material and Methods section.

were found in the Mexican Transition Zone, which includes the Sierra Madre del Sur, Trans-Mexican Volcanic Belt, Sierra Madre Oriental, and Chiapas Highlands biogeographical provinces, in addition to the Neotropical region, including the Pacific Lowlands, Veracruzan and Balsas Basin provinces (Morrone 2019). In turn, these provinces corresponded to the biogeographical provinces with the highest richness of nectar-feeding bats (Figure 4). The highest richness of consumed plant genera was concentrated in the northern part of the Pacific Lowlands province, the Trans-Mexican Volcanic Belt, and the Balsas Basin. These last two provinces showed the greatest overlap between the richness of plant genera and plant diet localities (Figure 5).

Discussion

The plant diet of nectar-feeding bats in México and the rest of their geographic range. The diet records of *Leptonycteris yerbabuenae*, *L. nivalis*, and *Glossophaga mutica* found are located mainly in Mexican territory, probably because these species are distributed mainly in México (Pfrimmer and Wilkins 1988; Cole and Wilson 2006; Calahorra-Oliart *et al.* 2021). Diet records of *Choeroniscus godmani* and *Glossophaga leachii* were also found mainly in México, although these species are also distributed in Central America and, in the case of *C. godmani*, in northern South America (Arita 2005).

Glossophaga commissarisi had a similar number of records within and outside of México, although the plant genera recorded for both areas were different. In México, the records correspond to the genera *Agave*, *Ceiba*, and *Cordia*; in the rest of its geographic distribution, records correspond to *Piper*, *Markea*, or *Mucuna*.

Anoura geoffroyi and *Hylonycteris underwoodi* have more records outside of México, and the recorded plant genera are different within and outside the country. For *A. geoffroyi*, the plant genera recorded most frequently in México were *Agave*, *Ceiba*, and *Ipomea*, while *Burmeistera* was the most recorded genus in the rest of the geographic range of this bat species. Separately, *H. underwoodi* has records of *Conostegia*, *Pseudalcantarea,* or *Spondias* in México, and of *Marcgravia*, *Markea,* or *Mucuna* in the rest of its distribution. The difference in the plant genera consumed by *A. geoffroyi, G. commissarisi*, and *H. underwoodi* within and outside of México may be due to the change of species in their plant diet throughout their geographic distribution. This pattern has also been observed in fruit bat genera distributed throughout the American continent (Saldaña-Vázquez *et al.* 2013).

Our results showed that the diet of *L. obscura* is poorly known across its distribution range, so further studies are needed to broaden the knowledge of this species. The analysis of plant diet is incomplete for all the species stud-

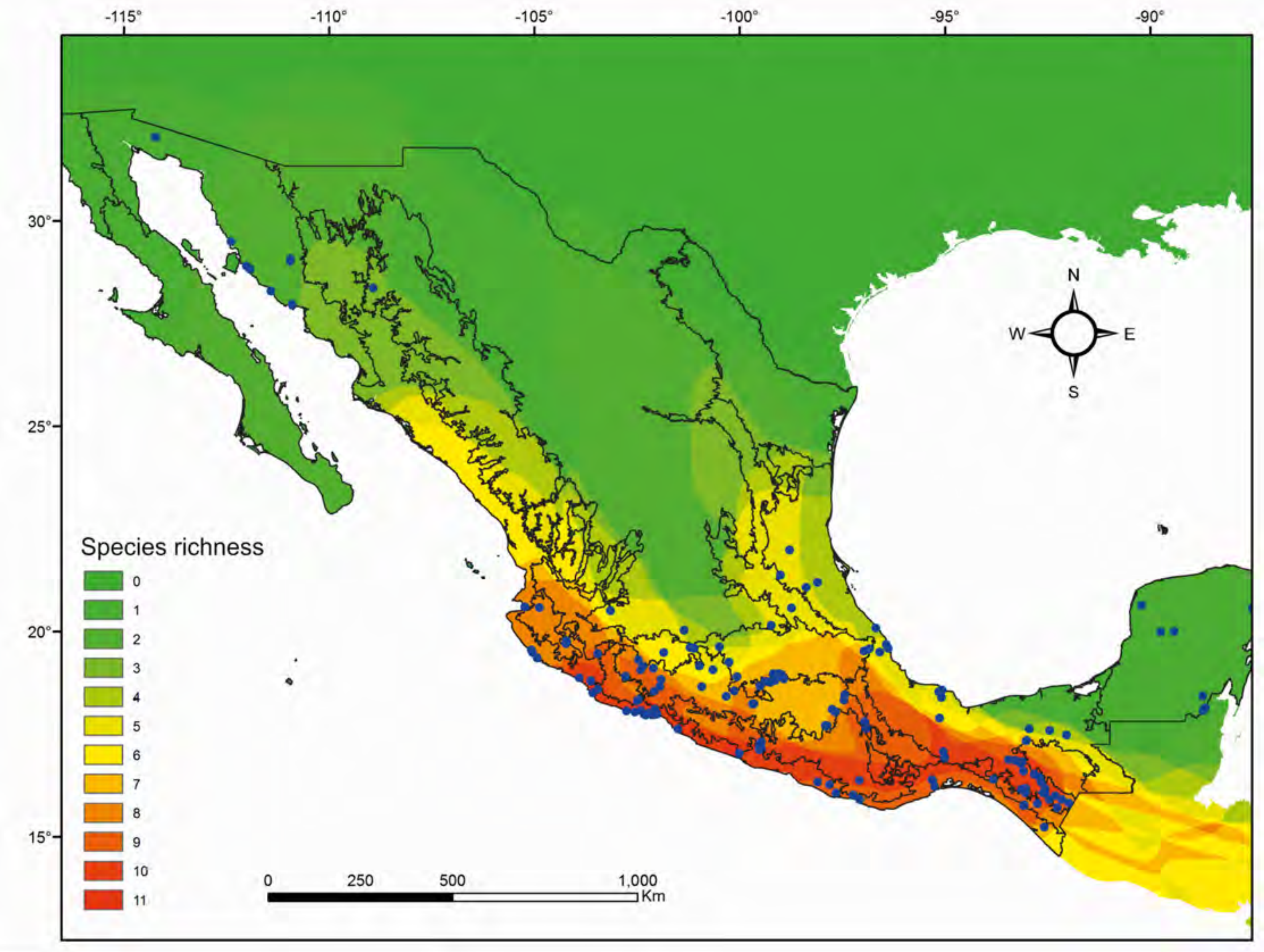

Figure 4. Map of unique plant diet localities (blue dots) in relation to the biogeographical provinces of Mexico (for further details of the provinces, refer to Morrone 2019), and the species richness of nectar-feeding bats in Mexico, shown in a decreasing (green) or increasing (red) gradient. Modified from Ortega Garcia 2018.

ied, but this study shows that there has been a greater effort to determine the plant diet of *Leptonycteris*, *Glossophaga*, and *Choeroniscus* in México than in any other country within their distribution area. In the case of *A. geoffroyi* and *H. underwoodi*, there is a need to increase the knowledge of these species in México relative to the rest of their geographic distribution.

Fleming *et al.* (2009) studied the evolution of bat pollination, including a list of 360 species of angiosperms visited by nectar-feeding bats of the family Phyllostomidae. The present study recorded 64.7 % of the plant species listed by Fleming *et al.* (2009). This suggests that the knowledge produced in México to date is significant. However, at the species level, our completeness results show that the dietary information is still limited for most nectar-feeding bats in the country.

The present study of the plant diet of Glossophaginae nectar-feeding bats also found records of fruit consumption (Supplement 2). Of the twelve species studied, only two had frugivory records: *L. yerbabuenae* and *H. underwoodi*. This may be due to various causes, such as the relative low frequency of frugivory in this group of nectar-feeding bats, or that these habits have been poorly studied. The available information shows that fruits are important elements in the diet of *L. yerbabuenae* (Rojas-Martinez *et al.* 2012); however, the contribution of fruits to the diet of the other species is unknown.

The knowledge of the plant diet of nectar-feeding bats summarized in this paper highlights the absence of research on key dietary habits for our ecosystems, especially in northern México.

Spatial analysis of diet localities. This study shows the information gaps in the plant diet of nectar-feeding bats in México. To understand the existence of these information gaps in México at the geographic level, we first tested whether the distribution of diet localities was associated with the presence of chiropterophilous plants, since a close relationship between some bat species and the plants on which they feed has been reported (Aguilar-Rodriguez *et al.* 2019). Our analysis of some species of the most consumed plant genera by these nectar-feeding bats showed a geographic overlap with the Trans-Mexican Volcanic Belt and

Balsas Basin provinces, but not with other high-richness areas such as the northern part of the Pacific Lowlands province.

The genus *Agave* is associated mainly with the Nearctic zone of México and the Valley of Tehuacán-Cuicatlán (Garcia Mendoza 2007; Morrone 2019), but most of the diet localities were found in the Neotropical region, where the Tehuacán-Cuicatlán Biosphere Reserve is also located. However, the number of unique localities in the Reserve is lower than in to other areas of the Neotropical region, as shown on the map (Figure 5).

This study did not find a geographic overlap in all sites with a high richness of these plant genera, and this finding may be because the association between bats and the plants on which they feed is not specialized in all the nectar-feeding bats in México. Plant-bat mutualism has been observed mainly for the bats *L. nivalis, L. yerbabuenae*, and *C. mexicana* (Arizaga *et al.* 2000; Arias-Coyotl *et al.* 2006), so an analysis by bat species may yield results differing from those reported herein. Additionally, the lack of overlap between diet localities and areas of high plant genus richness may be associated with a low research effort in these areas of high genus richness of chiropterophilous plants.

The superposition of the map of nectar-feeding bat richness with diet localities (Figure 4) showed a great degree of geographic overlap between the biogeographical regions with the highest number of diet localities and the areas with the highest richness of nectar-feeding bats, mainly associated with the Neotropical region. The relationship between the species richness of nectar-feeding bats and diet localities may be because the high coexistence of bat species leads to a greater number of plant-bat interactions, reflected in a greater number of plant diet localities reported. An exception to this pattern was observed in two regions of the country. One is located in the Yucatan Peninsula province and the second is in the Nearctic region, associated mainly with the Sonora province. As observed in other studies (refer to Guevara *et al.* 2015; Suárez-Castro *et al.* 2021), there are challenges in obtaining random samples, such as the range of activities of the researcher or the proximity to accessible areas, which bias the data distribution. Alternatively, the larger number of diet localities in sites with a high richness of nectar-feeding

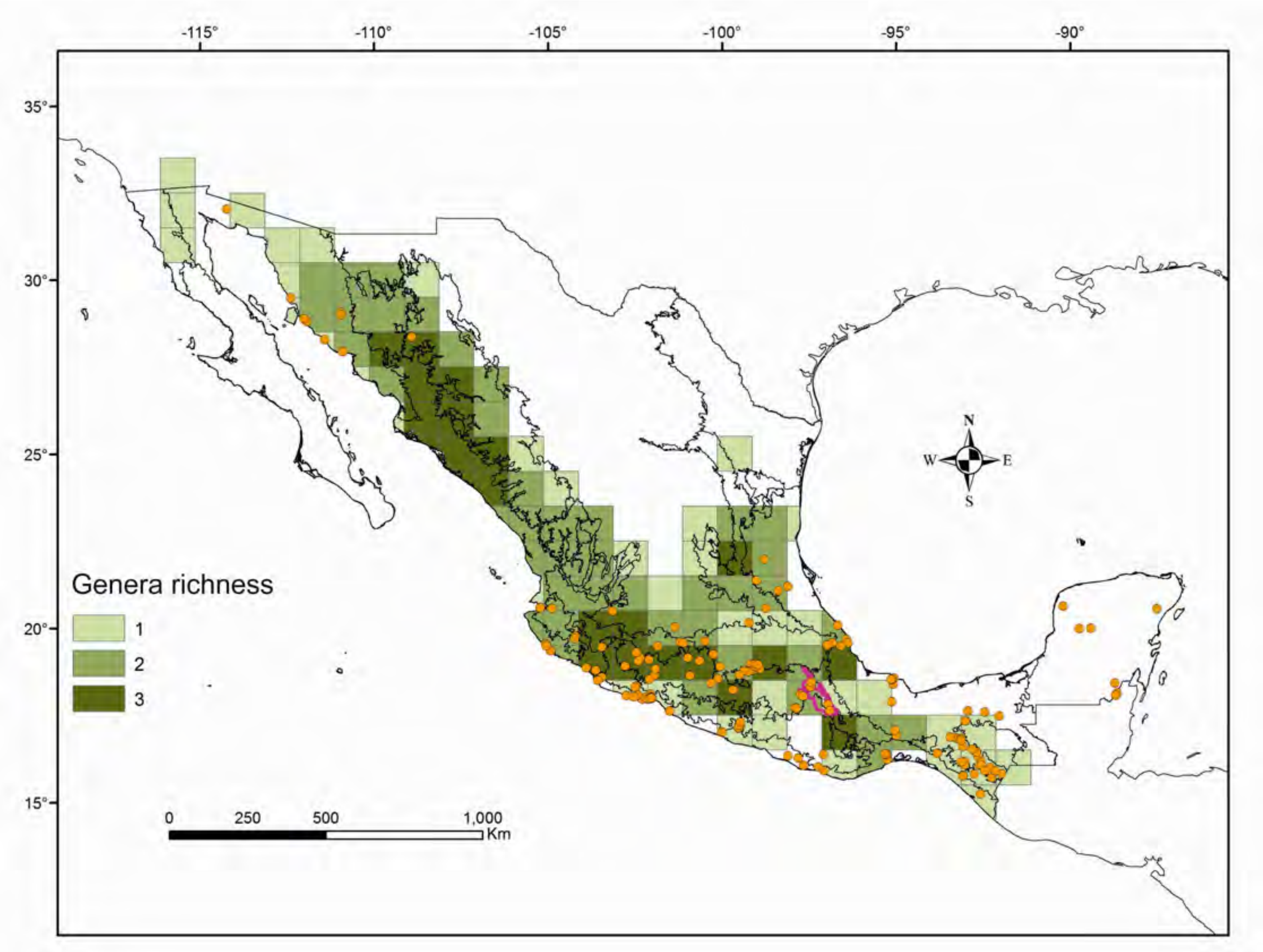

Figure 5. Map of unique plant diet localities (orange dots) in relation to the biogeographical provinces of Mexico (for further details of the provinces, refer to Morrone 2019), and the species richness of the genera *Agave*, *Ceiba*, and *Pseudobombax* in Mexico, shown in a decreasing (light green) or increasing (dark green) gradient. The Tehuacán-Cuicatlán Biosphere Reserve is marked in pink.

bats may be associated with the preference (or need) of many researchers to work in areas of high biodiversity. Therefore, the bias in the present knowledge of bat diet in these two areas is likely related to logistical rather than biotic variables.

In conclusion, the spatial analysis of the geographic distribution of plant diet localities showed that in the Trans-Mexicana Volcanic Belt and Balsas Basin, variables such as richness of plant genera consumed by bats and richness of nectar-feeding bats are importantly related to this plant-bat interaction, while in the rest of the territory, the presence of these localities is more closely related to the richness of nectar-feeding bats. However, the distribution of plant diet localities is also likely biased by logistical factors and a low research effort.

This study showed that the current knowledge of the diet of Glossophaginae nectar-feeding bats in México is far from complete, particularly for *L. obscura*, *H. underwoodi*, and *C. godmani*. We know that there is a decreasing trend in the occurrence and diversity of pollinators in northeastern Europe and North America, along with a lack of information on wild pollinators in several regions of the world, including Latin America (IPBES 2016). Pollinator decline has been linked to factors such as intensive agriculture, land-use change, and climate change, among others. For instance, Zamora- Gutierrez *et al.* (2021) used different future scenarios to analyze how co-occurrence patterns between pollinating bats and the plants pollinated could be disrupted due to the last two factors. These authors found that, in general, the number of plant-bat interactions may decrease between 34.1 % and 47.1 %, on average, under the pessimistic scenario for México.

The loss of pollinators has short- and long-term consequences (Ashworth *et al.* 2009). In the short term, there is a decrease in the food supply; in the long term, there are cascading effects related to the decline of plant diversity, air and water purification, nutrient cycling, and disease control, among others (Ashworth *et al.* 2009). If we are to preserve the permanence of bats, the plants they visit, and the ecosystem services they provide, advancing their study will guide us toward more effective conservation strategies.

Acknowledgments

We dedicate this study to the memory of Moisés Garcia Castillo, *Chiapaneco* at its heart, biologist, and nature lover. Part of the translation of the article was thanks to a grant sponsored by Dr. Robert Timm, and Universidad Iberoamericana Puebla

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Use of nanoparticle biosensors to evaluate carnivore health: A new approach

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The canine distemper virus (CDV) causes a common fatal disease in carnivores that has led to the local extinction of some species. Timely and effective monitoring and diagnosis of the spread of CDV are essential because these can contribute to containing and planning control strategies in early outbreak stages. The use of nanoparticle-based biosensors (NPs) is a novel alternative. A systematic analysis of NP design, use, and scope provides a clue on the potential application of this technology in CDV diagnosis and control. In this sense, the present paper outlines the most recent findings on the use of biosensors to diagnose canine distemper. We conducted a literature review of the use of biosensors to diagnose different viruses of human and zoonotic importance. Biosensors designed for the diagnosis and control of CDV were then searched and analyzed. Searches were conducted in scientific databases with the keywords "*Canine Distemper Virus*", "*nanoparticles*", and "*biosensors*". No studies on wildlife were found. We found 50 studies addressing the development and application of virus biosensors in domestic animals and under laboratory conditions. The use of metallic NPs with optical properties — for example, gold NPs and quantum dots — have been the main systems for the design of biosensors used in CDV diagnosis and control. Future steps include the application of this technology in widely distributed wildlife species, *e. g.,* coyote, by developing a device with a CDV-specific biosensor for *in-situ* detection, avoiding the need to capture individuals. Detecting CDV-infected populations in real-time will improve the management and containment of the disease to preserve the health of ecosystems.

La enfermedad causada por el virus del distemper canino (CDV) se presenta comúnmente en carnívoros con consecuencias mortales y ha causado la extinción local de algunas especies. Monitorear y diagnosticar la propagación del CDV de forma rápida y eficaz, es indispensable, ya que puede contribuir a contener y planificar estrategias de control en etapas tempranas. El uso de biosensores basados en nanopartículas (NPs) puede ser una novedosa alternativa. Sin embargo, un análisis sistemático del diseño, uso y alcance de estos dispositivos puede vislumbrar su potencial aplicación en el diagnóstico y control de la enfermedad. En este sentido, el presente trabajo compila los resultados más actuales del uso de biosensores para el diagnóstico de la enfermedad por el virus del distemper canino. Se realizó una revisión bibliográfica del uso de biosensores para el diagnóstico de diferentes virus de importancia humana y zoonótica. Después se buscaron y analizaron los biosensores diseñados para el diagnóstico y control del CDV. Las búsquedas se realizaron en bases de datos científicas con el conjunto de palabras "*Canine Distemper Virus*", "*nanoparticles*" and "*biosensors*". No se encontraron estudios con fauna silvestre. Se encontraron 50 estudios que muestran el desarrollo y aplicación de biosensores para virus en animales domésticos y en condiciones de laboratorio. Se encontró que el uso de NPs metálicas con propiedades ópticas, por ejemplo, las NPs de oro y puntos cuánticos; han sido los principales sistemas para el diseño de biosensores usados en el diagnóstico y control del CDV. Las perspectivas a futuro es que el uso de esta tecnología pueda trasladarse a la vida silvestre, en una especie de amplia distribución como el coyote, por medio del desarrollo de un dispositivo con un biosensor específico para CDV, para la detección *in situ* sin necesidad de capturar los individuos. El detectar a las poblaciones contagiadas con CDV en tiempo real, contribuirá a un mejor manejo y contención de la enfermedad para mantener la inocuidad de los ecosistemas.

Keywords: Biosensors; canids; canine distemper virus; ecology; nanoparticles; wildlife.

Introduction

Having fully healthy ecosystems is unfeasible. Zoonotic diseases are becoming increasingly common and more widespread in time and space due to the destruction and fragmentation of natural ecosystems caused by anthropogenic activities and climate change (Daszak *et al.* 2001; Stokholm *et al.* 2021). Understanding and identifying diseases that induce high wildlife mortality and morbidity is key for ecosystem management and conservation (Loots *et al.* 2017).

Ecological studies of diseases affecting carnivores emerged in the 1990s (McCallum and Dobson 1995). Populations infected with the canine distemper virus (CDV) were studied, finding that CDV was the primary cause of the local extinction of the lycaon (*Lycaon pictus*) in Serengeti, Tanzania (Creel and Creel 1998) and Masai Mara, Kenya (Alexander and Appel 1994), and of the black-footed ferret (*Mustela nigripes*) in Wyoming, United States (Williams *et al.* 1988). In 1994, Young concluded that the highest mortality in the tropics was due to disease in carnivores and food scarcity in herbivores; today, we know that intra- and inter-specific relationships are far more complex.

CDV belongs to the genus *Morbillivirus*, family *Paramyxoviridae* (Murphy *et al.* 2012). It is highly important for wildlife, particularly carnivores. It enters the body through the mucosae and spreads in the environment through urine and feces. Its rapid transmission among carnivores is related to their behavior, *i. e.*, communicating through smell, coupled with the ability of the virus to occur in all fluids of the infected organism, including aerosols from respiratory secretions (Barrett 1999; Ávila *et al.* 2015; Loots *et al.* 2017); besides, CDV can also spread from the consumption of infected organisms (Tahara *et al.* 2008).

Similar to any virus, CDV needs a host to replicate itself. As it is associated with cellular tropism, CDV-infected organisms suffer a range of systemic alterations affecting the respiratory system (breathing disorders, as the virus enters directly by air and replicates in the respiratory tract), digestive system (diarrhea), urinary system (kidney disorders), lymphatic system (lymphopenia and persistent immunosuppression), skeletal system (multiple sclerosis), skin disorders, and the central nervous system (leukoencephaltis; Lempp *et al.* 2014). Infected individuals usually die from the disease (Rendon-Marin *et al.* 2019). CDV does not only affect canids, as the name suggests, but has been recognized as a multi-host pathogen that has caused massive deaths, mainly in carnivorous species (Loots *et al.* 2017; Rendon-Marin *et al.* 2019). There are records of the adverse effects of this virus in felids such as lion (*Panthera leo*; Weckworth *et al.* 2020), tiger (*Panthera tigris*; Myers *et al.* 1997; Seimon *et al.* 2013), Iberian lynx (*Lynx pardinus*; Meli et al. 2010); hyenids: spotted hyena (*Crocuta crocuta*); ursids: giant panda (*Ailuropoda melanoleuca*; Feng *et al.* 2016); and phocids: crabeater seal (*Lobodon carcinophagus*; Bengston et al. 1991), Caspian seal (*Pusa caspica*; Forsyth *et al.* 1998) and nerpa (*Pusa sibirica*; Grachev *et al.* 1989). Martínez-Gutiérrez and Ruiz-Sáenz (2016) produced a literature compendium on the records of CDV-infected species, either naturally or experimentally, in all continents except Australasia. They found 217 articles that mention 12 families of the order Carnivora (*Ailuridae*, *Canidae* excluding the domestic dog, *Felidae*, *Hyaenidae*, *Mephitidae*, *Mustelidae*, *Odobenidae*, *Otariidae*, *Phocidae*, *Procyonidae*, *Ursidae*, and *Viverridae*), four of Rodentia (*Caviidae*, *Cricetidae*, *Muridae*, including *Mus musculus*; and *Sciuridae*), two of primates (*Cebidae* and *Cercopithecidae*), three of Artiodactyla (*Cervidae*, *Suidae* and *Tayassuidae*), and one of Proboscidea (*Elephantidae*).

The main factors impacting disease dynamics in carnivores are population size, density, and distribution (Dye *et al.* 1995). A suitable case study may be the coyote (*Canis latrans*) because it is a common species with a large amount of data and records, widely distributed in America, from Alaska to Central America. Some studies contend that its distribution may also include Colombia in South America (Monroy-Vilchis *et al.* 2020). The wide distribution of this species is due to its plasticity to adapt to different habitats and its varied diet (Gompper and Gittleman 1991). The broad geographic range of the coyote makes it hugely important as a model of disease dispersal in wild species. This species has shown a high prevalence of canine parvovirus, canine distemper, canine infectious hepatitis, and many other infectious pathogens (Gese *et al.* 1991; Gese *et al.* 1997; Bischof and Rogers 2005). The presence of these diseases was significant due to the transmission of many of these diseases to the populations of wolves in Yellowstone National Park, making their recovery slower than estimated (Gese *et al.* 1997).

The ability to monitor the spread of CDV quickly and sparing the need to capture individuals to collect blood samples — which involves the use of traps, chemicals, or containment instruments — may improve disease control and reduce time and costs. As an alternative to conventional sampling and detection methods, the use of biosensors based on advanced materials allows for indirect, non-invasive, and effective identification of the presence of the virus (Vidic *et al.* 2017). These biosensors are made from nanoparticles (NPs); in most cases, the operation principle is related to the intrinsic features of this nanomaterial, e.g., its optical properties (Maddali *et al.* 2020).

Biosensors can recognize, either qualitatively or quantitatively, a specific pathogen for which they are designed, thus avoiding interactions with other microorganisms or molecules. It functions through a bioreceptor with chemical qualities, i.e., a cell, an enzyme, a tissue, DNA, or RNA (Vidic *et al.* 2017). Biosensors are extensively used in biomedicine to detect and diagnose viral diseases, so their application in the detection of CDV in wild mammals seems feasible (Saylan *et al.* 2019). The objective of this paper is to establish an updated overview of the use of biosensors for monitoring and controlling viruses in wildlife, with special emphasis on the canine distemper virus.

Materials and methods

A comprehensive literature review was conducted in search of references addressing the use of biosensors for diagnosing viral diseases, including the canine distemper virus (CDV). A search and analysis of nanovaccines designed and used to control canine distemper was carried out. We used the search engines PubMed, Google Scholar, and Scopus, with the keywords "Virus", "Canine Distemper Virus", "nanoparticles", "NPs", "AuNPs", "QD", "Biosensor", and "Sensor".

Results

We found a total of 50 studies published in scientific journals in domestic animals. None of them refers to the use of biosensors for viruses in wild mammals because most are in the experimental and testing phase (Supplementary Material 1). Publications that had not been peer-reviewed, such as blogs, personal web pages, and social media, were discarded. The studies analyzed were sorted into three groups: use of biosensors to detect viruses, use of biosensors to detect the canine distemper virus (CDV), and use of NPs to develop vaccines.

Discussion

The publications analyzed were grouped as follows:

Use of nanoparticles in virus detection. The use of nanomaterials for the development of biosensors is a subject of extensive research in the biomedical area. For example, there is a wide variety of systems used for the detection and diagnosis of the human papillomavirus (Cheng *et al.* 2022), human immunodeficiency virus (Nandi *et al.* 2020), and SARS-CoV-2 (Deepshikha *et al.* 2022). A biosensor is a system made up of three main components: a transducer that can be an NP, a biological recognition element that must be selective to interact with the analyte of interest (enzymes, DNA, antibodies, cells, and others), and a detector (Saylan *et al.* 2019; Figure 1).

These systems stand out from other sensors because of their high selectivity, sensitivity, and direct real-time detection capability. For the manufacture of biosensors, several NP systems have been used as biological receivers and transducers, most importantly NPs with high optical, magnetic, electrochemical, or electroacoustic properties. Their morphological (shape and size) and functional properties confer quick and easy identification, even for people unfamiliar with biosensors (Ortega-Berlanga *et al.* 2021). It should be mentioned that there is no universal biosensor, *i. e.*, specific bionsensors should be designed and synthesized for the detection of a particular virus or analyte. Depending on the technique and NP used, biosensors can be highly selective and sensitive. However, there will always be limitations; for example, degradation of the biosensor by temperature or light, or saturation by interference with other biological agents that may induce false positives or affect detection limits and time (Wu *et al.* 2018). Our search found that systems with high optical properties have been the simplest and most effective devices for detecting viral diseases, which is why these systems are discussed in detail in this work.

Optical nanoparticles used as biosensors. The basic principle of an optical biosensor consists of inducing a change in the optical properties of the transducer associated with the analyte to be recognized, either through changes in fluorescence, light polarization, dispersion, or refractive index (Damborsky *et al.* 2016). Quantum dot-based (QD) optical biosensors and metallic NPs are the most suitable options due to several characteristics that make them superior to other types of NPs, including low manufacturing cost, high portability, high sensitivity and selectivity, short response time, and high spatial resolution (de La Rosa *et al.* 2020). Materials based on cadmium-selenium (CdSe), zinc sulfide (ZnS), selenium sulfide (SES_2), or gold nanoparticles (AuNPs) have boosted the development of biosensors for the detection of human and zoonotic diseases, making the detection and diagnosis of diseases more efficient and affordable, and with less impact on individuals (Bollella and Katz 2020).

We found a large number of studies on the use of biosensors in the detection of viral diseases, their main characteristics, and hosts (Supplementary Material 1); only the

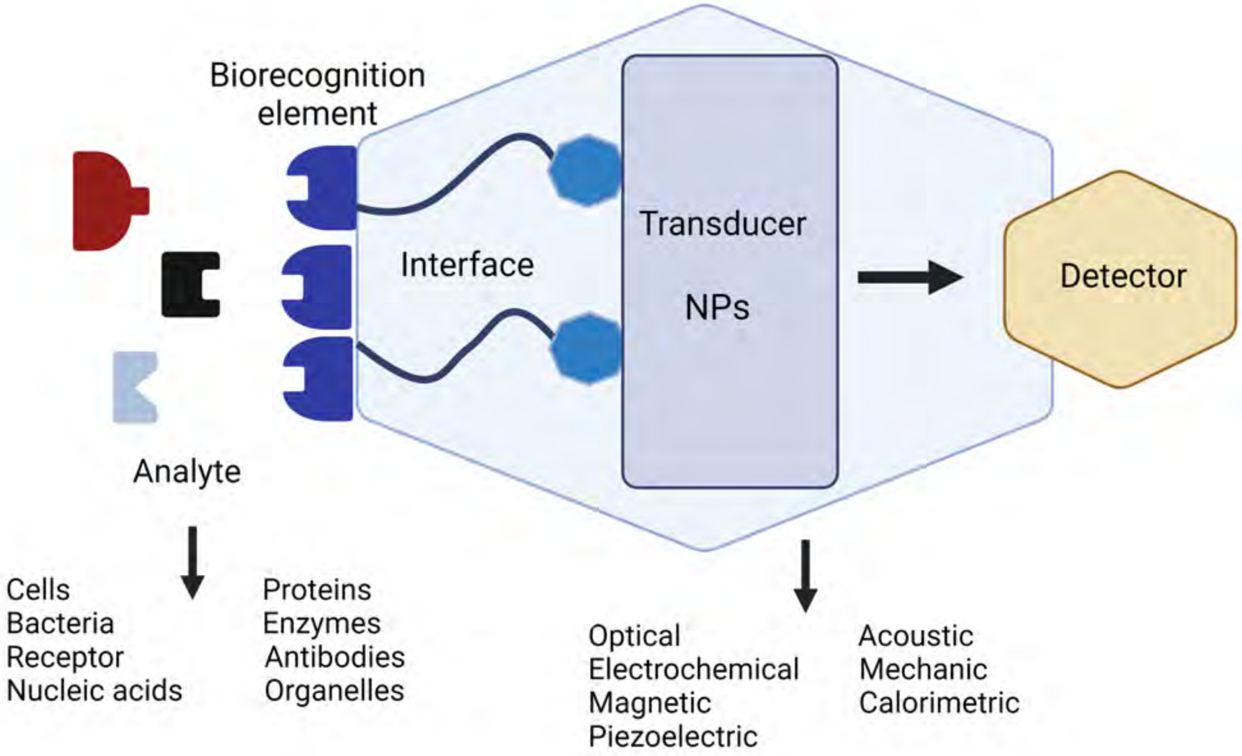

Figure 1. Schematic representation of a biosensor capable of selecting a specific analyte as its main characteristic.

Table 1. Development of common biosensors used for the detection of viral diseases.

System	Main characteristics	Virus detected	Host	Highlights	Reference
QD-LFIAS	QD-Lateral Flow Immunoassay Band Particle size: 20 nm	Sheep-and-goat plague (PPR)	Sheep and cattle	Diagnostic results are obtained within 15 minutes in a convenient and simple way, so the method shows great potential for detecting PPR in the field.	Cheng *et al*. 2017
QD@MHS NPs	QD + silica oxide Particle size: 6–14 nm	Hepatitis E virus, Norovirus	Crab-eating macaque (*Macaca fascicularis*)	Detection and diagnosis of hepatitis E virus equivalent to the RT-qPCR test.	Ganganboina *et al*. 2020
CdSe/ZnS QDs	QD Particle size: 157 nm	H1N1 bird flu	Human / zoonosis	Result equivalent to the PCR method; detection range 3.2 hemagglutination units/mL.	Jo *et al*. 2020
GO-QD	QD Particle size: 12 nm	H1N1 bird flu	Human / zoonosis	The virus was isolated and detected without interruption and impurities and can be used in complex biological samples.	Lee *et al*. 2018
AuNPs – PFAB	AuNPs – Optic fiber	SARS-CoV-2 coronavirus	Human	The portable biosensor can easily detect SARS-CoV-2 directly from saliva samples.	Murugan *et al. 2020*
CdZnSeS/ZnSeS + AuNPs	QD +AuNPs Particle size: 20–35 nm	Influenza virus	Human	Biosensors can be a suitable alternative for detecting a wide range of infectious viruses.	Nasrin *et al*. 2020
AuNPs	AuNPs + PCR Particle size: 1–100 nm	Canine coronavirus I and II	Canids	PCR using AuNPs was effective for the rapid detection of CCoV I and CCoV II.	Qin *et al*. 2021
CdTe QDs	QD Particle size: 3.1 nm	Zika virus	Human	The sensor is 100-fold more sensitive than traditional tests measuring the immune response of protein E. The immunofluorescent platform with QD can be considered convenient and simple for detecting Zika or monitoring the immune response in vaccine testing.	Ribeiro *et al*. 2019
CdSe/CdS/ZnS QD	QD Particle size: 7.8 nm	H1N1 and H3N2 influenza viruses	Human	Soluble QDs can be used to develop fluorescent sensors for the rapid diagnosis of influenza A in humans.	Nguyen *et al.* 2020
GO-AuNPs	AuNPs–Graphene Hybrid Particle size: 10–28 nm	Foot-and-mouth disease virus (HFMD)	Human / zoonosis	PCR detection is improved 1000-fold with AuNPs. High specificity and detection can be applied to diagnose the disease even in the early stages, so it can be a valuable tool in biomedical and clinical applications.	Kim *et al*. 2020
GO-AuNPs	AuNPs–Graphene Hybrid Particle size: 14 nm	Respiratory syncytial virus	Human	The graphene surface significantly increased the area of exposure of AuNPs. The immunoassay successfully detected the virus in artificial samples, showing great potential for commercial applications.	Zhan *et al.* 2021
AuNPs-ASOs	AuNPs–antisense oligonucleotides Particle size: 30–60 nm	SARS-CoV-2 coronavirus	Human	Sensors with modified AuNPs can diagnose positive COVID19 cases in less than 10 minutes from isolated RNA samples. The results are read at a glance with no need for specialized equipment.	Moitra *et al*. 2020
MNPs/AuNPs	Magnetic Nanoparticles—AuNPs Particle size: 15 nm	H1N1 influenza virus	Human	Sensor sensitivity better than the ELISA test. The technique is convenient, low-cost, and detects the H1N1 virus quickly and easily. The biosensor has great potential in clinical and pharmaceutical diagnosis.	Ye *et al*. 2021

most recent results, corresponding to the period 2017 to 2022, are shown in Table 1. The information found showed that, to date, the methodology has only been applied to domestic animals, with potential for use in wild mammals. The biosensors described can detect, in real time, diseases caused by a large number of viruses, such as the sheep-and-goat plague, hepatitis B, E, norovirus, Zika, or gumboro, among many others. Therefore, its replication in wildlife species seems feasible.

Diagnosis of CDV using biosensors. CDV has been diagnosed in different species of domestic and laboratory mammals using biosensors (Table 2). The results indicate that the virus can be detected either directly or through the antibodies produced by the host. The systems used most frequently include gold nanoparticles (AuNPs) as transducers, given their great versatility, allowing them to shift color in real time upon coming into contact with antibodies or virus particles. The color shift in AuNPs derives from the properties conferred by Localized Surface Plasmon Resonance (LSPR; Hidayah and Herbany 2020). LSPR is a physical phenomenon that consists of making free electrons on the NP surface oscillate coherently with the electrical field

Table 2. Development of biosensors for the diagnosis of canine distemper virus.

Type	Main characteristics	Host	Application	Lower detection limit	Hightlights	Reference
AuNPs	AuNPs + antibody - Antigen	Not specified	Diagnostic	0.7 ng CDV antigen/mL	Urine samples were analyzed and the results were visible (colorimetric) and obtained in an easy, simple and viable way at a low cost.	Basso *et al.* 2015
AuNPs	AuNPs - Antigen	Not specified	Diagnostic	$10^{7.5}$ TCID50 CDV antigen/mL	Flow bands with AuNPs can serve as *in-situ* diagnostic tools for the detection of distemper virus antibodies in dogs and felids.	Karki *et al.* 2022
AuNPs	AuNPs–antibody + Antigen	Rabbits	Diagnostic	635 µg CDV antigen/mL	The biosensor is very simple in showing CDV presence/absence results colorimetrically; therefore, it has potential for use in clinical tests.	Mazzu-Nascimento *et al.* 2017
AuNPs	AuNPs + PCR	Canids	Diagnostic	5.4 x 10^2 CDV primers/25 µL	The PCR using AuNPs was 100-fold more sensitive than conventional PCR tests. The technique allowed rapid and efficient CDV detection, with potential for use in the diagnosis of animal epidemics.	Wang *et al.* 2021

of incident light to form a plasma. This collective oscillation of electrons depends on electron density, effective medium, refraction index, NP shape and size, and separation between NPs. The maximum lambda spectral position (λ_{max}) indicates the position of the LSPR, and this can be tuned by modulating the size and shape of nanoparticles and changing the physicochemical properties of the surface and the medium where NPs are found (Aguilera-Juárez *et al.* 2022). The LSPR is composed of the extinction coefficient, which is the sum of the absorption coefficient and the scattering coefficient ($C_{ext} = C_{abs} + C_{scat}$), *i. e.*, of the total light photons interacting with the NP, one part is absorbed and the other is scattered mostly without apparent energy loss (elastic dispersion). In spherical nanoparticles between 40 and 80 nm, the LSPR is located within the visible spectrum. However, as nanoparticle size increases, the shape of NPs changes, or their surface chemistry changes, C_{ext} is dominated by the C_{scat} determined by a red shift of λ_{max}. This shift in the LSPR position induces the color shift in the biosensor and indicates the presence of the analyte anchored on the NP surface (Hernández-Adame *et al.* 2019).

Silver nanoparticles (AgNPs) have been studied as antivirals, *e. g.*, in the case of SARS-CoV-2 (Jeremiah *et al.* 2020). They also have optical properties via the LSPR; hence, they could be used to detect CDV. Unlike AuNPs, AgNPs are chemically unstable and sensitive to pH and temperature, which may cause nanoparticle degradation and release metal ions (Ag^{2+}), leading to interference and toxicity. However, stabilizing the surface of AgNPs with more stable ligands, *e. g.*, thiol terminal groups, would make their use safer.

These biosensors are convenient because the color shift is a qualitative indicator of the presence of viruses, thus sparing the need for additional equipment or techniques. Alternatively, by determining the wavelength at which the sample has the highest absorption (λ_{max}), its intensity in the spectrum may be used as a quantitative indicator of the viral load (Damborsky *et al.* 2016). These devices are efficient, portable, and highly efficient for diagnosing the presence of the disease in real-time using blood, feces, urine, or saliva samples from a potential host (carnivores, in this case).

Treatment of CDV with nanovaccines. The nanovaccines topic is beyond the scope of the present document. Mentioning it is nonetheless important because this area shows that the use of nanotechnology may contribute significantly to the diagnosis and control of diseases in mammals. Table 3 shows the CDV nanovaccine prototypes evaluated in various mammal species with encouraging results. Examples include the use of silver nanoparticles (AgNPs) for the treatment of CDV in the early stages of the disease (Bogdanchikova *et al.* 2016) or the use of proteins plus nanoparticles to enhance the immunological response of organisms against CDV (Dong *et al.* 2021; Wang *et al.* 2022).

The use of biosensors for the detection of diseases affecting ecosystem health is feasible through the NP methodology. A case of global importance because it affects several species is the CDV, which affects different groups of wild mammals and may be easily detectable. Carnivores susceptible to CDV include coyote (*Canis latrans*), a species for which this methodological technology can be developed. Monitoring coyote populations would be a valuable early indicator of the potential presence of CDV at some sites, which may affect various species and be transmitted to domestic ones, even to humans. Coyote can become a sentinel species for CDV because its populations are abundant and it not listed under a risk category on national and international lists (Kays 2018; DOF 2019). This species has a broad distribution, including all of North and Central America, probably reaching South America (Monroy-Vilchis *et al.* 2020). Besides, it inhabits different ecosystems, including urban landscapes, so it is a "bridge" species susceptible to infection of various diseases, including CDV, and capable of passing them from natural to peri-urban environments and vice versa (Gese *et al.* 1991; Bischof and Rogers 2005). Additionally, it has great vagility (Gompper and Glitterman 1991) and can rapidly transmit viruses to other mammals with populations under a risk category, as is the case of the Mexican

Table 3. Use of nanovaccines in disease biocontrol.

Type	Main characteristics	Host	Application	Hightlights	Reference
AgNPs	AgNPs	Canids	Treatment	Canids infected with CDV that had not yet developed symptoms of neurological damage recovered favorably from the disease after the administration of AgNPs.	Bogdanchikova, *et al.* 2016
NPs-CDV H	NPS with recombinant CDV H protein	Rodent	Biocontrol	Mice inoculated with the vaccine showed better immune responses versus the control group, so the H protein can be used for the development of vaccines against canine distemper virus.	Dong *et al.* 2021
NPs-HA-F	NPs + hemagglutinin CDV sequences + ferritin	Rodents	Biocontrol	All organisms inoculated with the nanovaccine showed an increased production of antibodies against CDV and were able to recognize and eliminate cells with antigens on their membrane. The nanovaccine has great potential for CDV biocontrol in domestic and wild organisms.	Wang *et al.* 2022

wolf (*Canis lupus baileyi*; Boitani *et al.* 2018), different species of fox, skunk, raccoon, or even felines, including jaguar (*Panthera onca*; Quigley *et al.* 2017).

Future perspectives. The health of ecosystems largely depends on the wellness of the wildlife populations in them. In increasingly fragmented ecosystems, the spread of diseases among organisms can occur more rapidly, so timely disease identification, management, and control are essential (Mathews 2009). As predators, carnivores are hosts for many pathogens that directly impact populations of other wild and domestic species, occasionally affecting humans (Bischof and Rogers 2005). The ecological importance of some species is worth highlighting, particularly the coyote. Besides being omnivorous and in contact with species of different trophic niches, this species inhabits a broad range of ecosystems, from pristine to the most disturbed ones. This is why it can act as a carrier of diseases across isolated environments.

The canine distemper virus (CDV) affects several species and families (Martínez-Gutiérrez and Ruiz-Sáenz 2016); it has been recorded in the orders Artiodactyla, Primates, Proboscidea, Rodentia, besides Carnivora (Martínez-Gutiérrez and Ruiz-Sáenz 2016). The extinction of local populations of carnivores such as the wolf has been recorded as a result of this virus (Gese *et al.* 1997), which highlights the importance of CDV for the health of ecosystems.

CDV is detected by blood antibody tests several days after the individual has been infected (Rendon-Marin *et al.* 2019). Carrying out these tests requires capturing the individuals, which implies the use of different trapping methods and having basic equipment and staff trained in carnivore handling. Blood samples should be processed in the laboratory, with results that are not readily available and can be expensive. These aspects restrain the implementation of this approach as a baseline for monitoring CDV in ecosystems.

The biosensors analyzed in domestic animals in this study have yielded highly promising results for the diagnosis of CDV, so these biosensors are likely suitable for use in wild mammals such as coyote. The current methodological progress allows for detecting CDV with a device containing an AuNP-based biosensor specific for this virus, which would work optically: when placing the sample in the device, the biosensor would shift to a reddish color if the virus is detected. Our review did not demonstrate the use of AuNPs for CDV detection by the Surface-Enhanced Raman Spectroscopy (SERS). We consider this option should be explored because star-shaped AuNPs can amplify the detection signal to diagnose CDV, even under very low viral loads.

The advantage of using this type of device with biosensors for CDV detection is that it works not only with blood samples but with any body fluids. This avoids the need to capture potential individuals to collect samples for testing and perform monitoring. One of the easiest indirect samples for testing is feces. CDV is a highly unstable virus outside its host, so its life expectancy in the environment is just a few hours, depending on the ambient temperature (Gilbert *et al.* 2015). The ideal scenario would be to track targeted locations assisted with predictive distribution models. Once the sampling site is selected, it should be searched for fresh feces. First, a fragment of feces should be dissolved in distilled water because the device would not accept solid samples; then, a single drop of the dissolved sample would be placed in the device. The biosensor would then detect the presence of the virus through a color shift. The system would allow immediate real-time detection of the health status of coyotes in the target area, which would likely also reflect the condition of several coexisting species. Also, the signal produced by the biosensor could be sent to a cell phone so that the information would be stored in a virtual space.

There are very few products commercially available; most are still in the development and research stages (Bollella and Katz 2020), so a market price for the device has not been established. One way to reduce costs could be the development of biosensors capable of detecting several analytes at a time (Haleem *et al.* 2021).

Biosensors have potential for use to assess the health status of ecosystems. Early detection of viruses in infected wild carnivores will allow for timely and effective CDV containment plans (Funk *et al.* 2001), avoiding high costs related to the long-term conservation, management, and protection of species and ecosystems. Above all, this strategy would avoid the spread of viruses between different species of mammals, including humans.

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