Therya Notes

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AMMAC
Asociación Mexicana de Mastozoología A.C.

THERYA NOTES tiene como propósito difundir exclusivamente notas científicas con información original e inédita relacionada con el estudio de los mamíferos en todas las disciplinas que contribuyen a su conocimiento. Es un foro abierto para profesores, investigadores, profesionales y estudiantes de todo el mundo, en el que se publican notas académicas en español e inglés. THERYA NOTES es una revista digital de publicación cuatrimestral (tres fascículos por año) que recibe propuestas para publicación durante todo el año. Tiene un sistema de evaluación por pares a doble ciego y es de acceso abierto.

En la Portada

El desarrollo de grandes infraestructuras como las carreteras ha colocado en grave amenaza a la biodiversidad debido a la mortalidad por atropellamiento de fauna silvestre. Una de las amenazas subyacentes es el fácil acceso para los cazadores; degradación del hábitat alrededor de la carretera por actividades agropecuarias, extracción de petróleo, gas y la minería. Lo anterior coloca en grave riesgo a muchas especies de mamíferos. De manera particular, para las poblaciones de primates causa que el 55 % del total de sus especies se encuentre en peligro de extinción, además de la disminución de sus poblaciones en un 75 % de sus especies.

(Fotografía de Pozo-Montuy et al. 2022)

El logo de la AMMAC: "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 del mono araña (*Ateles geoffroyi*), la especie de primate de más amplia distribución en México. "Es habitante de los bosques, sobre todo de los que están por donde sale el sol en Anáhuac. Tiene el dorso pequeño, es barrigudo y su cola, que a veces se enrosca, es larga. Sus manos y sus pies parecen de hombre; también sus uñas. Los Ozomatin gritan y silban y hacen 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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Editorial

Wildlife crossings and road impact on mammals

Highways are essential for countries' economic growth; therefore, the provision of road infrastructure services is undergoing rapid and permanent expansion. In current strategies, these communication routes are considered a fundamental element in economic reactivation, fostering many ambitious road modernization and expansion projects worldwide (CEPAL 2021). Despite their usefulness, roads are also a threat with significant environmental consequences. In recent decades, they have been considered one of the main threats to wildlife globally (van der Ree *et al.* 2015), mainly due to their impact by favoring habitat fragmentation and causing the mortality of multiple species of fauna due to collisions with vehicles.

In this issue, it is recognized that landscape connectivity is essential for maintaining the flow of mammalian individuals, their genetic variability, and allowing access to resources. An irruption of the connectivity brings, as a consequence, threats at the species and population level. However, despite the topic's relevance, there is little legislation on the maintenance of ecological connectivity. As indicated by Fernández-Buces *et al.* (2022), no laws, regulations, or agreements require means to maintain the connectivity of the landscape on the roads in a planned manner. As an example, there is an absence of specific laws to design wildlife crossings as a strategy to maintain said connectivity. It is necessary to understand that when building this type of communication routes, it is necessary to avoid or mitigate to a minimum the impacts that may be generated due to the construction activities and their subsequent operation.

Monitoring is another critical element that is part of a correct prevention and mitigation of the effects of roads. In this issue, we find studies that show the record of priority species that have suffered accidents against vehicular traffic and that show the need to take actions aimed at maintaining species as charismatic and relevant to natural ecosystems as the jaguar (*Panthera onca*; Rubio-Rocha *et al.* 2022b). We also found other infrequent species in the samplings due to the effect of the monitoring techniques used, but which are also affected by the existence of this infrastructure, as occurs with the spectral vampire (*Vampyrum spectrum*; Pacheco-Figueroa *et al.* 2022). In addition, reports are presented in which mammals present a differentiated use according to the different times of the year (Ruíz-Ramírez *et al.* 2022) or type of road (Ruíz-Ramírez *et al.* 2022; Rubio-Rocha *et al.* 2022a). All this is essential information for the design of mitigation measures (Cervantes-Huerta and Durán-Antonio 2022). Despite all the relevant information concentrated in this issue, there are still many information gaps on the effects of the roads, leaving great unknowns about what is happening on them due to the effects of run over.

On the other hand, monitoring wildlife crossings is another key element that can provide information on their effectiveness and the adaptation measures they require. This is evidenced in the study by González-Gallina *et al.* (2022), where the differentiated use of these structures is reported, according to the preferences of each species of mammal, as well as by the season of the year, or the time of existence of a fauna pass since it was built.

Also, some species clearly avoid highways, and easily adapt to the culverts used on these roads, even though they are not properly designed to be used as wildlife crossings; such is the case of the sereque or guatusa (*Dasyprocta punctata*) on the Inter-American Highway in Costa Rica (Monge-Velázquez and Sáenz 2022).

In the same way, we have evidence at the population level, which shows a reduction in a wildlife population when highway expansions were carried out, as occurred in Catazajá, Chiapas (Pozo-Montuy and Bonilla-Sánchez 2022), as well as a consequent and rapid adaptation to use of aerial wildlife crossings by howler monkeys (*Alouatta pigra*).

It is clear that roads remain a major threat to mammals. Current studies increasingly include the analysis of impacts on wildlife and analyze the effectiveness of the measures implemented in road projects. However, measures adapted to each region are not yet implemented, but they continue to use those that have been effective in temperate zones of the world.

The justification for the use of strategies in such areas is based on a cost-benefit analysis and are explicitly created for megafauna that is not distributed in tropical areas. Therefore, medium or small-sized fauna do not justify the construction of wildlife crossings for certain sectors of the population and decision-makers. In reality, it is not the economic cost of the accidents that should justify the construction of these structures to protect wildlife, but rather the environmental cost caused by the consequent loss of biodiversity. The measures must be created in a particular way for each region, especially in tropical zones, and analyze their cost-benefit for the recovered environmental services, considering a design adapted to the particular region. Faced with the current growth and modernization of communication routes globally, mammals will only have an opportunity if appropriate designs are implemented for each region and the species that inhabit it and that ensure the maintenance of landscape connectivity. Monitoring before, during, and after the construction of the infrastructure should be considered in the planning, in order to implement short, medium, and long-term measures that help maintain the diversity of mammals and their habitats adequately. Also, an effort to create an integrated legal framework that considers landscape connectivity as an essential element should be included. Road studies on the effects of communication routes on mastofauna are highly relevant. Collaboration between authorities, consultants, builders, researchers, and academia can lead to better informed and more efficient decision-making for the conservation of biodiversity and the ecosystems in which it is distributed.

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First records of road-killed mammals in the state of Sinaloa, México

Primeros registros de atropellamiento de mamíferos en el estado de Sinaloa, México

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The present research was conducted given the lack of data on the impact of motor vehicle traffic on wild animal populations. The present study aimed to assess the number of mammal road kills in Sinaloa, México. Roadkill records were obtained through field trips along roads, the NaturaLista web page, and citizen reports. Eleven mammal species were identified, importantly including some species listed in a conservation status, such as the jaguar (*Panthera onca*), ocelot (*Leopardus pardalis*), and badger (*Taxidea taxus*). Forty-five collision events were recorded on roads running across the south-central region of the state, from February 2019 to June 2021. The species with most records were the coati (*Nasua narica*), opossum (*Didelphis virginiana*), and lynx (*Lynx rufus*). This work is the first to record mammal mortality due to road collisions with vehicles and highlights the need to allocate greater resources to this line of research, which arises from the importance of wildlife conservation and the safety of road users.

Key words: Carnivores; collisions; mortality; road ecology; wildlife.

La ausencia de datos sobre el impacto que genera el tráfico vehicular en las poblaciones de animales silvestres motivó la presente investigación, que tuvo como objetivo conocer el número de muertes por atropellamiento de mamíferos en las carreteras de Sinaloa, México. Los registros de las colisiones se obtuvieron a través de transectos libres en carreteras, registros de la red social Naturalista y reportes ciudadanos. Se identificaron 11 especies de mamíferos, resaltando algunos en estado de conservación como el jaguar (*Panthera onca*), el ocelote (*Leopardus pardalis*) y el tlalcoyote (*Taxidea taxus*). Se obtuvieron 45 registros de colisiones en las carreteras de la región centro-sur del estado dentro del periodo de febrero de 2019 a junio de 2021. Las especies con más accidentes registrados fueron el coatí (*Nasua narica*), el tlacuache (*Didelphis virginiana*) y el lince (*Lynx rufus*). Este trabajo documenta por primera vez la mortalidad de mamíferos como resultado de las colisiones con el tráfico vehicular y resalta la necesidad de dirigir mayores recursos en esta línea de investigación que emerge ante la necesidad de conservar la fauna silvestre y la integridad de los usuarios de las carreteras.

Palabras clave: Carnívoros; colisiones; ecología de carreteras; fauna silvestre; mortalidad.

The coexistence between humans and wildlife has been recorded throughout history in caverns and rocks (Santos and Viñas 2005). Mammals have been of interest to humans due to their beauty and the services they provide as food sources, means of transportation, and companionship. This relationship has currently led to risk situations for wildlife inhabiting and moving through anthropic areas such as highways, roads, towns, and cities (Gottdenker *et al.* 2001; Filius *et al.* 2020).

Roads are indicators of socioeconomic development; they give rise to opportunities for productive activities and improve the communication between people and communities (Bañón and Beviá 2000). However, their construction and operation involve permanent adverse effects on ecosystems, including habitat fragmentation and destruction, and reduction of forest areas and natural biological corridors. This results in lesser dispersal capacity of wildlife individuals, thus threatening local populations (Dirzo *et al.* 2014; Suazo-Ortuño *et al.* 2018; Dean *et al.* 2019). The most conspicuous impacts include collisions between vehicles and wildlife, known as roadkill events (Gottdenker *et al.* 2001), which usually result in the death of animals when they attempt to cross the roads and are hit by vehicles. Road infrastructure also acts as a barrier for certain species and impairs the mobility and connectivity among wild populations, thus affecting their genetic diversity (Arroyave *et al.* 2006; Holderregger and Di Giulio 2010).

The impact of roads on wildlife has been documented for some regions of México. The vertebrates with the highest frequency of deaths by collision are mammals, followed by birds and reptiles (González-Gallina *et al.* 2013; Pacheco *et al.* 2016; Cupul-Magaña 2019; Canales-Delgadillo *et al.* 2020). Roadkill events can influence the decline of local populations (Puc-Sánchez *et al.* 2013), which is of particular concern considering the ongoing mass extinction of wildlife (Ceballos *et al.* 2015). The impact of vehicle traffic on animals in the state of Sinaloa, México, is currently unknown, although there are important records for some endangered or threatened mammal species (Rubio-Rocha *et al.* 2010), such as wild cats that are forced to cross roads as part of their natural displacement patterns. This research represents the first effort to quantify and report the impact of road traffic on wildlife in Sinaloa and documents the mortality of various groups of mammals due to collisions.

The state of Sinaloa is located in northwestern México, between coordinates 22° 31′ and 26° 56′ N, and 105° 24′ and 109° 27′ W. It comprises an area of 57,365.4 km^2 organized in 18 municipalities distributed from the Pacific coast to the Sierra Madre Occidental mountain range, according to data from the National Institute of Statistics and Geography (INEGI, in Spanish; INEGI 2017). The prevailing climate is warm sub-humid and semi-dry in 48 % of the territory, arid in 10 %, and temperate in only 2 %, with mean annual temperature and precipitation of 25 °C and 769 mm, respectively. Dry forests are the dominant vegetation type, and there are important extensions of medium-height tropical forests, pine-oak forests, and xeric shrubland (INEGI 2013).

The study area comprised nine municipalities located in the south-central region, from Culiacán city to Escuinapa city (Figure 1); these municipalities have state and municipal paved roads measuring approximately 6.5–7 m in width. These highways are crossed in some sections by the México 015 and the Maxipista México 015 D federal highways, whose paved cross-section is approximately 7 m wide, but can reach up to 17 m wide in some sections. State and federal highways have drainage zones at some points, which broaden the paved width by up to 1.5 m (IMT 2006). Records were gathered from February 2019 to June 2021 using three approaches. 1) Evidence found along free transects traveled by car whenever possible, during tours along the road infrastructure of the south-central region of Sinaloa, with the assistance of three persons. During 31 sampling days, a total distance of 4,328 km was traveled, 90 % on the two federal highways and the remaining 10 % along five state and four municipal roads. The average distance traveled per trip was 139.6 km; monitoring was conducted during daylight, usually between 11:00 and 18:00 hr, at an average driving speed of 80 km/hr. When a roadkill was spotted, data were gathered, including the road name or code, location (km #) and coordinates of the collision site, species and sex of the

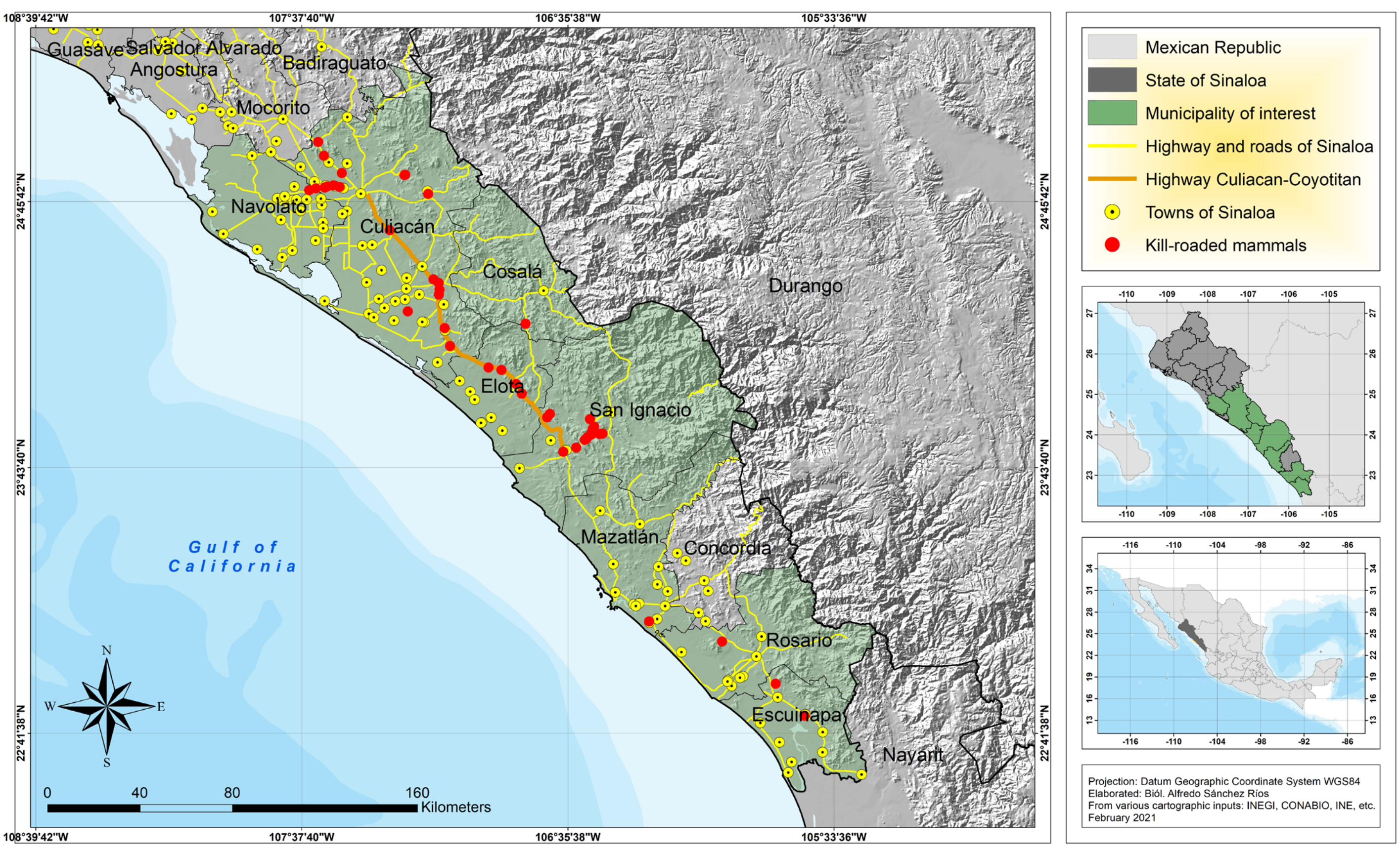

Figure 1. Study area and location of sites with reports of wild mammals roadkills on roads of Sinaloa, México, from 2019 to 2021.

mammal individual, photograph of the specimen, and habitat type. Species were identified at the collision site using field guides (Reid 1997). 2) Roadkill records gathered by users of the "Road Ecology of the state of Sinaloa" project website and posted at the NaturaLista web page. 3) Contributions of road users who photographed or lifted corpses and sent them to the facilities of the Fundación Sinaloense para la Conservación de la Biodiversidad A. C., reporting this to the Procuraduría Federal de Protección al Ambiente en Sinaloa. All records were added into a database.

A total of 45 mammal roadkills were recorded on the Sinaloa roads studied: 25 during road trips, 18 recorded on the NaturaLista web page, and 2 documented by road users (Table 1). Of these, 51.1 % of the collision events occurred on five state and four municipal highways, 44.4 % on the México 015 and 015D federal highways, and 4.4 % on dirt roads. The Culiacán-Coyotitan section of the México 015 federal highway, with a length of 145 km, showed the highest number of roadkills, with 14 records (Figure 1). The information reported herein corresponds to 8 of the 9 municipalities located in the south-central region of Sinaloa. The section of the México 015 federal highway connecting the municipalities of Culiacán, Elota, and San Ignacio showed the highest percentage of roadkills.

Eleven mammal species belonging to four orders were identified. The best-represented order was Carnivora, with 5 families: Felidae, Canidae, Mustelidae, Mephitidae, and Procyonidae. The orders Cingulata, Lagomorpha, and Didelphimorphia were each represented by 1 family with one species (Table 1). Carnivores were the group with the highest number of mammals roadkills (82 %); the families with the highest number of records were Procyonidae (*n* = 18), Felidae (*n* = 10), and Didelphidae (*n* = 9). The species most frequently involved in roadkills were the coati (*Nasua narica*), followed by opossum (*Didelphis virgiana*) and lynx (*Lynx rufus*). Seventy-three percent of the records occurred in 2 municipalities: Culiacán and San Ignacio. Figure 2 illustrates 8 of the road-killed species.

Three species listed in NOM-059-SEMARNAT-2010 (SEMARNAT 2019) were identified in this study; the ocelot (*Leopardus pardalis*) and jaguar (*Panthera onca*), classified as endangered species, and the badger (*Taxidea taxus*), listed as a threatened species. Specimens of these species were found in the Culiacán-Coyotitan section; the ocelot was recorded by a local inhabitant and the jaguar and badger, by the working group.

During this research, it was observed that the number of vehicles, high speed, and lack of roadside barriers are part of the threats and risks faced by mammals when crossing roads (González-Gallina *et al.* 2013; Puc-Sánchez *et al.* 2013; Pacheco *et al.* 2016; Filius *et al.* 2020). The maximum speed limit in federal and state highways is between 60 and 80 km/hr; however, this limit is frequently exceeded. According to the Secretariat of Communications and Transport (SCT, in Spanish), the average travel speed is about 100 km/hr (SCT 2018), which increases the risk of wildlife roadkills. The México 015 federal highway communicates important population centers that demand services from the main urban areas, Culiacán and Mazatlán cities, located at both ends of the road section studied. On average, 4,647 vehicles travel along these roads every day, of which 76 % are cars and the rest, motor carriers and trailer trucks (SCT 2017; SCT 2018). The trade and transport industries are the leading road users at the regional level (INEGI 2016); transport of passengers, freights, and various products to regional and international destinations takes place permanently throughout the year.

The Culiacán municipality, located at the center of the state, has a growing road and real-estate infrastructure, in addition to agricultural and forestry activities, which together account for 68 % of the deforestation compared to the rest of productive and service activities (INEGI 2016). The mean annual rate of vegetation cover loss in Sinaloa is one of the highest in the country (0.41 %), considering that the national average ranges between 0.35 % and 0.40 % (Monjardín-Armenta *et al.* 2017). This situation is likely

Table 1. Number of mammals struck on roads in Sinaloa, México, and conservation status. *NOM: Mexican Official Norm NOM-059-SEMARNAT-2010. P: extinction risk; A: threatened (SEMARNAT 2019). ** IUCN: Red list of threatened species of the International Union for Conservation of Nature. VU: vulnerable; LC: least concern (IUCN 2021). M: Number of records obtained by monitoring. N: Number of records obtained from the NaturaLista web page. A: Number of records from road users.

Family	Species	Common name	* NOM	** IUCN	M	N	A	Total records
Canidae	*Urocyon cinereoargenteus*	Gray fox		LC	3			3
Felidae	*Leopardus pardalis*	Ocelot	P	LC			1	1
Felidae	*Lynx rufus*	Bobcat		LC	3	5		8
Felidae	*Panthera onca*	Jaguar	P	VU			1	1
Mephitidae	*Mephitis mephitis*	Striped skunk		LC		1		1
Mustelidae	*Taxidea taxus*	American badger	A	LC	1			1
Procyonidae	*Nasua narica*	White-nosed coatí		LC	12	1		13
Procyonidae	*Procyon lotor*	Raccoon		LC	2	3		5
Dasypodidae	*Dasypus novemcinctus*	Nine-banded armadillo		LC	1	1		2
Dildelphidae	*Didelphis virginiana*	Virginia opossum		LC	2	7		9
Leporidae	*Lepus alleni*	Antelope jackrabbit		LC	1			1
Total					25	18	2	45

affecting the distribution, abundance, and interactions of mammals (Krebs 2014). Habitat fragmentation increases the edge and barrier effects and consequently, the risk of animals being struck while crossing roads in search of food (Fahrig 2003; Dirzo *et al.* 2014; Suazo-Ortuño *et al.* 2018; Abra *et al.* 2021). Nevertheless, it cannot be affirmed that the roads studied have the highest risk of wildlife roadkills in Sinaloa, but they are the scenario of road-killed animals as recorded by the working group and participants of the NaturaLista project (Naturalista 2021).

Roadkills allow verifying the presence of species with potential distribution in the area of collision sites, including common species, those considered rare or with a low frequency of observation, and species at risk (Krebs 2014; Abra *et al.* 2021). Roadkill records worth highlighting are those of endangered species, such as the jaguar and the ocelot; threatened species, such as the badger, and regional endemic species such as the antelope jackrabbit (*Lepus alleni*). A pregnant female raccoon (*Procyon lotor*) and young specimens of ocelot, lynx, and coati were also recorded. The jaguar cub was a male found on the road adjacent to the Meseta de Cacaxtla Flora and Fauna Protection Area, an ecological reserve demarcated by the México 015 federal highway in a 40 km stretch that goes from San Ignacio to Mazatlán, according to information from the Secretariat of the Environment and Natural Resources (SEMARNAT, in Spanish; SEMARNAT 2016). Vehicle traffic may be affecting the abundance and population structure of mammal species that move across its areas of influence, particularly in the case of rare species such as the badger (Bárcenas *et al.* 2009). This also occurs to endangered species such as felines for which roadkills have been reported throughout the country (González-Gallina and Hidalgo-Mihart 2018; Canales-Delgadillo *et al.* 2020).

According to previous reports, quantitative research addressing the impact of roads on wildlife is recent in México. Records of sporadic or fortuitous roadkills provide valuable data for recent studies on the subject. Three records of struck ocelots (González-Gallina and Hidalgo-Mihart 2018) have been found for Sinaloa. This information is valuable and reflects the need to focus efforts on the design of projects that generate systematic information on road ecology and assess the use and impact of infrastructure on wildlife (Canales-Delgadillo *et al.* 2020; Manteca-Rodríguez *et al.* 2021). The information recorded in the present study is the first effort to document wildlife roadkills occurring in the region. Academic and government organization should act coordinately to identify the sites or spots of greatest collision risk and the associated critical factors. Roadkill records can support the development of mitigation strategies to prevent wildlife deaths and injuries from collisions, which contribute to the loss of wildlife individuals and populations (Cáceres *et al.* 2010; Pacheco *et al.* 2016; Manteca-Rodríguez *et al.* 2021; Salom-Pérez *et al.* 2021). It is necessary to produce information to identify the critical wildlife crossing hotspots in the region to support road infrastructure design, construction, and management. It is equally important to provide resources for key aspects, such as proper signaling related to wildlife crossings that inform and raise awareness among road drivers to avoid hitting wild animals, which will contribute to wildlife conservation and the safety of road users.

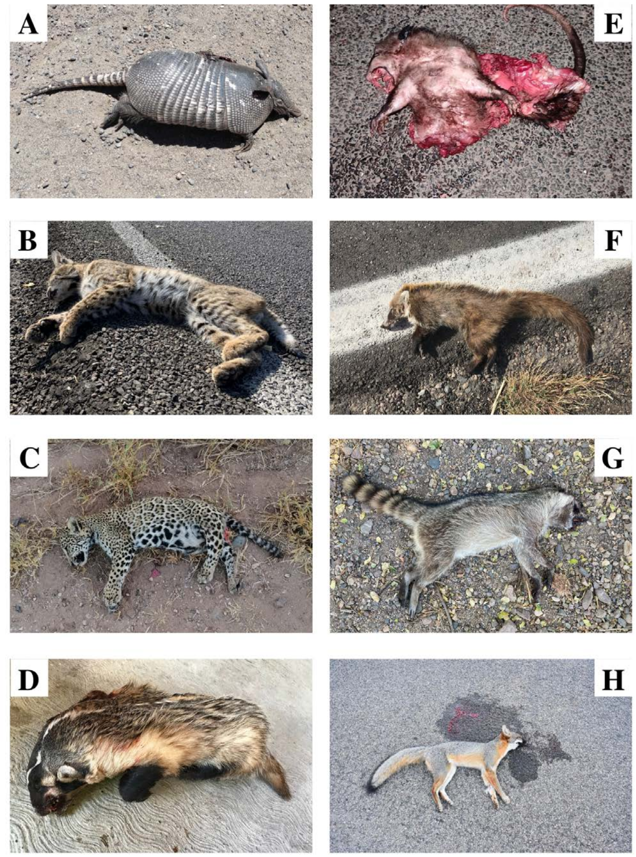

Figure 2. Mammals hit on roads of the south-central region of Sinaloa, México. A) *Dasypus novemcinctus* (Photograph by C. Anguamea), B) *Lynx rufus* (Photograph by B. Artigas), C) *Panthera onca* (Photograph by A. Loaiza), D) *Taxidea taxus* (Photograph by Y. Rubio), E) *Didelphis virginiana* (Photograph by C. Anguamea), F) *Nasua narica* (Photograph by Y. Rubio), G) *Procyon lotor* (Photograph by D. Alvarado), H) *Urocyon cinereoargenteus* (Photograph by Y. Rubio).

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Mammal use of some crossing structures in a Federal Highway in Jalisco, México

Uso por mamíferos de estructuras de cruce en una autopista federal en Jalisco, México

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Since the construction of Federal Highway 200 in the coast of Jalisco, México, there have been environmentally detrimental processes along its length. With no wildlife crossing structures built at the time, mammal connectivity has been "safely" maintained through streams and rivers and their corresponding bridges. We used photo-trapping to assess 2 bridges and 1 culvert in the vicinities of Careyes within the influence area of the Chamela-Cuixmala Biosphere Reserve from November 2020 to March 2021. We compared bridges with upstream cameras to see whether there were differences in species richness and relative abundance. With 736 camera / nights, we obtained a total of 421 mammal records, of which 252 were crossings of 14 mammal species, 12 native and 2 introduced beneath the highway, 241 through bridges, and 11 through the culvert. Upstream cameras showed higher mammal diversity values compared to the bridges. Streams and bridges serve to maintain mammal connectivity through the highway. Ungulates might cross the highway more often than carnivores due to their reticence of going near roads. Future wildlife crossing structures along this highway should keep this in mind.

Key words: Bridge; Careyes; Chamela-Cuixmala; drainage; underpass.

Desde la construcción de la carretera Federal 200 en la costa de Jalisco, México, se ha generado una serie de procesos de degradación a lo largo de ésta. Sin tener ningún paso de fauna construido, la conectividad "segura" de los mamíferos se ha mantenido a través de arroyos y ríos con sus correspondientes puentes. Utilizamos foto-trampeo para monitorear 2 puentes y 1 alcantarilla en las inmediaciones de Careyes, dentro del área de influencia de la Reserva de la Biosfera Chamela-Cuixmala de noviembre 2020 a marzo 2021. Comparamos los puentes con cámaras arroyo-arriba para ver si existen diferencias en riqueza de especies y abundancia relativa. Con un esfuerzo de 736 noches / cámara obtuvimos un total de 421 registros de mamíferos, de los cuales 252 correspondientes a 14 especies de mamíferos, 12 nativas y 2 introducidas, que cruzaron por debajo de la carretera, 241 a través de puentes y 11 por alcantarilla. Las cámaras arroyo-arriba mostraron una mayor diversidad de mamíferos comparadas con los puentes. Los puentes de los arroyos sirven para mantener la conectividad de los mamíferos a través de la carretera. Los ungulados pueden utilizar la carretera más frecuentemente que los carnívoros debido a su reticencia a acercarse a caminos. Futuras estructuras de paso de fauna en esta carretera deben tener lo anterior en mente.

Palabras clave: Careyes; Chamela-Cuixmala; drenajes; pasos de fauna; puente.

The environmentally most dangerous roads are those that penetrate relatively pristine regions (Laurance *et al.* 2009). New roads in forested areas can greatly increase deforestation because forest loss is spatially highly contagious (Boakes *et al.* 2010) and because an initial road often spawns networks of secondary and tertiary roads that can significantly increase the spatial extent of habitat disruption (Laurance 2015). The coast of Jalisco was practically isolated until the early 1970´s with the construction of Federal Highway 200. With the construction of the highway began a series of environmental deterioration processes with the following increase of human settlements, massive deforestation for agriculture and cattle grazing, and associated illegal hunting (Ceballos and García 1995). One of the few protected areas in that coast is the Chamela-Cuixmala Biosphere Reserve (CCBR; Ceballos and Garcia 1995), located on the coast of the State of Jalisco, protecting 13,142 ha of natural areas, mainly comprised of deciduous forest, gallery forest, and marshes. This place is home to 69 species of mammals out of which 16 species are considered endemic and 10 under protection (Instituto de Biología UNAM 2019). Most species are of terrestrial habits with only three strictly arboreal, and some other scansorial (Ceballos and Miranda 2000). The search for run over animals on several highways on the coast of Jalisco, including Highway 200, showed that the majority were mammals (57 %; Lara-Gómez 2008). The most frequent roadkills were opossums (*Didelphis virginiana*) and armadillos (*Dasypus novemcinctus*) with both covering 30 % of the records, followed by skunks (*Mephitis macroura*), raccoons (*Procyon lotor*), coatis (*Nasua narica*) and grey foxes (*Urocyon cinereoargenteus*; Lara-Gómez 2008).

No mitigation measures were built along this highway, but there are several bridges crossing the various streams and rivers of the area for topographic reasons. Even it has been observed that the use of a wildlife crossing structure does not necessarily equate to its effectiveness to maintain the connectivity on the landscape, several studies have demonstrated that a broad range of species use the structures, and the optimal design and placement of crossing structures is often species-specific and that crossing rates depend on both landscape and structural features (Van der Grift *et al.* 2013). Streams are important natural features that serve as biological corridors for most species. Many mammal species move through the landscape along riparian corridors and crossing structures along waterways will likely be optimal for them (Clevenger *et al.* 2002). Measuring the rate of use by wildlife is an important first step in almost every evaluation of wildlife crossing structures. In many studies, monitoring is limited to registering passing animals at the crossing structures, without measurements at control sites. Essential variables are often not measured, which hinders the interpretation of data and makes it impossible to compare the functioning of multiple structures (Van der Grift and Van der Ree 2015).

The present study objective assesses the use by mammals of some of the actual crossing structures along Federal Highway 200 to determine their potential role to facilitate the movement of medium and large size mammals between the coast and the upland area, in the influence area of Chamela-Cuixmala Biosphere Reserve. This study will serve as reference for further road projects and their mitigation efforts to maintain mammal connectivity in the area.

The study area is in the municipality of La Huerta, Jalisco, México, within the influence zone of the Chamela-Cuixmala Biosphere Reserve (CCBR), in the surroundings of Careyes residential area. We chose a 7 km segment of Federal Highway 200 connecting Melaque-Puerto Vallarta with Careyes as the center and monitored all existing crossing structures, consisting of 2 bridges, and 1 concrete culvert (Figure 1). The highway is paved and has two-way lanes and a width of 8 m. Each bridge was a concrete structure of *ca*. 15 m in length, 8 m wide and had a height of *ca*. 5 m (Figure 1A-B). The concrete arch culvert was of *ca*. 8 m length, 1 wide, 1.5 m high located 5 km south of the Careyes stream with rocky ground cover (Figure 1C). The terrain is rugged and hilly with seasonally dry streambeds, known locally as *arroyos*, separating the prominent hills. Streams are ephemeral, and during the dry season free water is found only in scattered, isolated pools in the *arroyos*. The study site has a dry tropical climate exhibiting a marked seasonality in precipitation, with 85 % of the 748 mm average annual rainfall occurring from June to November, and a prolonged drought from mid-February to late May (Bullock 1986). The dominant vegetation type is tropical dry deciduous forest, with semi-deciduous forest in the larger *arroyos* (Lott 1993).

Photo-trapping (Mixmart HC-800A) was used to record mammal crossings beneath the highway on the Careyitos and Careyes streams and a natural drain going through 1 concrete culvert. We set 5 camera trap stations in total. Two camera trap stations on each stream, located one under each bridge where the stream crosses the highway and one upstream further away from the highway (300 m in the Careyitos stream, and 1,000 m for Careyes stream); another in the entrance of the arch culvert. Structures were surveyed for approximately 5 months (November 2020 to April 2021), covering most of the dry season, when stream beds are dry, and mammals can walk freely without water hindering their motion. Cameras were programmed with a 16 MP resolution, high sensitivity in the motion sensor, to fire 3 shots for each triggering event with a one-minute delay.

Once retrieved from the memory cards, the photographs were stored and processed using Camera Base 1.7 (Tobler 2010). We excluded all the non-mammal pictures. We identified the photographed species using Ceballos and Miranda (2000). We considered independent events those consecutive photographs of the same species separated for at least 1 hr (Tobler *et al.* 2008). We used independent photographic events to approximate the abundance of each species (Ramesh and Downs 2015). To calculate the sampling effort per station, we obtained the number of camera days that each camera station functioned, counting the number of days from activation to the last photograph taken. We considered a camera night to be a period of 24 hr during which the camera was operating. Total sampling effort for each structure was obtained by adding the number of camera days that each camera station operated on each station (Ramesh and Downs 2015). For each target species, capture rate was calculated as an estimate of relative abundance: Capture rate = number of detections / number of camera trap nights *100 (Glen *et al.* 2014).

We performed all the data analyses with the independent events obtained as a proxy for species abundance for each station and then by stream (Careyes and Careyitos) comparing bridge stations against upstream stations. We did asymptotic diversity profiles, based on statistical estimation of the true Hill number for q = ^0D (species richness, Sobs), ^1D (Shannon´s exponential index, e^H) and ^2D (inverse Simpson´s index, 1 / D) for the different bridge and upstream stations using iNext software (Chao *et al.* 2016). We estimated diversities for standardized samples with a common sample size or sample completeness. Hill numbers, or the effective number of species, are increasingly used to characterize the taxonomic, phylogenetic, or functional diversity of an integrated assemblage curves. It is based on sampling theory that smoothly link rarefaction (interpolation) and prediction (extrapolation) and standard-

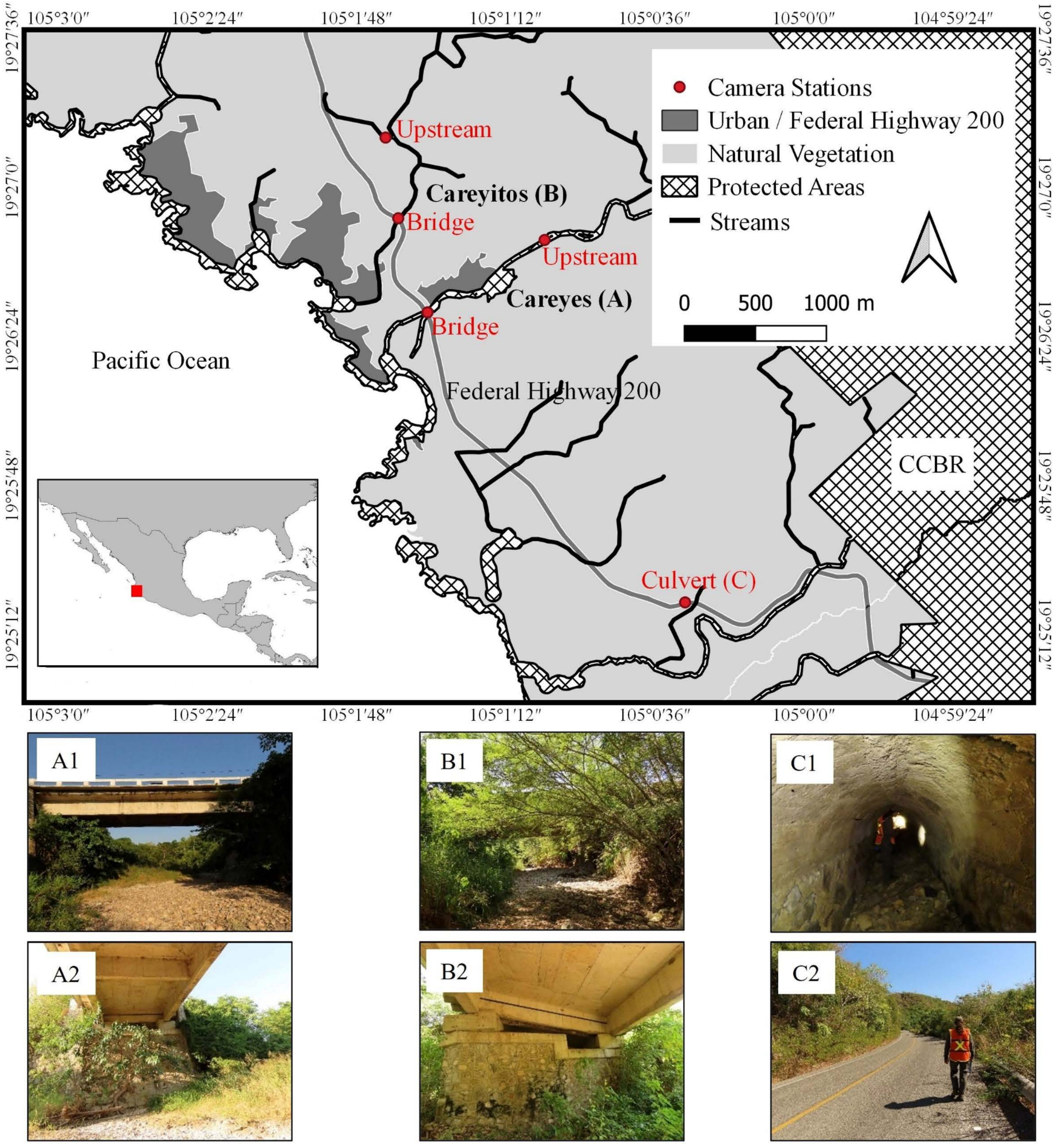

Figure 1. Location of surveyed drainage structures along Federal Highway 200, Jalisco, México. In map: Careyes (B1) and Careyitos (B2) streams with their bridges and their upstream cameras (U1 and U2) and concrete culvert with close-ups of the different streams and structures. A1) Careyes bridge viewed from stream and A2) under bridge; B1) Careyitos stream and B2) under bridge; C1) concrete culvert, C2) culverts location from highway at the end of the curve. All crossing structures crossed the whole width of the highways 8 m. Chamela-Cuixmala Biosphere Reserve and other protected areas appear with a grid, urban areas and highway in dark grey, streams in black, camera stations in red.

izes samples based on sample size or sample completeness and facilitate the comparison of biodiversity data (Chao *et al.* 2014). We did rank-abundance graphics for beta diversity and community structure to see how species changed in terms of photographic events recorded for each station.

With an effort of 736 camera / nights we recorded a total of 421 mammal records, of which 252 were crossings beneath Federal Highway 200, 241 through both bridges (158 for Careyes and 83 for Careyitos) and 11 through the culvert crossings (Table 1). A total of 14 mammal species were recorded, of which 3 are species under protection (NOM-059-SEMARNAT-2010; SEMARNAT 2010): the ocelots (*Leopardus pardalis*) and the jaguarundi (*Herpailurus yagouaroundi*) both considered endangered (P), and the pygmy

Table 1. Mammal species recorded crossings beneath Federal Highway 200 at Careyes and Careyitos bridges together with a nearby culvert in Jalisco, México. Each bridge had an upstream camera station on the same streams for comparison.

Family	Species	Endemic	NOM-059-SEMARNAT-2010	Careyes Bridge	Careyes Upstream	Careyitos Bridge	Careyitos Upstream	Culvert
Didelphidae	*Didelphis virginiana*			8	11	54	20	
Canidae	*Canis familiaris**			1	1			
	Canis latrans					1		
	Urocyon cinereoargenteus			1	4	3		
Procyonidae	*Nasua narica*			2	8	5	14	6
	Procyon lotor			9		1	1	
Mephitidae	*Spilogale pygmaea*	X	A		1			
Felidae	*Felis catus**			2				
	Herpailurus yagouaroundi		A					1
	Leopardus pardalis		P	5	19	2	46	2
	Puma concolor				7		9	
Leporidae	*Sylvilagus cunicularius*	X					1	
Cervidae	*Odocoileus virginianus*			77	7	14	5	
Tayassuidae	*Dicotyles angulatus*			53	0	3	15	2
Total records	421			158	58	83	111	11
* Invasive species								

skunk (*Spilogale pygmaea*) as threatened (A). When comparing bridges with the upstream cameras and their diversity profiles (q0 - q2) we found no significant difference (as confidence intervals overlap) speaking of species richness (q0 = 8.99 - 9.99). Nevertheless, differences appear (q1 - q2) where diversity values are lower under the bridge (3.47 - 3.84) than farther upstream (5.3 - 7.49; Figure 2). When comparing bridges against the culvert we found that the bridges allow far more species (q0 = 9.9 average) than the culvert (q0 = 4.23 average). Also, species composition (species related to their relative abundance) varied between the bridge and upstream camera stations (Figure 3). In general terms, species with the highest number of records using bridges were white-tailed deer (*Odocoileus virginianus*, 91), opossums (*Didelphis virginiana*, 62) and peccaries (*Dicotyles angulatus*, 56). While in upstream cameras, highest number of records were for ocelots (65), opossums (31), coatis (22), pumas (16), white-tailed deer (12), and peccaries (15) though not present in one upstream station (Tables 1 and 2).

We found differences in species composition and abundance in the same streams between the bridges and the upstream stations. The bridges had a higher ungulate presence while the upstream had more felines (pumas and ocelots). This could be explained by the reluctance of felines towards roads as seen by Conde *et al.* (2010), where jaguar probability of occurrence declines with increasing proximity to roads. If this is true, then ungulates might be closer to the highway as a safer zone for them. This does not mean that predators such as felines do not cross beneath

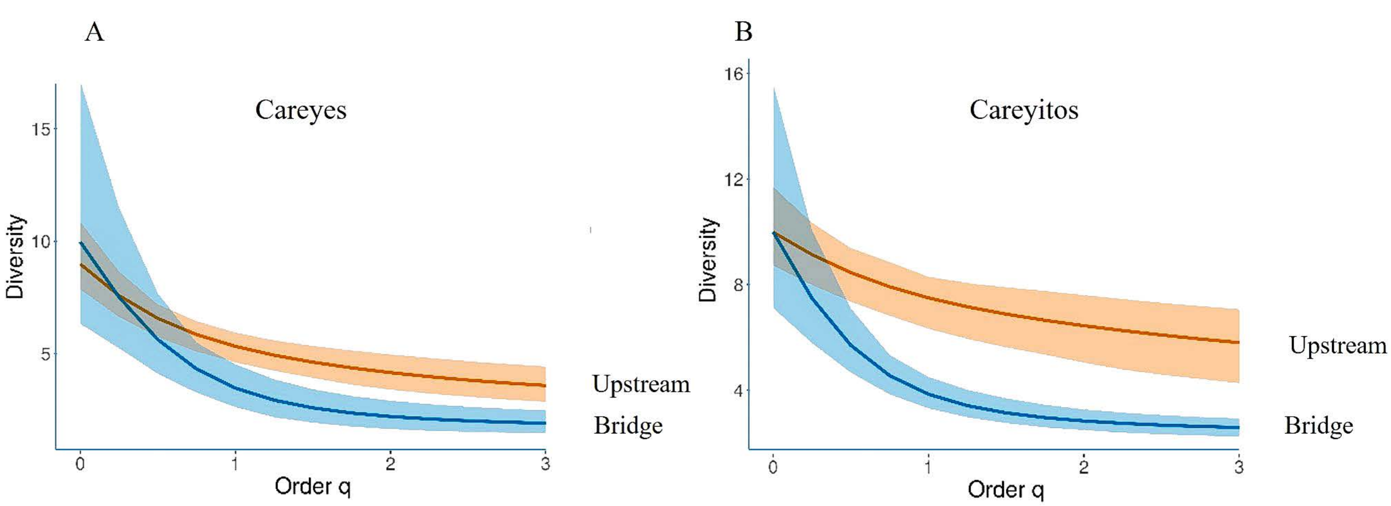

Figure 2. Diversity profile (q0 - q2) of Careyes and Careyitos streams. A) Careyes and B) Careyitos with bridge and upstream diversity profile, respectively. Species richness (q0), inverse Simpson (q1) and Shannon exponential (q2).

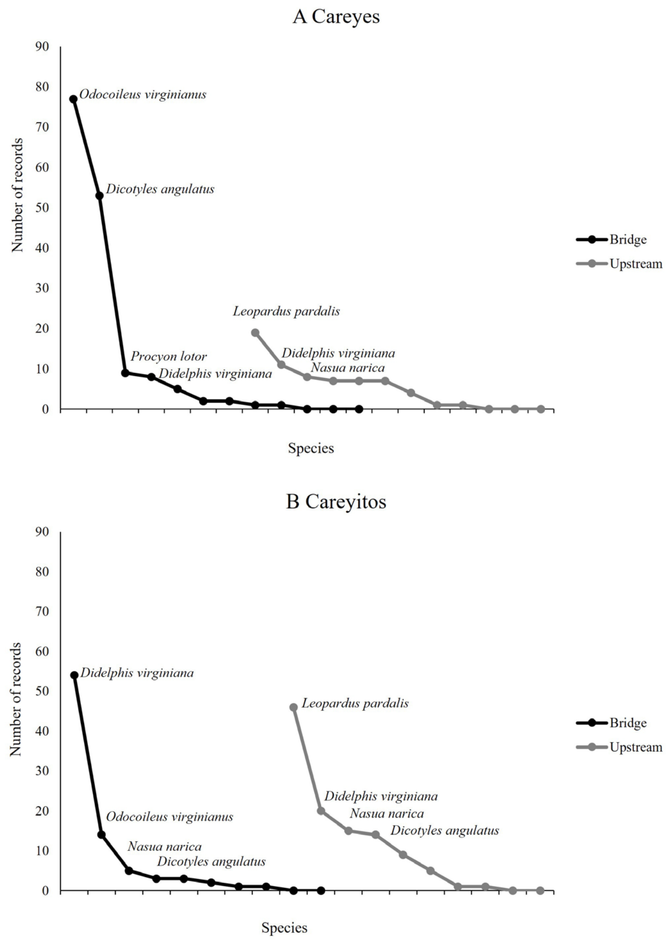

Figure 3. Mammal community assemblage per station in Careyes and Careyitos streams. A) Careyes and B) Careyitos with bridge community in black and upstream community in grey, with most abundant species labeled.

the highway, but they might be doing so in a lesser degree. In the case of ocelots, we have evidence they are going through both bridges and culverts, with bigger species such as pumas and jaguars. Even though we did not have records, there is evidence that these species can use these crossing structures (González-Gallina *et al.* 2018).

We found streams enable connectivity for ungulates across the highway, as we registered both white-tailed deer and collared peccaries of both sexes, adults and offspring crossing beneath the bridges. Till this point, it was unclear if these ungulates would use streams as a way of moving around the landscape. Previous studies by Mandujano *et al.* (2002) found in the CCBR that white-tailed deer preferred hillsides oriented to the north rather than streams and that peccaries formed bigger groups near streams for better protection against predators (5-12 individuals; Mandujano and Martínez-Romero 2002). Jaguars and pumas in the CCBR prefer streams and their associated vegetation (subperennifolia forests) using them to move through the landscape (Núñez-Pérez and Miller 2019).

Contrary to previous reports in México (González-Gallina *et al.* 2018), we recorded highway crossing of peccaries in bridges and culverts, indicating that there is probably some behavioral trait there that prevents them from using such structures in the Yucatán Peninsula. The fact that the González-Gallina *et al.* (2018) study was performed only a short period of time after the construction of the wildlife underpasses could prevented the use of this infrastructure by peccaries, different to what occurs in our study where the highway, the bridges and the culvert have been present more than 40 years. This could indicate that peccaries need a more extended habituation period to use wildlife crossing infrastructure.

Table 2. Capture rate for mammal species recorded crossing Federal Highway 200 through bridges and nearby culvert in Jalisco, México. Each bridge had an upstream camera station on the same streams for comparison.

Species	Careyes		Careyitos		Culvert
	Bridge	Upstream	Bridge	Upstream	
Didelphis virginiana	5.13	8.03	34.39	12.74	
Canis familiaris	0.64	0.73			
Canis latrans			0.64		
Urocyon cinereoargenteus	0.64	2.92	1.91		
Nasua narica	1.28	5.84	3.18	8.92	4.65
Procyon lotor	5.77		0.64	0.64	
Spilogale pygmaea		0.73			
Felis catus	1.28				
Herpailurus yagouaroundi					0.78
Leopardus pardalis	3.21	13.87	1.27	29.3	1.55
Puma concolor		5.11		5.73	
Sylvilagus cunicularius				0.64	
Odocoileus virginianus	49.36	5.11	8.92	3.18	
Dicotyles angulatus	33.97		1.91	9.55	1.55
Sampling effort (number of night traps)	156	137	157	157	129

When building a highway that crosses a stream that exceeds the need for a simple drainage structure, it's common practice to build a bridge. In Chamela, streams are usually ephemeral running with water for just a couple of months a year, which means most of the time they remain perfectly traversable trails across the landscape. Our results showed that at least during the dry season, these structures allow mammals to pass safely below the highway, either along with the riverine vegetation or through the stream bed.

Even though some medium sized mammal species are able to use the culvert, highway drainages cannot substitute bridges or proper wildlife underpasses in terms of mammal species crossings. González-Gallina *et al.* (2018) showed that large wildlife crossing structures allow more species and larger animals to cross through the highway in tropical areas. In order to increase the wildlife crossing capacity of the culverts along the highway, future retrofitting projects should consider increasing the size of these structures to allow more species and individuals to use them, and also prevent flooding during the rainy season, all structures could benefit from drift fencing to funnel wildlife through safe passages.

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Drainage culverts as a measure to avoid mammal roadkills in Costa Rica: the case of *Dasyprocta punctata*

Estructuras de drenaje como medida para evitar la mortalidad por atropello de mamíferos en Costa Rica: el caso de *Dasyprocta punctata*

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Wildlife passages are structures that connect habitats, populations and reduce wildlife mortality. In places with no road mitigation, wildlife can resort to using underground structures called drainage culverts to prevent direct road-crossing. To determine which mammal species generally benefit from these structures, we placed camera traps in 6 drainage culverts and compare the number of road-killed species by vehicle tours along a 30 km segment of the North Inter-American Highway in Costa Rica. We detected 14 mammal species using drainage culverts as wildlife passages. The Central American agouti (*Dasyprocta punctata*) showed the highest number of records. The absence of *D. punctata* roadkill records and the high culvert use rates suggests that these structures may be effective in roadkill mitigation for this species, allowing them to cross safely from one forest patch to another.

Key words: Animal behaviour; Central American agouti; Pan-American Highway; road ecology; wildlife passages.

Los pasos de fauna son estructuras que conectan hábitats, poblaciones y reducen la mortalidad de la vida silvestre. En sitios donde no hay mitigación vial, la fauna puede recurrir a estructuras de drenaje subterráneas llamadas alcantarillas, para evitar el cruce directo por la carretera. Para determinar de manera general qué especies de mamíferos se ven beneficiadas por estas estructuras, colocamos cámaras trampa en 6 alcantarillas y comparamos cuantificando las especies de mamíferos que mueren atropelladas mediante recorridos en vehículo a lo largo de 30 km de la carretera Interamericana Norte en Costa Rica. Detectamos 14 especies de mamíferos utilizando las alcantarillas como pasos de fauna, siendo el agutí centroamericano (*Dasyprocta punctata*) la especie con mayor número de registros. La ausencia de registros de atropello de *D. punctata* y la alta tasa de uso de alcantarillas sugiere que estas estructuras pueden ser efectivas en la mitigación de atropellamiento para esta especie y son un medio para cruzar de un bloque boscoso a otro.

Palabras clave: Carretera Panamericana; comportamiento animal; ecología de carreteras; guatusa centroamericana; pasos de fauna.

Roads running through national parks have multiple effects on wildlife, with mortality due to road collisions being the most researched impact (Andrews 1990; Bennett 1999). The construction of wildlife passages, including underpasses, overpasses, and oversized drains, is one of the most popular measures to reduce animal-vehicle collisions (mitigation) and connect habitats fragmented by roads (Beckmann *et al.* 2010).

Some studies have determined that the effectiveness of this type of mitigation varies depending on the size of the structure, the building material, and the location of the construction (Forman *et al.* 2003; Langton 2015). In addition, this mitigation is not equally effective for all taxa, as species use structure designs differently (Clevenger and Waltho 2000). Most roads in Latin America lack mitigation structures (Pinto *et al.* 2020), so wildlife crossing from one side of the road to the other resorts to non-specialized structures such as drainage culverts. These culverts are short ducts built at road intersections with ravines, streams, or rivers, and are usually designed according to the expected water flow (Carmona 2013) rather than on the local wildlife species (Torres 2011). In this work, we compared the species richness of both road-killed mammals and mammals that use drainage culverts to travel across roads in a segment of the North Inter-American Highway, to determine the potential relationship between roadkill rate and use of drainage culverts.

The North Inter-American Highway is one of the two sections of the Pan-American Highway that crosses Costa Rica. The study area comprises a 30 km segment that goes from Tempisquito River (10° 48′ 56.58″ N, 85° 32′ 38.25″ W) to the Santa Cecilia crossing (11° 2′ 46.64″ N, 85° 37′ 35.30″ W). This section crosses the Guanacaste Conservation Area (ACG, in Spanish), a site comprising 163,000 ha of Protected Wildlife Area belonging to 2 national parks: Guanacaste and Santa Rosa (Janzen and Hallwachs 2016; Figure 1). Nineteen drainage culverts were observed in this segment of the Inter-American Highway crossing the ACG.

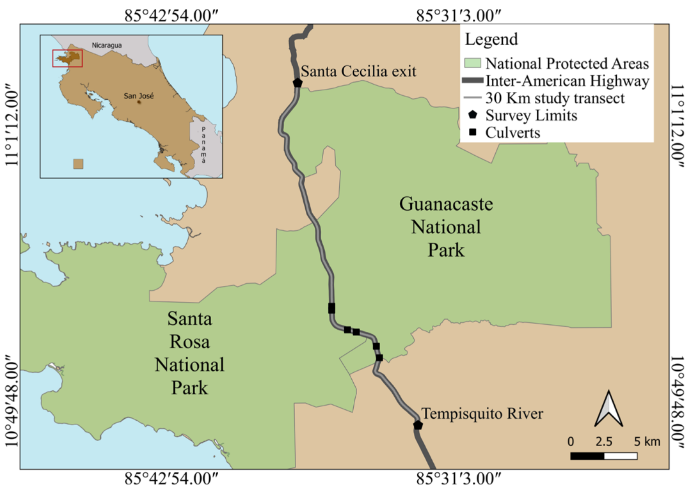

Figure 1. Study area (North Inter-American Highway) in Guanacaste, Costa Rica. The 30-kilometer segment used for road-killed mammal sampling is highlighted in grey, and black squares mark the location of culverts with a camera trap installed.

These structures vary in shape, building material, length, and number of entrances at each passage, and are used by multiple wildlife species (Torres 2011; Figure 2).

To identify the richness of species that use these structures, we placed one camera trap (SG570V; Scoutguard, Santa Clara, California) in 6 of the 19 underground culverts on the same road segment (Figure 1). Cameras were placed in culverts with low probability of flooding and theft, from November 2020 to September 2021. They were set to capture 2 photographs and a 5-second video when motion was detected through an infrared sensor, and remained operational 24 hours a day. An event was considered independent when the photograph or video had been captured 30 minutes apart from the next (O'Brien *et al.* 2003). The capture rate per species was calculated as the number of captures divided by the number of sampling days, multiplied by 100. To quantify the richness of road-killed species, we traveled the 30 km segment of the road in a vehicle with two observers at 25 km / hr. When a road-killed animal was spotted, we stopped the car to identify the species using field guides (Langen *et al.* 2007; Reid 2009) and recorded the event coordinates with a Garmin GPSMAP 64 (GPS). This procedure was performed for 3 consecutive days per month, from 19:30 to 22:00 hr and from 5:00 to 7:30 hr.

We captured 183 photographs of 14 mammal species using culverts as wildlife passages over 309 trap days. The species with the highest number of records (54 %) was the Central American agouti (*Dasyprocta punctata*), followed by the southern or black-eared opossum (*Didelphis marsupialis*), with 12 % of captures. On the other hand, we found 28 road-killed individuals of 14 mammal species, none of them belonging to *D. punctata* (Table 1).

Although the North Inter-American Highway lacks drainage culverts tailored to the local fauna, the absence of *D. punctata* roadkill records and the high culvert use rates suggest that these structures may be effective in roadkill mitigation for this species and allows crossing from one forest patch to another. This report is important since roads act as barriers for small and medium-sized mammals such as the Central American agouti, also affecting seed dispersal and forest regeneration (Lambert *et al.* 2014). Maintaining this connectivity is crucial as *D. punctata* is one of the main means of seed dispersal for many trees within its distribution range (Smythe 1978; Hallwachs 1986).

These results are consistent with those reported by Torres (2011), who also notes that the time of the day when these culverts are used by *D. punctata* is associated with the noise level on the road. Being a species with shy behavior (Smythe 1978), the agouti is expected to avoid roads due to traffic noise. According to the 2015 yearbook of traffic information (MOPT 2015), the North Inter-American Highway has an average daily traffic of 2,361 to 4,998 vehicles, which corresponds to a high-risk traffic level for wildlife (Clevenger *et al.* 2003).

Table 1. Number of photographs captured by camera traps placed in 6 drainage culverts and number of roadkill records in a 30-kilometer segment of the North Inter-American Highway, Costa Rica.

Species	Culvert use rate (% of captures)	Capture rate (No. of captures /No. of sampling days × 100)	Number of roadkill records
Dicotyles crassus	0.54	0.32	0
Odocoileus virginianus	1.09	0.32	0
Puma concolor	1.09	0.32	0
Nasua narica	1.09	0.64	1
Tapirella bairdii	1.09	0.64	1
Dasypus novemcinctus	2.18	1.29	1
Heteromys sp.	2.18	1.29	10
Cuniculus paca	2.73	1.61	0
Conepatus semistriatus	3.27	1.94	2
Leopardus pardalis	4.37	2.58	2
Saccopteryx sp.	4.37	2.58	3
Eira barbara	10.92	6.47	0
Didelphis marsupialis	12.02	7.11	8
Dasyprocta punctata	54.09	32.03	0

Figure 2. Culverts or drainage structures built on the 30-kilometer segment of the North Inter-American Highway. These structures are built according to the rivers and streams running across roads, rather than to animals that may cross the road.

Although culverts may play a mitigating role for *D. punctata*, building and adapting proper drainage in the underground sewer system is required. It has been reported that some species do not cross if the terrain is flooded or uneven (Jochimsen *et al.* 2004), as in the case of the structures in the study site. In addition, other taxonomic groups with high mortality rates, such as the cane toad (*Rhinella horribilis*) and other amphibian species not included in this note, are unable to detect the presence of nearby culverts to walk towards it (Bouchard *et al.* 2009; Cunnington *et al.* 2014). Hence, building and adapting culverts with accessory structures such as barrier fencing (2 – 2.5 m high wire mesh fences running parallel to the road; Littlewood *et al.* 2020) are essential to benefit other fauna species.

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Seasonal variation of mammal roadkill hotspots in the Sierra Madre Occidental, México

Variación estacional de los puntos críticos de atropellamiento de mamíferos en la Sierra Madre Occidental de México

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Roadkill hotspots are spatially aggregated sites that are not distributed at random. In the case of mammals, hotspots are used as a criterion to assess the locations of roadkill mitigation works, although these sites can vary at different time scales. The objective of this study was to identify the changes in mammal roadkill hotspots between two seasons of the year on a highway in the Sierra Madre Occidental, northeastern México. Mammal road-killed species were monitored through 2 vehicle tours per season, with 15 days of separation between them. The 40D highway (Durango-Mazatlán) was traveled in spring 2019 and 2020 and autumn 2018 and 2019. Mammal roadkill hotspots in spring, autumn, and both seasons combined were identified using geographic information systems. A total of 217 mammal roadkills were recorded during 8 road tours. Wildlife roadkill hotspots were not spatially consistent between stations or when all records were compared. The spatial aggregation of mammal roadkills varied over time, which could be related to changes in the movement of fauna and other factors. The seasonal variation of these hotspots should be considered for the implementation of mitigation measures, and systematic monitoring of road-killed fauna should be conducted.

Key words: Geographic information systems; road ecology; road-killed fauna; Sierra Madre Occidental; spatial distribution.

Los puntos críticos del atropellamiento son aquellos sitios agregados espacialmente que no corresponden al azar. Para el caso de mamíferos, los puntos críticos han sido considerados como una de las aproximaciones para la ubicación de las obras de mitigación del atropellamiento, aunque estos sitios pueden ser variables a escalas temporales. El objetivo de este estudio fue identificar los cambios de los puntos críticos del atropellamiento de mamíferos entre dos temporadas en una carretera en la Sierra Madre Occidental en el noreste de México. Para el monitoreo de las especies atropelladas se realizaron 2 recorridos en vehículo por temporada con 15 días de separación entre recorridos. Estos se realizaron en la primavera de 2019 y 2020 y otoño de 2018 y 2019 en la carretera 40D (Durango-Mazatlán). Utilizando herramientas de sistemas de información geográfica, se estimaron los puntos críticos de atropellamiento de mamíferos para la primavera, el otoño y ambas estaciones. Se obtuvieron 217 registros de mamíferos silvestres atropellados en 8 recorridos sobre la carretera. Los puntos críticos de atropellamiento de fauna no coinciden espacialmente entre las estaciones, ni al compararlos con todos los registros. La acumulación espacial del atropellamiento de mamíferos no fue coincidente en el tiempo, lo cual podría relacionarse con los cambios en el movimiento de la fauna y otros factores. Se debe considerar la variación estacional de estos puntos críticos para las obras de mitigación, así como realizar monitoreos sistemáticos de la fauna atropellada.

Palabras clave: Distribución espacial; ecología de carreteras; fauna atropellada; Sierra Madre Occidental; sistemas de información geográfica.

Roadkills represent an important mortality factor that threatens mammal species sensitive to anthropic disturbances (Forman and Alexander 1998; Havlick 2003; Benítez-López *et al.* 2010), since some animals may be attracted to roads when searching for food such as carrion, which may lead to wildlife-vehicle collisions and death (Forman and Alexander 1998; Spellerberg 1998; Arroyave *et al.* 2006; Monge-Nájera 2018). Therefore, it is essential to build safe wildlife passage structures to avoid animal-vehicle collisions (van der Grift *et al.* 2013). However, these generally involve high costs and there is controversy regarding their effectiveness (van der Ree *et al.* 2015). Understanding which wild mammal species are particularly vulnerable to collisions and their spatial and temporal distribution is essential for mitigating adverse road impacts. One of such impacts is the barrier effect of roads that reduce landscape connectivity for certain species and restrain their capacity to inhabit all available areas, with long-term consequences on the persistence and local viability of these populations (Forman *et al.* 2003; Filius *et al.* 2020).

The frequency of mammal roadkills is affected by structural aspects of the road, traffic flow, and ecological factors (Rytwinski *et al.* 2015). Vehicle collisions with animals tend to occur at certain times of the year, reflecting the life cycles of the species affected (Hothorn *et al.* 2015). For instance, mammal migration and dispersal movements increase the probability of encountering roads that limiting their free movement across the road or lead to a roadkill event (Arroyave *et al.* 2006; Zhang *et al.* 2018). Changes in traffic volume and speed, as well as the time of the day, also influence the collision rate; for example, the visual acuity of drivers can be reduced at night (Forman and Alexander 1998; Arroyave *et al.* 2006; Driessen 2021).

The sites with higher collision rates are segments where the paths traveled by animals are blocked by the road (Forman and Alexander 1998), including riverbeds, streams, or water runoff that cross the road through transverse structures (*e.g.*, major drainage structures; Forman *et al.* 2003) and tunnels. However, structures such as bridges and culverts can function as safe mammal passages, as long as access to the road is prevented with wire fences. On the other hand, if drainage structures and tunnels are not given proper maintenance, their access can be blocked by vegetation, forcing animals to cross over the road and contributing to their death by collision (Cervantes-Huerta *et al.* 2017).

The objectives of this work were to identify and quantify road-killed wildlife mammals, determine mammal roadkill hotspots cartographically, and compare the spatial pattern of collisions between two seasons. We hypothesized that the sites with the highest probability of collision events differ between seasons due to the variation in traffic volume and wildlife movements associated with dispersal and migration throughout the year. Therefore, mammal roadkill hotspots will change over time and space.

The study area was the 40D highway (Durango-Mazatlán), a two-lane road of 230 km long and 12 m wide bordered by road shoulders on both sides. The section studied runs from kilometer 37 (23° 58′ 32.19″ N, 104° 58′ 1.81″ W) in the Durango municipality to kilometer 155 (23° 32′ 37.4″ N, 105° 45′ 23.5″ W) in the Pueblo Nuevo municipality, both in the state of Durango. This section crosses the Sierra Madre Occidental, where the dominant types of vegetation are temperate pine-oak forest and, to a lesser extent, secondary vegetation and areas used for agricultural or livestock activities. The study area covers altitudes from 2,347 to 2,652 m; the prevailing climate is semi-cold sub-humid (INEGI 2008).

Roadkill records were obtained through 2 vehicle tours made in contrasting seasons in terms of water availability at the study sites: spring (the driest season) and autumn (the end of the rainy season; SMN 2010). Both sampling events at each season took place at least 15 days apart. A total of 8 tours were made in autumn 2018 and 2019 and spring 2019 and 2020. The vehicle tours were traveled at an average driving speed of 30 km / hr to look for mammal carcasses over the whole road width by a dedicated observer and a driver (Planillo *et al.* 2018). When a carcass was spotted, the species was recorded if the state of decomposition allowed it; otherwise, photographs were captured using reference scales for subsequent identification with the assistance of experts. In addition, the coordinates were recorded with a GPS (Garmin eTrex® 30x). Afterward, the carcass was removed to avoid recoding duplicate data in subsequent tours.

To identify mammal roadkill hotspots, *i.e.*, sites with a non-random accumulation of records, the Hotspot Analysis plugin (Oxoli *et al.* 2018) of the QGIS 3.16 software was used. The data of all mammals for spring and autumn were entered both separately and pooled together. To assess the hypothesis that all roadkill hotspots display a random spatial distribution, the Getis-Ord G_i* statistic was used to determine the degree of association between the points corresponding to records of mammal roadkills (Getis and Ord 2010). Positive (hotspots) and negative (coldspots) *Z*-values were obtained from this analysis. Then, confidence intervals were calculated to estimate whether the aggregation was random. Data for domestic mammals (*e.g.*, dogs and cats) were excluded from the analysis for being non-native fauna.

A total of 217 records of road-killed mammals were obtained during 8 tours. Eighty-three individuals were recorded in autumn and 134 in spring. The raccoon (*Procyon lotor*) showed the highest number of records, followed by rock squirrel (*Otospermophilus variegatus*), and eastern cottontail (*Sylvilagus floridanus*; Table 1). It is worth highlighting the roadkill records of 1 puma (*Puma concolor*) and 2 collared peccaries (*Dicotyles tajacu*; Table 1) since, due to their large body size, these species may pose a safety hazard for drivers. In addition, these species are of importance in hunting (SEMARNAT 2021), a key economic activity in the region.

Regarding the analysis of hotspots, the sites with the highest mammal roadkill rates varied between seasons; hotspots also differed when all records were combined (Figure 1). During spring, there were no hotspots with a confidence level greater than 90 %, according to *Z*-values (1.65 ≥ *Z* or *Z* ≥ 1.65); when data for both seasons were combined, some hotspots disappeared. Some of these hotspots coincide with sites where there are no major drainage structures and tunnels, but also with sites where these structures exist.

In this study, we compared the mammal roadkill hotspots identified from sampling in two different seasons. Our results suggest that mammal roadkill hotspots varied over the seasons and also when estimated with data for both seasons combined. Other studies also suggest spatial and temporal variations in roadkills (Canal *et al.* 2018; Bastos *et al.* 2019). In contrast with the observations in the present study, Bastos *et al.* (2019) reported a higher number of vertebrate roadkills in the rainy season. Also, Bueno and Almeida (2010) mentioned that the movements of mammals tend to increase during the dry season in search of resources, which explains the higher frequency of roadkill events in spring in our study. Multiple factors may affect the movement of wild animals, and hence the probability of death by collision; these include hydrological processes (*e. g*., rainfall, water runoff, infiltration) and the phenology of the local vegetation and wild mammals, including migration and dispersal (Arroyave *et al.* 2006; Bauni *et al.* 2017).

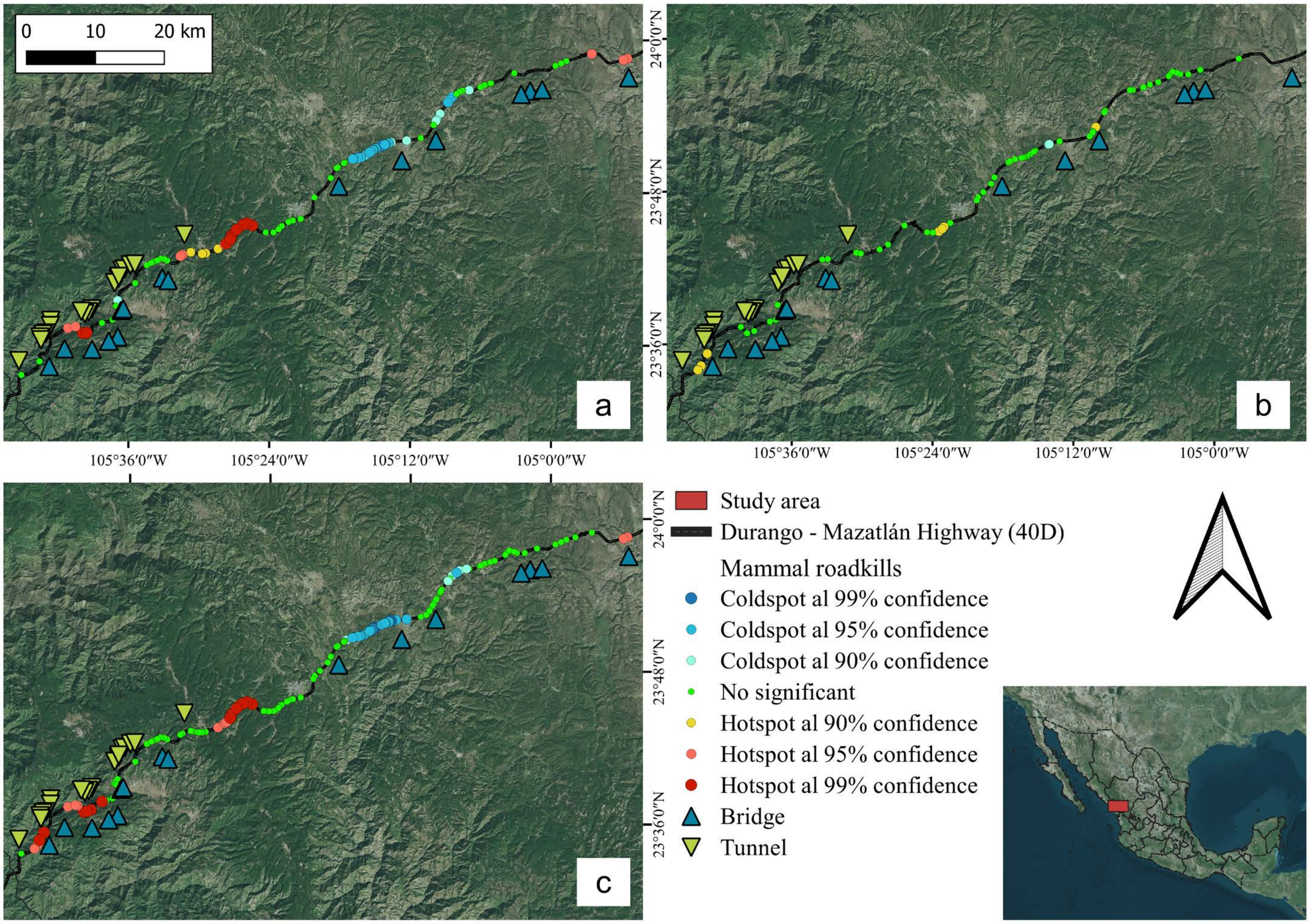

Figure 1. Study area. Location of critical points: hotspots (yellow and red circles), coldspots (blue circles). a) spring, b) autumn, c) both seasons, on 40D highway (Durango-Mazatlán), México, between years 2018 and 2020. Green (non-significant) circles correspond to sites of mammal roadkills with a random spatial clustering.

Although hotspots are neither static in time nor identical for all species, the identification of hotspots and the factors influencing their spatial and temporal patterns can support improved measures to mitigate adverse road impacts on wildlife (Monge-Nájera 2018; Bíl *et al.* 2019). This should be conducted along with studies to gain a deeper understanding of the distribution, abundance, and movement patterns of wild animals in the area surrounding the road (Ascensão *et al.* 2019).

Similar to other roads of México, major drainage structures such as bridges, culverts, and tunnels are considered wildlife passages without being designed for this purpose or maintained properly to ensure that wild animals can use these structures to cross the road safely (van der Grift *et al.* 2013; Cervantes-Huerta *et al.* 2017). In this study, hotspots were observed mainly in sites far from major drainage structures and tunnels, but also in sites where these have been built, suggesting that these structures are ineffective for reducing mammal roadkills. The streams flowing and the vegetation growing in and around structures can prevent free crossing through drainage structures considered wildlife passages (SCT 2012), so it is recommended to provide regular maintenance to sewers.

Road-killed fauna and wildlife passages should be systematically monitored to apply the most appropriate mitigation measures (Bauni *et al.* 2017) and identify those sites where wild animals can cross paved roads, to build barriers aiming to prevent animal crosses (van der Grift *et al.* 2013; Cervantes-Huerta *et al.* 2017). On the other hand, seasonal variation in the location of hotspots represents a challenge for the application of these and other mitigation measures (Iuell *et al.* 2003; Bauni *et al.* 2017) because they are expensive; thus, if they are not sufficiently effective, their application becomes feasible. More effective methods should be developed to identify these sites, which would support the effective mitigation of adverse effects of roads on wild mammals, considering their biological characteristics and other aspects of the local vegetation and the terrain that could serve for the identification of optimal sites for implementing these measures. The current policies of the Secretariat of Communications and Transportation (SCT, in Spanish) and the office of Federal Roads and Bridges (CAPUFE, in Spanish) of México do not support research on road ecology. Therefore, conducting studies on this field requires strengthening the links between academic researchers and construction managers. Although significant progress has been achieved, much remains to be done.

Table 1. Total records of road-killed mammals by order and family on the Durango-Mazatlán 40D highway, México during spring and autumn between years 2018 and 2020.

Order	Family	Scientific name	Records
Artiodactyla	Tayassuidae	*Dicotyles tajacu*	2
Carnivora			59
	Canidae		19
		Canis latrans	2
		Canis lupus familiaris	12
		Canis sp.	2
		Urocyon cinereoargenteus	3
	Felidae		10
		Felis catus	7
		Lynx rufus	2
		Puma concolor	1
	Mephitidae		14
		Conepatus leuconotus	1
		Mephitis macroura	5
		Mephitis sp.	7
		Spilogale gracilis	1
	Mustelidae	*Mustela frenata*	1
	Procyonidae		14
		Nasua narica	1
		Procyon lotor	13
	Unidentified		1
Chiroptera	Unidentified		1
Didelphimorphia	Didelphidae	*Didelphis virginiana*	8
Lagomorpha	Leporidae	*Sylvilagus floridanus*	12
Rodentia			72
	Cricetidae		9
		Neotoma mexicana	1
		Neotoma sp.	4
		Peromyscus sp.	4
	Sciuridae		20
		Otospermophilus variegatus	12
		Sciurus nayaritensis	8
	Unidentified		43
Mammalia			82

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Population decline of an endangered primate resulting from the impact of a road in the Catazajá wetlands, Chiapas, México

Reducción poblacional de un primate en peligro por el impacto de una carretera en los humedales de Catazajá, Chiapas, México

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The construction of large infrastructures such as roads poses a major threat to biodiversity due to wildlife roadkills. Some of the underlying threats are easy access for hunters, habitat degradation around the road caused by agricultural activities, oil and gas extraction, and mining. Altogether, these result in serious risk for primates as 55 % of the total species are endangered and 75 % have declining populations. This study is the first in the Americas addressing the population decline of an endangered arboreal primate resulting from the impact of road expansion in Chiapas, México. In the study area, the population of *Alouatta pigra* underwent a 56 % decline from 2012 to 2017, with 37 roadkills. The most affected age group was immature animals (both sexes), with an age:sex ratio that changed from 1.2 immature individuals per adult female in 2012 to 0.5 in 2017. This work highlights the dramatic population decline resulting from the poor conduct of Environmental Impact Studies combined with an inadequate application of mitigation measures on the road studied.

Key words: Arboreal primates; environmental impact; howler monkeys; mitigation; road ecology.

El desarrollo de grandes infraestructuras como las carreteras han colocado en grave amenaza a la biodiversidad debido a la mortalidad por atropellamiento de fauna silvestre. Una de las amenazas subyacentes es el fácil acceso para los cazadores; degradación del hábitat alrededor de la carretera por actividades agropecuarias, extracción de petróleo, gas y la minería. Lo anterior coloca en grave riesgo a los primates, con 55 % del total de sus especies en peligro de extinción y disminución de sus poblaciones en un 75 % de sus especies. Este trabajo es el primer estudio en América con un primate arborícola en peligro de extinción y que examina la reducción poblacional sufrida por el impacto de una ampliación de carretera existente en Chiapas, México. La población de *Alouatta pigra* se redujo en el área de estudio en un 56 % entre el periodo 2012-2017 con 37 atropellamientos. La proporción sexo-edad más afectada es la de inmaduros (de ambos sexos) pasando de 1.2 en 2012 a 0.5 inmaduros por cada hembra adulta en el 2017. Este estudio resalta la disminución poblacional drástica provocada por no realizarse de manera adecuada los estudios de impacto ambiental y las medidas de mitigación en la carretera de estudio.

Palabras clave: Ecología de carreteras; impacto ambiental; mitigación; monos aulladores; primates arborícolas.

In México, road construction underwent accelerated growth over 21 years, reporting 817,966 km in 2021. The longest roads run through the states of Chihuahua, Sonora, Veracruz, Jalisco, Chiapas, Oaxaca, Durango, Michoacán, Tamaulipas, and Guerrero (RNC 2022). México is habitat to only 3 species of arboreal primates (*Alouatta pigra, A. palliata,* and *Ateles geoffroyi*). The southeastern region of México (Veracruz, Oaxaca, Chiapas. Tabasco, Campeche, Yucatán, and Quintana Roo), within the distribution range of primates, has 20,000 km of roads (2.8 % of the total road infrastructure in México), and 98 % lacks mitigation structures to protect these primates (own data). Additionally, the Maya Train (a government megaproject consisting of the construction of freight and tourism railroads in the Yucatán Peninsula, eastern Tabasco, and northern Chiapas) started construction works in 2020 and will comprise approximately 1,600 km; thus, a primate protection program should be a priority to prevent roadkills.

The most recent global review, limited to reports in English and social networks, recorded 46 species of wild primates affected in 368 roadkills and 16 species from South America with 72 roadkill records (Hetman *et al.* 2019). Most of these reports inform less than 10 primate roadkills, a figure below death by predation or natural causes (Hetman *et al.* 2019). However, in Southern Asia, a higher number of deaths by vehicle collision have been reported for 4 species (*Nycticebus bengalensis, Macaca leonina, Trachypithecus phayrei,* and *Macaca mulatta*), with 17 events in 2 National Parks in Bangladesh (Al-Razi *et al.* 2019). These reports suggest that roads are one of the main causes of mortality of primates in tropical forests.

An 18-year study of primate species (*Colobus angolensis palliatus, Cercopithecus mitis albogularis, Chlorocebus pygerythrus hilgerti,* and *Papio cynocephalus cynocephalus*) along a 10 km stretch of road on the outskirts of Diani, Kenya, recorded 705 roadkills and 1,896 reports of conflicts between humans and monkeys (Cunneyworth and Duke 2020). There were 3 primate-vehicle collisions per month on average (range 0–10), and 83 % resulted in primate deaths.

Finally, another recent study reported 29 roadkills of *Piliocolobus kirkii* on Zanzibar Island off the coast of East Africa (Olgun *et al.* 2021). This study describes that while natural predators select young, old, or sick animals, vehicles kill individuals from populations irrespective of their health condition or age. In addition, even non-lethal collisions cause adverse effects on animals through an increase in glucocorticoid production, leading to physiological stress.

In Latin America, 4 roadkills of *Sapajus cay* were recorded in Brazil (Brum *et al.* 2018) and 4 of *A. palliata* in Costa Rica (Artavia *et al.* 2015). In Panamá, howler monkeys (*A. coibensis trabeata*) have been killed by vehicle collisions in the Azuero Peninsula (Méndez-Carvajal *et al.* 2013; Méndez- Carvajal 2020). In México, 8 road-killed individuals of *Alouatta pigra* have been reported in the Usumacinta region in Tabasco (Pozo-Montuy *et al.* 2008; Candelero-Rueda and Pozo-Montuy 2011).

However, research on road ecology in Tabasco is at a very early stage despite recent contributions addressing road impacts (Pacheco-Figueroa *et al.* 2014; Pacheco-Figueroa *et al.* 2021). There is an urgent need for information on primate roadkills to support mitigation measures in the construction of new roads and the modernization of the existing ones.

This study represents the first effort in America to assess the impact of road expansion work on the population of black howler monkeys, *Alouatta pigra*, inhabiting patches of vegetation at both sides of the road. The present report outlines behavioral observations, a population census, and recommendations to mitigate primate roadkills in southeastern México.

We monitored and followed up a population of black howler monkeys inhabiting a tropical forest crossed by a stretch of road at the Emiliano Zapata Tabasco–Usumacinta bridge within the Catazajá Lagoon System, a Protected Natural Area in the state of Chiapas comprising 47,058.77 ha. The Villahermosa–Escárcega road was originally C-type (6 m wide, no shoulder lanes), built before an additional 6 m-wide protected area was declared. In 2012, it was upgraded to an A2 superhighway measuring 12 m in width with 2 m of shoulder lane and a 60 m right-of-way, due to increased traffic volume, particularly of heavy trucks traveling to and from the Yucatán Península. The C-type road ran across secondary vegetation with trees reaching 30 m in height and natural tunnels formed by the canopy used as corridors by animals (Figure 1).

The study area is a 10 km stretch between km 135 + 660 to km 145 + 800 (17° 46′ 24.89″ N, 91° 49′ 41.96″ W) running from the Emiliano Zapata junction to the toll booth of the Usumacinta bridge (17° 51′ 15.73″ N, 91° 47′ 12.53″ W). In this stretch, an 800 m wide polygon was drawn in Google Earth, 400 m on each side of the road, where complete population censuses of howler monkeys were carried out (total count of individuals identified by body marks within an area), considering the daily movements of individuals of this species (Pozo-Montuy *et al.* 2013; Figure 2).

A complete population census was performed in February 2012 (Pozo-Montuy *et al.* 2021), during the first stages of the road expansion work, when land clearing, deforestation, and slope construction operations were carried out. During the study, each patch of vegetation at both sides of the road was traveled along a 10 km stretch by 5 persons over 6 hours, between 7:00 and 13:00 hr. The data collected included the geographic coordinates and sex and age of the individuals observed. Sex and age:sex ratios were calculated as the number of adult females divided by the number of adult males and the number of immature organisms (infants and juveniles of both sexes) divided by the number of adult females, respectively. Also, average group sizes were computed.

In July 2014, 2 years after the start of the road expansion work but before completing it, a second population census was performed in the same area and with the same methodology. Finally, in August 2017, a third population census was conducted after the road expansion work was completed, when the stretch was released and wildlife overpasses expressly made for these primates were installed.

Roadkill monitoring was performed continuously from 2012 to 2017. Roadkills were recorded and reported by the Saraguato Wildlife Management Unit staff, who perform daily tours along this stretch at different times, mainly in the morning (7:00–11:00 hr) and afternoon (15:00–18:00 hr). Additionally, residents and taxi drivers of Villa Chablé reported howler roadkills to the study team. At the collision sites, the data gathered included the geographic coordinates and sex, age, and photographs of roadkilled specimens; afterward, carcasses were removed from the road.

During population censuses and site visits at 2-month intervals since 2012, *ad libitum* observations were made to collect information on the behavior of *A. pigra* along the road. Data on responses to environmental noise, foraging, and activity patterns were recorded. A roadkill event witnessed by our team was also described.

Finally, as part of the primate roadkill mitigation measures following a Conservation Plan submitted to the General Direction for Environmental Impact and Risk of the Secretariat of the Environment and Natural Resources (SEMARNAT, in Spanish) one year after the start of the expansion, the Secretariat of Communications and Transportation (SCT, in Spanish) installed 4 wildlife bridges including galvanized steel cables, concrete poles, and green plasticized cyclone wire. The effectiveness of each of these bridges was assessed once per month by a camera trap (Bushnell Trophy Cam HD) from August to December 2016.

Figure 1. Stretch of the Villahermosa-Escárcega highway from km 135 + 660 to km 145 + 800 in Playas de Catazajá, Chiapas, México. a-b) before road expansion (2010); c-d) after road expansion (2012)

The initial 2012 census revealed a total population of 57 howler monkeys in the study area in 12 social groups plus 5 solitary males living in the roadside forest (5 groups on one side of the road and 7 on the other). The average group size was 4.3 individuals (Table 1). The adult female:male ratio was 1.9:1, and the immature:adult female ratio was 1.2:1.

The 2014 census (during the road construction) revealed a population reduction to 35 individuals in 9 social groups plus 5 solitary adult males inhabiting the roadside forest, representing a 39 % reduction from 2012 to 2014. The average group size was 3.3 individuals (Table 1). The adult female:male ratio was 0.9:1 and the immature:adult female ratio was 0.8:1.

The 2017 census, once the road expansion work was completed, showed that the population of howler monkeys was further reduced to 25 individuals in 5 social groups plus 2 solitary adult males living in the roadside forest. Five specimens in 2017 showed evidence of bruises and wounds, likely being survivors of non-fatal vehicle collisions. The population was reduced by 56 % versus the initial one. The average group size was 4 individuals (Table 1). The adult female:male ratio was 0.8:1 and the immature:adult female ratio was 0.5:1.

During the 2012–2017 monitoring, 37 roadkill events were recorded; most of them involved immature individuals ($n = 20$), although the number of adults was also noticeable ($n = 17$). There were no significant differences ($P > 0.05$) between sexes in the number of roadkills, with 19 males and 18 females hit (Figure 3). However, the immature:adult female ratio showed that immature monkeys are the most affected age group as the ratio decreased from 1.2:1 at the start of the road expansion work to 0.5:1 after completion.

The overall roadkill rate was 37 individuals per 10 km. At the start of the road expansion work (2012), there were 12 roadkills; subsequently, the number dropped to 10 in 2013 and remained around 5 from 2014 to 2016; finally, only one roadkill was recorded in 2017. With a sampling effort of 480 camera trap days, we confirmed that only 2 of the 4 bridges were used by black howler monkeys (Figure 3).

The *ad libitum* observations confirmed that howler monkeys display the normal pattern of daily activities reported for the species (Pozo-Montuy and Serio-Silva 2007), *i. e.*, a long resting time (70 %) followed by foraging (20 %) and moving around (10 %). In the study area, black howler monkeys feed on guácimo (*Guazuma ulmifolia*), ceiba (*Ceiba pentandra*), pichi (*Enterolobium cyclocarpum*), caracolillo

(*Albizia guachapele*), cantemó (*Albizia niopoides)*, macuili (*Tabebuia rosea*), jobo (*Spondias mombin*), and a variety of unidentified vines. In addition to arboreal locomotion and road crossings from 6:00 to 9:00 hr and from 16:00 to 19:00 hr, black howler monkeys descend to the ground to move between patches, feed on seedlings, and drink water from pools between road bridges.

Of the total visits (n = 24), play behavior was rarely observed; this is typical of the species since social interactions are rare. However, there was a considerable number of intergroup vocalizations (2 per day) from 7:00 to 9:00 hr and from 17:00 to 18:00 hr to keep the distance between groups. At the start of the road expansion work, the groups showed behavioral signs of stress, moving more than the usual rhythm reported for the species (*i. e.*, moving in resting hours between 11:00 and 16:00 hr) and exchanging vocalizations between groups (vocalizations at any time). During a roadkill event observed by the team, behavioral stress was recorded: a juvenile male that was hit remained lying on the road; this caused distress in the rest of the group, mainly in the alpha male, which emitted vocalizations and attempted to descend to approach the lying corpse. The first author removed the body from the road and carried it close to a tree trunk near the group of monkeys, which remained in the tree until the next day.

This is the first study in America reporting the impact of roadkill events on the population size and structure of howler monkeys. Immature individuals (infants and juveniles) were most affected, reducing the immature:adult female ratio from 1.2:1 to 0.5:1, thus affecting reproductive success (Días *et al.* 2020). One of the implications of the higher mortality of immature individuals is the lesser adequacy of the species associated with the loss of genes inherited during reproduction. The most recent studies on primate roadkills in Africa and Asia reported collisions by species without estimating the remaining population in the road-effect zone (Al-Razi *et al.* 2019; Cunneyworth and Duke 2020; Olgun *et al.* 2021).

A study on primate roadkills in Asia and Africa estimated that the road-effect zone varies in extent, depending on the size or body mass of the primate species, from 1 km to as much as 7 km for large primates (Perumal *et al.* 2021). In a detailed analysis by Andrasi *et al.* (2021) on chimpanzees (*Pan troglodytes verus*) in east Africa, these authors calculated the road-effect zones, estimating a range of 4.9 to 5.8 km for secondary roads (6 m wide) and 15.8 to 18.6 km for highways, with the species and type of road as key attributes. In the case of the black howler monkey, an arboreal species, the effect zone may be smaller, depending on landscape connectivity and the ability of this species to move across the ground, which has been reported as 350 m in the Usumacinta region (Pozo-Montuy *et al.* 2013). However, food availability and tree cover on roadsides (within the right of way) may be acting as an ecological trap for individuals who colonize these zones, sometimes increasing roadkill rates by including between 15 and 25 species of plants as sources of food (road attraction effect; Benitez *et al.* 2021a). In the road stretch studied, 37 howler monkeys were road-killed, but the annual roadkill rate decreased due to the deterrent effect of the road, which induces animals to avoid it, and the behavior of the species.

Behavior is another aspect that has been poorly investigated. This work confirmed that animals maintained their daily activity patterns with long resting periods. They also moved on the ground, as reported to occur in disturbed sites (Pozo-Montuy *et al.* 2013). Besides, this report describes for the first time the reaction of a group of howler monkeys to the roadkill of one of its members. An evaluation of the movement and home range of gibbons (*Hylobates lar* and *H. pileatus*) estimated a probability of road crossing of 35 % by tracking them over 90 full days and recording their movements every minute (Asensio *et al.* 2021). These authors state that although roads divide the home range of these primates, these were not impassable, and gibbons had crossing points on the road. It is necessary to continue performing detailed studies analyzing the

Table 1. Complete censuses and percent population reduction of the black howler monkey, *Alouatta pigra*, at the start, during, and after the road expansion work of the Villahermosa-Escárcega highway in Chiapas, México. AM, adult male; AF, adult female; JM, juvenile male; JF, juvenile female; IM, infant male; IF, infant female; ind, individuals.

Class sex age	Initial census In 2012	Census during road expansion work in 2013	Census after road expansion work in 2017
AM	16	13	11
AF	19	12	9
JM	7	4	2
JF	8	3	2
IM	4	2	1
IF	3	1	0
Total	57 ind	35 ind	25 ind
Social groups	12	9	5
Solitary individuals	5 males	5 males	2 males
Average size	4.3	3.3	4
% Population reduction	0.00	39 %	56 %

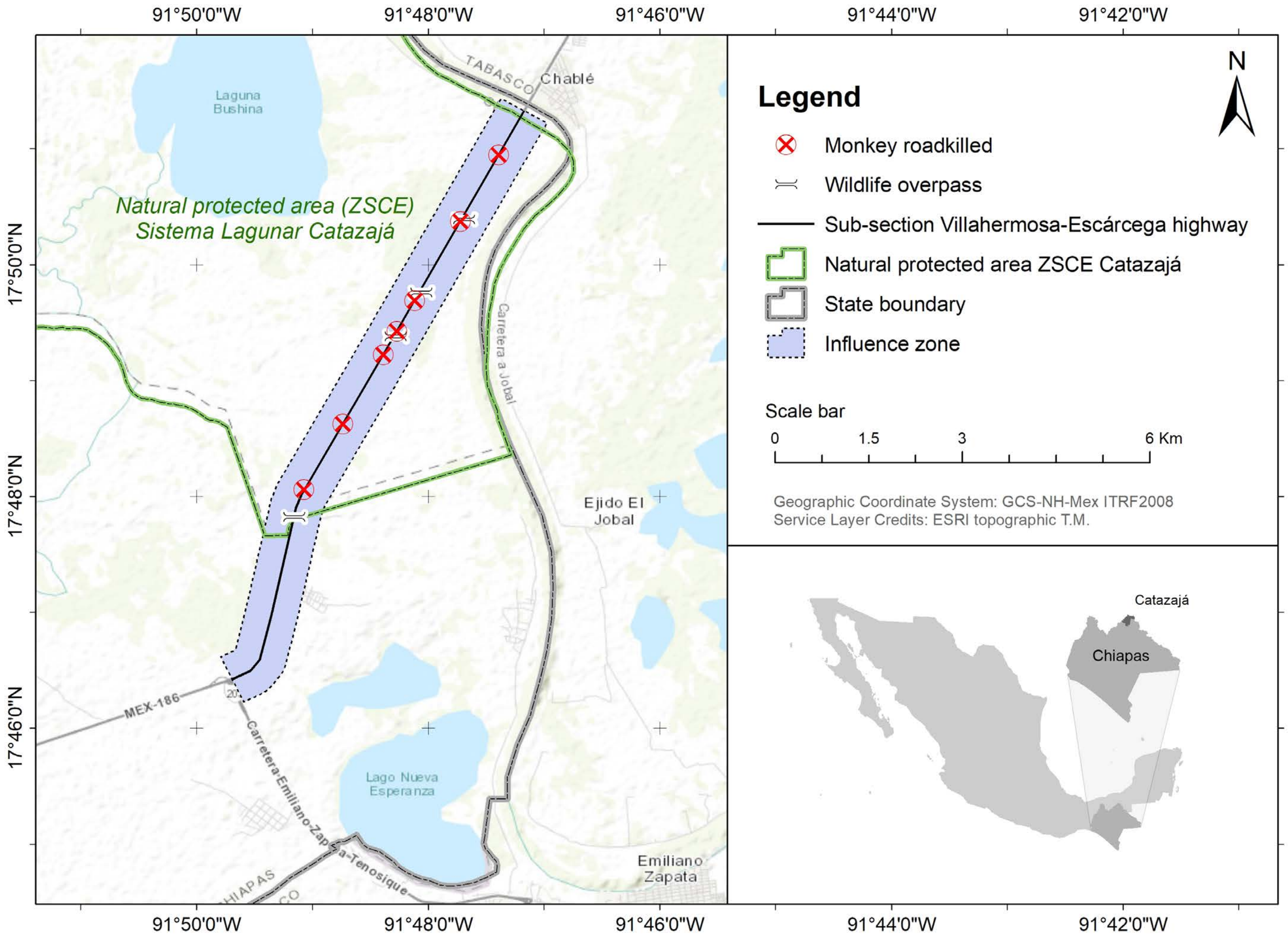

Figure 2. Roadkill area of black howler monkeys, *Alouatta pigra*, on the Villahermosa-Escárcega highway (blue stripe), state of Chiapas, México. The sites of *A. pigra* roadkills (2012-2017) are marked with red crosses. The Catazajá Lagoon System Protected Natural Area is demarcated with a green polygon. Drafted by I. Castañeda Guerrero.

behavior and movements of howler monkeys, addressing the sink and ecological-trap effects, in addition to studies on the physiological effects of vehicle collisions impacting howler monkeys, and estimate the main crossing points for the construction of arboreal wildlife overpasses.

First, it is recommended that environmentalists and environmental authorities be particularly careful when performing and reviewing Environmental Impact Assessments to avoid overlooking species at risk, as this may have major consequences; this is the case of the black howler monkey, an endangered species, which has undergone a 56 % population reduction (Estrada 2017; IUCN 2021). Although there is literature reporting the presence of black howler monkeys in the area (Bonilla-Sánchez *et al.* 2010) and it is a species listed as endangered in NOM-059-SEMARNAT-2010 (SEMARNAT 2010), consultants and environmentalists failed to report their presence. Despite this omission, the environmental authorities approved the road expansion project. It should be noted that in the road stretch studied, other protected species are also affected by roadkills, which were not considered in mitigation actions (wildlife overpasses), including the anteater (*Tamandua mexicana*), otter (*Lontra longicaudis*), common green iguana (*Iguana iguana*), black iguana (*Ctenosaura similis*), and freshwater turtles such as the narrow-bridged musk turtle (*Staurotypus triporcatus*) listed as a threatened species (NOM-059-SEMARNAT-010; SEMARNAT 2010; GBIF 2021).

Finally, the mitigation measures were implemented by SCT after the local population of howler monkeys was reduced by half (which could have been avoided). Bridges may have worked better if a formal study on the movements of howler monkeys had been performed and, mainly, if overpasses had been installed in sites with trees growing on both sides of the road or with the presence of these primates (Benitez *et al.* 2021b). During our assessment, we confirmed that half of the bridges were built in sites devoid of vegetation on one side. In addition, the Federal Electricity Commission (CFE, in Spanish) installed high-voltage lines close to bridges, which pose an additional risk for primates and hamper the functionality of these bridges. This can be addressed with reforestation programs to reconnect the vegetation on the treeless side of the road, and by isolating the CFE cables. Bridges work as linear corridors that restore the connectivity between forest patches (Teixeira *et al.* 2013). However, assessing the effectiveness of bridges requires investigating whether barrier effects and mortality

Figure 3. Roadkills of black howler monkeys, *Alouatta pigra*, of different sex and age, and wildlife overpasses on the Villahermosa-Escárcega highway in the Emiliano Zapata-Chable stretch, Chiapas, México. a) Adult female with a male infant; b) juvenile female; c) proper placement of a bridge connecting two wooded roadsides; d) the use of the bridges by howler monkeys is confirmed when these are placed and built correctly.

are reduced and whether the population remains stable. A review paper confirmed that most studies on wildlife passages have documented that mitigation can be deemed successful for individual animals, but the implications on persistence at the population level should also be monitored (van der Ree *et al.* 2015).

In this sense, it is recommended that the impact of roads on biodiversity be legislated, including mandatory guidelines on good construction practices of linear communication infrastructure and mitigation measures. These actions should be addressed with proper planning, approved budgets, and considering subsequent maintenance and monitoring.

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Comparison of road-killed mammals on roads of different types of jurisdictions and traffic volume in Veracruz, México

Comparación de mamíferos atropellados en vías de diferentes tipos de jurisdicción y flujo vehicular en Veracruz, México

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Road projects are key for human development but also pose a risk to wildlife. The objective of this work was to determine the number of mammal road-kills on 3 types of roads according to their jurisdiction and traffic volume in the northern part of the state of Veracruz, México. Two roads were selected according to their jurisdiction (federal, state, and municipal) considering 2 types of traffic volume (high and low). Road-killed wild mammals were recorded through systematic sampling. The inventory was enriched with non-systematic records. Non-parametric tests were used to analyze the differences in mammal roadkills between types of roads and traffic volume. We found 49 road-killed individuals of 4 mammal species and 1 genus not identified to species, in addition to 3 species from incidental records. The species with the highest number of roadkills was *Didelphis marsupialis.* There were no significant differences in the rates of collisions between types of roads, but we found a significantly higher number of records related to high traffic volume. The non-systematic records allowed to enrich the inventory of road-killed species in the area. Two of the mammal species recorded are listed under a risk category. The biological characteristics of the species influence the extent of the impact of roads on them; traffic volume is a key driver of the impact of roads on wildlife. Non-systematic records can contribute to improving roadkill inventories and provide better tools for assessing the effects of roads on wildlife.

Key words: Environmental impact; mammals; road ecology; Veracruz.

Los proyectos viales son un elemento importante de desarrollo humano, pero a la vez representan un riesgo para la fauna silvestre, por lo que el objetivo del trabajo fue determinar el número de mamíferos atropellados en tres tipos de caminos en función de su jurisdicción y flujo vehicular en el norte del estado de Veracruz, México. Se seleccionaron dos caminos por tipo de jurisdicción (federal, estatal y municipal) en dos tipos de flujo vehicular, alto y bajo, registrando de manera sistemática todos los mamíferos silvestres atropellados y enriqueciendo el inventario con registros no sistemáticos. Se emplearon pruebas no paramétricas para analizar las diferencias en los atropellamientos de mamíferos en función del tipo de camino y flujo. Se registraron 49 individuos atropellados pertenecientes a 4 especies y 1 género de mamíferos sin determinar a nivel específico, además de 3 especies por registros incidentales, donde *Didelphis marsupialis* representa la especie más atropellada. No se encontraron diferencias significativas en las tasas de atropellamientos en función del tipo de camino, pero sí con relación al flujo vehicular, con mayores registros durante el flujo alto. Los registros no sistemáticos permitieron enriquecer el inventario de especies atropelladas en la zona, además de incluir el registro de 2 especies de mamíferos bajo alguna categoría de protección. Las características biológicas de las especies condicionan su grado de afectación por las carreteras; la afluencia vehicular es un factor importante que determina el impacto de las carreteras sobre la fauna silvestre. Los registros no sistemáticos pueden ayudar a mejorar los inventarios y brindar mejores herramientas para evaluar el efecto de las carreteras sobre la fauna silvestre.

Palabras clave: Ecología vial; impacto ambiental; mamíferos; Veracruz.

Road projects are an important element of human development as they represent economic and social benefits by improving the life quality of inhabitants. However, the construction and opening of roads have adverse effects on the environment, including loss and habitat fragmentation, pollution, deforestation, dispersal of exotic species, impaired animal behavior, creation of barriers for populations, limited access to resources, and wildlife mortality from collisions; the ultimate consequence is a significant decline of biodiversity (Trombulak and Frisell 2000; Jaeger *et al.* 2005; Arroyave *et al.* 2006; Colchero *et al.* 2011).

The probability of death by collision is related to road characteristics, such as traffic volume and road width (De la Ossa and Galván 2015), as well as to behavioral modifications in the local species, either because wild animals are attracted by roads, avoid them, or need to cross them.

For instance, roads are a source of food for some mammals and birds, especially scavengers. Reptiles such as snakes and lizards, which need to regulate their body temperature by absorbing heat from the environment, approach roads both during daytime and nighttime hours due to the heat absorbed by the pavement, thereby increasing the probability of roadkill events (Arroyave *et al.* 2006; Rojas 2010; Eloy-Seijas *et al.* 2011).

México is a megadiverse country as it is home to great biodiversity of multiple taxa. It ranks second globally in reptile species richness, third in mammals, fifth in amphibians, and eighth in birds (Llorente-Bousquets and Ocegueda 2008). At the same time, it has an extensive road network of approximately 780,509 km in length, including 174,779 km (22 %) of paved roads (federal, state, and municipal), 78,385 km (10 %) of urban roads and connections, and 527,345 km (68 %) of unpaved roads (INEGI 2020).

Veracruz is one of the 3 states with the greatest biodiversity in México; it ranks third in species richness of amphibians (Parra-Olea *et al*. 2014) and reptiles (Flores-Villela and García-Vázquez 2014), second in birds (Navarro-Sigüenza *et al.* 2014), and third in mammals (Sánchez-Cordero *et al.* 2014). Nevertheless, most of the current knowledge about wildlife has focused on the central and southern areas of the state, particularly the Los Tuxtlas region, an important biosphere reserve at the state level (Gaona *et al.* 2003).

One of the earliest studies on wildlife roadkills in Veracruz was conducted in Los Tuxtlas. During 24 sampling periods of 5 days each, 8 km of paved road were traveled over 2 years, recording 468 carcasses of 73 species; reptiles were the most affected group (Morales-Mávil *et al.* 1997). Another survey was performed on the Amozoc-Cantona-Perote highway. During 34 days of sampling, 14 km of highway were traveled in 2 environments (xeric shrubland and grasslands), recording 58 road-killed species of vertebrates; mammals were the most affected group with 82.87 % of the total roadkills, followed by birds (9.64 %) and reptiles (7.8 %; González-Gallina *et al.* 2013).

Therefore, and due to the lack of assessments of road impact on wildlife in northern Veracruz, the objectives of this work were to assess potential differences in the number of road-killed mammals between 3 types of roads according to their jurisdiction (federal, state, and municipal), and between 2 levels of traffic volume (high and low), and to identify the species listed in a risk category in the Mexican regulations (SEMARNAT 2010).

The study area is located in northern Veracruz and comprises the municipalities of Tuxpan, Tihuatlán, and Álamo. The fieldwork was conducted along a 35 km-stretch of 2 federal highways, a 20 km-stretch of 2 state highways, and a 4 km-stretch of 2 municipal highways.

The stretches of federal highways were a) the México–Tuxpan 130D highway, from the Río Pantepec section to the connection with the Tampico–Poza Rica 180 highway, consisting of 4 paved lanes surrounded by grasslands, secondary vegetation, and areas near paddocks, and b) the Tampico–Poza Rica 127 highway, from Tihuatlán to Alamo, comprising 2 paved lanes surrounded by secondary vegetation, paddocks, and some populated areas.

The state highways were: a) the México 106 Highway, from Alamo to Manantial, consisting of 2 paved lanes bordered by paddocks with water bodies, orange tree plantations, and populated areas, and b) the Tuxpan–Tamiahua highway, comprising 2 paved lanes bordered by grasslands, paddocks, groves, and water bodies.

The municipal roads studied were: a) the road to La Barra Norte from the Lázaro Cárdenas Boulevard in Tuxpan, located 1 km from the coast and consisting of 2 narrow lanes with paved but sandy sections, adjacent to grasslands, halophytic vegetation, secondary vegetation, and sparsely populated areas and, b) the API Tuxpan–Santiago de la Peña road, comprising 2 paved and sandy lanes traveled by heavy trucks, adjacent to landscapes including halophytic vegetation, mangroves, and a nearby water body.

To assess the effect of traffic volume on roadkills, tours were made in 2019 on each type of road in periods of 2 different traffic volume levels: high, during the Easter (April) and summer (July) holiday periods; low, during March and June. To calculate the traffic volume, 3 equidistant observation points in each section were considered on all sampling dates; the vehicles traveling through the observation point in both directions were quantified for 1 minute using a digital counter. In each period of high / low traffic volume, 2 tours were conducted over the course of 2 weeks each month on the 3 types of roads, for a total of 48 tours.

Road tours were conducted systematically in both directions (one every day of the week), between 11:00 and 18:00 hr, to take advantage of daylight and prevent accidents, at an average driving speed of 40 km / hr. Two observers participated in each tour. When a carcass was spotted, preventive signs were placed on the road, the collision event was recorded, and the corpse was removed from the road to avoid duplication of records. Mammal specimens were identified using specialized literature and field guides (Aranda 2012; Reid 2009). At the collision sites, the data gathered included date and time, road type, and whether the corpse was found on a curve or straight stretch; the coordinates were read with a GPS (Garmin Etrex 10). To supplement the inventory of road-killed species in the area, road-killed animals spotted during random encounters were also recorded; these non-systematic records were considered valid if they corresponded to the same road and sampling period studied and provided a photograph of the specimen was included along with its approximate location.

The relationship between the roadkill rate (number of road-killed individuals divided by the total number of kilometers traveled), type of road, and traffic volume was assessed using a Kruskal-Wallis test (Zar 2014). The relationship between the species richness of road-killed mammals and the average traffic volume for each road type was explored through a Spearman correlation test (Zar 2014). These analyses were selected due to the non-parametric distribution of the data.

Systematic sampling led to the identification of 4 species (*Didelphis marsupialis, D. virginiana, Dasypus novemcinctus,* and *Sciurus aureogaster*) and 1 unidentified mammal species (*Didelphis* sp.), in addition to 4 domestic cats (*Felis catus*) and 9 dogs (*Canis lupus familiaris*) that were excluded from the analyses. Opossum (*Didelphis marsupialis*) was the most affected species (Figure 1).

Regarding the type of road, no significant relationship was found with the roadkill rate ($K = 2$; $P = 0.36$). However, significant differences were found between traffic volume and roadkill rates ($K = 5.91$; $P = 0.01$), which were higher for high traffic volume. A positive and significant correlation was also found between species richness and mean traffic volume by road type ($S = 2.53$; $P = 0.007$; $rho = 0.92$; Figure 2).

The non-systematic sampling resulted in 12 validated records involving 5 mammal species, including 3 species not recorded in the systematic sampling: raccoon (*Procyon lotor*), jaguarundi (*Herpailurus yagouaroundi*), and anteater (*Tamandua mexicana*). It is worth highlighting that the latter 2 species are protected by the Mexican Official Norm (NOM-059-SEMANART-2010; SEMARNAT 2010), listed as threatened and endangered of extinction, respectively.

Based on the results obtained in the present study, mammals are among the most affected groups by wildlife roadkills. This pattern is consistent with the reported by several studies globally (Puc-Sánchez *et al.* 2013). However, it may be influenced by the methodology used (roads traveled at constant speed), the environmental characteristics of the study area (few water bodies), and the sampling periods based on traffic volume instead of climate season. Other works conducted in Veracruz with different methodology (walking tours) and climatic and environmental conditions (Morales-Mávil *et al.* 1997) reported different results, finding amphibians as the second most affected group. Recent studies have described that in Latin America, amphibians have the highest average roadkill rate (0.2 ind / km / year; Pinto *et al.* 2020). Therefore, we suggest conducting further studies on this topic in the study area to confirm the actual effect of roads on different groups of vertebrates.

Opossums, particularly *D. marsupialis*, were the most affected mammals, not only for recording the largest number of road-killed individuals but also because these were recorded in almost all types of roads and traffic volume levels. This may be associated with their reproductive season, which ranges from January / February to June / July, when young and inexperienced individuals are more abundant and might be attracted by food lying on the roads or at roadsides. This factor, alongside their generalist habits, great displacement capacity, and defense response when threatened (paralyzing), makes them more vulnerable to roadkills (McManus 1974; Puc-Sánchez *et al.* 2013).

The results reported in this study, namely the higher roadkill rates in periods of higher traffic volume and the correlation between species richness and traffic volume according to the type of roads, demonstrate that traffic is

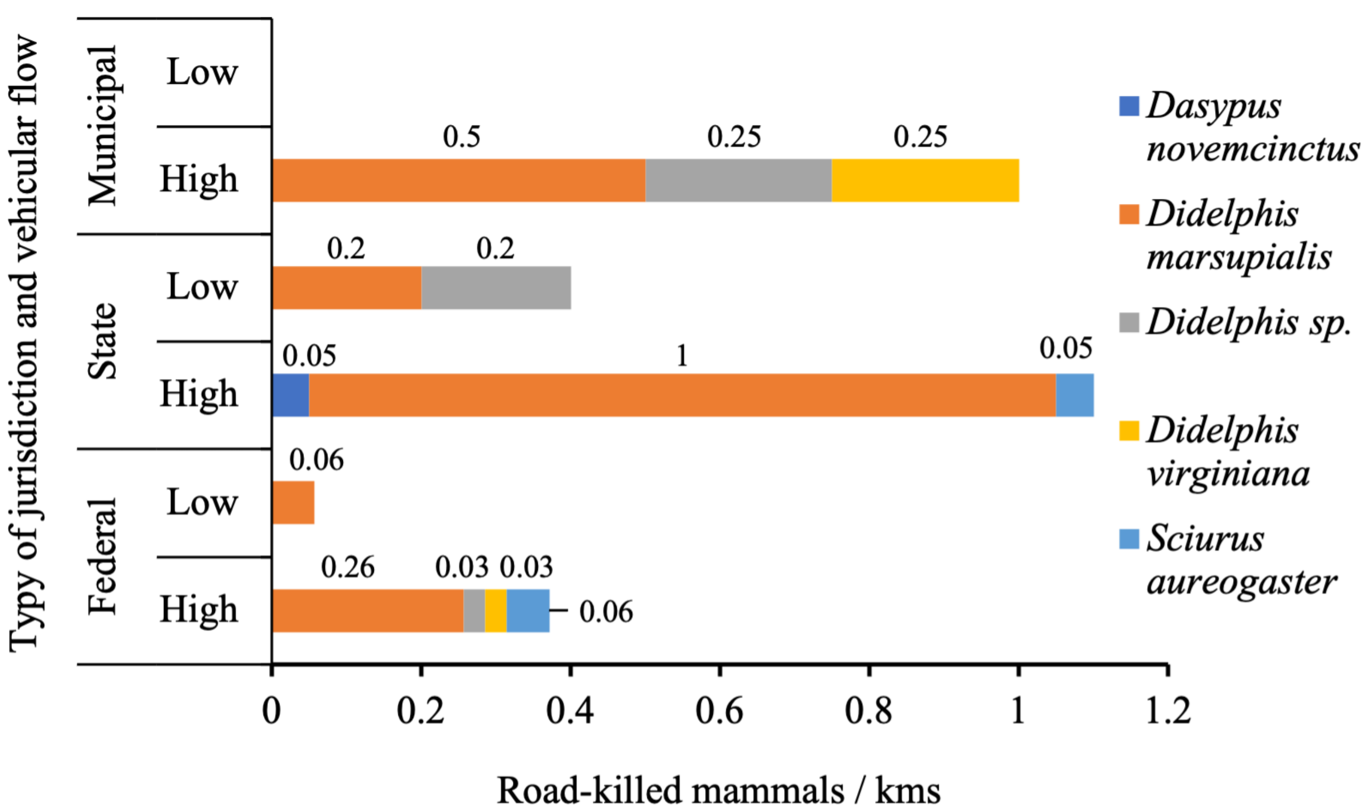

Figure 1. Diagram showing the number of road-killed mammal individuals per kilometer traveled and by type of road and traffic volume on roads in northern Veracruz, México.

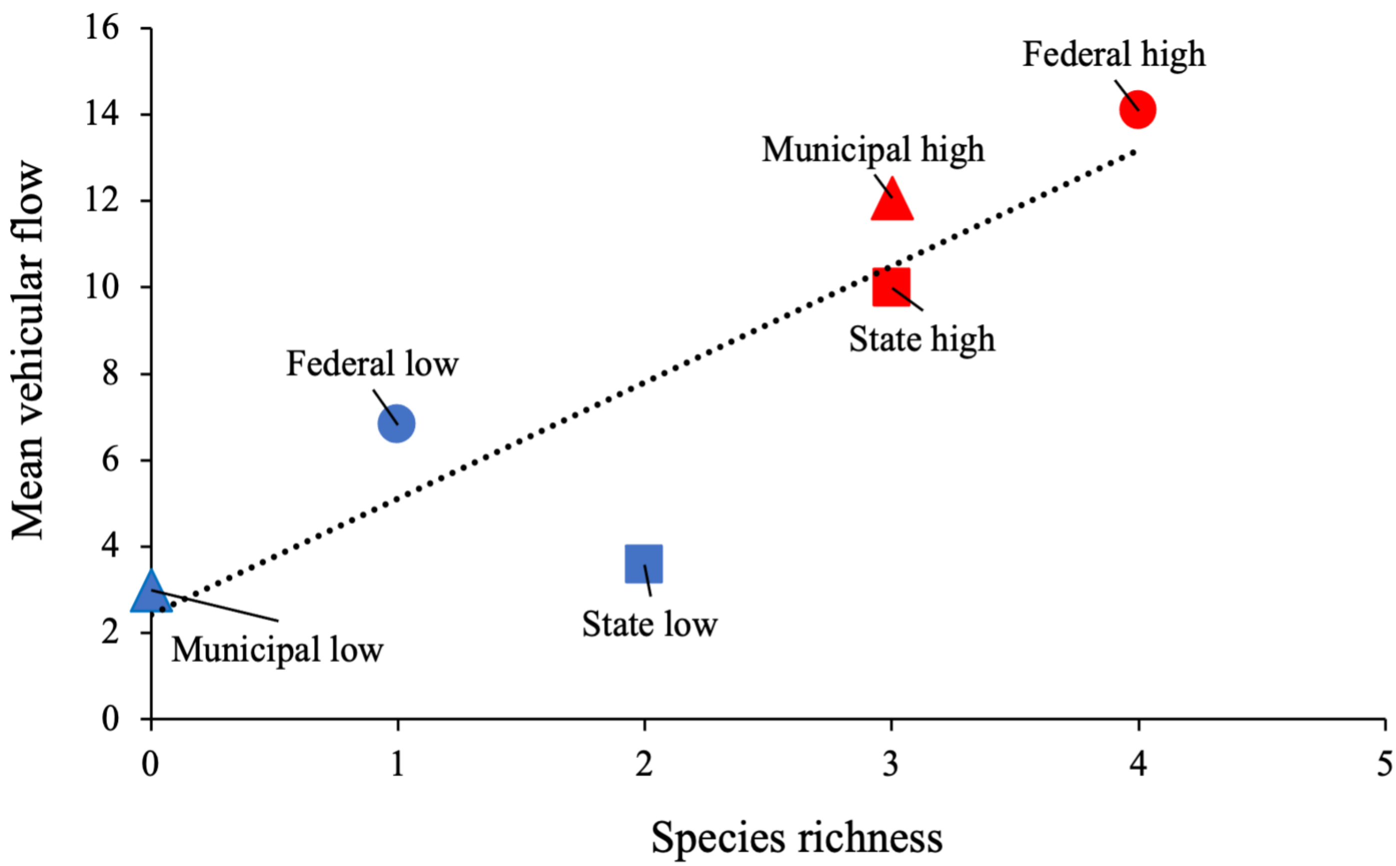

Figure 2. Relationship between richness of mammal road-killed species and mean traffic volume and type of road on roads in northern Veracruz, México.

a key factor in wildlife roadkills. Therefore, it is important to develop a permanent conservation program to reduce the environmental impact of roads, which should include, at least (SCT 2020): proper road signs to make users aware of the presence of wildlife, particularly during the seasons of high traffic volume, when the highest roadkill rates were found; drainage works retrofitted in areas near water bodies to allow water flow and the displacement of slow-moving aquatic fauna (*e.g.*, amphibians); seasonal passages for migratory invertebrates because high mortality of the crab *Gecarcinus lateralis* was observed in one of the sites studied (road to La Barra Norte) during a sampling period coinciding with its breeding season (July; Capistrán-Barradas *et al.* 2003); and reforestation of areas parallel to the roads affected by Hurricane Grace in August 2021.

Non-systematic records added 2 species of protected mammals: jaguarundi and anteater. This demonstrates the importance of this type of records, many of them reported by citizens, which can reduce biases in systematic studies, mainly when digital platforms are used to share information (Di Cecco *et al.* 2021), in addition to helping improve the assessment of the real impact of roads on biodiversity.

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Ecological connectivity and wildlife passages on roads: a reflection for México

Conectividad ecológica y pasos para fauna en carreteras: una reflexión para México

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The construction of wildlife passages is a globally growing trend, supported by adequate legal frameworks and practical experiences that have assessed their effectiveness and efficiency to reduce roadkills; particularly for mammals. In México, however, this field is still incipient, with a few wildlife passages implemented and a non-existent legal framework, only regulated through authorization resolutions of environmental impact assessments related to road construction projects. Fauna passages play an important role in biodiversity conservation and recovery of landscape connectivity; however, studies are still required worldwide to assess the relevance of these passages on wildlife populations and communities conservation through time and space. This paper summarizes current considerations in terms of connectivity and fragmentation, and their relationship with wildlife passages construction on roads, as a strategy to mitigate wildlife impacts, encouraging the reader to reflect on the current status of these topics in México and the importance of implementing them through a legal framework as part of the commitments of México toward the global conservation of biodiversity.

Key words: Artificial corridor; biological corridor; fragmentation of ecosystems.

La construcción de pasos para fauna silvestre es una tendencia creciente en el mundo, fundamentada en marcos jurídicos adecuados y experiencias prácticas que han dado como resultado la valoración de su eficacia y eficiencia en reducir el atropello, particularmente de mamíferos. No obstante, en México el tema es aún incipiente, con pocos ejemplos ejecutados y un marco legal específico inexistente; quedando su ejecución obligada a través de los resolutivos de autorización en impacto ambiental de los proyectos de infraestructura carretera. El papel que los pasos para fauna tienen en la conservación de la biodiversidad y recuperación de la conectividad en el paisaje es de gran relevancia; sin embargo, hacen falta a nivel mundial, planificar estudios que permitan cuantificar su relevancia en la conservación de comunidades y poblaciones de especies en el tiempo y espacio antes y después de su construcción. Esta nota resume algunas de las consideraciones actuales en materia de conectividad y fragmentación, y su relación con la construcción de pasos para fauna silvestre en la infraestructura vial como una forma de mitigar el daño ejercido, invitando a la reflexión sobre el estado que guarda México con respecto a estos temas y la importancia de retomarlos en la práctica y a través de un marco legal, como parte de los compromisos que México ha adquirido en materia de conservación de la biodiversidad a nivel mundial.

Palabras clave: Corredores artificiales; corredores biológicos; fragmentación de ecosistemas.

Conserving ecosystems connectivity to protect the natural and cultural diversity of México is a commitment to present and future generations (Sarukhán 2017). Ecosystems have supported the livelihood of human populations since the early stages of history, providing goods and services (Balvanera *et al.* 2009) that are essential for the development of human societies (MEA 2005). Biodiversity represents a supporting ecosystem service, as it participates in processes such as pollination, seed dispersal, climate regulation, carbon sequestration, and pest control (Orijel *et al.* 2008).

In terms of ecosystem connectivity, the transformation and fragmentation of ecosystems resulting from the construction of road infrastructure represent a stress factor that splits and alters the ecological functions, leading to biodiversity loss (Mendoza-Sánchez and Marcos-Palomares 2016). The conservation and recovery of ecological connectivity allows to reverse fragmentation effects and, additionally, fight the threats of climate change through the recovery of ecosystem functions (Sarukhán 2017). In this context, it is evident that road projects need to be design under a comprehensive territorial planning proposal that meets the communication and mobility requirements of society without threatening the conservation of biodiversity and ecosystem services (Tsunokawa and Hoban 1997; Seiler 2001; Daigle 2010).

Nowadays, strategies such as the conservation and recovery of ecological corridors and the construction of artificial corridors perpendicular to road works, known as wildlife passages, are feasible options to preserve ecological connectivity and reduce the adverse effects of ecosystem fragmentation; they provide a continuous habitat that represents a safe passage for animals as they move through the landscape (Conservation Corridor 2020). This document reviews the situation in México regarding the implementation of diverse strategies for the conservation and recovery of landscape connectivity, as a way to mitigate ecosystem fragmentation caused by roads, particularly considering the construction of fauna passages as a measure to recover connectivity.

Current context on ecosystem fragmentation. Ecosystem fragmentation is on the rise, mainly the one caused by the lack of land use control and strategic planning for the use of natural systems. In México, urban development, livestock increase and agriculture practices are drivers of this issue. Additionally, in the early 20th century, the creation of transport infrastructure exacerbated this problem, initially comprising railway networks only, and later, with the increase in highways, roads, and country roads (López-Feldman 2012; INEGI 2017). The construction of roads represents an economic and anthropic symbol of progress that enables mobility and the logistical capacity to plan and improve our life quality. However, life quality also depends on ecosystem services and these, in turn, on the connection and balance of its components (MEA 2005).

Road infrastructure represents significant movement barriers for mammals, resulting in increasingly smaller, isolated populations with greater difficulty for obtaining food, water, or mating sites to conserve genetic diversity, species continuity, and ecosystem integrity (Arroyave *et al.* 2006). Additionally, the construction of road infrastructure has increased the risk of wildlife roadkills and is currently considered one of the leading causes of mammals mortality, as well as other fauna inhabiting the road neighboring area. This issue has been of great concern since the 1970s in various parts of the world, and efforts have been focused on developing solutions for safe wildlife crossing (Mendoza-Sánchez and Marcos-Palomares 2016).

There are few studies in México investigating alternatives to prevent the impacts to fauna caused by road infrastructure construction, and those that have been conducted resulted from environmental impact assessments of road projects. Also, there is no clear quantification of roadkills on various roads in the country, nor records of accidents resulting from collisions or drivers wildlife dodges. There are initiatives such as the Observatory of Mobility and Fauna Mortality on Roads of México (*Observatorio de Movilidad y Mortalidad de Fauna en Carreteras de México*), supported by the Mexican Institute of Transport (*Instituto Mexicano del Transporte;* IMT, in Spanish) with the collaboration of citizens and centers of the Secretariat of Communications and Transport (SCT, in Spanish). The objective of the observatory is to build a database through WATCH MX, a platform created for the monitoring of fauna that crosses roads in México to develop mitigation strategies and prevent accidents or collisions between vehicles and wildlife; the earliest results of this initiative were published by Mendoza-Sanchez and Palomares (2016). The project is promising and can support research studies to help mitigate, compensate, or avoid damage to wildlife, but it requires further support to remain functional and promote the use of the WATCH MX platform by different sectors and users who contribute by reporting wildlife-vehicle incidents on roads in the country.

Connectivity and wildlife passages on roads. The concept of biological or ecological connectivity is complex but in general terms include the way in which organisms can move among particular natural landscape elements or the number of connections between habitat fragments relative to the maximum number of potential connections or interrelations of key processes within and between ecosystems in multiple scales (Fisher and Lindenmayer 2007). The maintenance and construction of ecological corridors represent an effective strategy worldwide to increase such connectivity in landscapes or ecosystems.

The International Union for Conservation of Nature (IUCN) defines a biological or ecological corridor as "A clearly defined geographical space that is governed and managed over the long term to maintain or restore effective ecological connectivity" (Hilty 2020). According to their functionality, there are different types of ecological corridors. In the case of roads, wildlife passages are currently considered artificial corridors that enhance connectivity and are highly important to reduce the damage caused by road infrastructure (Panthera 2020).

Although wildlife passages were initially built in the 1970s in other countries (Clevenger 2007), they are a relatively new subject in México and still lack a specific legal framework. Although there are studies supporting that these structures can increase ecosystem connectivity (Bissonette and Adair 2008), the connectivity mentioned within the Mexican legal framework mainly refers to natural or large corridors whose objective is conservation, particularly of protected natural areas (SEMARNAT *et al.* 2017). However, no reference is made to wildlife crossings as related to the connectivity of ecosystems and the conservation of mammals and other species, hence the importance of including it within the legal framework. As human capacity to create technology generally involves ecological impacts, it can also represent the key to face environmental challenges, including the fragmentation of landscapes and ecosystems through knowledge and technology development (Maass and Equihua 2015). The incorporation of wildlife passages to the legislation may foster sustainable practices in México, as long as they are built at strategic sites and under an integrated management approach of landscapes and ecosystems, besides being preventive rather than corrective (Iuell 2003; Bissonette and Adair 2008; Correa *et al.* 2016; SCT 2021).

The selection of the best route among several alternatives should prevent affecting ecologically relevant areas.

For instance, in conserved areas, a single type of wildlife passage out of the eight described as a mitigation measure in the Manual for the Design of Road Wildlife Passages (*Manual de Diseño de Pasos para Fauna Silvestre en Carreteras*; SCT 2021) may be insufficient to provide the permeability required by different species in a given habitat (Rytwinski *et al.* 2016) since these structures should be built according to their biological and ecological needs. Thus, it is worth to emphasize that the best wildlife crossing is the one that does not need to be built, *i.e*., when the road or railway has been designed in order to avoid ecosystem fragmentation.

On the other hand, even though other countries have implemented fauna passages, each case has its own experiences and requires particular alternatives, considering the needs of the area in terms of its fauna and ecosystems (Rytwinski *et al.* 2015) and the political and social contexts. An example is the wildlife passages built in the temperate region of the United States and Canada, where the presence of large mammals and their displacement in herds requires the construction of large wildlife passages over the roads and the fencing of the entire right of way. These measures reduce wildlife-vehicle collisions and the high costs of driver compensation and insurance payments (the primary motivation for constructing these crossings). In São Paulo, Brazil, collisions between vehicles and wildlife have produced annual costs to society of USD 25,144,794.00 (Abra *et al.* 2019). In this sense, the cost-benefit balance justifies the profitability of the construction (Lee *et al.* 2013; Ascensão *et al.* 2021). However, in countries with diverse tropical fauna, usually small-sized and abundant, as is the case of México, the construction of large structures as passages, with long distances between them, would not solve the fragmentation issues. The ecological characteristics of the animals living in these regions require a greater density of passages with shorter distances between them in order to allow the animals to move across the roads, which differs from requirements for wildlife in North America. These features reduce construction costs, even allowing to include structures for multiple species (Sijtsma *et al.* 2020). Additionally, it is feasible to consider the use of road structures as passages, like drainage works that can be adapted for a dual use: water flow and wildlife passage (SCT 2021).

An important aspect in the construction of wildlife passages is the implementation of actions to protect the habitat of wildlife species, that is, to connect these structures with the surrounding ecosystems, thereby maintaining connectivity and ecosystem services at the same time. Some studies report that purchasing agricultural land for ecological restoration is more effective and efficient for species conservation and connectivity than constructing large crossings as the only mitigation measure (Queiroz *et al.* 2014).

It is important to note that vehicle collisions with medium-sized or small fauna usually causes no damage to cars, unless the driver attempts to dodge it, which can result in a serious car accident; therefore, it is difficult to perform collisions cost analyses with small animals to justify for the construction costs of wildlife passages under these circumstances. This makes it necessary to assess the construction costs of wildlife crossings based on a cascade of issues and adverse effects that result from the loss of biodiversity; therefore, construction of such wildlife passes should be perceived as part of the ecosystem support services that provide long-term benefits for the quality of human life. To this date the economic quantification of this type of ecosystem services benefits is a scarcely developed parameter (Sijtsma *et al.* 2020), given the complexity and abstraction involved (Bartkowski *et al.* 2015).

Importance of the legal framework and its application through public policies aimed at promoting ecological connectivity and fauna passages. Globally, the implementation of legislation and public policies at the spatial and temporal levels is related to the idiosyncrasy, culture, development, and economy of each region. According to 147 documents about the regulatory framework of countries in Europe, North America, and Latin America in terms of connectivity, fragmentation, and wildlife conservation, there is a marked difference in the application of environmental policies between the governments of developed and developing countries. One of the most advanced regions in environmental regulations and with broad experience in preserving their ecosystems and fauna is the European Union. The protection of fauna, flora, landscapes, and ecosystems is a comprehensive part of its political agenda, and the actions to support its natural heritage have acquired increasing importance over the past 30 years.

In North America, each country has its own regulations and programs to preserve and manage wildlife species and ecosystems. To protect biodiversity affected by the vigorous development of the region, the Canada/México/United States Trilateral Committee for Wildlife and Ecosystem Conservation and Management was established in 1995. Its goals focus on promoting a comprehensive approach to the conservation and sustainable use of biological resources, contributing to the maintenance of the ecological integrity of its ecoregions, and the conservation of biodiversity.

Additionally, México has signed a significant number of international, bilateral, and multilateral environmental treaties and agreements, providing the bases for developing our environmental legislation. The commitments adopted by México by joining these treaties have led, at different times, to the issuance of new laws addressing different areas of human activities, as well as a series of amendments to the existing regulations, from the Constitution to the regulatory provisions of secondary laws.

Finally, it is important to note that, although the Mexican environmental legal system addresses the proper protection of flora and fauna, it is not necessarily managed holistically. This is the consequence of the lack of linkage between the different planning and management instruments, and ineffective implementation in terms of governance and conservation economics.

Today, there are few environmental regulations and enforcement instruments in matters of ecological connectivity and fauna passages in the Mexican environmental legislation. Although México has worked in subjects related to connectivity and biological corridors for over two decades, integrating the definitions and associated regulations in the General Law of Ecological Balance and Environmental Protection (LGEEPA, in Spanish) is still pending. The LGEEPA does mention the "landscape" concept from an aesthetic standpoint as part of the establishment of Natural Protected Areas (article 47 bis, Fracc. II, section e); however, it includes neither a comprehensive vision of landscape as an environmental entity, nor the relationship with connectivity, biological corridors, ecosystems, and conservation of flora and fauna. The only federal law that defines biological corridors and refers to ecological connectivity is the General Law on Climate Change, issued on 6 June 2012. The General Wildlife Law (LGVS, in Spanish), issued in 2000 and with its latest amendment published in 2020 (SEMARNAT 2021), was the first of its kind to address the establishment of biological corridors within the framework of Management Units for Wildlife Conservation (UMAs, in Spanish); however, no legal policies were developed for the protection and conservation of biological corridors addressing the displacement of mammals or other wildlife species.

México is committed to preserving ecosystems and their connectivity by 2030 in response to the international agreements signed regarding a Sustainable Development, particularly in relation to biodiversity conservation, as part of the Aichi targets on the Strategic Plan for Biodiversity 2011-2020, through the document "National Vision for Integrated Landscape Management and Connectivity", intended to build a national policy of integrated landscape management and connectivity under a coordinated approach (SEMARNAT 2017). However, no regulatory framework in the legal system is still available that mandates the construction of artificial corridors (herein referred to as wildlife passages on roads and railways) as a measure to foster connectivity and reduce fragmentation caused by such infrastructure.

Location, design, construction, and monitoring initiatives such as those presented in the Manual for the Design of Road Wildlife Passages (SCT 2021) are essential to standardize monitoring activities and eventually define the efficiency and effectiveness of the different types and sizes of passages, as well as the economic profitability of the construction in terms of conservation and recovery of ecological connectivity. There have been some legislative initiatives promoting the regulation of these topics in road construction. An example is the one addressed by Deputy G. E. Ralis Cumplido in 2018: "That amends article 2 and adds article 25 Bis to the Law of Roads, Bridges, and Federal Motor Transportation in 2018" and "With an agreement, by which the SCT is encouraged to issue, with the support of SEMARNAT, the Mexican Official Standard (NOM, in Spanish) that sets forth the technical specifications to build wildlife passages on federal roads and highways". Another is the initiative of 25 March 2021, where the Chamber of Deputies unanimously approved the "Addition of article 22 Bis to the Law of Roads, Bridges, and Federal Motor Transportation, which establishes that for the construction of new roads and highways, as well as in the modernization of the existing ones, the Secretariat of Communications and Transport, seeking the protection and conservation of ecosystems, shall include the implementation of fauna crossings in its design and conservation plan". The latter was forwarded to the United Commissions on Communications and Transport and Legislative Studies of the Senate on 6 April 2021 and is currently pending approval in the Senate. In both instances, the agreements that establish mandatory technical guidelines for implementing the construction of fauna passages from the planning stage of roads, as a necessary measure to reduce fragmentation and foster connectivity in landscape and ecosystems, have not yet permeated the Mexican legislation.

Although the implementation of wildlife passages in road infrastructure is a growing trend worldwide, México currently lacks regulations and public policies in this regard. Therefore, initiatives should be put forward to develop the corresponding legal framework aimed at building increasingly sustainable road projects in the country. A first step taken in this direction is the development of the first Manual for the Design of Road Wildlife Passages (SCT 2021); this document is expected to serve as a guide from the project planning and decision-making stages regarding the design, types, and number of wildlife passages, to the evaluation and monitoring of their effectiveness and efficiency.

Also, additional studies should be conducted on the efficiency, effectiveness, and role of wildlife passages in the conservation and recovery of connectivity, biodiversity, and associated environmental services on which we all depend. The "wildlife passage" figure should be institutionalized in the respective regulatory bodies in a coordinated and integral manner, aiming to promote the creation of sustainable projects in México.

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Impact of the roadkill of a jaguar cub (*Panthera onca*) on social networks and the inhabitants of Sinaloa, México

Impacto del atropellamiento de una cría de jaguar (*Panthera onca*) en las redes sociales y las comunidades de Sinaloa, México

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Vehicle traffic on roads is a risk factor for wildlife; the death of wild animals by collision involves direct adverse effects at the population level, especially for endangered species such as the jaguar (*Panthera onca*). This note reports the first roadkill record of a jaguar cub and its impact on the inhabitants of southern Sinaloa, México. The collision occurred in November 2020 on the road running adjacent to the Meseta de Cacaxtla Ecological Reserve. Information on the jaguar cub roadkill was distributed by the people that rescued the corpse and media awareness. About 50 thousand people were reached or informed through social networks. Environmental education workshops, conferences, and webinars were conducted in several villages across the region to raise awareness and for drivers to take precautions to prevent accidents threatening the well-being of wild fauna and road users.

Key words: Conservation; feline; mortality; roads; rural communities; social networks.

El tráfico vehicular en carreteras se ha evidenciado como un factor de riesgo más para la fauna silvestre; las muertes por atropellamiento de individuos pueden tener impactos negativos directos a nivel poblacional, sobre todo para aquellas especies en peligro de extinción, como es el jaguar (*Panthera onca*). El objetivo de este documento es dar a conocer el primer registro de atropellamiento de una cría de jaguar y el impacto que generó en comunidades del sur de Sinaloa, México. El accidente ocurrió en noviembre de 2020 en una carretera colindante a la reserva ecológica Meseta de Cacaxtla. Las personas involucradas en el rescate del cuerpo y los medios de comunicación dieron a conocer la muerte de la cría. Cerca de 50 mil personas fueron alcanzadas / informadas en redes sociales; en los pueblos de la región se llevaron a cabo talleres de educación ambiental, conferencias y webinarios, dirigidos a sensibilizar y tomar precauciones para evitar accidentes que comprometan el bienestar de la fauna y los transeúntes en carretera.

Palabras clave: Carreteras; comunidades rurales; conservación; felino; mortalidad; redes sociales.

The jaguar (*Panthera onca*) is the most admired feline throughout the history of humankind in America (Saunders 2005; Olivier 2016). It plays a key role in the structure of the ecosystems where they thrive (Miller and Rabinowitz 2002; Ripple *et al.* 2014; Cruz *et al.* 2021) and is the focus of research and conservation efforts at continental and national levels (Medellín *et al.* 2016; Paviolo *et al.* 2016; Ceballos *et al.* 2021a).

In México, the original distribution range of the jaguar used to comprise a vegetation continuum ranging from dry forests in Sonora to tropical rainforests in the southeast (Ceballos *et al.* 2016). However, this area has been reduced, and jaguar populations are currently threatened by multiple factors such as poaching, habitat destruction by changes in land use, cattle-jaguar conflicts, and the construction of infrastructure (de la Torre *et al.* 2017; Peña-Mondragón *et al.* 2017; Quigley *et al.* 2017; SEMARNAT 2019). The latter includes the opening of roads that impairs the connectivity and fragments natural landscapes, creating barriers that reduce the ability of wild animals to move in the habitat to meet their needs (food, shelter), ultimately leading to their death. Several feline species involved in roadkills have been recorded, including the ounce (*Herpailurus yagouaroundi*) and lynx (*Lynx rufus*; González-Gallina and Hidalgo-Mihart 2018). The anthropization of nature alters essential habitats for wildlife (Ceballos *et al.* 2021b), including the construction of infrastructure for the production and transportation of goods and people (González-Gallina and Hidalgo-Mihart 2018; Filius et al. 2020).

Wildlife death from road accidents can have direct impacts on their populations. Road mortality has been reported as one of the most serious hazards for many threatened species, such as ocelots (*Leopardus pardalis*) and Florida panthers (*Puma concolor coryi*) in the United States, whose mortality is as high as 32 % (Clevenger *et al.* 2008). In Latin America, research addressing this issue is at a very early stage (Abra *et al.* 2021); in México, knowledge is being generated regarding the impacts of vehicles (González-Gallina and Hidalgo-Mihart 2018; Cupul-Magaña 2019; Canales-Delgadillo *et al.* 2020; Preciado and Romero 2020) and actions are being taken to prevent wildlife losses on roads (González-Gallina *et al.* 2013; Pacheco *et al.* 2016; Manteca-Rodríguez *et al.* 2021).

In some regions of México, scientific research has been supplemented by cultural activities and actions to promote the dissemination of information and environmental education (Briones-Salas *et al.* 2011; Peña-Mondragón *et al.* 2017; Castillo *et al.* 2020; Zamudio *et al.* 2020). These measures have raised awareness among individuals and social groups, who have recognized the jaguar as an important species in the contemporary natural and cultural landscapes of México (Morales and Morales 2018). This note describes the effect of a roadkill event involving a jaguar cub on a number of actions to conserve this species in Sinaloa (DCS 2020).

The present research was conducted in the San Ignacio municipality, in the southern region of the state of Sinaloa, México (Figure 1). This area comprises 4,651 km^2 with landscapes of the Pacific coastal plains and high mountains in the Sierra Madre Occidental. The prevailing climate in the study area is warm sub-humid, being semi-dry on foothills and coastal plains and temperate in mountainous areas. The mean annual temperature ranges between 22 ºC and 26 °C, and the mean annual precipitation is between 700 and 1,000 mm. There are two clearly defined seasons: rainy in summer and part of autumn and dry the rest of the year. Dry forests are the dominant vegetation, and pine-oak forests are distributed in high-mountain temperate areas (Rzedowski 2006). The towns with the largest number of inhabitants are Estación Dimas, Piaxtla, Coyotitán, and San Ignacio (the municipal capital); the total population in the San Ignacio municipality is 23,355 inhabitants (INEGI 2010).

The jaguar cub was road-killed in the early morning hours of 5 November 2020. A. Loaiza, a local inhabitant, found it at 6:30 hr lying on the road at km 64 of the México 015 federal highway, Coyotitán-Mazatlán section, Sinaloa (23° 47′ 18.86″ N, 106° 36′ 42.02″ W; Figures 1 and 2). This

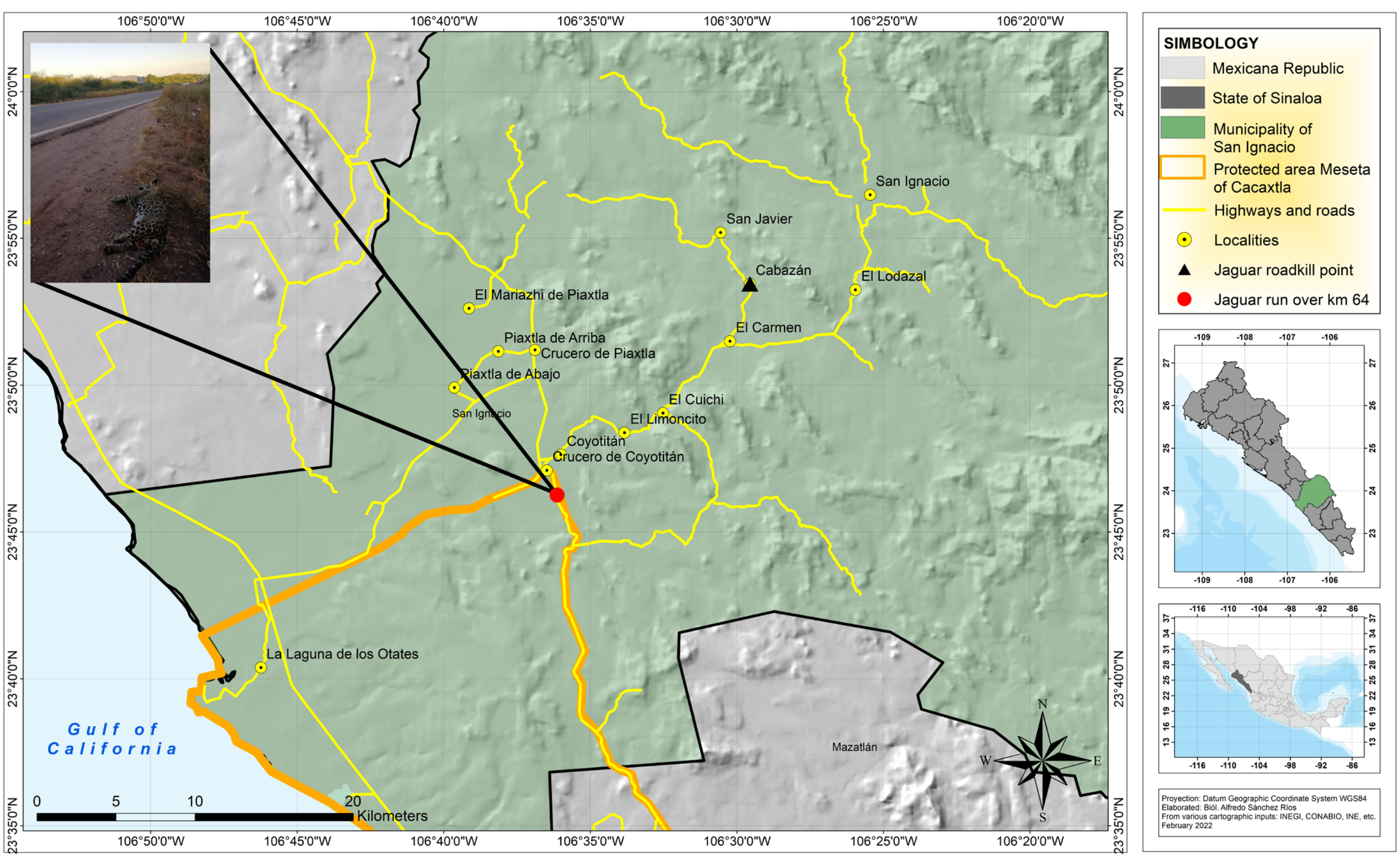

Figure 1. Location map of the San Ignacio municipality, Sinaloa, México, and site of the jaguar cub roadkill event. Prepared by A. Sánchez.

work is the first to record in detail the roadkill of a jaguar cub on a highway in México. The specimen was a male of approximately 100 days of age, with a weight of 8.5 kg and a body length of 1.5 m from the nose to the tip of the tail. This event was informed to the staff of the Museo del Jaguar (Jaguar Museum), the Procuraduría Federal de Protección al Ambiente (Federal Attorney's Office for Environmental Protection; PROFEPA, in Spanish), and the Comisión Nacional de Áreas Naturales Protegidas (National Commission of Natural Protected Areas; CONANP, in Spanish), particularly the direction of the Meseta de Cacaxtla Flora and Fauna Protection Area (APFFMC, in Spanish). The road section where the roadkill occurred is part of the border delimiting the polygon of the APFFMC protected area, which comprises 50,000 ha of tropical deciduous forest and is a key habitat for the conservation of populations of jaguar and other felines at risk, such as the ocelot, margay (*Leopardus wiedii*), and jaguarundi (*H. yagouaroundi*; Rubio-Rocha *et al.* 2010).

In years prior to the jaguar cub roadkill (Armenta 2020; Redacción/Sin Embargo 2020), anecdotal evidence of the roadkills of 4 adult jaguars and 2 cubs was found. These 6 specimens include one adult that was road-killed in 2014 at kilometer 59 of the México 015 highway, at the periphery of the APFFMC (G. Corrales-Herrera, com. pers.). On 25 July 2016, a CONANP staff recorded the roadkill of 1 female jaguar with 2 cubs at kilometer 36 of the Mazatlan-Culiacán highway (México 15D) and 1 male jaguar at kilometer 32 of the same road on 19 September of the same year (M. Amador-Medina, webinar of the third Road Ecology Workshop). In 2017, the death of 1 adult male in the Concordia municipality was reported (Entreveredas 2017; Rasnoticias 2017).

Sinaloa has a road network of 16,837 km, of which 4,866 km are paved roads plus 11,971 km of gravel rural and dirt roads (INEGI 2017). The México 015 highway comprises 656 km and runs throughout the state, from the north in the Ahome municipality to the south in Escuinapa; the length of the section that crosses the San Ignacio municipality is 41 km (INEGI 2017). This information gives an idea of the extent of the paved road network and the potential risk for the conservation of the jaguar and the wildlife that crosses it (Canales-Delgadillo *et al.* 2020). Some animals are attracted to roadsides where grass and food resources consumed by deer and other herbivores grow, posing the risk of being hit and killed by vehicles (Gottdenker *et al.* 2001; Toro-Garay *et al.* 2021). Certain species, such as the jaguar, are rarely observed after a collision event. Sometimes, corpses are removed (legally or illegally) before the road maintenance crew finds them (Abra *et al.* 2021) for the economic value of the skin and fangs; in other cases, the animal affected does not die immediately and seeks shelter in the surrounding vegetation. Abra *et al.* (2021) conducted thorough research on the roads of Sao Paulo, Brazil, over 10 years of monitoring and did not record jaguar roadkills. However, these authors found one report of 2 dead jaguar cubs in the databases of the company in charge of managing the roads and estimated more than 37 thousand road-killed mammals in 27 10-km sections out of a total of 199,371 km of road.

The road-killed jaguar cub caught the attention of society, even though it occurred during the SARS-CoV-2 pandemic (DCS 2020; Ortiz 2020), triggering the launch of the #IBrakeForTheJaguar campaign. The people of the communities of San Ignacio municipality, following the health protocols and with the collaboration of several institutions, organized educational activities to raise awareness about wildlife-vehicle collisions. On November 12, 2020, within the framework of the International Jaguar Day, the campaign # IBrakeForTheJaguar (Museo del Jaguar 2020a) was launched on the media and digital platforms, with the support of the Alianza Nacional para la Conservación del Jaguar (National Alliance for Jaguar Conservation) and Biofutura organization. During this event, with the collaboration of local inhabitants, 150 posters were affixed at various road crossings, starting in the Crucero de Piaxtla community (Figure 3). As a result, more than 150 drivers were informed about the jaguar cub roadkill, the importance of slowing down when traveling through signposted wildlife passages, and being alert to spot the crossing of any animal.

Additionally, 6 exhibitions were held, including environmental education workshops, in the communities of Ajoya, La Labor, El Carmen, Cabazán, San Javier, and San

Figure 2. Road-killed jaguar cub on the México 015 federal highway at km 64 of the Coyotitán-Mazatlan section, Sinaloa, México. Photograph by A. Loaiza.

Ignacio municipal capital, with the participation of around 400 children and adults (Leyva 2020; Tenorio 2020). With the appropriate permits, taxidermy was performed on the specimen, which was exhibited in the Jaguar Museum of Cabazán (Museo del Jaguar) to attract attention and raise awareness about the impacts of road accidents.

Informative videos were produced on the ecological and cultural importance of the jaguar, the threats that put it at risk, and the actions to support the conservation of its populations. Online meetings were promoted on digital platforms (Facebook and Zoom). On the Museo del Jaguar Facebook page, news of the road-killed cub was posted, reaching a large number of people. The digital administrator statistics show that the news was seen by 48,884 users and shared 272 times; 2,198 comments were also posted, and 7,531 clicks were recorded in digital posts (Museo del Jaguar 2020b).

As a result of the jaguar cub roadkill event, the Direction of the APFFMC Reserve monitored 107 km of roads on 12 occasions and recorded 92 roadkill events of 31 species, including 11 raccoons, 8 boas, and 5 rattlesnakes (S. González-Palacios, webinar of the third Road Ecology Workshop). These findings reflect the extent of the issue for wild mammals and other vertebrate species (Leyva 2021).

Roads in Sinaloa pose a hazard for jaguar and other wildlife populations; the vast majority of roads do not include wildlife passages and signaling to guide and inform about the possibility of animals crossing the road. An additional risk factor is the poaching facing these natural predators because they are considered potential threats to livestock, even without actual evidence (Inskip and Zimmermann 2009; Puc-Sánchez *et al.* 2013). Therefore, it is necessary to promote new forms of human-jaguar coexistence. Socio-environmental assessments should be performed to define participatory strategies that consider the needs of people and wildlife to mitigate wildlife species loss, including America's big cat — the jaguar (Castillo *et al.* 2020; Ceballos *et al.* 2020; PNUMA 2021). Jaguar roadkills are seemingly sporadic events in the study region. Nonetheless, the adverse effect of the loss of even a single specimen is serious enough for the local jaguar population, which is extremely small, from 0.75 to 3.3 individuals per 100 km^2 (Chávez *et al.* 2016). Therefore, measures for its long-term conservation are of paramount importance and urgent.

The use of digital strategies and communication actions is an instrument that allows sharing information quickly over large distances to a large number of people, objectively and assertively in the best case (Larena 2010). The dissemination of socio-environmental news, such as the jaguar cub roadkill, can foster changes in social perception and behavior (Peña-Mondragón *et al.* 2017; Whitehouse-Tedd *et al.* 2021) to avoid or reduce road-related risks, which claim lives of wildlife and road users (Ament *et al.* 2021).

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Figure 3. Informative poster about the sites identified as wildlife crossings and participants in the #IBrakeForTheJaguar campaign in the San Ignacio municipality, Sinaloa, México. Photograph by A. Pérez.

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Documentation of a road-killed spectral bat (*Vampyrum spectrum*) and first report of the species in Tabasco, México

Documentación de un murciélago espectral (*Vampyrum spectrum*) atropellado en carretera y primer reporte de la especie en Tabasco, México

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Roads are human infrastructures with adverse effects on wildlife; in the case of mammals, bats are rarely mentioned as road-killed animals. This work describes the record of a road-killed spectral bat (*Vampyrum spectrum*) on the El Bellote–Santa Cruz section of the 180 highway in Paraiso, Tabasco, México, the first report of the species in the state. The spectral bat is the largest bat species in the Americas, considered locally rare. The finding was recorded in 2015 during a wildlife mortality sampling tour. The specimen was collected and deposited in the National Mammals Collection of Universidad Nacional Autónoma de México. The corpse of a bird (Tyrannidae) was also found during the sampling trip. This is the first documented record of a road-killed spectral bat. The unique flight characteristics of the spectral bat, such as flying near obstacles and at ground level, make it vulnerable to vehicles and collisions. Mitigation measures should be applied to reduce the risk of collision for this species in particular and all bats in general.

Key words: Bats; mammals; road ecology; roadkill.

Las carreteras son infraestructuras humanas con efectos negativos para la fauna silvestre; en el caso de los mamíferos, los murciélagos pocas veces se mencionan como víctimas de atropellos. En este trabajo, se presenta el registro de un murciélago espectral (*Vampyrum spectrum*) atropellado en la carretera 180 del Tramo El Bellote-Santa Cruz, Paraíso, Tabasco, México, representando el primer reporte de esta especie en el estado. El murciélago espectral es el más grande del Continente Americano, el cual es considerado como una especie localmente rara. El hallazgo ocurrió en 2015 durante un recorrido de un muestreo de mortandad de fauna silvestre. El ejemplar fue recolectado e incorporado a la Colección Nacional de Mamíferos de la Universidad Nacional Autónoma de México. Al momento de la colecta se encontró también el cuerpo de un ave (Tyrannidae). El presente registro corresponde al primero de atropellamiento de murciélago espectral del que se tenga documentación. Las características particulares de vuelo del murciélago espectral de volar cerca de obstáculos y a ras del suelo, lo hace vulnerable a los vehículos y a las colisiones. Es necesario tomar medidas de mitigación que disminuyan los riesgos de colisión para esta especie y murciélagos en general.

Palabras clave: Atropello; ecología de carreteras; mamíferos; murciélagos.

Roads are the type of anthropic infrastructure with the most adverse effects on wildlife, mainly from collisions with vehicles and the impact of roads on habitat loss, fragmentation, and alteration (van der Ree *et al.* 2011; van der Ree *et al.* 2015; Schwartz *et al.* 2020; Benítez *et al.* 2021). Roadkills account for 6.25 % of vertebrate mortality (Hill *et al.* 2019), with large mammals as the most recorded and vulnerable taxa, followed by birds, amphibians, and reptiles (Rytwinski and Fahrig 2012; Silva *et al.* 2021).

Mammals are a group of great concern because of their vulnerability to local extinction due to roadkills (Grilo *et al.* 2021) and the human and economic costs of these accidents (Ascensão *et al.* 2021). The impact of roads on wild mammals has been focused mainly on ungulates, large carnivores, and small non-flying mammals (Novaes *et al.* 2018; Pinto *et al.* 2020).

Bats are rarely detected in roadkill studies compared to birds and amphibians (Berthinussen and Altringham 2012; Medinas *et al.* 2019; de Figueiredo Ramalho *et al.* 2021a; de Figueiredo Ramalho *et al.* 2021b). This group of mammals is vulnerable to roadkills due to the barrier effect limiting their displacement from one site to another.

Despite its low detectability and scarce documentation, they are assumed to be at high risk of collision with motor vehicles given their flight habits by preferring to forage in the linear elements of the landscape (Bennett and Zurcher 2013; Claireau *et al.* 2021). They go unnoticed due to their small size, their ability to hide out among the vegetation, rapid decomposition of the corpse, and predation by scavengers (Russell *et al.* 2009; Teixeira *et al.* 2013; Altringham and Kerth 2016; Delgado *et al.* 2019). Since bats are less frequently detected than most vertebrates in road studies, collisions with vehicles might be an important cause of bat mortality (Lesiński *et al.* 2011).

The spectral bat (*Vampyrum spectrum*) is the largest bat species in the Americas, with a wingspan from 101 to 110 cm and a weight of approximately 180 gr (Navarro and Wilson 1982; Gardner 2008). It belongs to the family Phyllostomidae and is an apex predator with a wide home range and small scattered populations (Timm *et al.* 1989; Ceballos and Oliva 2005). This species inhabits caves or tree cavities and lives as solitary individuals or in family groups of about 5 members (Solari *et al.* 2019).

The spectral bat has a wide distribution in the Neotropical region, from Veracruz in México to Brazil, Perú, and Bolivia (Hall 1981; Medellín *et al.* 2008; Solari *et al.* 2019). This species has been found in tropical forests and secondary and riparian vegetation (Vehrencamp *et al.* 1977; Solari *et al.* 2019), and recorded in elevations from 4 m to 1,600 m (Timm *et al.* 1989; LaVal and Rodríguez 2002).

Vampyrum spectrum is a carnivorous bat (Gual-Suárez and Medellín 2021) that consumes rodents, other bats, probably insects, and birds of 20 gr to 150 gr. Up to 18 bird species have been identified in their diet, mainly trogons (Trogonidae), columbids (Columbidae), motmots (Momotidae), cuckoos (Cuculidae), wrens (Troglodytidae), and icterids (Icteridae; Timm *et al.* 1989; Reid 2006; Reid *et al.* 2010). *Vampyrum spectrum* is considered locally rare (Reid 2006). In México, it is classified as endangered (SEMARNAT 2010), while the International Union for Conservation of Nature listed it as Near Threatened (IUCN 2021). The main threats to their populations are fragmentation, habitat loss (Solari 2018), and anthropic destruction of their shelters due to negative perceptions influenced by myths and ignorance (Gómez-Nísino 2006).

This note reports for the first time one specimen of *V. spectrum* inhabiting the Tabasco plain that was road-killed from a collision with a vehicle on a road in the Paraiso municipality.

This information was obtained during the Wildlife Mortality Project in the Tabasco Plain conducted by researchers of the Universidad Juárez Autónoma de Tabasco (UJAT). The objective of this project was to identify critical sites of wildlife collisions and to define mitigation measures in the Tabasco Plain.

The prevailing climate in the study area is warm humid, with annual precipitation ranging from 1,500 to 2,000 mm and annual mean temperatures above 26 °C, with maximum values between 30 °C and 34 °C in May and minimum between 20 °C and 22 °C in January (Hernández-Santana *et al.* 2008). Dominant winds flow from E to NE at an average speed of 4 to 6 m/s (Hernández-Santana *et al.* 2008). There are three clearly defined seasons in a year: dry, rainy, and winter rainy seasons (Moguel and Molina-Enríquez 2000). The dominant habitats are mangrove forests, wetlands, cultivated pastures, and water bodies (INEGI 2016).

The prevailing land use at the collision site is permanent rainfed agriculture (INEGI 2016). The study section is a paved state road consisting of two C-type lanes with an average crown width of 18 m. The speed limit is 80 km/h, and the mean traffic volume is 103 vehicles per day, of which 83.6 % are cars. The Annual Mean Daily Traffic (TDPA, in Spanish) was 3,489 vehicles in the year of the road-kill event (SCT 2016).

The corpse was photographed, collected, and brought to the Landscape Ecology and Global Change Laboratory of the Biological Sciences Division at UJAT for identification. Afterward, it was frozen and sent to the National Mammal Collection (CNMA, in Spanish) of the Institute of Biology at Universidad Nacional Autónoma de México (UNAM), where its identification was confirmed. The specimen was conventionally prepared (skin and skeleton) and listed with the corresponding coordinates. The following measurements were recorded in millimeters (mm): total length (LT), tail length (LC); rear right leg length (LPDT); and right ear length (LOD). Additionally, the total weight was reported.

The sites where this species has been documented in México were gathered from a survey in the literature, the Global Biodiversity Information Facility (GBIF 2021), and the NaturaLista platform (2021). The data obtained was visualized on a map of the region.

On 16 May 2015, at 6:00 hr, a male road-killed *V. spectrum* bat was observed during a monitoring trip (Figure 1). The animal was found lying on the El Bellote-Santa Cruz section of the 180 highway in Chiltepec village, Paraiso municipality, Tabasco. It was recorded during the rainy season (May to October) when precipitation exceeds 120 mm. The collision occurred on a stretch of the straight road surrounded by secondary vegetation associated with fruit trees: mango (*Magnifera indica*), banana (*Musa paradisiaca*), coconut (*Cocos nucifera*), and gliricidia (*Gliricidia sepium*).

The bat specimen was recorded under catalog data CNMA 47872, male; sampling locality 0.64 km W Aquiles Serdán, Paraíso municipality, Tabasco (18° 24′ 46.89″ N, 93° 0′ 57.74″ W), on the Chiltepec-Vicente Guerrero highway, at 1 m above sea level. The measurements of the specimen (mm) were: LT 144; LC 0; LPDT 32.0; LOD 42. total weight was 182.9 gr.

The bat specimen was collected with bird feathers in the mouth. The headless body of a bird of the family Tyrannidae was also observed (Figure 1). The head was not found near the site, so the bird was likely captured by the bat.

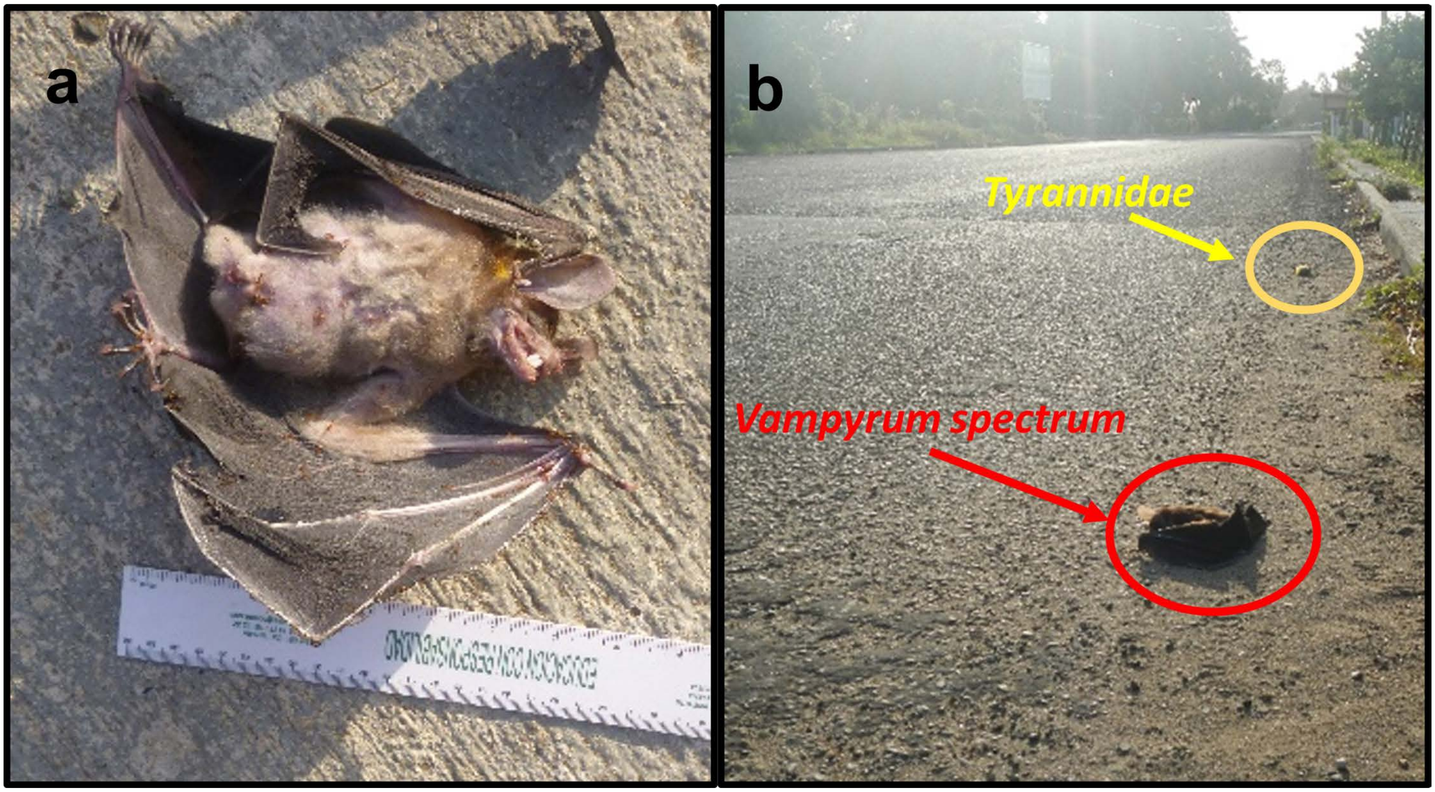

Figure 1. a) *Vampyrum spectrum* individual at the collision site. Ants are observed preying on the specimen, and b) location of the spectral bat and a bird specimen of the family Tyrannidae at a distance of approximately 2 meters, possibly preyed upon by the bat.

Only 13 previous records of *V. spectrum* were found (Figure 2): 3 in Veracruz (Goldman 1917; Navarro 1979; GBIF 2021), 2 in Oaxaca (Alfaro *et al.* 2005; Santos Moreno *et al.* 2010), 3 in Chiapas (Hernández-Mijangos *et al.* 2008; López *et al.* 1998; Peña-Cuéllar *et al.* 2012), 4 in Campeche (Hernández-Huerta *et al.* 2000; Escalona-Segura *et al.* 2002; NaturaLista 2021a, 2021b), and 1 in Quintana Roo (NaturaLista 2021c). Therefore, this is the first documented record of the species for Tabasco, which is also a roadkill report.

Although bats are a well-studied group in Tabasco (Sánchez and Romero 1995; García-Morales *et al.* 2014; Hidalgo-Mihart *et al.* 2016), the presence of *V. spectrum* had not been previously reported in the literature. According to Solari *et al.* (2019), its distribution in México comprises southern Veracruz, Oaxaca, Chiapas, and the southern Yucatán Península, so this record adds Tabasco to its distribution range.

Bats are among the rarest organisms reported in roadkill studies (de Figueiredo Ramalho and Aguiar 2020; de Figueiredo Ramalho *et al.* 2021a; Vargas-Contreras *et al.* 2021), but an underestimation is plausible due to the poor detectability of these individuals. Furthermore, specific studies have reported that roads cause significant adverse effects on this group, particularly on insectivorous bats (Berthinussen and Altringham 2012; Medinas *et al.* 2019). The families most commonly recorded are Vespertilionidae and Phyllostomidae (de Figueiredo Ramalho and Aguiar 2020; Novaes *et al.* 2018).

In México, bat records in road ecology studies are scarce (González-Gallina and Badillo 2013; González-Gallina *et al.* 2013; Pacheco-Figueroa *et al.* 2021; Vargas-Contreras *et al.* 2021), probably because of the sampling techniques applied. As mentioned by Vargas-Contreras *et al.* (2021), corpses lying on the road are difficult to spot since the speed of the vehicle forces bats out of the road area and into the vegetation. Therefore, this is likely the first record of a road-killed *V. spectrum* (González-Gallina and Badillo 2013; González-Gallina *et al.* 2013; Ceron *et al.* 2017; Cervantes-Huerta *et al.* 2017; de Figueiredo Ramalho and Aguiar 2020; de Figueiredo Ramalho *et al.* 2021a).

Furthermore, the flight strategy influences the risk of collision (Vargas-Contreras *et al.* 2021). Species flying at less than 10 m high, such as members of the family Phyllostomidae, face a higher risk of vehicle collisions (de Figueiredo Ramalho and Aguiar 2020); this family includes the spectral bat, the largest in the Neotropics. The spectral bat uses smell to locate its prey (Veherencamp *et al.* 1977) and flies slowly, near obstacles, and at ground level (Khan 2015), making it particularly vulnerable to vehicles and collisions. Spectral bats are likely to move across roads continuously, being frequently hit by vehicles, but road surveys are not efficient enough to record them.

The impact of roads on bats remains poorly documented and with unknown effects on species that are rarely observed. Most of the available research focuses on four-lane, high-traffic roads, and large or charismatic species. Low-to-moderate traffic roads are considered to have negligible impacts. However, small roads run across landscapes all over the world, with poorly studied species incidentally found in collision events on these roads.

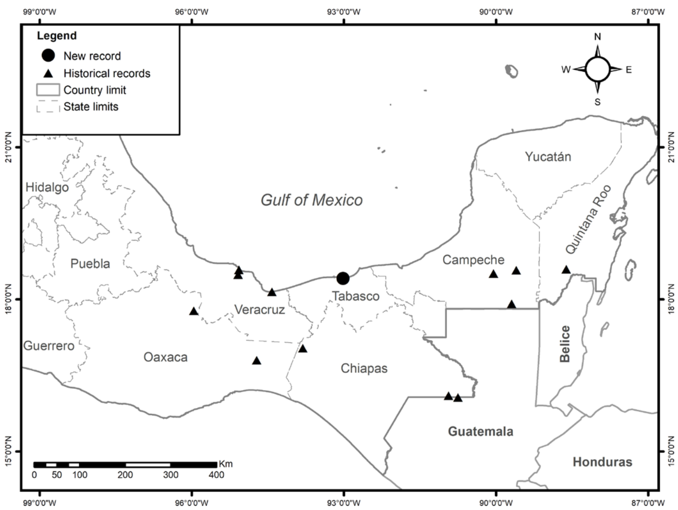

Figure 2. Historical and current records of the spectral bat (*Vampyrum spectrum*) near Paraíso, Tabasco, México.

The spectral bat is threatened by habitat loss and the negative perception of bats among people (Ceballos and Oliva 2005; Gómes-Nísimo 2006; Solari 2018). This species prefers little-disturbed habitats; however, as Vleut (2013) mentioned, it can also take advantage of secondary vegetation, as long as there are forests that provide roost sites and shelter. Bats are most frequently hit by vehicles on roads close to vegetation that offers shelter, food, and roost sites (Rusell *et al.* 2009; Roemer *et al.* 2020). The road where the killed spectral bat was found is surrounded by natural vegetation dominated by mangrove trees, providing shelter and roost sites (Timm *et al.* 1989). Besides, the tyrannid bird, which apparently was caught as prey, suggests that these bats forage in patches adjacent to the road. The road studied represents high-risk conditions for the spectral bat to get hit by vehicles.

The road section where the bat was found showed a marked increase in the TDPA from 5,768 vehicles per year in 2015 to 6,977 vehicles per year in 2016. Although these values dropped in the following years (6,002 vehicles per year in 2019; SCT 2021), the risk of collision persists because this road is part of the Modernization and Expansion Plan of the Sánchez Magallanes – Paraíso – Frontera Highway (GET 2019), which involves expansion works and increased traffic flow, particularly cargo trucks.

Since *V. spectrum* is a threatened species, it should be considered in mitigation measures to reduce the impact of roads on this species. Without proper control and measures, isolated populations of *V. spectrum* in Tabasco may suffer adverse effects without even having an estimate of their populations.

Regarding mitigation measures, green bridges allow safe passage of bats (de Figueiredo Ramalho and Aguiar 2020), but proper measures must be applied to guide bats towards these structures. Underpasses such as large bridges and culverts are considered effective measures (Claireau *et al.* 2019); however, these passages are implemented mainly in temperate climates, so there is no evidence of their effectiveness in tropical areas (Vargas-Contreras *et al.* 2021). Therefore, these measures must be applied along with a monitoring program allowing for future adaptations.

The most effective measure is the reduction of speed limits in areas with tree cover surrounding the road because these are the sites with the highest probability of vehicle-wildlife collisions. Structural modifications should be implemented by planting trees or installing fences that force bats to fly higher (Vargas-Contreras *et al.* 2021). Continuous artificial light affects the behavior of nocturnal species (MITECO 20219); therefore, if the road infrastructure requires lighting, it should be kept to a minimum (Garin *et al.* 2016).

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Confused identities: the case of the *chauve–souris septième* or *chauve–souris brun–blanchâtre* of Azara (1801)

Identidades confundidas: el caso del *chauve–souris septième* o *chauve–souris brun–blanchâtre* de Azara (1801)

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Félix de Azara described several species of bats in his seminal work on mammals of Paraguay in 1801. One of these bats, the *chauve–souris septième* or *chauve–souris brun–blanchâtre*, was used by É. Geoffroy St–Hilaire (1806) to describe his *Vesp*[*ertilio*]. *villosissimus*, a name currently applied to the southern hoary bat, *Lasiurus* (*Aeorestes*) *villosissimus*. The latter, in addition, is the type species of the genus *Aeorestes* Fitzinger 1870. In this contribution, through a reevaluation of the description provided by Azara (1801), we demonstrate that the *chauve–souris septième* or *chauve–souris brun–blanchâtre* is clearly referable to the subgenus *Dasypterus* W. Peters, 1870. Strict application of the rule of priority in this case would cause considerable and undesirable nomenclatural upheaval, including the synonymy of *Nycticejus Ega* Gervais, 1856 with *Vesp*[*ertilio*]. *villosissimus* É. Geoffroy St.–Hilaire, 1806 and the need of a new genus (or subgenus)-level name for hoary bats. We thus recommend preservation of current usage by declaring a neotype for *Vesp*[*ertilio*]. *villosissimus* É. Geoffroy St.–Hilaire, 1806 and proposing a new type species for *Aeorestes* Fitzinger, 1870.

Key words: *Aeorestes*; *Dasypterus*; Hoary Bats; Lasiurine bats; *Lasiurus*.

Félix de Azara describió varias especies de murciélagos en su trabajo seminal sobre los mamíferos del Paraguay en 1801. Uno de estos murciélagos, el *chauve-souris septième* o *chauve-souris brun-blanchâtre* fue utilizado por É. Geoffroy St-Hilaire (1806) para describir *Vesp*[*ertilio*]. *villosissimus*, un nombre que se aplica actualmente al murciélago escarchado gris del sur, *Lasiurus* (*Aeorestes*) *villosissimus*. Esta última, además, es la especie tipo de *Aeorestes* Fitzinger 1870. En esta contribución, a través de una reevaluación de la descripción proporcionada por Azara (1801), demostramos que el *chauve-souris septième* o *chauve-souris brun-blanchâtre* es claramente referible al subgénero *Dasypterus* W. Peters, 1870. La aplicación estricta de la regla de prioridad en este caso causaría una alteración nomenclatural considerable e indeseable, incluida la necesidad de sinonimizar *Nycticejus Ega* Gervais, 1856 bajo *Vesp*[*ertilio*]. *villosissimus* É. Geoffroy St-Hilaire, 1806 y la necesidad de un nuevo nombre genérico o subgenérico para los murciélagos escarchados grises. Por lo tanto, recomendamos la preservación del uso actual a través de la designación de un neotipo para *Vesp*[*ertilio*]. *villosissimus* É. Geoffroy St-Hilaire, 1806 y proponiendo una nueva especie tipo para el taxón *Aeorestes* Fitzinger, 1870.

Palabras clave: *Aeorestes*; *Dasypterus*; Lasiurinae; *Lasiurus*; murciélagos canosos.

Félix de Azara (1742–1821) was a Spanish military engineer and pioneer in natural history studies in southern South America (Contreras 2010). Charged with delimiting of the borders of the Spanish and Portuguese crowns on the continent, he was sent to Buenos Aires and Asunción (at this time within the limits of the Virreinato del Río de La Plata), in representation of the Spanish king. When his Portuguese equivalent failed to show, he focused his attention on documenting the fauna, culture and geography of his new home, a broad area that he defined as "Paraguay and La Plata", that includes modern day eastern Argentina, Paraguay, extreme southern Brazil and Uruguay. A detailed chronology of his life, movements and work was provided by Mones and Klappenbach (1997) and Contreras (2010).

The two volume "Essais sur l'histoire naturelle des Quadrupedes de la Province du Paraguay" (Azara 1801) was perhaps the first serious attempt to describe the mammal fauna of the Southern Cone of the continent. Much to the chagrin of the author, the French version (translated by L. E. Moreau–Saint–Méry) was an incomplete draft that he had sent to Europe for opinion, only to find out later that it had in fact been published. This prompted comments in the prologue of the Spanish version of the text "Apuntamientos para la historia natural de los quadrúpedos del Paragüay y Río de la Plata" (Azara 1802) to the effect that this Spanish version should be considered the finished item, as it contained corrections to the errors and additional species not figured in Azara (1801). Nonetheless, many of the scientific names coined by European academics (including É. Geoffroy St–Hilaire, Desmarest, Oken and Olfers among others), that were derived from Azara's text (as Azara did not employ Linnean binomials), were based on this French version, even though the specimens described (the types) did not always correspond to those in the Spanish version (although not in this case).

Though Azara's descriptions are well written for the time, a lack of biological training, coupled with a global underestimation of the extent of zoological variation in the 19th century, meant that not all of the animals in his descriptions have been identified conclusively, or correctly. Reviews of his texts on the didelphids (*e. g*., Voss *et al.* 2009) and rodents (Tate 1932; Contreras and Teta 2003; Pardiñas *et al.* 2007) have been published, but the descriptions of the bats have been largely assumed to be correct (given that many original scientific names were based upon them). However, the description of the *chauve–souris septième* or *chauve–souris brun–blanchâtre* (Azara 1801; Tome 2: 284), currently regarded as referring to the Southern Hoary Bat, and the basis of *Lasiurus villosissimus*, originally described as *Vesp*[*ertilio*]. *villosissimus* É. Geoffroy St–Hilaire, 1806, clearly does not refer to a hoary bat. A re–evaluation of the identity of this bat takes on renewed significance, given the recognition that this form, formerly considered a subspecies of *Lasiurus cinereus* (Palisot de Beauvois 1796) by Sanborn and Crespo (1957), is in fact a different species (Baird *et al.* 2015). In addition, *Vesp*[*ertilio*]. *villosissimus* is the type species of the genus level name *Aeorestes* Fitzinger, 1870 by original designation.

Disagreement as to the identity of *Vesp*[*ertilio*]. *villosissimus* is not new (see Allen 1901, 1905; Thomas 1901, 1902, 1910). Though Allen (1901) originally considered the name to refer to *Vespertilio bonariensis* Lesson and Garnot, 1827 (at this time, the name applied in part to the South American "red bats"), he later associated it with *Dasypterus* W. Peters, 1870 (Allen 1905). On the other hand, Thomas (1901, 1902, 1910) consistently maintained that the bat described by Azara (1801) corresponded to *L. cinereus* (at this time, the name used for the South American "hoary bats"). Allen (1905) provided a convincing argument to link Azara's *chauve–souris septième* or *chauve–souris brun–blanchâtre* with *Dasypterus*, but Thomas (1910) did not accept the suggestion, although he did concede it was "a little more plausible". Allen (1905:190–191) reasoned: "(1) That it [i.e., *Vesp*[*ertilio*]. *villosissimus*] was of about the size of *L. borealis*, having a wing span of about 317 mm. (2) That it had a rather long, pointed ear, much larger and differently shaped than the ear of *Lasiurus sensu lato*. (3) That the naked edges of the interfemoral membrane, do not exist in the *L. borealis* group, and are only slightly seen in the *L. cinereus* group. (4) That the extreme softness, great length, and the color of the pelage, does not agree in any respect with that of *L. borealis*, nor very well with that of *L. cinereus*, but does agree in length and softness with the *Dasypterus ega* group." Subsequent authors (*e. g*, Cabrera 1958; Sanborn and Crespo 1957; Gardner and Handley 2007) did not discuss this issue again, tacitly accepting the opinion of Thomas (1910).

In this note, we discuss the identity of *Vesp*[*ertilio*]. *villosissimus*, through a re-evaluation of the original texts of Azara (1801, 1802) and application of our current knowledge of the genus *Lasiurus*. In addition, we include some notes on the type locality and the nomenclatural implications of possible name changes.

Notes on the identity of the Azara's chauve–souris septième *or* chauve–souris brun–blanchâtre.

Azara (1801, 1802) described his *chauve–souris septième* or *chauve–souris brun–blanchâtre* as a species with extremely soft hair, an overall whitish-brown coloration and moderately long, pointed ears (Appendix 1–3). Azara (1801, 1802) also remarked that the uropatagium was hairy, except at its border, and of the same color as the body pelage (Appendix 1–3). The description of the furred uropatagium confirms that, within the context of the Paraguayan fauna, this bat is a species of *Lasiurus* Gray, 1831 *sensu lato*. The genus *Lasiurus* includes 3 subgenera (sometimes treated as genera), for which the names *Lasiurus* (broadly referred to as the red bats), *Dasypterus* (yellow bats), and *Aeorestes* (hoary bats) are used. Three species, representing the three recognized subgenera, are documented for Paraguay (De La Sancha *et al.* 2017): *Lasiurus* (*Lasiurus*) *blossevillii* (Lesson, 1826), *L.* (*Aeorestes*) *villosissimus* (É. Geoffroy St–Hilaire, 1806) and *L.* (*Dasypterus*) *ega* (Gervais, 1856) (López-González 2005). However, while both *L. blossevillii* and *L. villosissimus* have their uropatagia covered by hairs almost to the border, *L. ega* has it furred only in the proximal half.

Though the description of this bat is brief, there are no grounds for associating a bat described as having "whitish–brown" pelage with the clade of hoary bats, which are distinctively–colored with frosted dark grey–and–white body fur and an obvious yellowish "balaclava," or the red bats, which are characterized by a reddish coloration frosted with white (*e. g*., Shump and Shump 1982a, 1982b; Kurta and Lehr 1995; Díaz *et al.* 2011). In turn, *L. ega* has a yellowish–olivaceous to brownish coloration (cf. López-González 2005), more in accordance with the description made by Azara (1801, 1802). Finally, *L. ega* has more pointed and moderately long ears, contrasting with the shorter and more rounded pinna of the other two species (cf. Barquez *et al.* 1999). That Azara (1801, 1802) is indeed describing an individual of the species currently referred as *L. ega* is supported circumstantially by the fact that the morphometrics are consistent (though not diagnostic) with the measurements of *L. ega* (Table 1) and the fact that this species is a much more common bat than *L. villosissimus* in Paraguay (López-González 2005).

Notes on type locality.

Azara (1801, 1802) does not provide a type locality for the *chauve–souris septième*, *chauve–souris brun–blanchâtre*

Table 1. External measurements (mean ± standard deviation, and range) for *Vesp*[*ertilio*]. *villosissimus* and the 3 species currently included within *Lasiurus* found in Paraguay (cf. López-González 2005). Measurements for *V. villosissimus* were converted into mm using the French inch ("pie de París" o "pie de Rey") and line (1 inch= 27.07 mm, 1 line = 2.23 mm).

	Lasiurus blossevillii	***Lasiurus ega***	***Vesp*[*ertilio*]. *villosissimus***	***Lasiurus villosissimus***
Total length	Male: 103.4 ± 8.70	Male: 119.7 ± 4.80	117.28	Male: 130.3 ± 1.53
	(89.0–110.0; *n* = 7)	(107.0–127.0; *n* = 35)		(129.0–132.0; *n* = 3)
	Female: 107.2 ± 5.27	Female: 56.5 ± 3.72		Female: 131.6 ± 4.50
	(97.0–119.0; *n* = 16)	(117.0–139.0; *n* = 31)		(127.1–136.0; *n* = 2)
Tail length	Male: 46.1 ± 7.22	Male: 53.5 ± 4.28	51.88	Male: 55.3 ± 6.81
	(33.0–55.0; *n* = 7)	(45.0–61.0; *n* = 35)		(50.0–63.0; *n* = 3)
	Female: 48.6 ± 3.35	Female: 56.5 ± 3.72		Female: 56.3 ± 3.21
	(45.0–56.0; *n* = 16)	(48.0–63.0; *n* = 31)		(54.0–60.0; *n* = 3)
Wingspan	~350–450	335–355	311.31	~430
Ear length	Male: 12.0 ± 1.53	Male: 17.5 ± 4.55	15.79	Male: 15.7 ± 0.58
	(10.0–14.0; *n* = 7)	(13.0–21.0; *n* = 35)		(15.0–16.0; *n* = 3)
	Female: 12.3 ± 1.35	Female: 16.5 ± 1.63		Female: 16.0 ± 1.00
	(9.0–14.0; *n* = 16)	(13.0–21.0; *n* = 31)		(15.0–17.0; *n* = 3)

or *murcielago blanquizco*, noting merely that he had "several identical specimens". However, Azara (in Agacino 1941) stated that his Morcielago 5[to] (= murcielago blanquizo) was captured in the house of Dr. Ignacio Pazos during February and that the specimen had been given to Azara. From the near identical text and the identical measurements, it is clear that this refers to the same species as the *chauve–souris septième* or *chauve–souris brun–blanchâtre*. The restriction of the type locality of *L. cinereus villosissimus* made by Cabrera (1958) to Asunción is thus accurate, based on the available data. Ignacio Pazos (born La Coruña, Spain 1760, died Cádiz, Spain 3 October 1804) was a naval officer and geographer, who formed part of an expedition to delimit the Spanish territories in South America, and worked closely with Azara during his time in Paraguay. The type specimen is now lost (Carter and Dolan 1978).

Nomenclatural implications.

Vesp[*ertilio*]. *villosissimus* É. Geoffroy St.–Hilaire, 1806 is based on the *chauve–souris septième* or *chauve–souris brun–blanchâtre* of Azara (1801). However, as demonstrated here and by Allen (1905), *Vesp*[*ertilio*]. *villosissimus* É. Geoffroy St.–Hilaire, 1806 is in fact a senior synonym of *L. ega* and has date priority over *Nycticejus Ega* Gervais, 1856, the current valid name for the Southern Yellow Bat. Strict application of the rule of priority in this case would cause considerable and undesirable nomenclatural upheaval and may be summarized as follows:

1) The date priority of *Vesp*[*ertilio*]. *villosissimus* E. Geoffroy St.–Hilaire, 1806 over *Nycticejus Ega* Gervais, 1856 means that the scientific name of the Southern Yellow Bat would become *Lasiurus* (*Dasypterus*) *villosissimus* (E. Geoffroy St.–Hilaire, 1806), with type locality in Asunción, Paraguay. Such a change would be unfortunate, since *D. ega* is the name that has been applied to the southern yellow bats for more than 150 years (see Gardner and Handley 2007).

2) The species actually known as *Lasiurus* (*Aeorestes*) *villosissimus*, the Southern Hoary Bat, would be called by the next available name; *i. e*., *Lasiurus* (*Aeorestes*) *grayi* Tomes, 1857: 40, with type locality "Chili" (= Chile).

3) The genus-level name *Aeorestes* Fitzinger, 1870 (dated 13 October 1870), with *Aeorestes villosissimus* as type species, would become a senior synonym of *Dasypterus* W. Peters, 1870 (dated 22 December 1870, with *Lasiurus intermedius* as type species) and thus be applicable to the Yellow Bats, not the Hoary Bats.

4) No valid supraspecific names are available for the Hoary Bats and they would require a new name, either for use as a genus or subgenus.

In order to avoid all these undesirable and destabilizing nomenclatural changes, the authors of this note will formally request the International Commission on Zoological Nomenclature (ICZN) to use its plenary powers to conserve both the supraspecific and specific names for both species affected (*i. e*., those currently referred as *L. ega* and *L. villosissimus*). We thus recommend preservation of current usage by declaring a neotype for *Vesp*[*ertilio*]. *villosissimus* É. Geoffroy St.–Hilaire, 1806 and proposing a new type species for *Aeorestes* Fitzinger, 1870.

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Appendix 1

Transcription of the original French text of (Azara 1801; Tome 2, pp. 284–285) upon which the name *Vesp*[*ertilio*]. *villosissimus* E. Geoffroy St.–Hilaire, 1806 is based.

CHAUVE–SOURIS SEPTIÈME

ou

CHAUVE–SOURIS BRUN–BLANCHÂTRE

J'ai possé dé divers individus de cette espèce, et ils étoient identiques entre eux.

Longeur, 4 pouces i tiers (11 centimètres 2 tiers)

Queue, 23 lignes (5 centimètres)

Envergurs, 11 pouces et demi (31 centimètres un tiers).

Le poil est extrémement doux, plus long que d'ordinaire, et d'un brun très–blanchâtre. C'est de la même coleur qu'est la membrane de la queue ; cette dernière est velue, excepté dans sa bordure.

L'aile est couleur de mure, excepté les doigts et le voisinage du bras et du corps, que sont brun–blanchâtres. L'aile est unie au métatarse, et la membran que va à l'extrémité de la queue nait un peu plus haut ; les vertèbres de la queue sont très–longues et minces.

L'oreille est comme celle du rat ; elle est haute de 7 lignes (1 centimètre et demi) ; elle présente presque son ouverture en avant ; elle est un peu aiguë à sa pointe, un peu inclinée vers le front, et encore vers le côté, et de son intérieur nait une pointe aiguë comme celle d'une épée.

Le museau n'est pás aigu, et il est divisé, à son extrémité pelée, par un canal, comme celui de la Chauve–Souris précédente.

La mâchoire supérieure excède un peu ; mais cet excédant, et même un peu plus d'espace encore, manque d'os, et par conséquent d'incisives, et peut se retrousser facilement ; cependant plus en dedans est une longue canine de chaque côté, jointe à laquelle et vers la partie extérieure, est une petite dent aiguë, que l'on pourroit appeller incisive, si elle n'étoit pas démesurément éloignée de son analogue de l'autre côté.

Dans la mâchoire inférieure, l'on n'apercoit point d'incisives, mais le tact les découvre, et l'on voit, de chaque côté, une canine un peu plus grande que celle d'en haut.

Appendix 2

Translation of the French text of Azara (1801) (see above, Appendix 1).

SEVENTH BAT

or

WHITISH–BROWN BAT

I have possessed various individuals of this species, and they were identical to each other.

Length, 4 inches and a third (11 2/3 centimetres)

Tail, 23 lines (5 centimetres)

Wingspan, 11 and a half inches (31 1/3 centimetres)

The hair is extremely soft, longer than usual, and of a very whitish brown. The membrane of the tail is of the same color; the latter is hairy, except at its border.

The wing is blackberry–colored, except for the fingers and the area around the arm and the body, which are whitish–brown. The wing is united to the metatarsus, and the membrane that goes to the end of the tail inserts a little higher; the vertebrae of the tail are very long and thin.

The ear is like that of the rat; it is 7 lines high (1 1/2 centimetres); it almost presents its opening forward; it is a little sharp at its point, a little inclined towards the forehead, and again towards the side, and from its interior arises a sharp point like that of a sword.

The muzzle is not sharp, and it is divided, at its bare end, by a canal, like that of the previous bat.

The upper jaw protrudes a little; but this excess, and even a little more, lacks bones, and consequently lacks incisors too, and thus can be easily pushed in. Internally there is a long canine on each side, and next to it externally, is a small sharp tooth, which one would call an incisor, if it were not disproportionately distant from its equivalent on the other side.

In the lower jaw, it is not possible to see any incisors, but they can be felt, and on each side, there is a canine, a little larger than that above.

Appendix 3

Transcription of the original Spanish text of Azara (1802; Tome 2, pp. 303–304).

NUM. LXXVII.

DEL PARDO BLANQUIZCO.

He pillado varios idénticos. Longitud 4 1/3 pulgadas: cola 1 11/12: braza 11 ½. El pelo es suavísimo, mas largo de lo regular, y pardo muy blanquizco. De este color es la membrana de la cola, que tiene pelos, ménos en la borda. El ala morada; y los dedos y la inmediacion del brazo y del cuerpo pardos blanquizcos. Dicha ala une al metatarso, naciendo poco mas arriba la membrana que va al fin de la cola. Oreja de Raton, alta 7 líneas, casi presenta delante su abertura, algo aguda en la punta, un poco inclinada para la frente y hácia el costado; y de lo interior sale una punta aguda como de espada. El hocico no agudo: la mandíbula superior excede un poco; pero este exceso y algo mas carece de hueso, y por consiguiente de incisivos, y se arremanga fácilmente: mas adentro hay en cada lado un colmillo largo, y junto á él hácia fuera un dientecillo agudo, que podría llamarse incisivo si no distase demasiado del del otro lado. No se ven incisivos abaxo, pero los encuentra el tacto; y hay un colmillo en cada lado algo menor que el de arriba.

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Establishing the availability of the recently erected binomen *Phyllotis pehuenche* (Rodentia, Cricetidae, Sigmodontinae)

Estableciendo la disponibilidad del binomio recientemente erigido *Phyllotis pehuenche* (Rodetia, Cricetidae, Sigmodontinae)

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In 2021, Jayat *et al.* propose a new species of sigmodontine rodent, named *Phyllotis pehuenche,* for the populations of the *P. xanthopygus* species complex from southwestern Mendoza and western Neuquén provinces, Argentina. The formal description of the species, published in a modifiable electronic supplementary material, do not fulfill the requirements established by the Amendment on e-publication (ICZN 2012) of The Fourth Edition of the International Code of Zoological Nomenclature (ICZN 1999), rendering the name *Phyllotis pehuenche* unavailable. The objective of this note was to comply with the provisions of the code and make the name available. Here we offer a summarized version of the original description of this species. In this note, we provide a taxonomic account for *P. pehuenche*, including its synonymy, type locality, holotype and paratypes, providing the etymology of the specific epithet, and offering a diagnosis for the species and comments regarding its geographic distribution. With the provided information, we comply with the provisions of The International Code of Zoological Nomenclature (ICZN 1999, 2012) making the name available.

Key words: Argentina; nomenclature; Phyllotini; Sigmodontinae; taxonomy.

En 2021, Jayat *et al.* propusimos una nueva especie de roedor sigmodontino, nombrada *Phyllotis pehuenche*, para las poblaciones del complejo de especies de *Phyllotis xanthopygus* del sudoeste de la provincia de Mendoza y el oeste de la provincia de Neuquén, Argentina. La descripción formal de la especie, publicada en un material suplementario electrónico modificable, no cumple con los requerimientos establecidos en la enmienda sobre publicaciones electrónicas (ICZN 2012) de la Cuarta Edición del Código Internacional de Nomenclatura Zoológica (ICZN 1999), haciendo que el nombre no esté disponible. El objetivo de esta nota es cumplir con los requerimientos del código y hacer el nombre disponible. Aquí ofrecemos una versión resumida de la descripción original de esta especie. En esta nota, ofrecemos un tratamiento taxonómico para *P. pehuenche*, incluyendo su sinonimia, localidad tipo, holotipo y paratipos, proveyendo la etimología del epíteto específico, y ofreciendo una diagnosis para la especie y comentarios acerca de su distribución geográfica. Con toda la información provista, cumplimos con los lineamientos del Código Internacional de Nomenclatura Zoológica (ICZN 1999, 2012) haciendo el nombre disponible.

Palabras clave: Argentina; nomenclatura; Phyllotini; Sigmodontinae; taxonomía.

In 2021, Jayat *et al.* published a morphologic and molecular review of the *Phyllotis xanthopygus* species complex of the central and southern Andes, proposing a new species, *Phyllotis pehuenche*, for the population from southwestern Mendoza and western Neuquén provinces, Argentina. The study, published in an electronic only journal, do not fulfill the article 8.1.3.2, concerning the accessibility of electronic copies with fixed content and layout. The inclusion of the definition or description of the new taxon and the fixation of a holotype were addressed in a modifiable (word type file) supplementary material instead of within the main text of the contribution, being therefore the name unavailable. However, all the conclusions reached by Jayat *et al.* (2021) about species limits and taxonomic status, remain valid. In this note we comply with the provisions of The International Code of Zoological Nomenclature (ICZN 1999, 2012) in order to solve these deficiencies and make the name *Phyllotis pehuenche* available. The information provided below was mainly taken from the Supplementary Material S6 of Jayat *et al.* (2021). This published work have been registered in ZooBank (LSID urn:lsid:zoobank.org:pub:151D639A-B722-4150-BDB5-25F50FF9F579) and the online version of this work will be archived and available at Zenodo (https://zenodo.org/).

We offer a summarized version of the original description of this species (see supplemental material 6 of Jayat *et al.* 2021). More important, we provide information about the holotype and paratypes specimens, the type locality, the etymology of the specific epithet, the diagnosis for the species, and comments regarding its geographic distribution.

Acronyms for the mammal collections of Argentina mentioned in text are as follow: Instituto Argentino de Investigaciones de Zonas Áridas (CMI), Mendoza; Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-Ma), Ciudad Autónoma de Buenos Aires; Centro Nacional Patagónico (CNP), Puerto Madryn.

Taxonomy

Subfamily Sigmodontinae Wagner, 1843

Tribe Phyllotini Vorontsov, 1959

Genus *Phyllotis* Waterhouse, 1837

Phyllotis pehuenche, new species

LSID urn:lsid:zoobank.org:act:F518FEC6-FE85-44BF-90A6-F34BDBBED764R, (Figures 1 and 2).

Phyllotis darwini vaccarum: Pearson, 1958:419; part.

Phyllotis darwini xanthopygus: Pearson, 1958:420; part.

Phyllotis darwini rupestris: Hershkovitz, 1962:302; part.

Phyllotis xanthopygus vaccarum: Steppan, 1998:574; part.

Phyllotis xanthopygus (clade centro-2): Riverón, 2011:39.

Phyllotis xanthopygus (mesw, nqnw, and nqso geographic aggregations): Teta *et al.* 2018:70.

Phyllotis sp. 2 clade: Ojeda *et al.* 2021:10.

Phyllotis pehuenche Jayat, Teta, Ojeda, Steppan, Osland, Ortiz, Novillo, Lanzone, and Ojeda, 2021:689 (name unavailable).

Holotype.— An adult (age class 3) male (CMI 6791), including skin, skeleton, and tissues, collected on 23 February 2004 by R. A. Ojeda (original field number RAO 126). An associated 801 base-pair sequence of the *cytochrome-b* (*cytb*) gene has been deposited in GenBank with accession number MT 776482 (see Ojeda *et al.* 2021). See Table 1 for measurements of the holotype.

Type locality.— Argentina, Provincia de Mendoza, Departamento Malargüe, 10 km al S de Las Leñas, margen del Río Salado, 1,900 m. Geographic coordinates of the collecting site (wrongly reported as 35° 10′ 55″ S, 70° 42′ 00″ W in the original publication; see Supplementary Material S6 in Jayat *et al.* 2021) are 35° 11′ 41″ S, 70° 2′ 53″ W (Figure 3).

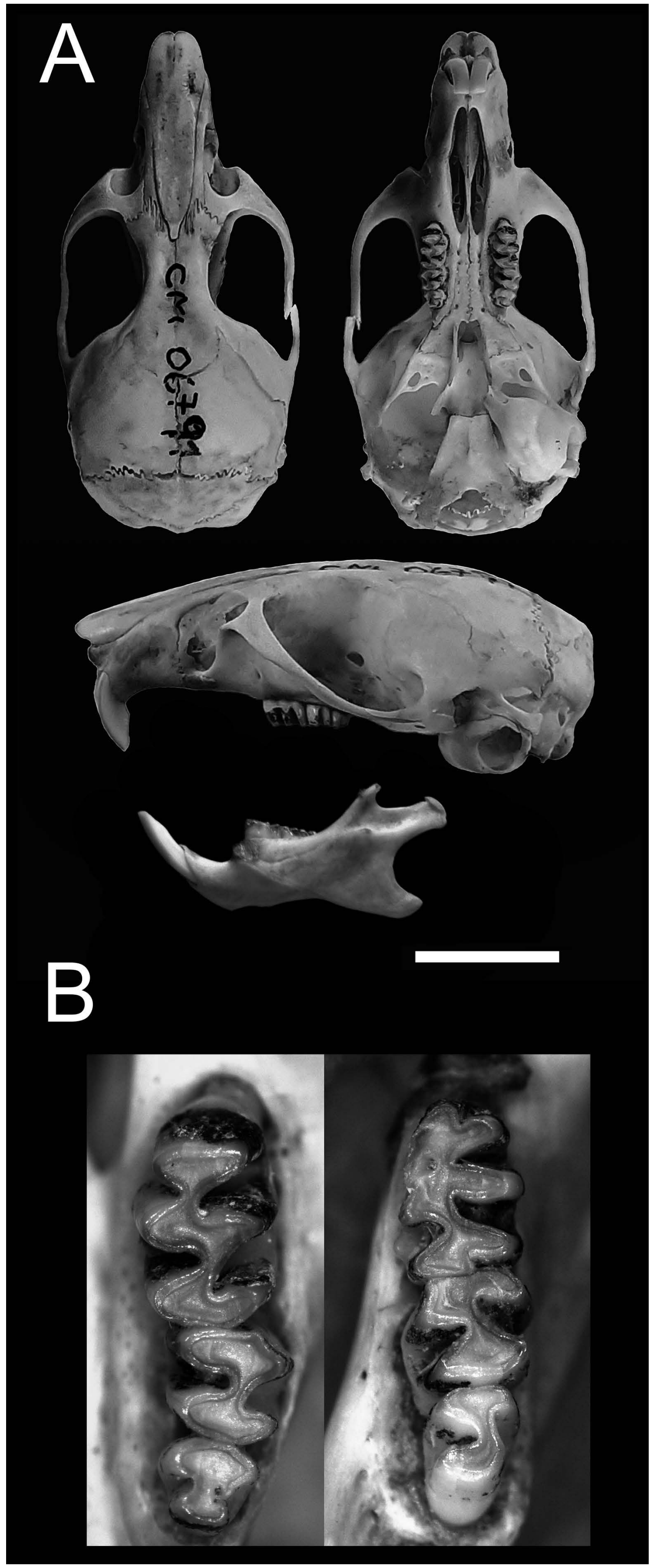

Figure 1. *Phyllotis pehuenche* new sp. Holotype (CMI 6791). A) dorsal (upper left), ventral (upper right) and lateral (middle) views of skull and labial view (bottom) of mandible. Scale bar = 10 mm. B) left upper (left) and left lower (right) molar rows (not in scale).

Diagnosis.— *Phyllotis pehuenche* new sp. can be distinguished from all other species of *Phyllotis* by the following combination of characters: medium size for the genus but somewhat large-sized in the context of the *P. xanthopygus* species complex (see Table 1 in Jayat *et al.* 2021). Fur with dorsum light yellowish ochre, smoothly spattered with black; flanks with a slightly developed yellowish-orange fringe; venter predominantly whitish gray, washed with buff in some specimens. Ears covered by sparse light orangish hairs (Figure 2B). Hind foot comparatively large. Tail generally shorter than, or approximately of the same length, of the length of the head and body, and not strongly bicolored (light brown above and grayish-white below). Manus and pes completely covered with short white hairs (Figure 2A). Skull not so heavily constructed for the genus, with a narrow rostrum, and rounded (not sharp-edged) inter-orbital region. Nasals anteriorly not much widened. Fronto-parietal suture mostly "U" shaped. Zygomatic arches comparatively not well expanded. Zygomatic notches narrower and less excavated than other species of the genus. Postero-palatal pits large and located ahead of the anterior border of the mesopterigoyd fossa (Figure 1A). Upper incisors generally orthodont, frontally covered by orange enamel. Upper and lower molar series comparatively large but thin.

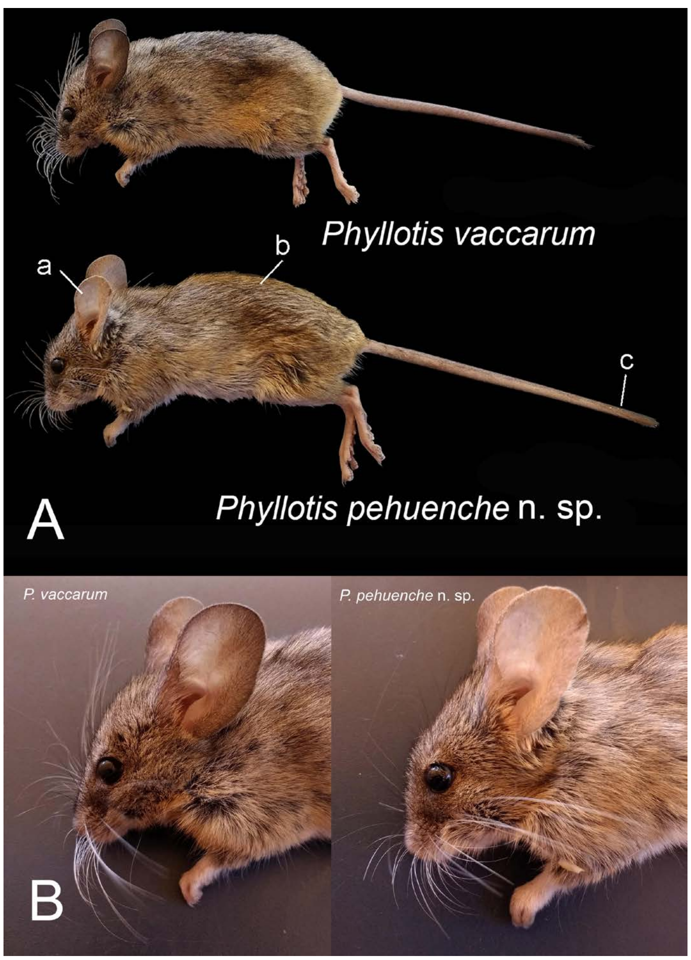

Figure 2. Comparison of the external characteristics in *P. vaccarum* and *P. pehuenche* new sp. showing: A) the clearer ears (a), the light-colored skin pattern (b) and the tail without the black tip (c) in the new species; and B) the detail of the ear coloration in *P. vaccarum* (ears covered with blackish hairs) and *P. pehuenche* new sp. (ears covered with orangish hairs).

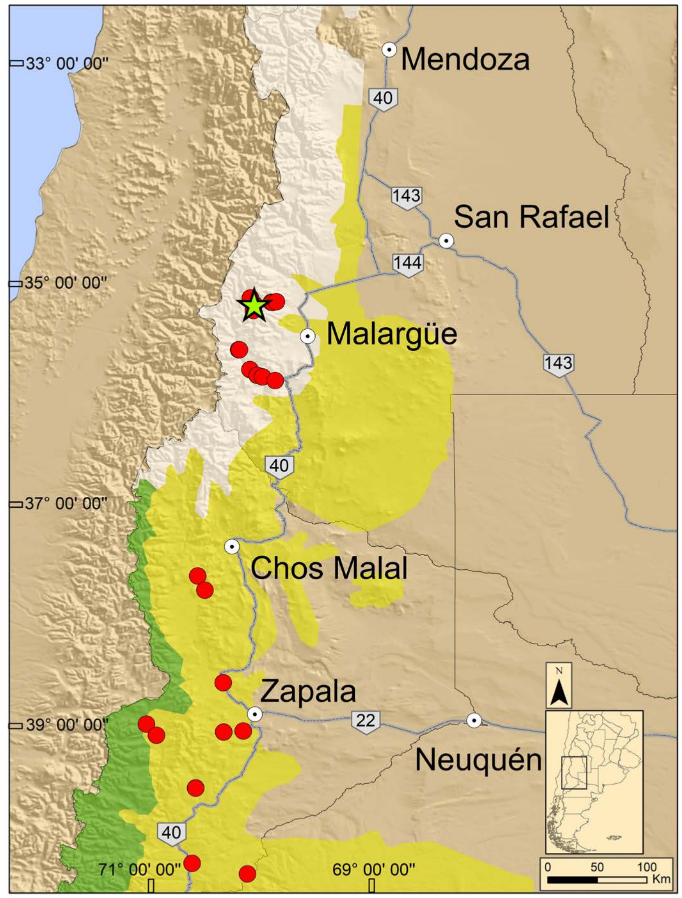

Figure 3. Map showing the type locality (green star) and additional records (red dots) of *P. pehuenche* new sp. in central western Argentina (Mendoza and Neuquén provinces). Shaded areas indicate ecoregions (white = Altos Andes; yellow = Estepa Patagónica; green = Bosques Patagónicos) *sensu* Burkart *et al.* (1999).

Simplified enamel molar pattern on the upper molar series, with a very shallow paraflexus on M2 and no trace of mesoloph complex on M1 and M2 (Figure 1B). Karyotype characterized by $2n = 38$, FN = 71-72.

Paratypes.— Three specimens collected at the type locality. CMI 6795 is a juvenile female (age class 1), including skin, skeleton, and tissues (GenBank MT 776481) collected on 23 February 2004 by R. A. Ojeda (original field number RAO 130). CMI 6792 is a juvenile male (age class 2), including skin, skeleton, and tissues (GenBank MT 776484) collected on 23 February 2004 by R. A. Ojeda (original field number RAO 125). CMI 6790 is a juvenile unknown sex (age class 2), including skin, skeleton, and tissues (GenBank MT 776483) collected on 23 February 2004 by R. A. Ojeda (original field number RAO 138).

Other referred specimens.— Fifty-four specimens from Mendoza (2.2 km E Los Molles, CMI 5275; 3.2 km E Los Molles, CMI 5249; a 7 km al Norte de Las Loicas, por R 226, 1,626 m, CMI not cataloged (field catalog number A. Ojeda 450), CMI not cataloged (field catalog number A. Ojeda 460); a 20 km al O de Bardas Blancas, R 145, 1,502 m, CMI 7651 - 7659; a 36 km al O de Bardas Blancas, R 145, 1,536 m, CMI 7625, CMI 7627 - 7629, CMI 7631 - 7632, CMI 7634 - 7639, CMI 7643, CMI 7645 - 7646, CMI 7661 - 7664, CMI 7672, CMI 7676, CMI 7679, CMI 7689; Arroyo El Seguro, 1,800 m, CMI 7421), and Neuquén (18 km S, 3 km E ,by road, Lonco Luan, a lo largo

Table 1. External and craniodental measurements (in mm) for the holotype specimen and all age classes of *P. pehuenche* new sp. *n* = sample size; X̄ = mean; *SD* = standard deviation; *r* = range. Measurement abbreviations are listed in Materials and Methods of Jayat *et al.* (2021).

	P. pehuenche new sp. Holotype	*P. pehuenche* new sp. age class 1			*P. pehuenche* new sp. age class 2			*P. pehuenche* new sp. age class 3			*P. pehuenche* new sp. age class 4			*P. pehuenche* new sp. age class 5		
		n	**X̄ ± *SD***	***r***	***n***	**X̄ ± *SD***	***r***	***n***	**X̄ ± *SD***	***r***	***n***	**X̄ ± *SD***	***r***	***n***	**X̄ ± *SD***	***r***
TBL	280	15	205 ± 19.89	170–240	17	231 ± 18.12	202–266	7	246 ± 13.37	230–280	2	249 ± 15.56	238–260	4	264 ± 12.07	251–278
T	135	15	100 ± 11.69	65–112	18	112 ± 9.55	95–132	7	117 ± 8.04	112–135	2	117 ± 10.61	109–124	4	124 ± 11.74	113–140
HF	33	15	29 ± 1.72	25–31	20	31 ± 1.67	26– 33	7	29 ± 3.31	25–33	2	31 ± 2.12	29–32	5	31 ± 1.79	28–32
E	26	15	22 ± 1.46	20–25	20	24 ± 1.97	20–27	7	25.34 ± 1.04	24–27	2	24.5 ± 0.71	24–25	5	26 ± 1.10	25–27
W	70	13	35 ± 11.68	19–58	18	48 ± 11.61	34–80	7	59 ± 14.24	36–74	2	56.5 ± 6.36	52–61	5	60 ± 17.37	31–75
TLS	33.56	14	28.82 ± 1.21	27.15–31.03	20	31.02 ± 1.13	29.11–32.87	7	31.64 ± 1.21	30.29–33.56	3	32.05 ± 1.01	30.95–32.94	4	33.07 ± 1.26	31.77–34.25
CIL	30.97	14	26.10 ± 1.29	24.12–28.44	20	28.41 ± 1.17	26.58–30.41	7	29.16 ± 1.11	27.77–30.97	3	29.85 ± 0.88	28.85–30.48	5	31.06 ± 1.15	29.53–32.19
BL	28.31	14	23.87 ± 1.26	22.11–26.21	20	26.19 ± 1.16	24.38–28.46	7	26.84 ± 0.95	25.69–28.31	3	27.66 ± 0.86	26.73–28.43	5	28.75 ± 1.11	27.24–29.74
PL	17.35	15	14.75 ± 0.71	13.84–16.15	21	16.03 ± 0.70	14.67–17.27	8	16.40 ± 0.53	15.74–17.35	3	16.83 ± 0.70	16.03–17.30	5	17.50 ± 0.67	16.48–18.14
DL	8.99	15	6.94 ± 0.44	6.33–7.98	22	7.83 ±0.49	6.95–8.71	8	8.19 ± 0.51	7.65–8.99	3	8.40 ± 0.25	8.11–8.56	5	8.79 ± 0.15	8.60–8.97
PB	5.86	15	5.50 ± 0.32	5.05–6.06	21	5.76 ± 0.32	5.01–6.33	8	5.74 ± 0.29	5.14–6.17	3	5.64 ± 0.15	5.49–5.79	5	5.84 ± 0.46	5.08–6.21
MTRL	5.59	15	5.88 ± 0.13	5.66–6.07	22	5.87 ± 0.16	5.65–6.20	8	5.84 ± 0.21	5.59–6.11	3	5.76 ± 0.17	5.60–5.94	5	6.09 ± 0.39	5.53–6.61
BLLT	5.86	14	5.79 ± 0.33	5.08–6.30	20	6.03 ± 0.23	5.67–6.51	7	6.16 ± 0.24	5.86–6.46	3	6.17 ± 0.20	6.05–6.40	5	6.28 ± 0.35	5.81–6.64
BuB	5.26	14	4.99 ± 0.20	4.65–5.35	20	5.22 ± 0.19	4.91–5.51	7	5.25 ± 0.22	4.86–5.57	3	5.10 ± 0.18	4.91–5.27	5	5.27 ± 0.21	4.99–5.49
IFL	8.20	15	6.59 ± 0.41	6.04–7.32	22	7.29 ± 0.40	6.63–8.29	8	7.44 ± 0.35	7.11–8.20	3	7.88 ± 0.30	7.57–8.17	5	8.17 ± 0.33	7.76–8.49
AW1	5.99	15	5.70 ± 0.16	5.35–5.96	22	5.86 ± 0.18	5.43–6.15	8	5.93 ± 0.25	5.55–6.32	3	5.56 ± 0.03	5.54–5.60	5	5.79 ± 0.12	5.70–5.99
AW2	5.33	15	5.02 ± 0.23	4.60–5.44	22	5.28 ± 0.20	4.92–5.59	8	5.57 ± 0.19	5.33–5.84	3	5.31 ± 0.17	5.12–5.44	5	5.51 ± 0.32	5.10–5.94
ZL	17.49	14	14.82 ± 0.73	13.53–16.27	22	16.13 ± 0.63	15.14–17.30	8	16.69 ± 0.61	15.96–17.49	3	16.51 ± 0.65	15.91–17.20	5	17.41 ± 0.64	16.44–18.25
ZP	3.45	15	3.19 ± 0.23	2.83–3.62	22	3.54 ± 0.26	3.20–4.36	8	3.59 ± 0.16	3.31–3.81	3	3.83 ± 0.06	3.76–3.87	5	3.98 ± 0.07	3.91–4.07
ZB	16.54	14	14.85 ± 0.73	13.92–16.26	20	15.85 ± 0.42	15.26–16.98	8	16.16 ± 0.51	15.01–16.54	3	16.25 ± 0.54	15.81–16.86	5	16.95 ± 0.40	16.67–17.64
BB	14.46	14	13.53 ± 0.29	13.20–14.13	20	13.89 ± 0.35	13.30–14.68	7	13.99 ± 0.39	13.41–14.46	3	13.72 ± 0.32	13.45–14.08	5	14.33 ± 0.33	14.00–14.83
IOC	4.31	15	4.16 ± 0.14	3.81–4.39	22	4.12 ± 0.16	3.85–4.43	8	4.24 ± 0.13	4.05–4.44	3	4.05 ± 0.26	3.80–4.32	5	4.10 ± 0.21	3.85–4.43
RW2	5.63	15	4.72 ± 0.25	4.34–5.26	22	5.08 ± 0.31	4.46–5.53	8	5.08 ± 0.33	4.67–5.63	3	5.27 ± 0.15	5.10–5.36	5	5.62 ± 0.21	5.41–5.91
NL	14.75	15	12.31 ± 0.76	11.35–13.71	22	13.46 ± 0.60	12.45–14.48	8	13.92 ± 0.56	13.11–14.75	3	14.21 ± 0.89	13.19–14.83	4	14.37 ± 0.57	13.86–15.04
RL	13.33	15	11.07 ± 0.59	10.20–12.09	22	12.16 ± 0.51	11.30–13.23	8	12.38 ± 0.58	11.67–13.33	3	12.59 ± 0.73	11.77–13.16	4	13.08 ± 0.73	12.30–13.77
OL	11.41	14	9.73 ± 0.47	9.22–10.82	21	10.52 ± 0.30	9.96–10.97	8	10.85 ± 0.46	10.26–11.42	3	11.03 ± 0.29	10.71–11.29	5	11.12 ± 0.40	10.57–11.62
OCW	7.30	13	6.92 ± 0.32	6.33–7.31	20	7.05 ± 0.24	6.70–7.56	7	7.21 ± 0.15	7.01–7.39	3	7.01 ± 0.08	6.93–7.10	5	7.20 ± 0.15	7.02–7.35
ML	18.54	15	15.47 ± 0.64	14.66–16.96	22	16.71 ± 0.64	15.27–17.82	8	16.99 ± 0.77	16.15–18.54	3	17.12 ± 0.58	16.54–17.71	5	18.44 ± 0.72	17.35–19.09
mTRL	5.76	15	5.73 ± 0.19	5.45–6.12	22	5.77 ± 0.16	5.44–6.00	8	5.81 ± 0.15	5.55–5.96	3	5.77 ± 0.16	5.59–5.87	5	5.99 ± 0.30	5.65–6.33

ruta 23, CML 3636; 2 km S Lonco Luan, along Hwy 23, 3,860 ft, CML 3635; Cerrito Piñón, Ea. Collón Curá, 608 m, CNP 1945, 1946 - 1948; El Huecú, MACN-Ma 20980; Las Coloradas, CNP 1951, MACN-Ma 13482, 13627, 14568, 15527, 15528, 17724; Parque Nacional Laguna Blanca, MACN-Ma 14900, 23589; Piedra del Águila, Ea. Yuncón, 628 m, CNP 1941) provinces.

Distribution.—We studied specimens of *P. pehuenche* from several localities in southwestern Mendoza (Malargüe Department) and western Neuquén (Aluminé, Catan Lil, Collón Curá, Ñorquín, Picunches, and Zapala Departments) provinces, Argentina, south to the Rio Limay. Most known localities of *P. pehuenche* new sp. are associated to mountain Andean ranges between 1,000 and 2,300 m (see Figure 1 in Jayat *et al.* 2021 and Figure 3). The new species appears to be sympatric (and syntopic) with *P. vaccarum* (see Jayat *et al.* 2021 for a revalidation of the specific status of this nominal form); both species were caught in the area of Bardas Blancas (Malargüe Department, Mendoza Province), but in contact areas representatives of *P. vaccarum* seem to prefer lower elevation habitats of the Monte de Valles y Mesetas ecoregion (*sensu* Burkart *et al.* 1999).

Etymology.— The specific epithet (a noun in apposition) honors the "Pehuenches", native South American Andean people belonging to the Mapuche culture, which inhabits both sides of the Andes mountain range, in south-central Chile and southwestern Argentina. The geographic distribution of the new species mostly coincided with the northern geographic distribution of the Pehuenche people in Argentina.

Additional comments.— Phylogenetic relationships of *Phyllotis pehuenche* new sp. with the other species of the genus *Phyllotis* can be found in Ojeda *et al.* (2021). Additional information for the species, including an extended morphological description (and comparisons with other species of the genus), karyotype, and natural history can be found in the Supplementary Material S6 of Jayat *et al.* (2021).

With the information provided in this note, we comply with the provisions of the article 8.1.3.2 of The International Code of Zoological Nomenclature (ICZN 1999, 2012). This article, concerning the accessibility of electronic copies, established that for a work be regarded as published it must have been produced in an edition containing simultaneously obtainable copies by a method that assures widely accessible electronic copies with fixed content and layout. This criterion, which was not accomplished in the original publication of Jayat *et al.* (2021), is fulfilled here, which make the name available. As such, the year of publication and availability of *Phyllotis pehuenche* new sp. is 2022 (with the exact date corresponding to the date when this publication is being published in the sense of the Art. 21 of the Code) and as such they must be referred in future works.

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We express our gratitude to C. Sciocia for her valuable assistance in nomenclatural issues. The comments of A. R. Percequillo and three anonymous reviewers substantially improve a first version of this work. G. D'Elía warned us about an error in the coordinates of the type locality reported in the original publication. Finally, we also want to correct an oversight made by Jayat *et al.* (2021); in that work, we forgot to thank the valuable collaboration of the curators and technical staff of the mammal collections studied, mainly to S. Gamboa, S. Lucero, and B. Bender. We apologize for the inadvertent omission.

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Ecological interaction between *Artibeus jamaicensis* and *Microdesmia arborea* in a deciduous forest of central México

Interacción ecológica entre *Artibeus jamaicensis* y *Microdesmia arborea* en un bosque caducifolio del centro de México

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Seed dispersal is essential for many plant species, so they can colonize new sites. Among dispersers, fruit bats have been considered essential for ecosystem functioning. Although the role of bats as seed dispersers has been widely proved, many of the mutualistic interactions between plants and frugivorous bats are still unknown and little direct evidence of seed dispersal has been provided. As part of continuous monitoring conducted in the Sierra de Huautla Biosphere Reserve, we found a small colony of *Artibeus jamaicensis* resting in a cave near a series of crops used by local people to grow food. Inside the cave, we found many growing cotyledons of *Microdesmia arborea*, just below the resting places used by the bats. These cotyledons germinated from the seeds of the fruits on which the individuals of *A. jamaicensis* fed. To our knowledge, this is the first record reporting the association between *A. jamaicensis* and *M. arborea*. The growing cotyledons we found demonstrate the importance of *A. jamaicensis* for the dispersal and germination of certain plant species, even in unfavorable conditions like inside a cave. According to Mexican legislation, *M. arborea* is listed as threatened. So, the seed dispersal by bats may be vital for conserving this plant species. Seed dispersal is of great significance in an environment surrounded by crops, where forest regeneration may play a critical role in maintaining natural vegetation.

Key words: Chrysobalanaceae; deciduous forest; seed dispersal; Sierra de Huautla.

La dispersión de semillas es esencial para una gran cantidad de plantas, de manera que estas puedan colonizar nuevos sitios. Entre los dispersores, los murciélagos frugívoros han sido considerados esenciales para el funcionamiento del ecosistema. Aunque el papel de los murciélagos como dispersores de semillas ha sido ampliamente probado, muchas de las interacciones mutualistas entre las plantas y los murciélagos frugívoros aún se desconocen y se ha proporcionado poca evidencia directa de la dispersión de semillas. Como parte de un monitoreo continuo que hemos realizado en la Reserva de la Biosfera Sierra de Huautla, encontramos una pequeña colonia de *Artibeus jamaicensis* descansando en una cueva cerca de una serie de cultivos utilizados por la población local para producir alimento. En el interior de la cueva encontramos una gran cantidad de cotiledones de *Microdesmia arborea* (Chrysobalanaceae) en crecimiento, justo debajo de los lugares de descanso utilizados por los murciélagos. Estos cotiledones germinaron a partir de las semillas de los frutos de los que se alimentaron los individuos de *A. jamaicensis*. Hasta donde sabemos, este es el primer registro que reporta la asociación entre *A. jamaicensis* y *M. arborea*. Los cotiledones en crecimiento que encontramos demuestran la importancia de *A. jamaicensis* para la dispersión y germinación de ciertas especies de plantas, incluso en condiciones desfavorables como el interior de cuevas. De acuerdo con la legislación mexicana, *M. arborea* se encuentra catalogada como amenazada, por lo que la dispersión de sus semillas por parte de los murciélagos puede ser de vital importancia para su conservación. La dispersión de semillas tiene una gran importancia en un ambiente rodeado de cultivos, donde la regeneración forestal debe desempeñar un papel fundamental en el mantenimiento de la vegetación natural.

Palabras clave: Bosque caducifolio; Chrysobalanaceae; dispersión de semillas; Sierra de Huautla.

Seed dispersal by birds and mammals is fundamental for ecosystem functioning and forest regeneration processes (Mello *et al.* 2005; Rocha *et al.* 2018). Many studies have shown that among mammals, fruit-eating bats contribute successfully to seed dispersal and the reforestation and restoration of tropical forests (Brändel *et al.* 2020; Enríquez-Acevedo *et al.* 2020). In the New World, bats of the family Phyllostomidae are the main representatives of seed dispersers among mammals (Dumont 1999; Melo *et al.* 2009; Trevelin *et al.* 2013). Phyllostomid bats disperse ~559 plant species in the neotropical region (Lobova *et al.* 2009). However, many of the mutualistic interactions between plants and frugivorous bats are still unknown. The study and determination of these relationships become essential, especially when the relationship involves species that have been severely affected by human activities (Moran *et al.* 2009).

In the neotropical region, with its 12 species currently described, bats of the genus *Artibeus* are one of the main components of the mammal fauna (Redondo *et al.* 2008; Larsen *et al.* 2013). *Artibeus jamaicensis* is one of the most widespread and ecologically diverse frugivorous bat species in this genus. It occurs from northern México southward to Central America to northwestern Argentina (Ortega and Arita 2000). Roosting sites used by the species include caves, tree holes, and human-made structures that in some cases are located within its foraging areas (Kunz *et al.* 1983; Handley and Gardner 1991; Kunz and Diaz 1995). Commuting distances between roosting sites and foraging areas have been determined by food availability and can range from 1 to 8 km (Morrison 1978). *Artibeus jamaicensis* is a specialized fig-eater (*Ficus* sp.); however, its diet includes a wide variety of fruits encompassing 44 plant genera (August 1981; Handley and Gardner 1991; Ortega and Castro-Arellano 2001).

The role of *A. jamaicensis* as seed disperser of many plant species has been inferred mainly from scatological evidence (*e.g.*, Andrade *et al.* 2013; Kasso and Balakrishnan 2013; Horsley *et al.* 2015). However, such evidence is indirect and provides partial proof of its effectiveness as seed dispersal. For example, to be an effective disperser, the seeds need to be dispersed in optimal sites for their gemination (Schupp *et al.* 2010). In this sense, many bat-plant interactions remain to be determined. Here we present an observation of *A. jamaicensis* presumably feeding on the cacahuananche (*Microdesmia arborea*) and the seed dispersal and germination of this plant species inside a cave located in a tropical dry forest in Central México.

As part of a continuous wildlife monitoring in the Sierra de Huautla Biosphere Reserve, we visited the "Cueva del Toro" cave located in the community of Chimalacatlán in the state of Morelos, in central México (18º 27´43.176" N, 99º 6´ 0.801" W). We conducted a single visit in December 2021. The vegetation surrounding the cave is composed of tropical dry forest with species from the Burseraceae, Bombacaceae, Moraceae and the Chrysobalanaceae families, and an extensive area of crops (Alba-Zúñiga *et al.* 2009; Ebergenyi and León 2015). We recorded the ambient temperature and humidity inside the cave using a temperature-humidity meter (model 971, Fluke Corporation). We found a maternity colony of *A. jamaicensis* roosting inside the cave. We determined at sight the size of the colony and the presence of pregnant females and pups. Underneath roosting sites, we found germinated seeds which were collected (8 seedlings and 3 seeds) for their identification. Seedlings and seeds were measured and weighted. Species identification was done following Rios-García (2018).

Inside the cave, the average ambient temperature was 27.8 ºC, and the relative humidity was 71 %. We observed at least 100 pregnant females and 50 young bats of *A. jamaicensis* and some individuals of the nectar-feeding bat *Glossophaga soricina*. No signs from another mammal or bird were found. We also identified the germinated seeds as *M. arborea* (Chrysobalanaceae; Sothers *et al.* 2016). We found seeds under the roosting area of *A. jamaicensis* of ~16 m^2 (~ ¼ of the total area of the cave), ~ 10 m inside the cave with their respective cotyledons. The etiolated cotyledons had a mean height ± standard deviation of 340 ± 1.2 mm (*n* = 8). Seeds (*n* = 3) measured 19.5 ± 0.6 mm long and 12.2 ± 1.1 mm wide and weighted 1.4 g ± 0.7 gr.

The large number of germinated seeds of *M. arborea* we found under the resting places used by *A. jamaicensis* as the only frugivore capable of seed dispersion inside the cave, strongly suggests an ecological interaction between these species. This interaction is an important association of a fruit bat with a plant species categorized as threatened by the Mexican legislation (SEMARNAT 2010). Although it has been suggested that *A. jamaicensis* feeds on fruits produced by plants of the *Microdesmia* genus (Ortega and Castro-Arellano 2001), to our knowledge, this is the first record reporting this specific association. *Microdesmia arborea* is a native American plant species distributed from México to Panamá (Carpio-Malavassi 2003). In México, this plant species has been traditionally used for a variety of purposes which may include household construction, fuel, live fences, and for many medicinal uses (Ríos-García *et al.* 2014, 2017; Ríos-García 2018). However, with an exemption of some protected areas where the species can be relatively safe from human disturbance (López *et al.* 2012), the management and exploitation plans along the territory for this threatened plant species are scarce (Ríos-García *et al.* 2014, 2017). Up to date, there are no reforestation plans in the places where *M. arborea* is extensively used. In these places, the ecosystem services provided by frugivorous bats are crucial to decrease the impact of humans on the ecosystems. This is especially important in tropical dry forests, one of the most threatened ecosystems in México (Trejo and Dirzo 2000; CONANP 2006).

Our observation also highlights the amazing ability of *M. arborea* to germinate and develop cotyledons in unfavorable low-light conditions inside a cave. *Microdesmia arborea* is distributed in sub-deciduous and sub-evergreen forests. The flowering and fruiting occur from October to July (Palacios 2006; Ríos-García *et al.* 2017). Like other species of the family Chrysobalanaceae, *M. arborea* is a secondary succession plant (Finegan 1996) that requires dispersing agents like birds and bats (Horseley *et al.* 2015). The germination of *M. arborea* is hypogeal (*i.e.*, it takes place under the ground), and this species roots preferably in sandy soils (Palacios 2006; Ríos-García 2018). Although the cave presents favorable conditions like the humidity for its germination, we believe that the cotyledons of *M. arborea* should not be successful because of the low-light conditions inside the cave and the strict sunlight requirements of this tropical plant species (Poorter *et al.* 2005). This could be interpreted as if *A. jamaicensis* could not be an effective disperser for *M. arborea* in our study site (Schupp *et al.* 2010). However, the successful germination of *M. arborea* may occur when bats roost in trees immersed in the vegetation or near the crops or while navigating in their foraging areas. Although there is no information on the feeding sites used by *A. jamaicensis* out of their resting places in tropical dry forests, in the humid forests of Costa Rica, this species has been observed using feeding roosts in primary forests and abandoned plantations. In these sites, bats have been reported depositing seeds of *Ficus insipida* below the places where they fed, contributing to the forest regeneration process (Lopez and Vaughan 2004).

The size of the seeds we found strongly suggests that the dispersal of *M. arborea* is epizoochoric rather than endozoochoric. This means that bats used their resting place to feed at night after transporting the fruit and deposited the seed once they consumed the pulp. In this regard, it has been reported that *A. jamaicensis* is a generalist fruit-eating bat (Gardner 1977) that carries the fruit to feeding roosts that can be located close to the parental tree or several hundred meters away (Fleming and Heithaus 1981). In these places, bats drop the seeds on the ground once they have fed (Bizerril and Raw 1998; Charles-Dominique and Cockle 2001).

In this work, we provide a direct observation of the seed dispersal capacity of the frugivorous bat *A. jamaicensis* living in the tropical dry forest of central México. In this ecosystem, bats may play a fundamental role in habitat regeneration. Seed dispersal is especially important for threatened plant species like *M. arborea* which is distributed in an environment surrounded by crops, where human activities have affected the ecosystem functioning in one of the most vulnerable places on the earth.

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Home range and movement ecology of the woolly opossum (*Caluromys derbianus*) in a Neotropical rainforest of Costa Rica

Ámbito hogareño y ecología del movimiento del zorro de balsa (*Caluromys derbianus*) en un bosque Neotropical de Costa Rica

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Understanding animal movement is critical to elucidate how non-sessile species survive and reproduce, as well as their influence in evolutionary and ecological processes and patterns. By characterizing the spatial movements of a Neotropical mammal, we aimed to generate information regarding the home range and movement ecology of the woolly opossum, *Caluromys derbianus*. Fieldwork was conducted in a Neotropical Rainforest of Costa Rica where animals were captured and fitted with collar mounted radio transmitters. Data on spatial locations were then analyzed to estimate the home range and the activity areas. Mean home range and activity areas of the opossums were concentrated in small patches (< 2 ha for home range, ≤ 1.1 ha for foraging areas and < 0.5 ha for core use areas). Overall, our results contribute to the growing knowledge on the natural history of Neotropical marsupials, as well as highlights that, as expected, *C. derbianus* is likely a species that might meets its most critical requirements within small activity areas. So, we argue that an opportunistic and omnivorous diet, and a well conserved habitat contribute to the observed patterns of animal movement. Further efforts should focus on increasing sample size and tracking periods to better comprehend habitat and resource use patterns.

Key words: Didelphidae; local convex hulls; marsupials; minimum convex polygon.

Comprender el movimiento de los animales es fundamental para dilucidar cómo las especies no sésiles sobreviven y se reproducen, así como su influencia en los procesos y patrones evolutivos y ecológicos. Al caracterizar los movimientos espaciales de un mamífero neotropical, nuestro objetivo fue generar información con respecto al ámbito hogareño y a la ecología del movimiento del zorro de balsa, *Caluromys derbianus*. El trabajo de campo se realizó en un bosque Neotropical lluvioso de Costa Rica donde los animales fueron capturados y equipados con un transmisor de radio montado en un collar. Los datos sobre las localizaciones espaciales fueron luego analizados para estimar el ámbito hogareño y las áreas de actividad para los animales. Las áreas de actividad y área promedio del ámbito hogareño de los zorros de balsa se concentraron en parches pequeños (< 2 ha para el ámbito hogareño, ≤ 1.1 ha para áreas de alimentación y < 0.5 ha para áreas de uso principal). En general, nuestros resultados contribuyen al conocimiento sobre la historia natural del zorro de balsa neotropical, además de destacar que, como se esperaba, *C. derbianus* es una especie que probablemente puede cumplir con sus requisitos más críticos dentro de las áreas con una actividad reducida. Así, argumentamos que una dieta oportunista y omnívora, además de un hábitat bien conservado contribuye a los patrones de movimiento del zorro de balsa observado. Los siguientes esfuerzos deben centrarse en aumentar el tamaño de la muestra y los períodos de rastreo para así mejorar la comprensión de patrones en el uso del hábitat y de los recursos.

Palabras clave: Didelphidae; marcos convexos locales; marsupiales; polígono mínimo convexo.

Non-sessile animals survive and reproduce by finding critical resources such as food, shelter, and mates at the same time as they avoid predators (Nathan *et al.* 2008). So, animal movement patterns are likely shaped by evolutionary and ecological processes that influence important phenomena such as density, ecological interactions, competition and ultimately diversity of the ecosystems (Patterson *et al.* 2008; Kays *et al.* 2015). Therefore, is not surprising that animal movement studies have been increasing (*i.e.*, more research) and enhancing (*i.e.*, smaller and more accurate devices such as "tags" equipped with Global Position System (GPS), implanted electronics, animal-mounted small cameras; Kays *et al.* 2015).

Among vertebrates such as mammals, multiple studies have been contributing to the knowledge on the movement ecology of hundreds of species around the globe, but many more efforts should be carried to document ecological information of highly diverse taxonomic clades with myriad of species (Kays *et al.* 2015). For instance, among highly diverse clades, and usually small-bodied size, such as bats, rodents and marsupials, surprisingly few information is available out there, which limits our understanding of basic biological aspects of unique species and their interaction with the environment (Kays *et al.* 2015).

The woolly opossum, *Caluromys derbianus* (Waterhouse 1841), is a 300 gr nocturnal and arboreal Neotropical marsupial (Figure 1A; Bucher and Hoffmann 1980). The natural history, ecology, and behavior of *C. derbianus* is poorly known (Bucher and Hoffmann 1980). Few observations indicate that the species are likely solitary and strictly arboreal, using symmetrical gaits in locomotion and their prehensile tail for balance and grip (Hall and Dalquest 1963). The natural diet seems to be comprised of fruits (Salas-Durán 1974; Bucher and Hoffmann 1980; Steiner 1981; Rasmussen 1988; Timm *et al.* 1989), nectar and pollen (Salas-Durán 1974; Steiner 1981; Tschapka and von Helversen 1999); and insects such as cockroaches, moths, cicadas and katydids (Salas-Durán 1974; Steiner 1981; Rasmussen 1988).

Through its distribution in lowland and highland rainforest from San Luis Potosí, south-central Veracruz to western Colombia and norther Ecuador, there is no information regarding the movement ecology of *C. derbianus* or many other animals (Pérez-Gracida and Serna-Lagunes 2021). Therefore, in order to contribute to the growing knowledge on the movement ecology of poorly understood mammals, our main was to document and understand the home range and the movement behaviors of the Neotropical marsupial *C. derbianus* in a Neotropical Rainforest of Costa Rica.

Fieldwork was conducted from March until September 2015, January to March 2016 on Tirimbina Biological Reserve (hereafter: Tirimbina), Sarapiquí, Heredia Province, Costa Rica (TBR; 10° 25′ 2″ N, 84° 7′ 32″ W). The study site is covered with tropical wet forest (Holdridge 1967). Elevation ranges from 40 to 150 m, and the average temperature and precipitation is 25.3 °C and 3,900 mm, respectively (McDale and Hartshorn 1994). Tirimbina is approximately 345 ha, composed of primary and secondary forest and a small proportion of abandoned cacao plantation (*Theobroma cacao*) surrounded by a complex matrix composed of man-made structures (*i.e.*, La Virgen de Sarapiquí town), pastures, diverse kinds of plantations (*e.g.*, trees, banana, pineapple) and tropical forest patches of differing size.

Woolly opossum were captured using Tomahawk live traps (6151 U.S. Hwy 51 Hazelhurst, WI 54531 USA) baited with ripe bananas and placed at 25 to 30 m aboveground using a pulley system. Upon capture, we used leather gloves to extract individuals from the traps and place them in soft cloth bags to further processing. Woolly opossums were classified as reproductive adults or immatures based on the sequence of eruption of the premolars and molars (Tyndale-Biscoe and McKenzie 1976) and on several external morphological variables such as body size and coloration. Reproductive status was determined based on enlarged testes on males and presence of litters on the pouch of females. Animals selected for radiotracking were adults of both sexes, and in case of females we only used individuals without litters. These animals were fitted with a 15 gr collar mounted radio transmitter (Figure 1A; model RI-2D, Holohil Systems, Carp, Ontario, Canada) which represented ≤ 7 % of the body mass of the smallest monitored woolly opossum. Individuals were then released at the site of capture within 30 min of seizure. We used a TRX-1000WR tracking receiver and a 5 element directional yagi antenna (Wildlife Materials, Murphysboro, Illinois, USA) to monitor the spatial movement of animals at night, usually between 17:30 hr and 00:00 hr. Azimuths from the position of the observer to the bearing determined with the directional antenna were measured to the nearest degree using a compass (Suunto, Helsinki, Finland) and a global position system (Garmin Corporation, Olathe, Kansas).

Observers frequently moved to improve radio signal strength and proximity to the animal activity in order to reduce computational errors in determining spatial locations. When radio contact with a focal animal was lost, we quickly re-established radio contact by walking in the direction of the disappearing radio signal. We categorized the activity of animals in 2 behaviors: locomotion and stationary. Both behaviors were distinguished by detection of varying signal strength (*i.e.*, locomotion) or of a steady signal at a fixed directionality for at least 1 min (*i.e.*, stationary). Radio-fixes were recorded every 10 min in a digital voice recorder (Olympus VN-3100PC; Olympus Europa Holding GmbH, Hamburg, Germany). Each radio-fix included records of time, signal strength from an analogue meter, gain setting from gradations calibrated on the gain dial, the GPS position of the observer, and the activity of the opossum.

Spatial locations were estimated from single azimuths along with distance which was calculated using the relationship between signal strength and gain (Law and Lean 1999; Bonaccorso *et al.* 2015). Estimated relationship of signal strength to distance (± 30 m error) was calibrated from transmitters set at 15 m aboveground at standardized gain settings along measured reception distances to maximum 300 m. Azimuths were recorded always under the upper limit of reception from the transmitting animal (*i.e.*, 310 m) while avoiding topographical features potentially causing refraction or reflection of radio signals. Spatial locations of animals were calculated using the following equations: 1) $BN = (ON + D) \sin\Theta$; and 2) $BE = (OE + D) \sin\Theta$, where BN is the northing UTM location of the animal, ON is the northing UTM location of the observer, D is the estimated distance based on signal strength, Θ is the azimuth in radians from the observer to the animal, BE is the easting UTM location of the animal, and OE is the easting UTM location of the observer. Finally, all locations were then analyzed with the adehabitatHR package (Calenge 2015) in R software (R Development Core Team 2021). We employed the minimum convex polygon method (MPC; Mohr 1947; Harris *et al.* 1990) and the nonparametric kernel method (adaptive local convex hulls; Getz *et al.* 2007) to estimate the home range and the regions in space with different probabilities of usage (hereafter: isopleths). In this sense, we used 95 % of the data to identify the home range areas, the (90 %) isopleths to identify foraging areas and the (50 %) isopleths for core use areas.

We tagged 4 individuals (3 males and 1 female) during our study period. Individual woolly opossums were monitored between 3 and 12 consecutive nights (7 ± 2.12, *n* = 4) in which we obtained between 24 and 88 telemetry locations (51.25 ± 17.18, *n* = 4; Figure 1B). Mean home range area obtained through the minimum convex polygon method was 1.91 ± 0.56 ha, meanwhile the a-LoCoH method generated a mean area of 1.30 ± 0.35 ha (Table 1). Foraging and core use areas of opossums ranged between 0.52 and 1.33 ha (1.10 ± 0.27, *n* = 4) and between 0.093 and 0.46 ha (0.31 ± 0.08, *n* = 4), respectively (Table 1; Figure 2A-D). Available information did not allow us to compare movement areas between males and females. Moreover, based on plotted data of level (*i.e.*, amount of data) against movement areas, none of the monitored individuals has reached a stable home range area (Appendix 1).

This work represents an important piece of natural history information about a poorly known Neotropical mammal. Specifically, our work represents the first effort in studying the movement ecology of *C. derbianus* through its entire geographic distribution and one of the few endeavors to document and understand the spatial movements of any member of the genus *Caluromys* (but see Julien-Laferriere 1995; Lira *et al.* 2007).

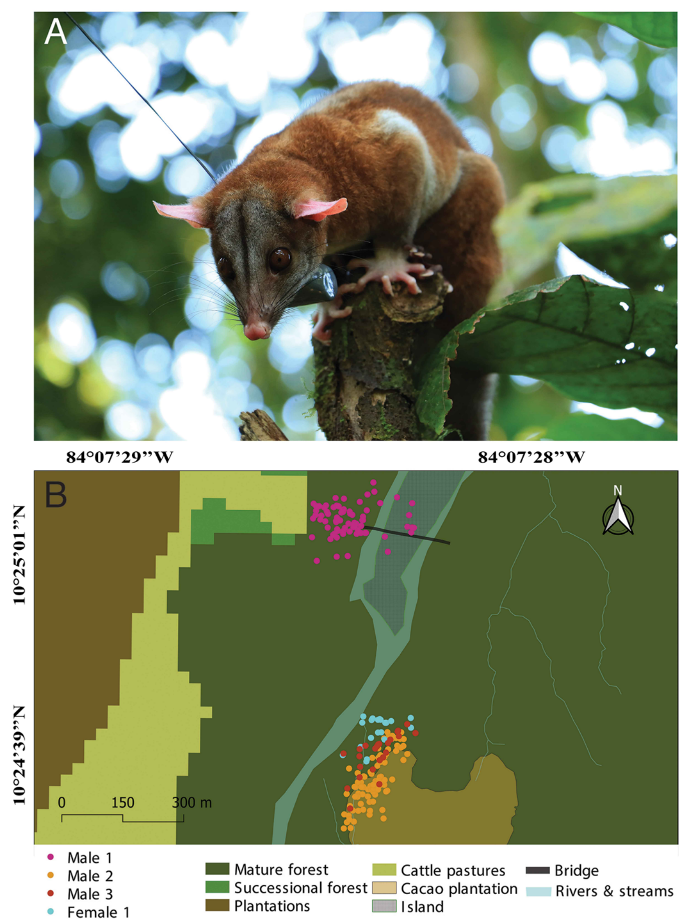

Figure 1. An adult woolly opossum (*Caluromys derbianus*) fitted with a radio-collar (A); and the telemetry location of the 4 radio-tracked individuals (B) at Sarapiquí, Costa Rica.

However, what ecological information can we gain from home range and activity areas estimations? Considering that any estimate of home range is, at best, a limited model of reality and that no universal home range estimation exists (Powell and Mitchell 2012), our data provide information on how *C. derbianus* uses its resources and where its willing to go to meet its requirements. For instance, most of our monitored animals (except for male 1) performed their activities on the cacao plantation and surroundings areas (Figure 1B), suggesting that despite the vegetation in this area is slightly dominated by one species (*i.e.*, *Theobroma cacao*), animals are still able to find their key resources (including food, potential mates, safe sites) to survive. These might also be related with the fact that most of the core use areas of these 3 animals (Figure 2B-D) were associated with the mature forest of TBR or the boundaries between these forests and the cacao plantations, habitats that likely provide more resources (*e.g.*, food) and vertical structure for locomotion than other types of vegetation (*e.g.*, pineapple or cattle pastures; Figure 2B-D).

Although food availability was not measured in this study, small home ranges, and activity areas (*i.e.*, foraging and core use areas) can also be related with few limitations on the food supply at Tirimbina. In this sense, considering the extent of information regarding feeding habits of *C. derbianus*, it seems that an opportunistic and omnivorous diet based on fruits (Timm *et al.* 1989; M. M-L pers. obs.), nectar and pollen (Tschapka and von Helversen 1999; E. R-V pers. obs.), insects (E. R-V pers. obs.) and even small vertebrates (D. V-C pers. obs.) might allow individuals to fulfill their food requirements in smaller, more numerous and more evenly distributed food patches.

Overall, the behaviors and food choices of *C. derbianus* in Costa Rica resembles the ecological information obtained by Julien-Laferriere (1995) regarding *Caluromys philander*. Here we argue that as both marsupials species are ecologically and morphologically similar, in addition to co-exist with other arboreal mammals such as *Potos flavus* (Julien-Laferriere 1995; Lira *et al.* 2007), their resemblance might not be a coincidence but most likely the result of analogous pressures and similitudes of their habitats. Additional information will be needed to understand the influence of other species on the movement patterns observed for *C. derbianus*, as well as the similitudes or differences between *C. derbianus* and *C. philander*. Finally, available data on overlapping home range areas among males and between males and females (Figure 1B) might point out that, as similar as other didelphids (Gentile *et al.* 1997; Pires and Fernandez 1999; Cáceres 2003; Martins 2004; Moraes and Chiarello 2005), *C. derbianus* may show a promiscuous mating system (Ostfeld 1990; Krebs and Davies 1996; Gentile *et al.* 1997) where the high overlapping home range suggests an absence of territorial behavior (Sandell 1989).

In conclusion, by providing novel information about the movement ecology of *C. derbianus* at a rainforest of Costa Rica, we contributed to the growing knowledge of the natural his-

Table 1. Size of movement areas used by 4 *Caluromys derbianus* in the Rainforest of Sarapiquí, Costa Rica. a-LoCoH = adaptive local convex hull method; MCP = minimum convex polygon method. Size of areas is displayed in hectares (ha). S. E. = standard deviation.

Sex-Ind.	Month/Year	Tracking nights (n)	Locations (n)	Isopleth's size- a-LoCoH						MCP (95 %)
				50 %	60 %	70 %	80 %	90 %	95 %	
Male 1	Apr/May-2015	12	73	0.45	0.71	0.71	1.33	1.78	2.27	3.38
Male 2	Jan/Mar-2016	9	88	0.46	0.68	0.71	1.00	1.30	1.34	2.20
Male 3	Jan/Mar-2016	4	24	0.25	0.44	0.37	0.58	0.83	0.59	1.24
Female 1	May-2015	3	20	0.093	0.28	0.21	0.52	0.52	1.26	0.84
	Mean ± S. E.	7 ± 2.12	51.25 ± 17.18	0.31 ± 0.08	0.44 ± 0.14	0.50 ± 0.12	0.85 ± 0.19	1.10 ± 0.27	1.30 ± 0.35	1.91 ± 0.56

tory of an elusive and poorly understudied neotropical marsupial. Although useful, we are also aware of the limitations of our data to reach strong conclusions regarding multiple aspects of the ecology of *C. derbianus* (*e.g.*, intraspecific differences, differences between males and females). Therefore, further steps should focus on increasing the sample size and the monitoring periods of the species (Appendix 1), as well as incorporating potential ecological and environmental pressures that might be influencing the documented behaviors (*e.g.*, food availability, predation, moon phase, reproductive season).

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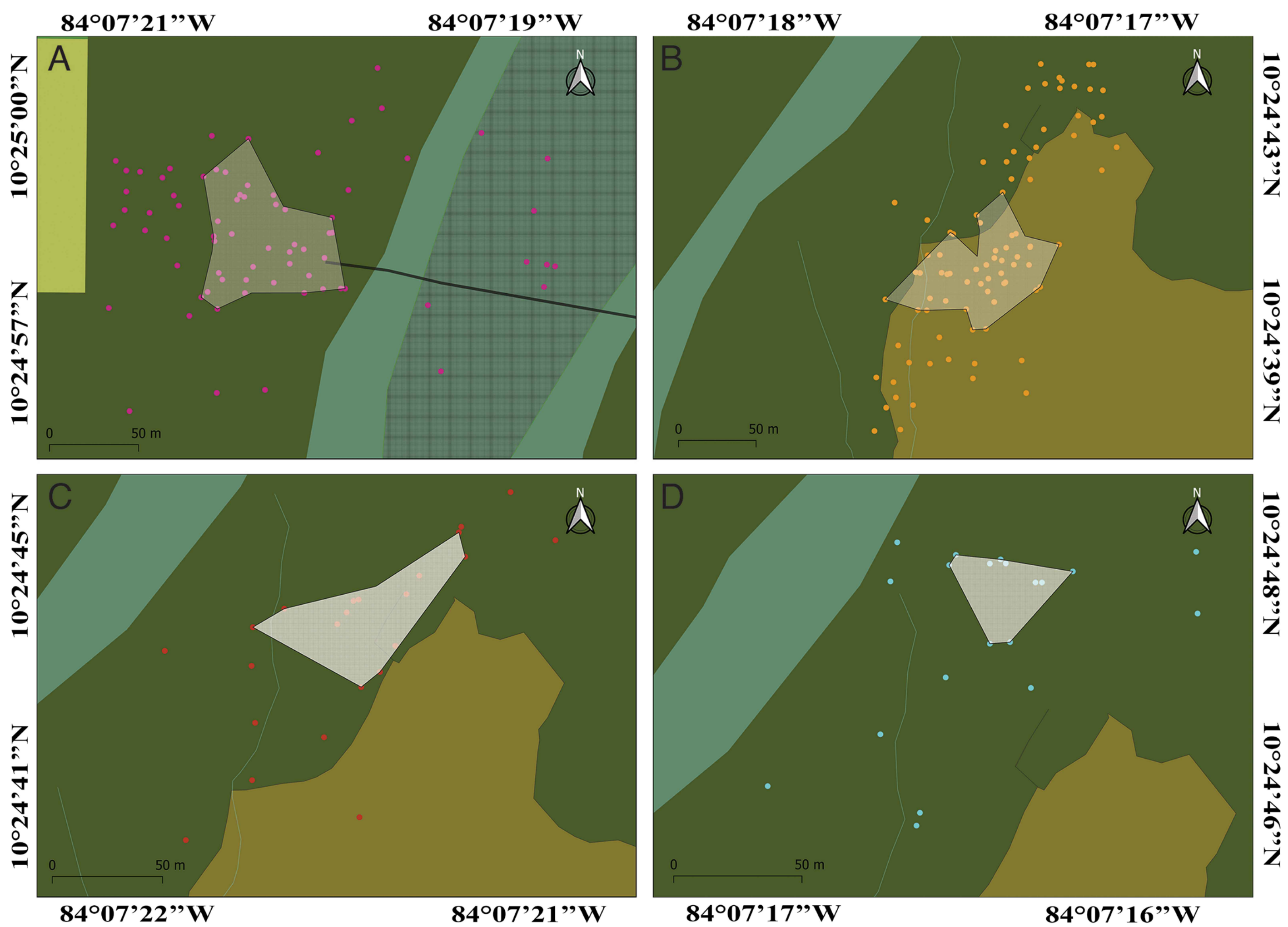

Figure 2. Telemetry locations and core use areas (a-LoCoh 50 %) of the 4 radio-tracked *Caluromys derbianus* at Sarapiquí, Costa Rica. Male 1 (A); Male 2 (B); Male 3 (C) and Female 1 (D).

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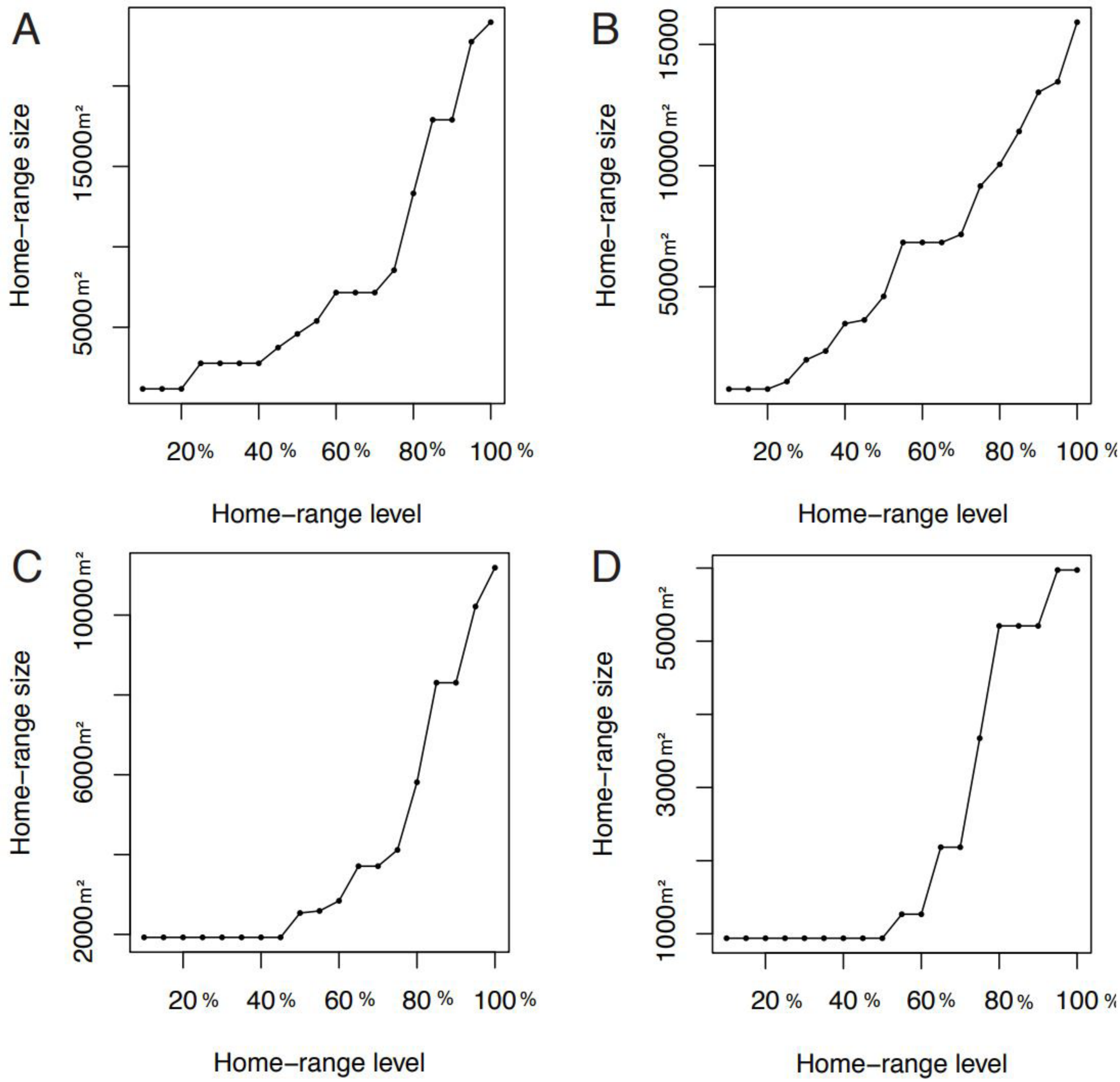
Appendix 1

Home-range level (*i.e.*, percentage of calculated home range used) plotted against home range size (*i.e.*, size of the areas) of the 4 radio-tracked woolly opossums at Sarapiquí, Costa Rica. Male 1 (A); Male 2 (B); Male 3 (C) and Female 1 (D).



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Diet of *Lontra longicaudis* in La Sangría Lagoon, México

Dieta de *Lontra longicaudis* en la Laguna La Sangría, México

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There are records of Neotropical otters, *Lontra longicaudis* in Campeche, but ecological information is scarce. The diet of this species inhabiting La Sangría Lagoon, México, was analyzed to record feeding preferences over a year through the identification of undigested remains. Data were obtained from feces and feeders. Once per season (winter rainy season or *nortes* 2016, dry, and rainy 2017), 11.9 km of the shoreline was traveled. One hundred twenty-six feces and one feeder were collected in 52 sites. These samples were analyzed by assessing the percentage of occurrence (PO) and ecological niche width (Levin's index) through the identification of undigested remains to the finest taxonomic level possible. Seven major groups of prey were identified: fish, crustaceans, mollusks, mammals, birds, and others. From a total of 16 prey categories, fishes were the best represented with a higher PO and number of genera, including janitor fish (invasive species), followed by crustaceans and mollusks. There were no variations in the frequency of prey consumption by season, but there were statistical differences in the diversity of species consumed. Three native fish species and 4 potential new records of fish were confirmed as prey of Neotropical otters. Levin's index showed that La Sangría Lagoon has a niche width with a trend toward specialization for the otter (0.2078). Comparing the diet observed herein with literature reports for other localities confirms the trophic adaptability and highlights the ecological value of the Neotropical otter.

Key words: Campeche; feeding habits; janitor fish; Neotropical otter; Palizada River.

En Campeche hay registros de nutria neotropical, *Lontra longicaudis*, pero la información ecológica es escasa. Se analizó la dieta de esta especie que habita la laguna La Sangría para conocer las preferencias alimentarias a lo largo de un año identificando los restos encontrados en las heces. Los datos se obtuvieron de la revisión de heces y comederos. Una vez por temporada (*nortes* 2016, secas y lluvias 2017), se recorrieron 11.9 km de la ribera. Se analizaron 126 heces y un comedero colectados en 52 sitios, evaluando el porcentaje de aparición (PA) y amplitud de nicho ecológico (índice de Levins) identificando los restos hasta el máximo nivel taxonómico posible. Se identificaron 7 grupos principales de presas: peces, crustáceos, moluscos, mamíferos, aves, mamíferos y otros. De un total de 16 categorías de presas, los peces fueron los mejor representados al tener un mayor PA y número de géneros, incluida la presencia de pez diablo (especie invasora), seguida de crustáceos y moluscos. No existió variación en las frecuencias de consumo de las presas por temporada climática, pero sí hubo diferencia estadística en la diversidad de especies consumidas. Se confirmó la presencia de 3 especies nativas y 4 posibles nuevos registros de peces como presas. El índice de Levins indicó que la laguna La Sangría presenta una amplitud de nicho para la nutria que tendió a la especialización (0.2078). Al comparar esta dieta con la registrada por otros autores en otras localidades, se evidencia la adaptabilidad trófica de la nutria neotropical y se resalta su valor ecológico.

Palabras clave: Campeche; hábitos alimentarios; nutria neotropical; pez diablo; Río Palizada.

The Neotropical otter, *Lontra longicaudis*, inhabits from northwestern México to Uruguay, Paraguay, and northern Argentina (Larivière 1999; Gallo-Reynoso and Meiners 2018). In México, the subspecies *L. l. annectens* is distributed throughout the Sierra Madre and the Gulf of México (Gallo-Reynoso 1997; Sánchez and Gallo-Reynoso 2007; Hernández-Romero *et al.* 2018). There are various records of this species in the state of Campeche, but the ecological information is scarce (Vázquez-Maldonado *et al.* 2021). There were only 2 records of *L. l. annectens* for the Palizada River prior to this work. The first is a report by Gallo-Reynoso (1997), who found tracks and burrows. The second, 19 years later, is an otter skin donated to the Mastozoological Collection of the Center for Studies in Sustainable Development and Use of Wildlife (*Centro de Estudios en Desarrollo Sustentable y Aprovechamiento de la Vida Silvestre* CEDESU-UACAM, in Spanish) in 2016.

The Neotropical otter feeds mainly on the available species in its environment. Fish comprising a high species diversity are its main prey, including introduced species such as trout; crustaceans like shrimps, crabs, and prawns are also consumed. Additional prey types include invertebrates such as bivalve mollusks and insects and vertebrates, including amphibians (frogs and toads), reptiles (lizards, iguanas, snakes, and turtles), birds (including aquatic birds), and small rodents, in addition to vegetation such as leaves, fruits, and seeds (Gallo-Reynoso 1996; Macías-Sánchez and Aranda 1999; Arellanes-Licea and Briones-Salas 2003; Díaz-Gallardo *et al.* 2007; Gallo-Reynoso *et al.* 2008; Monroy-Vilchis and Mundo 2009; Briones-Salas *et al.* 2013; Duque-Ávila *et al.* 2013; Rangel-Aguilar and Gallo-Reynoso 2013; Santiago-Plata *et al.* 2013; Guerrero *et al.* 2018; Grajales-García *et al.* 2019; Lavariega *et al.* 2020; García-Silva *et al.* 2021).

Therefore, the Neotropical otter is recognized as a generalist and opportunistic carnivore and a top predator (Sánchez and Gallo-Reynoso 2007; Gallo-Reynoso *et al.* 2008; Rheingantz *et al.* 2017). Its feeding habits allow determining the diversity of otter prey in its local environment (Casariego-Madorell *et al.* 2008; Monroy-Vilchis and Mundo 2009; Rheingantz *et al.* 2017; García-Silva *et al.* 2021).

Assessing the niche width (Levin's index) to better understand the ecological role of the Neotropical otter in this fluvial-lagoon system, the objective of this study was to record the presence and percentage of occurrence (PO) of preys over a year through the identification of undigested remains collected from one feeder and feces.

La Sangría Lagoon is located in the state of Campeche (18° 26' 49.9", 18° 29' 19.3" N; 91° 47' 44.7", 91° 49' 10.34" W; Figure 1). The study area comprises approximately 2.41 km^2 and is adjacent to the Palizada River and the town of Boca Chica (to the west). The Palizada River is located southwest of Laguna de Términos, which is part of the Laguna de Términos Natural Wildlife Protected Area (APFFLT, for its acronym in Spanish; Ocaña and Lot 1996). This river is the first branch of the Usumacinta River Delta (Coll de Hurtado 1975). It is part of the Palizada–Del Este fluvial-lagoon system, with a mean salinity range of 0 ppm to 8 ppm, temperature between 22 ºC and 31 °C (Ayala-Pérez 1989), and water transparency of 1.0 ± 0.23 m (Muciño-Márquez *et al.* 2017). The surrounding vegetation is a well-developed mangrove forest with a tree height from 10 m to 25 m, dominated by black mangrove (*Avicennia germinans*), red mangrove (*Rhizophora mangle*), and, to a lesser extent, white mangrove (*Laguncularia racemosa*; Jardel *et al.* 1987).

Samples were collected in 3 different seasons of the year between 2016 and 2017: winter rainy season (*nortes* 2016), characterized by strong winds from the north and northeast and frequent rainfall from October to January; dry season (2017), with less frequent and intense rains from February to May; and rainy season (2017), characterized by heavy showers almost daily, particularly in the afternoon and night from June to September (Yáñez-Arancibia and Day 1982, 1988). We conducted 3 surveys for a total of 11.9 kilometers traveled along the lagoon shoreline. Since the study area is floodable and with dense vegetation, all surveys were made on board a 7.6 m long IMEMSA-type boat with a 60 HP outboard motor. We looked for indirect evidence (feeders, feces, tracks, latrines, vocalizations, genital secretions, and burrows) of the presence of Neotropical otters (Aranda-Sánchez 2012). Each evidence was georeferenced with a GPS (Garmin map 78s) and photographed with a digital camera (Ricoh WG-4 SR). Two kayaks were used when sailing with a boat was unfeasible. Surface temperature, water depth (Seahawk manual echo sounder), and salinity (ATAGO refractometer) were recorded in all the sites where feces and feeders were found (sites with food leftovers around burrows, logs protruding above the water surface, and latrines, along with other traces such as otter tracks; Spínola-Parallada and Vaughan-Dickhaut 1995).

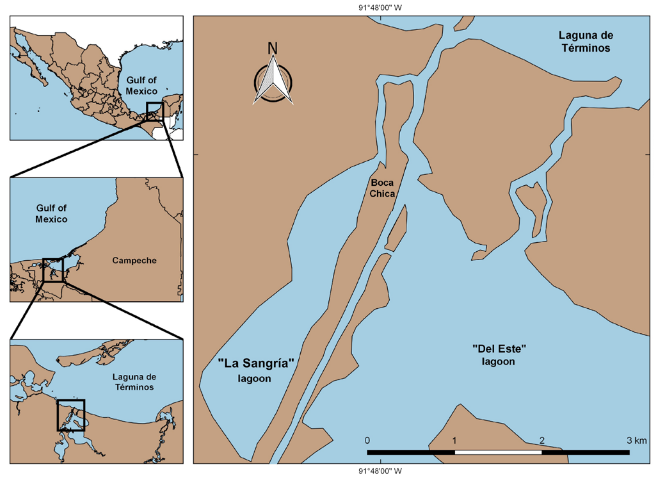

Figure 1. Location of the study area: La Sangría Lagoon, adjacent to the Palizada River, Campeche, México (Laguna de Términos Wildlife Protection Area).

Samples were collected manually and placed in properly labeled watertight bags stored in a fridge to be transported to the Laboratory 1 of the Faculty of Natural Sciences (FCN, for its acronym in Spanish) of the *Universidad Autónoma del Carmen* (UNACAR), where they were kept refrigerated until processing and analysis.

Feces samples and the materials collected from feeders were processed following the method by Santiago-Plata *et al.* (2013), with some modifications. Samples were washed with a 1:10 soap solution and left for 24 hours to remove any adhered fecal residues and decaying organic matter. Afterward, residues were washed in a constant flow of tap water using a sieve (0.5 mm mesh). Materials were placed on aluminum foil trays and stove-dried (Felisa FE - 292AD) at 61 °C. The materials were then placed in previously labeled manila paper envelopes. Any identifiable components and structures in each sample were sorted considering the zoological groups reported (to the finest taxonomic level possible), using a stereo microscope (Iroscope model Es - 24 PLIT) and tweezers (R - 1).

Small-sized components, such as scales, bones, hairs, feathers, teeth, and other fragments, were stored in properly labeled plastic vials. Larger structures such as partial fish skeletons, found mainly in feeders, were stored in paper envelopes.

One of the elements of greatest interest was otoliths; these were cleaned with a wet towel to remove adhered remains and determine whether their condition was suitable for identification. It is worth highlighting that the identification of each otolith in a pair (left and right) was conducted following the main characteristics mentioned by Ramírez-Pérez *et al.* (2019).

We identified species based on otoliths using three types of reference materials: 1) photographic catalogs from the *Formes d'Otòlits* database (AFORO BASE 2018, Catalan acronym; http://aforo.cmima.csic.es/index.jsp), *Sagitta* Otolith Catalog of Fishes of the Gulf of México (*Catálogo de otolitos* Sagitta *de peces del Golfo de México*) by Ramírez-Pérez *et al.* (2019), and Otoliths: A Way to Identify Fishes (*Otolitos:*

una manera de identificar a los peces) by Rosas Arevalo *et al.* (2021); 2) A list of fish species recorded for the Palizada–Del Este and Laguna de Términos system obtained from the Hydrological and Ichthyological Atlas of Laguna de Términos (*Atlas hidrológico e ictiológico de la Laguna de Términos*) by Ramos-Miranda *et al.* (2006) and Marine and Coastal Ichthyofauna of Campeche (*Ictiofauna Marina y Costera de Campeche*) by Ayala-Pérez *et al.* (2015), and 3) otoliths from the Otolith Collection of the Faculty of Natural Sciences at UNACAR. Once otoliths were identified to the finest taxonomic level possible, they were deposited following the Collection guidelines.

Ecological niche width was calculated through Levin's index (Levins 1968; Jaksic and Marone 2007), including the analysis of the remains of 98 samples of organisms identified to the finest taxonomic level possible:, where *B* is Levin's Index, and *Pi* is the relative frequency of the species using the resource *i*. The standardized Levin's index $B_{est} = (B - 1) / (n - 1)$ was used to express the niche width on a scale ranging from 0 (narrow niche) to 1 (wide niche; Krebs 1999).

Percentage of Occurrence was calculated with the formula: $PO = fi/ft \times 100$, where *fi* is the number of feces samples containing a prey category and *ft*, the total number of occurrences of all prey categories in the total number of feces samples (Macías-Sánchez and Aranda 1999). Finally, the Statistica Software version 7.0 (VinceStatSoft 2002-2007) was used to perform non-parametric Kruskal-Wallis tests and assess whether the consumption of prey groups (mollusks, crustaceans, fish, reptiles, birds, and mammals) varied significantly between the three seasons (Díaz-Gallardo *et al.* 2007).

We analyzed 126 samples of Neotropical otter feces and one feeder collected at 52 sites (Figure 2) in the three seasons. The dry season showed the highest abundance of feces ($n = 71$, 56.3 %), followed by the winter rainy season ($n = 40$, 31.7 %) and the rainy season ($n = 15$, 11.9 %). The latter record was likely the result of heavy rains prior to sampling, washing away the feces on some mangrove trunks.

Seven major groups were identified from feces. Fish showed the highest percentage of occurrence (59 %), followed by crustaceans (23 %), mollusks and mammals (5 % each), reptiles (4 %), birds (1 %), and others (3 %, including insects, polychaetes, and unidentified materials). Throughout the study, 4 types of indirect evidence were recorded: feces, latrines, vocalizations, and feeders (Figure 2A-C), with the winter rainy season (*nortes*) showing the greatest diversity of indirect evidence (Figure 2A). Only one feeder was recorded in the winter rainy season (*nortes*; Figure 2A). Bone remains were found in the feeder, allowing the identification of two fish genera: 1) *Pterygoplichthys* (janitor fish) and 2) *Cathorops* (dark sea catfish, *C. melanopus*; Table 1).

Of a total of 101 otoliths, only 29 (28. 7%) were identifiable due to their good state of conservation. Seven fish families were identified in feces: Ariidae, Batrachoididae, Gobiidae, Paralichthyidae, Cichlidae, Poeciliidae, and Loricariidae (Table 1). The genus *Pterygoplichthys* (janitor fish) was additionally identified using not only otoliths but also structures such as vertebrae, spines of pectoral and dor-

Table 1. Prey organisms identified in feeders and feces of the Neotropical otter in La Sangría Lagoon, Campeche. * Native species (Ramos-Miranda *et al.* 2006; Ayala-Pérez *et al.* 2015; Rosas Arevalo *et al.* 2021). ** Possible new records for the study area. + Taxonomic hierarchy according to Fishbase (Froese and Pauly 2022). ++ Taxonomic hierarchy according to the World Register of Marine Species (WoRMS 2021). FO = Frequency of Occurrence. PO = Percentage of Occurrence.

Phylum / Subphylum	Class	Order	Family	Genera	Species	FO	PO
Mollusca ++	Bivalvia	Ostreida	Ostreidae	*Crassostrea*	*Crassostrea* sp.	7	3.68
		Myida	Dreisseni-dae	*Mytilopsis*	*Mytilopsis* sp.	4	2.11
	Gastropoda		Unidentifiable			13	6.84
Arthropoda ++ / Crustacea	Malacostracea	Decapoda	Portunidae	*Callinectes*	*Callinectes* sp.	86	45.3
			Penaeidae	*Penaeus*	*Penaeus* sp.	21	11.1
	Hexanauplia	Balano-morpha	Balanidae	*Balanus*	*Balanus* sp.	1	0.53
Chordata / Vertebrata	Actinopterygii+	Batrachoi-diformes	Batrachoi-didae	*Opsanus*	*Opsanus beta* *	1	0.53
		Cicliformes	Cichlidae	*Cichlasoma*	*Cichlasoma urophthalmus* *	1	0.53
		Gobiifor-mes	Gobiidae	*Awaous*	*Awaous* sp. **	9	4.74
		Silurifor-mes	Loricarii-dae	*Pterygo-plichthys*	*Pterygo-plichthys* sp.**	19	10.0
			Ariidae	*Cathorops*	*Cathorops melanopus**	4	2.11
		Pleuronec-tiformes	Paralich-thyidae	*Cyclopsetta*	*Cyclopsetta chittendeni* **	1	0.53
		Cyprino-dontiformes	Poeciliidae	*Poecilia*	*Poecilia* sp. **	1	0.53
	Reptilia	Squamata Suborder: Serpentes		---		8	4.21
	Aves		Unidentifiable			1	0.53
	Mammalia	Rodentia		---		13	6.84
					TOTAL	190	100

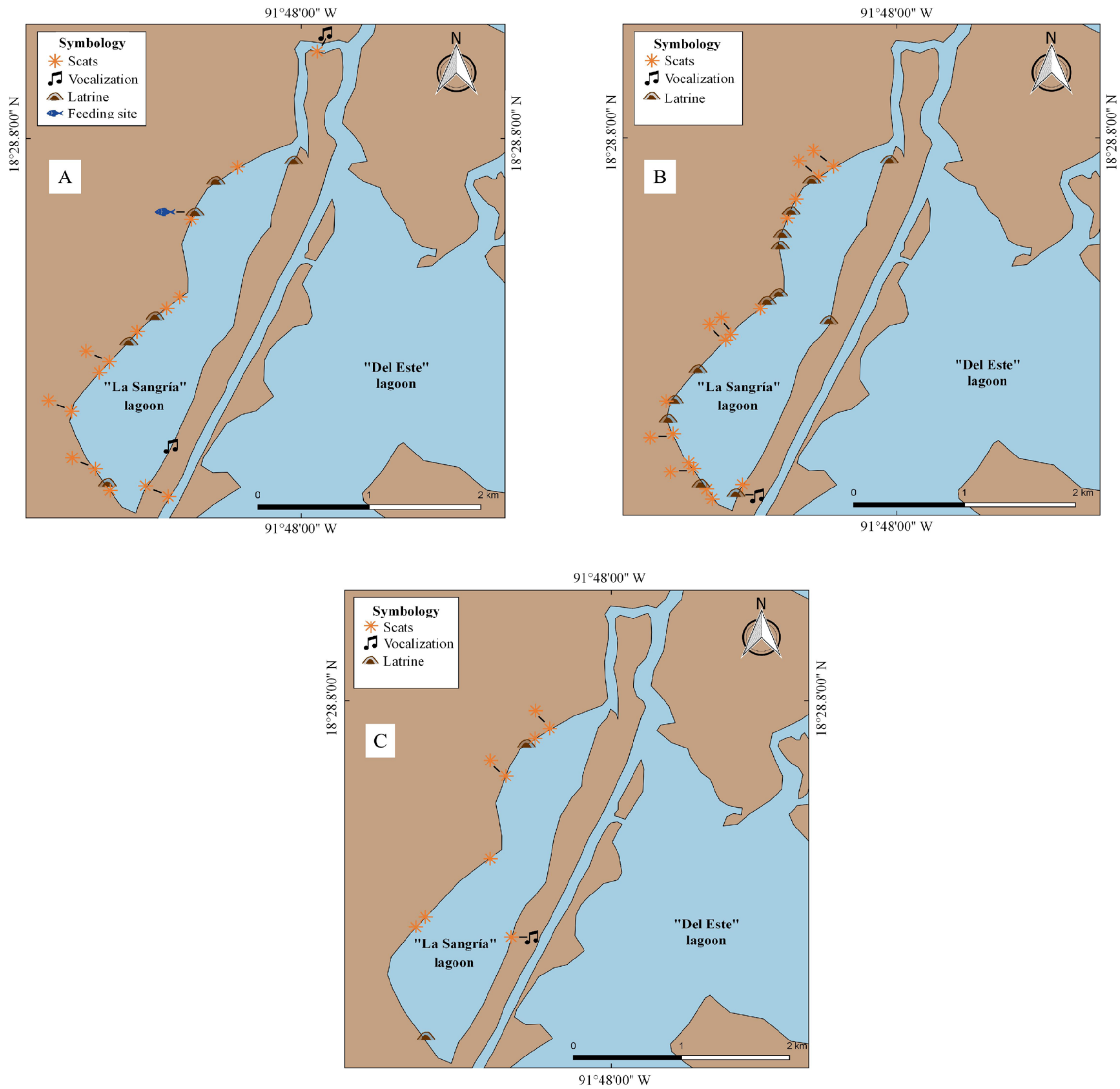

Figure 2. Spatio-temporal distribution of indirect evidence of Neotropical otter in La Sangría Lagoon, Palizada, Campeche, México. A) Winter rainy season (*nortes* 2016). B) Dry season (2017). C) Rainy season (2017).

sal fins, and, particularly, dermal plates. The janitor fish showed a total percentage of occurrence of 14.28 % during the sampling period, with the highest percentage in the rainy season (26.70 %), followed by the winter rainy season (*nortes*; 15 %) and the dry season (11.30 %).

Two families of crustaceans were identified (Portunidae: *Callinectes*; and Penaeidae: *Penaeus*). Regarding mollusks, 2 classes were recorded: Gasteropoda and Bivalvia, the latter with two genera (*Crassostrea* and *Mytilopsis*), both representative of the area (García-Cubas and Reguero 2007). Only the Order Rodentia was identified for mammals, while the reptiles identified belong to the Order Squamata, particularly the Suborder Serpentes. Finally, birds could only be identified to class (Table 1).

Considering the organisms identified in Table 1, the standardized Levin's index showed that the niche width of *L. l. annectens* tends to specialization in La Sangría Lagoon (B_{est} = 0.2078; Krebs 1999). The statistical analysis of the feeding habits by season revealed no variation in the consumption frequency of prey groups (H = 5.421, *d.f.* = 2, $P >$ 0.05; Table 1). However, there were statistically significant differences in the diversity of species consumed (H = 46.3, *d.f.* = 2, $P < 0.01$).

Fish were the main prey group in the three seasons (53 % rainy, 50 % winter rainy, and 71 % dry). The rank of percentages for the main prey groups were fish > crustaceans > mollusks = mammals, with fish > crustaceans in the three seasons.

The food resources of the Neotropical otter recorded in the feeder and feces comprised diverse groups (7 in total: fish, crustaceans, mollusks, mammals, reptiles, birds, and others). However, the low standardized Levin's index value (0.2078) in La Sangría Lagoon may suggest that the Neotropical otter presents a niche width with a specialist tendency. This work confirms that species with a wide distribution, such as the Neotropical otter (Ramírez-Bravo 2010), will shift their trophic condition from generalist to specialist, depending on the site where preys are located (Monroy-Vilchis and Mundo 2009) or exposure to intense disturbances (Briones-Salas *et al.* 2013), feeding preferentially on the most abundant and available resource, in this case, fish, including the janitor fish (*Pterygoplichtys* spp.). Briones-Salas *et al.* (2013) mentioned that classifying the Neotropical otter as a specialist should not depend solely on the Levin's index value; its ability to consume a wide range of food items and its plasticity to adapt to new conditions should be assessed too. Therefore, further studies should address the abundance, frequency, and other ecological aspects of prey species in the study area.

Several authors have described *L. longicaudis* as an opportunistic carnivorous species that can consume prey according to their availability (Gallo-Reynoso 1996; Macías-Sánchez and Aranda 1999; Arellanes-Licea and Briones-Salas 2003; Díaz-Gallardo *et al.* 2007; Casariego-Madorell *et al.* 2008; Santiago-Plata *et al.* 2013; Rheingantz *et al.* 2017; Grajales-García *et al.* 2019; García-Silva *et al.* 2021). The hypothesis that diet analysis is a key factor in classifying the Neotropical otter as an opportunistic species (defined as a species that has greater adaptability by not specializing in any particular food) is not consistent with the results of this work. However, all the studies on the subject should be considered, and some authors report that the Neotropical otter is an opportunistic species with a flexible diet (Chanin 1985; Briones-Salas *et al.* 2013; Rheingantz *et al.* 2017).

Of the 7 groups of prey identified in feces, fish was the main group in most cases. The presence and consumption of *Pterygoplichtys* spp. were recorded in the three climatic seasons, suggesting that the Neotropical otter may play a role in regulating janitor fish populations, as reported by Amador del Ángel *et al.* (2014) and Vázquez-Maldonado *et al.* (2018). In this work, it was not possible to identify janitor fish species with otoliths. Two species are reported in the region, *P. pardalis* and *P. disjunctivus*, and hybridization between both species also has been documented (Li-Wei *et al.* 2011; Amador del Ángel *et al.* 2014). It is worth highlighting that the lowest percentage of occurrence (11.3 %) for *Pterygoplichtys* spp. was recorded in the dry season when the highest salinity values were observed with a maximum concentration of 5 ppm (parts per million). It has been reported that specimens of *P. pardalis* kept at a salinity level of 0.2 PSU (practical salinity units) can survive a sudden (acute) exposure to salinities up to 10 PSU for 10 days (experimental evaluation criterion of 240 hr; Capps *et al.* 2011) with low mortality. La Sangría Lagoon is under the hydrological influence of Laguna de Términos. The latter is characterized by brackish to marine water masses, and salinity values above 0 ppm prevail in the dry season, given the lack of inputs of fluvial and pluvial fresh water (Ayala-Pérez 1989). It has been mentioned that the hybridization between *P. pardalis* and *P. disjunctivus* has given rise to hybrid superiority, so hybrid specimens are more adaptable when they invade new habitats (Jumawan *et al.* 2011). Further studies are required to understand its resistance and abundance (*i.e.*, higher salinity values are associated with a lower percentage of occurrence of janitor fish in feces, likely due to lower consumption from lower prey abundance).

The identification of fish to species based on otoliths was complex. It is worth mentioning that some otoliths also did not allow identification to family, mainly for three reasons: 1) otoliths were highly degraded due to the digestion process, 2) they were not the *Sagitta* otoliths commonly used for fish identification, and 3) they were only small fragments that did not allow observing morphological structures essential for proper identification. Since species identification directly impact Levin's index, the presence of potential prey, including fish and other taxa, should be addressed in future studies. This issue is not unique to this study but affects any results reporting that otoliths remained and passed through the digestive tract, as in the case of common bottlenose dolphins (*Tursiops truncatus*; Naranjo Ruíz *et al.* 2019). However, this index gives insights into understanding niche width, which should not depend solely on the Levin's index value for the area, as suggested by Briones-Salas *et al.* (2013).

Three of the prey fish species identified are native to the area (*Cathorops melanopus, Opsanus beta*, and *Cichlasoma urophthalmus*), according to Ramos-Miranda *et al.* (2006), Ayala-Pérez *et al.* (2015), and Rosas Arevalo *et al.* (2021). Four likely new fish records for the area were also obtained (*Awaous* sp., *Cyclopsetta chittendeni*, *Poecilia* sp., and *Pterygoplichthys* sp.). Nonetheless, more detailed analyses should be performed since no reference catalog of otoliths is currently available for the region or the genera inhabiting the study area.

Regarding the ranking of the percentages of the main prey groups recorded in this study (fish > crustaceans > mollusks and mammals), a new relationship emerged versus studies from other regions of México, even relative to those where fish are highlighted as the main prey of otters (Table 2).

Our results in La Sangría Lagoon showed that the consumption percentages are related to prey availability each season. This is consistent with the reports by authors such as Chemes *et al.* (2010) and Santiago-Plata *et al.* (2013), the latter for a locality (La Veleta) very close to our study area in Laguna de Términos region, Campeche.

The Neotropical otter plays an important role in the ecosystems where it thrives. When its feeding habits in La Sangría Lagoon are compared with those recorded elsewhere, its trophic adaptability and ecological value are evidenced. Accordingly, otters are considered umbrella species (Bifolchi and Lodé 2005) and top predators, highlighting their potential to maintain the functioning of aquatic environments (Sánchez and Gallo-Reynoso 2007; Gallo-Reynoso *et al.* 2008; Grajales-García *et al.* 2019; García-Silva *et al.* 2021), particularly through the consumption of exotic and invasive species, such as the janitor fish (Capps *et al.* 2011; Amador del Ángel *et al.* 2014).

The fact that otters use logs of different mangrove species illustrates the functional links of this species with other components in the same ecosystem, as previously described by Bifolchi and Lodé (2005), confirming that the Neotropical otter is a key umbrella species in its ecosystem.

Table 2. Percentage of occurrence of the main prey groups in feces of *Lontra longicaudis annectens* in México. *Insects, arachnids, diplopods, and gastropods. **Laguna de Términos Wildlife Protection Area.

Order	Location	Citation
Crustaceans > fishes > insects	Ríos Ayuta, Copalita and Zimatán, costa de Oaxaca	Casariego-Madorell *et al.* 2006
		Casariego-Madorell *et al.* 2008
	Río Zimatán, costa de Oaxaca	Briones-Salas *et al.* 2013
*Invertebrates > fishes > plants	Río San Diego, en la quebrada de Galindo, Durango	Cruz-García *et al.* 2017
Crustaceans > fishes > mollusks	Zona costera de Tuxpan, Veracruz	Grajales-García *et al.* 2019
Fishes > amphibians and insects	Río Bavispe-Yaqui, Sonora	Gallo-Reynoso 1996
Fishes > insects > mammals	Río Bavispe-Yaqui, Sonora	Rangel-Aguilar and Gallo-Reynoso 2013
Fishes > crustaceans > insects	Sierre Madre del Sur	Gallo-Reynoso 1989
	Río Los pecadores, Veracruz	Macías-Sánchez and Aranda 1999
	Río San Cipriano, Tabasco	Ramón 2000
	Río Ayuquila, Jalisco	Díaz-Gallardo *et al.* 2007
Fishes > insects > amphibians	Temascaltepec, Edo. de México	Monroy-Vilchis and Mundo 2009
Fishes > amphibians, arachnids, crustaceans	Río Mezquital-San Pedro, Durango	Charre-Medellín *et al.* 2011
Fishes > crustaceans > reptiles	Camino La Veleta, APFFLT**, Campeche	Santiago-Plata *et al.* 2013
Fishes > reptiles > insects	Río Grande, Reserva de la Biosfera Tehuacán-Cuicatlán (Puebla-Oaxaca)	Duque-Dávila *et al.* 2013
Fishes > insects > reptiles	Reserva de la Biosfera Tehuacán-Cuicatlán (Puebla-Oaxaca)	Lavariega *et al.* 2020
	Río Santiago, Nayarit-Jalisco	Guerrero *et al.* 2018
Fishes > crustaceans > mollusks and mammals	Laguna La Sangría, Palizada**, Campeche	This work

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Macroscopic morphology of the male genitalia of *Caenolestes caniventer* (Caenolestidae: Paucituberculata)

Morfología macroscópica del genital masculino de *Caenolestes caniventer* (Caenolestidae: Paucituberculata)

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The anatomy of the penis in the Paucituberculata has received little attention. The only treatise addressing the genitalia and prostate in this group of marsupials comes from a century ago. The present work describes the macroscopic morphology of the male genitalia of the gray-bellied shrew opossum, *Caenolestes caniventer*, a structure that can be useful in the taxonomic, reproductive, and evolutionary fields. The genitals were removed (*n* = 2) through a basal incision during the taxidermy of specimens and preserved in a 90 % alcohol solution. In the laboratory, genitals were prepared following eversion protocols and stained to highlight the structures. The penis and its structures were described. The penis has a long-wide glans with two distinctive prongs at distal end. A medium spermatic groove extends to the prongs. The prongs are 1.6 times longer than the glans, relatively cylindrical and striated, progressively tapering to a hook-shaped distal tip. Prong length and penile spines may be key to reproductive efficiency. We hypothesize that the family Caenolestidae generally displays similar penis shapes, with differences between species. These results are useful in taxonomic, reproductive, and evolutionary fields.

Key words: Genitals; glans; penile spines; phallus; spermatic sulcus.

La anatomía del pene en paucituberculados ha merecido poca atención. El único tratado que aborda la genitalia y próstata en este grupo de marsupiales proviene de hace un siglo. El presente trabajo describe la morfología macroscópica de la genitalia masculina del ratón marsupial de vientre gris, *Caenolestes caniventer*, estructura que puede ser de utilidad en el campo taxonómico, reproductivo y evolutivo. Los genitales fueron removidos (*n* = 2) con una incisión basal durante la taxidermia de los especímenes y conservados en una solución de alcohol al 90 %. En el laboratorio los genitales fueron preparados siguiendo protocolos de eversión y tinturado los genitales para resaltar las estructuras. Se describió el genital y sus estructuras. El pene presenta un glande largo y ancho, con dos distintivos apéndices ubicados en su extremo. Un surco medio espermático se extiende hasta los apéndices. Los apéndices son 1.6 veces más largos que la longitud del glande, de forma relativamente cilíndrica y estriada, estrechándose progresivamente hasta terminar en una punta distal con aspecto de garfio. El largo de los apéndices y las protuberancias en forma de espina podrían ser clave para la eficiencia reproductiva. Hipotetizamos que la familia Caenolestidae presenta de manera general formas similares del pene, pero con diferencias entre especies. Los resultados son de utilidad en el campo taxonómico, reproductivo y evolutivo.

Palabras clave: Falo; genitales; glande; surco espermático; tubérculos espinosos.

Knowledge of penile morphology has been moderately documented, mainly for medium-sized and large mammals (Mollineau *et al.* 2006; Amezcua *et al.* 2009; Carneiro *et al.* 2010; Rocha *et al.* 2017) and, to a lesser extent, for small mammals, such as marsupials (Didelphidae) and rodents (Cricetidae and Muridae; Hooper and Musser 1964; Voss and Linzey 1981; Nogueira 1988; Martinelli and Nogueira 1997; Nogueira *et al.* 2004). For the Order Paucituberculata, a group of marsupials endemic to the New World (Goin *et al.* 2009; Ojala-Barbour *et al.* 2013; Patterson 2016; Voss and Jansa 2021), knowledge of this trait is scarce, as well as its anatomical, morphological and functional adaptations. The only work dealing with the genitalia and prostate in Paucituberculata comes from *Caenolestes fuliginosus*, studied by Osgood (1921) a century ago. Rodger (1982) and Woolley (1987) provided an anatomical approach to the seminiferous tubes, epididymis, *rete testis*, and efferent and deferens ducts of this species.

This work describes the macroscopic morphology of the penis of *Caenolestes caniventer* (Figure 1), a species restricted to the western Andes of Ecuador and northwestern Perú (Patterson 2016) and classified by IUCN as Nearly Threatened (Solari and Martínez-Cerón 2015). This is the first work that fully illustrates the gross morphology

Figure 1. Adult male specimen of *Caenolestes caniventer* (MECN 4821). Chillacocha, Cordillera de Chila, Provincia de El Oro, Ecuador. Photograph: J. Brito.

of a male genital of Paucituberculata, which can be useful in taxonomic, reproductive, and evolutionary fields.

Two adult specimens of *C. caniventer* were captured in Chilla (3° 30' 9.35" S, 79° 37' 32.49" W, 3,338 m), a locality in the Provincia de El Oro, Ecuador, using Sherman traps (7.5 × 9 × 27 cm; H. B. Sherman Traps, Tallahassee, Florida) baited with a mixture of oat and coconut oil. Specimens were handled following the guidelines of Sikes *et al.* (2016). The voucher specimens (catalog numbers: MECN 4821 and MECN 4841, head-body length (total) 125 mm and 120 mm, respectively) are deposited in the mastozoological collection of the Instituto Nacional de Biodiversidad (MECN), Quito, Ecuador.

The genitals were removed through a basal incision during the taxidermy and preserved in a 90 % alcohol solution. The penises were prepared based on reptile hemipenis eversion protocols (Pesantes 1994; Betancourt *et al.* 2018), modified according to the anatomical structures of specimens. The genitals were immersed in a sodium dodecyl sulfate solution for 24 hr. Petroleum jelly was melted with a laboratory burner, and red vegetable dye was added for staining. Once confirmed that organ tissues were soft and flexible, organs were slowly inflated with the stained petroleum jelly using 2 ml insulin syringes, mainly for the glans prongs. The procedure was repeated until the organs reached their maximum expansion. The description of the penis and its structures is based on direct observations of the sexual organs, following the considerations of Osgood (1921).

The measurements used (with their respective abbreviations) to describe each anatomical structure and its proportions include phallus length (PL), measured from the base to the tip where it splits into prongs; glans width (GW), measured horizontally along the widest segment of the glans; length of the right glans prong (LRP), measured from the insertion base at the outer edge of the phallus to the tip; length of the left glans prong (LLP); length of the distal right glans prong capsule (LRC), measured from the base of the capsule to the distal region of the capsule; length of the distal left glans prong capsule (LLC); basal width of the right glans prong (BWRP), measured at the widest base of each glans prong; basal width of the left glans prong (BWLP); distal basal width of the right glans prong (DWRP), measured at the distal base of the glans; distal basal width of the left glans prong (DWLP). All measurements were taken with a Buffalo Tools digital calibrator to the nearest 0.1 mm.

The phallus is longer than wide, with two distinctive prongs at the glans tip (Figure 2A). The phallus has an average length of 17.27 mm, with an average glans width of 6.8 mm (Table 1). The glans prongs emerge from the lateral tips of the penis and are 1.6-fold longer than the phallus length. They are relatively cylindrical and striated, progressively tapering to a hook-shaped distal tip (Figure 2A, 2B, 2D, 2E).

The average glans prong length is 27.72 mm, with an average basal width of 5.52 mm, tapering to 3.21 mm at the base of the distal prong tip. The sagittal glans surface exhibits a middle spermatic groove (Figure 2C) that stretches along the ¾ parts of the prongs; the distal quarter is formed by a sub-distal capsule that wraps around the middle of the distal tip, conspicuously showing the spermatic groove (Figure 2E). Both the capsule and the distal tip are covered by penile spines (Figure 2F).

Male genitalia of the genus *Caenolestes* was partially studied by Osgood (1921) based on *C. fuliginosus*, who most probably did not evert the genitals to fully visualize the two glans prongs. The anatomy of the male genitalia of *Caenolestes* displays a unique structure, such as the presence of a spermatic sulcus and a bifurcated glans, a condition that has also been reported in didelphid marsupials, birds, and reptiles (Tyndale-Biscoe and Renfree 1987; Ricaurte-Galindo 2006; Passos and Lynch 2010).

Differences in the shape and proportions of male genitalia in some mammal groups are related to a certain extent to body proportions and evolutionary processes (Amezcua *et al.* 2009). However, the large dimensions of the glans prongs, the filiform structure of the penis, the hook-shaped glans prongs tips, and the presence of penile spines in *C. caniventer* may suggest that these are adaptations to deposit the sperm as close uterus as possible. This has also

Table 1. Morphometric measurements (mm), $\bar{X}$ mean and ± standard deviation (SD) of the male genital of 2 adult specimens of *Caenolestes caniventer*. Abbreviations of measurements are described in the text.

Measurements	MECN 4821	MECN 4841	$\bar{X}$	± SD
PL	15.14	19.39	17.27	3.01
GW	6.76	6.83	6.80	0.05
LRP	28.66	26.63	27.65	1.44
LLP	28.86	26.58	27.72	1.61
LLC	4.92	6.06	5.49	0.81
LRC	4.95	6.08	5.52	0.80
BWRP	3.04	3.37	3.21	0.23
BWLP	3.37	3.34	3.36	0.02
DWRP	1.14	1.16	1.15	0.01
DWLP	1.12	1.26	1.19	0.10

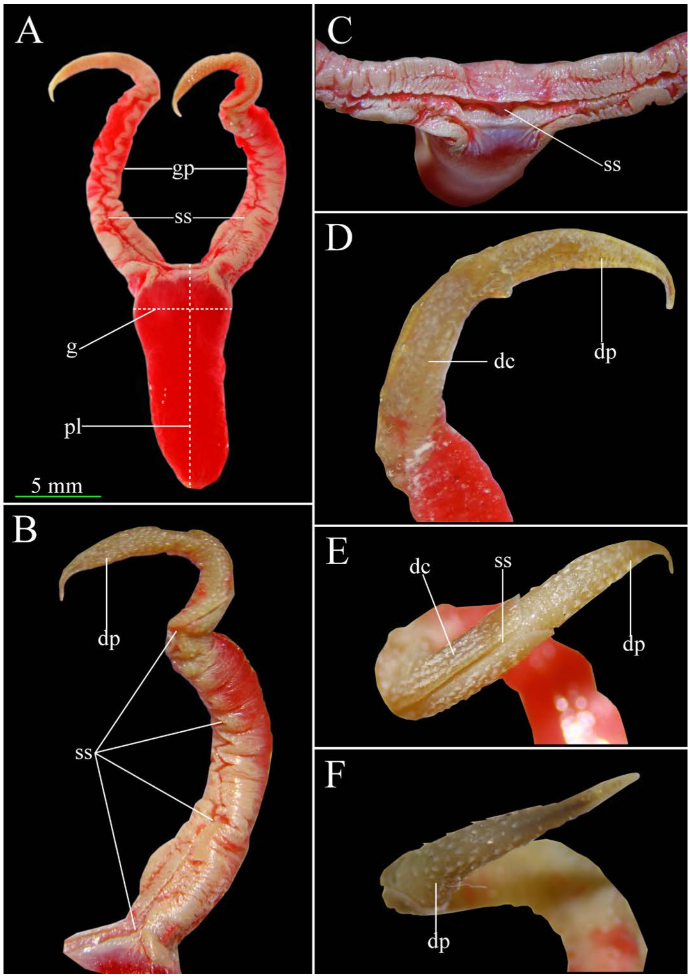

Figure 2. Male genital of an adult specimen of *Caenolestes caniventer* (MECN 4841). A) Ventral view of the genital: (pl) phallus length; (g) glans; (gp) glans prongs; (ss) spermatic sulcus. B) Enlargement of the left glans prong, showing the spermatic sulcus (ss) and distal prong tip (dp). C) Anterior view of the glans at the bifurcation of the prongs. D) Detail of (dp) and the subdistal capsule (dc). E) Detail of the (dc), showing the (ss). F) Detail of (dp). Note in E and F the penile spines around the (dc) and (dp). Scale = 5 mm. Photographs: M. Yánez-Muñoz.

been observed in some marine mammals and ungulates with long and filiform penises (Bonet *et al.* 2013; Brennan 2016; Orbach *et al.* 2017) and in reptile species that have deeply bifurcated hemipenises with hooks and spines, such in *Thamnophis radix* (King *et al.* 2009).

Penile spines are also associated with sensory functions during the coitus, promoting stimulation between the male organs and the vaginal wall (McLean *et al.* 2011; Orbach *et al.* 2017). In rodents and primates, these spines appear to play a crucial role. Dixson (1986) showed as the experimental removal of these structures resulted in a lesser stimulus, with males requiring a longer time for penetration and ejaculation. Mammals with spineless penises have prolonged copulations so that the sensory stimulation lengthens, and the presence of penile spines no longer seems essential (McLean *et al.* 2011). The presence of these structures in the glans prongs of *C. caniventer* suggests that the copulation is probably short-lasting, and according to this reproductive behavior, the penile spines increase the stimulation and the probability of successful deposit sperm in the female genital tract.

The coevolution of male and female genitalia plays a central role in the shape of the penis (Brennan *et al.* 2007; Masly 2012; Orbach *et al.* 2017). The morphology of the penis must match the female anatomy (following the key-lock hypothesis) of the species to avoid intromission into females of other species; in turn, the female must display reproductive characters unique to its species (Hershkovitz 1966; Brennan *et al.* 2007; Masly 2012; Brennan and Prum 2015). These interspecific morphological variations have been reported for multiple small mammal species (Hooper and Musser 1964; Hershkovitz 1966; Langguth and Neto 1993). Therefore, we hypothesized that the Caenolestidae species would display similar genital morphology and structures, albeit with interspecific differences. However, only the comparison between genitalia of different species would allow determine if there is a significant anatomical variation and its intrinsic value in the taxonomy.

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Arthropofauna consumed by the Andean night monkey (*Aotus lemurinus*) in a forest fragment at the Cordillera Central Colombia

Artropofauna consumida por el mono nocturno andino (*Aotus lemurinus*) en un fragmento de bosque en la Cordillera Central de Colombia

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Arthropods are important food for primates such as night monkeys (*Aotus* spp.) because they provide easily digestible protein and fat. However, the record of their consumption has been underestimated due to the opportunistic nature of their capture and difficulties in identification. We describe arthropod consumption by a group of Andean night monkeys (*Aotus lemurinus*) in a fragmented forest in Colombia. Between March and June 2016, we collected scat samples from nocturnal monitoring and visits to the resting site from a group of 4 individuals of the Andean night monkey. We preserved the samples in 96 % alcohol and subsequently visualized them under a stereoscope. The scats were inspected for arthropod fragments that were later determined at the taxonomic level of order and family. In 12 of the 15 scats analyzed, we found 48 arthropod fragments, among which we classified 38 at the order level and 16 at the family level. In total, we identified 7 arthropod orders (6 insects and 1 arachnid) and 9 insect families (5 Coleoptera, 3 Hemiptera and 1 Hymenoptera). The orders with the highest number of fragments in the scats were Coleoptera (21), Hemiptera (5) and Orthoptera (5) and the families were Elateridae and Curculionidae with 5 and 4 numbers of fragments, respectively. We report for the first time the consumption of the order Isoptera, as well as 2 new families of Hemiptera (*i.e.*, Coreidae and Pentatomidae) and 5 families of Coleoptera (*i.e.*, Chrysomelidae, Coccinellidae, Curculionidae, Elateridae and Staphylinidae) in the diet of night monkeys (*Aotus* spp.). The large number of beetle fragments in the scats of Andean night monkeys could be due to the chitinous composition of their exoskeletons that makes them difficult to degrade. This study provides novel information on the variety of arthropods consumed by the Andean night monkey and compiles all the information on the consumption of this item for the entire genus.

Key words: Chitin; Coleoptera; Isoptera; scats.

Los artrópodos son alimento importante para primates como los monos nocturnos (*Aotus* spp.) debido a que proveen proteínas y grasas fáciles de digerir. No obstante, el registro de su consumo ha sido subestimado por la naturaleza oportunista de su captura y por las dificultades en la identificación. Describimos el consumo de artrópodos por parte de un grupo del mono nocturno andino en un bosque fragmentado de Colombia. Entre marzo y junio de 2016, recogimos muestras fecales a partir de seguimientos nocturnos y visitas al dormidero de un grupo de 4 individuos del mono nocturno andino (*Aotus* spp.). Preservamos las muestras en alcohol al 96 % y posteriormente las visualizamos a partir de un estereoscopio. Se inspeccionaron las heces en búsqueda de fragmentos de artrópodos que posteriormente fueron determinados a nivel taxonómico de orden y familia. En 12 de las 15 heces analizadas encontramos 48 fragmentos de artrópodos, de los cuales se clasificaron 38 a nivel de orden y 16 a nivel de familia. En total, identificamos 7 órdenes de artrópodos (6 insectos y 1 arácnido) y 9 familias de insectos (5 Coleoptera, 3 Hemiptera y 1 Hymenopera). Los órdenes con mayor cantidad de fragmentos en las heces fueron Coleoptera (21), Hemiptera (5) y Orthoptera (5) y las familias más frecuentes fueron Elateridae y Curculionidae con 5 y 4 fragmentos, respectivamente. Reportamos por primera vez el consumo del orden Isoptera, así como 2 nuevas familias de Hemiptera (*i.e.*, Coreidae and Pentatomidae) y 5 familias de Coleoptera (*i.e.*, Chrysomelidae, Coccinellidae, Curculionidae, Elateridae y Staphylinidae) en la dieta de los monos nocturnos (*Aotus* spp.). La gran cantidad de fragmentos de escarabajos en las heces del mono nocturno andino podría ser debido a la composición quitinosa de sus exoesqueletos que los hace difícil de degradar. Este estudio aporta información novedosa sobre la variedad de artrópodos consumidos por el mono nocturno andino y recopila toda la información sobre el consumo de este ítem para todo el género.

Palabras clave: Coleoptera; heces; Isoptera; quitina.

Primates must select a diverse array of feeding items in order to fulfil their energetic and nutritional requirements (Felton *et al.* 2009). Obtaining nutrients such as sugars, fats and proteins can be challenging, and primates must balance the consumption of different feeding sources based on their preferences and habitat-wide availability (Felton *et al.* 2009). In particular, fruits are important sources of sugars and carbohydrates, while leaves and insects provide proteins not readily available in fruits. Arthropods are one of the most important feeding items of small and medium-bodied primates, partly because they provide proteins and fats easier to absorb compared to traditional foods such as fruits and leaves (Rothman *et al.* 2014; Bryer *et al.* 2015). Nonetheless, the systematic recording of feeding on arthropods might be underestimated due to the opportunistic nature of capturing them and the difficulties associated with identifying arthropod prey in arboreal primates (Pickett *et al.* 2012).

The diet of night monkeys (*Aotus* spp.) has been recorded for several species of this broadly distributed primate genus. Night monkeys rely on ripe and fleshy fruits, and complement their diet with flowers, leaves and arthropods (Puertas *et al.* 1992; Fernandez-Duque 2007; Wolovich *et al.* 2010; Guzmán *et al.* 2016; Bustamante-Manrique *et al.* 2021; Montilla *et al.* 2021) and occasionally with small vertebrates and bird eggs (Puertas *et al.* 1992). Studies on night monkeys living both in the field and in captivity have recorded a few arthropod orders in their diet. For example, in a study on *Aotus azarae,* they were observed preying on Hemipterans (Fernandez-Duque 2007), while *A. nigriceps* has been observed eating several arthropods orders including Araneae, Coleoptera, Hymenoptera, Lepidoptera and Orthoptera (Wright 1985, 1986). In captivity, *A. nancymaae* was recorded eating both flying and crawling insects (Blattodea, Coleoptera, Diptera and Lepidoptera) as well as spiders (Araneae) and millipedes (Diplopoda; Wolovich *et al.* 2010). Castaño *et al.* (2010) observed *A. lemurinus* consuming arthropods of the orders Araneae, Hemiptera and Lepidoptera, and Grajales-Suaza *et al.* (2021) recorded the consumption of beetles (Coleoptera) of the genus *Phyllophaga*. Although most authors who have described the diet of night monkeys have reported arthropod consumption (Wright 1986; Arditi 1992; Puertas *et al.* 1992; Castaño *et al.* 2010; Wolovich *et al.* 2010, Guzmán *et al.* 2016; Bustamante-Manrique *et al.* 2021; Montilla *et al.* 2021), none have been able to establish quantitative estimates of this item within the diet (Fernandez-Duque 2007) mainly because of the challenges imposed by the nocturnal and arboreal nature of these primates, which makes it difficult to determine the arthropods consumed.

The Andean night monkey (*Aotus lemurinus*) is distributed in the Andes mountains of Colombia, Ecuador, and Venezuela between 1,000 and 3,200 m (Link *et al.* 2021). It has been recorded in primary and secondary forests, as well as in small degraded forest fragments, coffee plantations and forests immersed within urban areas (Castaño *et al.* 2010; Defler 2010; Montilla *et al.* 2017). Currently, this species is considered as Vulnerable according to the International Union for Conservation of Nature (IUCN), due to habitat loss and forest fragmentation, mainly as a result of agricultural and illicit crops expansion (Link *et al.* 2021). In the last decade, the diet of *A. lemurinus* has been described by Castaño *et al.* (2010), Guzmán *et al.* (2016), Bustamante-Manrique *et al.* (2021) and Montilla *et al.* (2021) who have recorded that they spend approximately between 12 % of their feeding time foraging and eating arthropods. However, none of these previous studies has provided a detailed account of arthropods consumed by night monkeys. Here, we describe the consumption of arthropods by the Andean night monkey in a fragmented forest of the Andes Central Cordillera of Colombia, providing novel information on their dietary habits.

This study was carried out in a fragmented forest known as Bosque de Las Martejas located at El Billar farm, in the municipality of Pijao, Quindío, Colombia (4° 19' 58" N, 75° 42' 39" W; 1,750–1,800 m). This forest which extends over approximately 6 ha is immersed in a complex mosaic of small forest fragments and banana, coffee, and avocado plantations. The forest canopy has a height of approximately 25 m and retains the typical characteristics of an Andean forest with large trees such as the genus *Poulsenia* and *Ficus* as well as pioneer trees of the genus *Cecropia* and *Miconia*. Within the forest, there are also several groupings of bamboo (*Guadua angustifolia*) that cover approximately 10 % of the entire fragment. Between March and June 2016, we collected scat samples from a single group of Andean night monkeys consisting of 4 individuals (*i.e.*, an adult male, an adult female and a subadult and a juvenile, both of undetermined sex). Scats were obtained from nocturnal monitoring of the group of monkeys, or directly at their resting sites (Figure 1). For nocturnal monitoring, we followed the night monkeys at least once a week during the entire sampling period, taking advantage of the hours of greatest activity proposed for these primates (*i.e.*, between 18:00 and 21:00 hr and between 03:00 and 06:00 hr; Castaño *et al.* 2010) and when weather conditions permitted. Samples were collected and preserved in 2 mL Eppendorf tubes with 96 % alcohol. Subsequently, we inspected all the fragments from the samples under the stereoscope (Zeiss Stemi dv4) in the laboratory of Universidad del Quindío and used the field guide of Snodgrass (1993) to identify arthropods contained in each sample to the highest taxonomic category.

Overall, we analyzed 15 scat samples, from which we found arthropod fragments in 12 of them. Based on the visual inspection of scat samples, we were able to identify 48 arthropod body parts (*e.g.*, legs, wings, antennae, exoskeletons), and taxonomically classify 38 of them at the order level and 16 at the family level.

We identified 7 orders of arthropods (6 insects and 1 arachnid) in Andean night monkey scat samples. We report for the first time the consumption of termites (order Isoptera), in the feeding ecology of night monkeys (*Aotus* spp.; Table 1; Figure 2a). The order with the highest number of fragments in the scats was Coleoptera (43.8 %), followed by Hemiptera (10.4 %) and Orthoptera (10.4 %). We found 9 different insect families (5 Coleoptera, 3 Hemiptera and 1 Hymenoptera) in the diet of the Andean night monkey (Table 2). The beetles' families Elateridae and Curculionidae

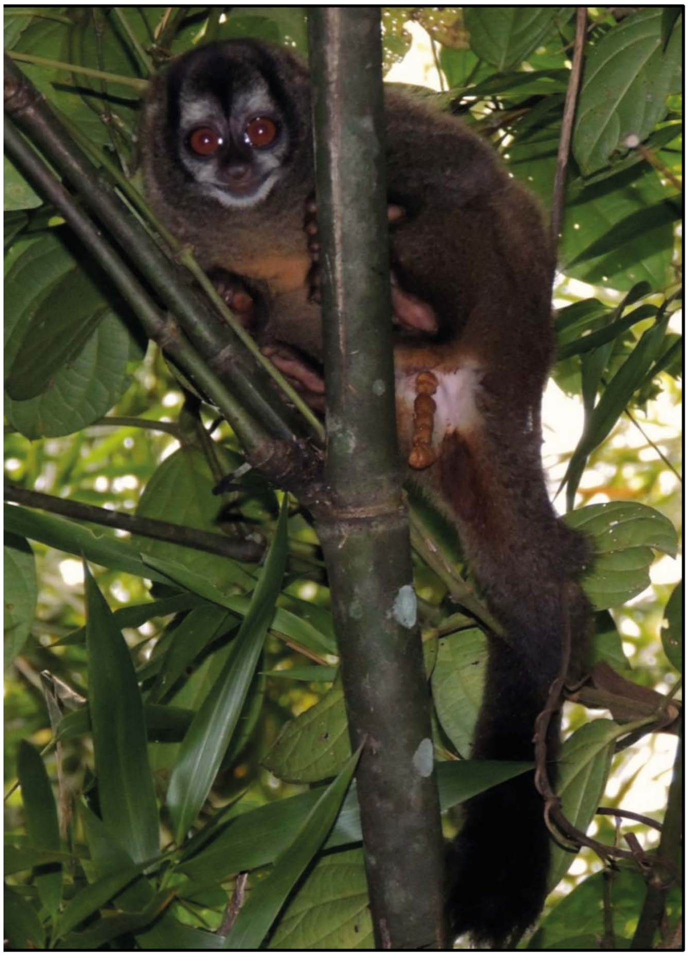

Figure 1. Andean night monkey, *Aotus lemurinus*, defecating at Bosque de Las Martejas of Pijao, Colombia.

(Figure 2b-c) had the highest number of fragments with 10.4 % and 8.3 %, respectively. The remaining orders and families were represented in less than 8 % of the fragments found in the samples.

The results of this study highlight how Andean night monkeys include a wide variety of arthropods in their diet. Despite our small sample size of scats, we detected the consumption of at least 7 arthropod orders and 9 families. Six of the arthropod orders recorded in our study have been also reported in the diet of night monkeys, including the Andean night monkey (Wright 1986, 1994; Fernandez-Duque 2007; Castaño *et al.* 2010; Wolovich *et al.* 2010; Bustamante-Manrique *et al.* 2021; Grajales-Suaza *et al.* 2021; Montilla *et al.* 2021). Earlier studies have documented the consumption of cycads (Hemiptera), which belong to the family Cicadellidae (Fernandez-Duque 2007; Castaño *et al.* 2010) and other studies report the consumption of beetles of the genus *Phyllophaga*, which belong to the family Scarabaeidae (Coleoptera; Grajales-Suaza *et al.* 2021) as well as ants of the family Formicidae (Hymenoptera; Wright 1985, 1994). In our study, we detected the consumption of Cicadellidae insects and added 2 new families of Hemiptera (*i.e.*, Coreidae and Pentatomidae) and 5 families of Coleoptera (*i.e.*, Chrysomelidae, Coccinellidae, Curculionidae, Elateridae and Staphylinidae) in the diet of Andean night monkeys. It is likely that the number of families and orders of arthropods consumed by Andean night monkeys and other night monkey species will increase from studies that include longer time series, ideally a year as well as a larger number of night monkey groups that inhabit forests with different degrees of anthropogenic impact.

Although Coleopterans are the most abundant insects in our samples, the high number of fragments found in the scats of the Andean night monkey may be a biased result inherent to the roughness and challenge to degrade their exoskeleton (Lambert 1998). Among insects, beetles are one of the groups that have more chitin in their exoskeletons, a very difficult compound to degrade that would require longer retention times in order to facilitate microbial fermentation and break down of this polysaccharide (Lambert 1998; Janiak 2018). The composition of the exoskeleton of these arthropods could explain why so many fragments remain intact after passing through the gastrointestinal tract of Andean night monkeys (Zeale *et al.* 2010). Indeed, based on observations in the scats of another nocturnal primate species in Madagascar (*Cheirogaleus medius*), it was evidenced the preference for the consumption of the order Coleoptera (Hladik *et al.* 1980); however, when performing DNA metabarcoding analysis, they found up to 32 different families that had not been found before in the scats (Rowe *et al.* 2021). Additionally, it has been suggested that the order Coleoptera has the highest abundances at ground level compared to the middle and upper canopy

Table 1. Comparison of the orders of arthropods consumed by night monkey species, *Aotus* spp.

Order of Arthropods	Species ***A. azarae*** **(Fernandez-Duque 2007)**	***A. nigriceps*** **(Wright 1985, 1986)**	***A. nancymae*** **(Wolovich *et al.* 2010)**	***A. lemurinus*** **(Castaño *et al.* 2010)**	***A. lemurinus*** **(Grajales-Suaza *et al.* 2021)**	***A. lemurinus*** **(This study)**
Araneae		X	X	X		X
Blattodea			X			
Coleoptera		X	X	X	X	X
Diptera			X			X
Diplopoda			X			
Hemiptera	X			X		X
Hymenoptera		X				X
Isoptera						X
Lepidoptera		X	X	X		
Orthoptera		X				X

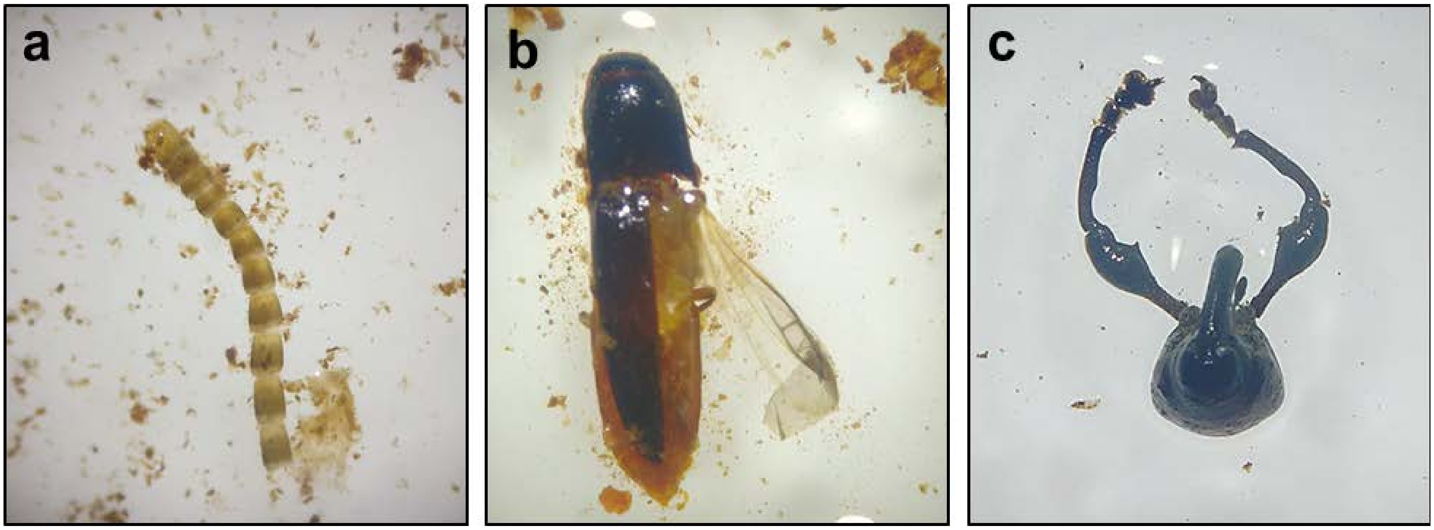

Figure 2. Fragments of arthropods found in the scats of the Andean night monkeys, *Aotus lemurinus*. a) Isoptera (the moniliform antennae are typical of terminate), b) Elateridae (Coleoptera), and c) Curculionidae (Coleoptera).

in tropical forests (Sutton and Hudson 1980; Sutton *et al.* 1983; de Souza *et al.* 2022), so that consumption of large amounts of beetles is unlikely because night monkeys forage mainly in a middle stratum (Wright 1994). Further, few studies have attempted to study the influence of arthropod availability in the canopy on the diet of primate species (Bryer *et al.* 2015; Fonseca *et al.* 2019); however, future studies should include these different approaches that improve our understanding of the feeding ecology and dietary preferences of primate species.

A similar pattern of detection bias in scats could occur with the order Hemiptera, which together with the order Orthoptera, ranked second in the number of fragments found in Andean night monkey scats. In this sense, it has been suggested that the order Hemiptera may also be more easily detected in scats (Zeale *et al.* 2010). However, this order may have a high abundance at upper canopy levels in tropical forests (de Souza *et al.* 2022) where Andean night monkeys move frequently during the night (Wright 1994). Despite the possible bias of higher detection of the order Coleoptera and Hemiptera in the scats of the Andean night monkey, these orders have been frequently recorded in the diet of different species of night monkeys including the Andean night monkey from field observations (Wright 1985; Fernandez-Duque 2007; Castaño *et al.* 2010; Grajales-Suaza *et al.* 2021).

Studies on the Andean night monkey have shown that it is mainly frugivorous, but they complement their diet with arthropods being the second most important dietary element for this species (Castaño *et al.* 2010; Montilla *et al.* 2021). The benefits of including arthropods in their diet are very high; because insects have a high protein and fat content compared to other sources such as leaves (Rothman *et al.* 2014; Bryer *et al.* 2015). It is important to note that these benefits could be reflected only if the consumption of arthropods is higher due to their small size of them. In *A. azarae* it has been suggested that nutritional levels obtained from the number of insects consumed could influence the reproductive seasonality (Fernandez-Duque *et al.* 2002; Rothman *et al.* 2014). However, the availability of arthropods can vary among different forest types, influencing the foraging strategies that the monkeys must use in order to find and eat this resource (Fernandez-Duque 2007; Fernandez-Duque *et al.* 2008; Fonseca *et al.* 2019). This could explain the differences in arthropod consumption by the Andean night monkey in different forest types such as coffee agroecosystems and rural-urban forests (Guzmán *et al.* 2016; Bustamante-Manrique *et al.* 2021) as well as in fragmented forests (Castaño *et al.* 2010; Montilla *et al.* 2021).

In conclusion, this study provides novel information on the variety of arthropods consumed by the Andean night monkey, among which we highlight the orders Coleoptera, Hemiptera and Orthoptera. Our results suggest that perhaps the variety of insects and arachnids this primate

Table 2. Percentage of arthropod fragments (orders and families) found in the scats of a group of Andean night monkeys, *Aotus lemurinus,* in the Bosque de Las Martejas of Pijao, Colombia. *Indicates new records of orders and families for night monkeys.

Order	Percentage of fragments in scats (%)	Family	Percentage of fragments in scats (%)
Araneae	2.1		
		Chrysomelidae*	2.1
		Coccinellidae*	2.1
Coleoptera	43.8	Curculionidae*	8.3
		Elateridae*	10.4
		Staphylinidae*	2.1
Diptera	4.2		
		Cicadellidae	2.1
Hemiptera	10.4	Coreidae*	2.1
		Pentatomidae*	2.1
Hymenoptera	2.1	Formicidae	2.1
Isoptera*	6.3		
Orthoptera	10.4		
Undetermined orders	20.8	Undetermined	66.7
Total	100	Total	100

includes in its diet could be higher if future studies include techniques like DNA metabarcoding that can detect the remains of DNA of soft-bodied organisms such as larvae, spiders, flies and even ectoparasites like acari that are not possible to find in the scat samples. We highlight the importance of insects in the diet of Andean night monkeys, as it is the second most important item in their diet. This raises important questions in the feeding ecology of this species as for example we still do not know how arthropod availability over time and the disturbance of their habitat might influence foraging, or if this primate species has an ecological role in controlling insect populations in the canopy, vital information to understand their role and conservation importance in the ecosystems. Finally, another relevant finding was that by comparing all the information published so far on the consumption of different orders of arthropods by night monkey species, we were able to report the first time the consumption of insects like termites (order Isoptera) by this group of neotropical primates.

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First record of predation by the Andean white-eared opossum *Didelphis pernigra* on the red swamp crayfish *Procambarus clarkii* in Colombia

Primer registro de depredación de la zarigüeya *Didelphis pernigra* sobre el cangrejo rojo de río *Procambarus clarkii* en Colombia

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The Virginia opossum (*Didelphis virginiana*) has been identified as a predator of the red swamp crayfish (*Procambarus clarkii*) in North America. In the regions where this crayfish has been introduced, other mammals have been identified that include it in their diet. The present paper is the first record of predation by *D. pernigra*, a species that is endemic to the Andean Mountain range, on the invasive exotic crayfish *P. clarkii* in Colombia. We carried out 100 m transects in a riverside forest in the middle basin of the Chicamocha River, Colombia. We collected feces and recorded tracks, and afterwards, obtained photographic records of individual opossums in the area. We weighed the feces, then dissolved them and separated them in Petri dishes. The tracks and the individuals photographed were identified using a specialized bibliography. We found structures in the feces that allowed the identification of 5 individuals of the species *P. clarkii*, an odonate, as well as Poaceae seeds. The tracks and the specimens photographed belong to the species *D. pernigra*, a species previously recorded in the region. The colonization and reproduction of *P. clarkii* in the area represents a new resource that the species *D. pernigra* has integrated into its diet. *D. pernigra's* predation on *P. clarkii* may be related to the nocturnal behaviour of both species, which increases the probability of predation. It is likely that this South American species of the genus *Didelphis* may modify their diet if the invasion area of *P. clarkii* increases.

Key words: Decapods; diet; feces; invasive species.

La zarigüeya de Virginia (*Didelphis virginiana*) ha sido identificada como depredador del cangrejo rojo de río (*Procambarus clarkii*) en Norteamérica. En las regiones en donde ha sido introducido este cangrejo se han identificado otras especies de mamíferos que lo integran en su dieta. El presente trabajo es el primer registro de depredación de *D. pernigra,* especie endémica de la cordillera de los Andes, sobre el cangrejo exótico invasor *P. clarkii* en Colombia. Se realizaron transectos de 100 m en bosque ribereño de la cuenca media del río Chicamocha, Colombia, en donde se recolectaron las heces y se registraron huellas, posteriormente se obtuvieron registros fotográficos de individuos de zarigüeya en la zona. Las heces se pesaron, disolvieron y separaron en cajas de Petri. Las huellas e individuos fotografiados se identificaron empleando bibliografía especializada. En las heces se encontraron estructuras que permitieron identificar 5 individuos de la especie *P. clarkii*, un odonato y semillas de Poaceae. Las huellas y ejemplares fotografiados corresponden a la especie *D. pernigra,* una especie registrada previamente en la región. La colonización y reproducción de *P. clarkii* representa una nueva oferta de recursos que la especie *D. pernigra* ha integrado a su dieta. La depredación de *P. clarkii* por parte de *D. pernigra* puede estar relacionado con el comportamiento nocturno de las 2 especies, que aumenta la posibilidad de depredación. Probablemente las especies del género *Didelphis,* en Sudamérica, puedan modificar su dieta, si aumenta el área de invasión de *P. clarkii*.

Palabras clave: Decápodos; dieta; especie invasora; heces.

The marsupial genus *Didelphis* contains 6 species distributed throughout the American continent (Lemos and Cerqueira 2002; Cerqueira and Tribe 2007). In Colombia, *D. marsupialis* is distributed mostly between 0 and 2,000 m, and *D. pernigra,* between 2,000 and 3,900 m (Alberico *et al.* 2000; Dias-Rocha *et al.* 2021), although Medina *et al.* (2021) note a record of *D. marsupialis* cohabiting with *D. pernigra* in the higher altitude range. The latter is the species of the genus *Didelphis* that predominates in the middle basin of the Chicamocha River in Colombia (GBIF 2021). Species belonging to the *Didelphis* genus are an omnivorous and opportunistic group that include plants, rodents, birds, reptiles, and insects in their diet, and although in North America *D. virginiana* is a natural predator of river crayfish, it is not common for *Didelphis* species in South America to consume crustaceans (Knudsen and Hale 1970; Hobbs 1993; Leite *et al.* 1996; Cáceres 2002; Durant 2002).

A species introduced into a new habitat may become a food source for species already established there. For example, in the Iberian Peninsula, mammals such as the red fox (*Vulpes vulpes*), the common genet (*Genetta genetta*), the Egyptian mongoose (*Herpestes ichneumon*), and the otter (*Lutra lutra*) quickly included the red swamp crayfish into their diet, which is one of the most widely introduced species in the world (Correia 2001; Ruiz-Olmo and Clavero 2008; Oficialdegui *et al.* 2020). The first records of *P. clarkii* in high Andean ecosystems were in 2004 (Campos 2005). As from 2016, their presence was identified in the middle basin of the Chicamocha river in the towns of Sotaquirá and Paipa (Colombia). Notwithstanding, before now, the predation upon this introduced species by South American native mammals had not been documented.

The objective of the study was to record the incorporation into the diet of the Andean white-eared possum (*D. pernigra*) of the introduced species, the red crayfish (*P. clarkii*) in Colombia.

We conducted the study in the rural area of El Salitre, in the town of Paipa (Colombia), at an altitude of 2,500 m, at the coordinates 5° 45' 29.60" N, 73° 9' 33.27" W. We searched for feces, foraging areas and tracks of opossums in 10 100 m transects, parallel to the edges of a riverside forest on the Chicamocha river. We obtained photographs of opossums *in situ* during a mammal monitoring carried out in 2022 in the same study zone.

During the sampling, we found a foraging area with feces and tracks in the sediment on the edge of a stream. We collected the feces in resealable plastic bags and photographed the tracks after placing a measuring tape as a reference. In the laboratory, we dissolved the feces in Petri dishes using 70 % alcohol and separated and identified the items found using a ZEISS stemi 305 stereo microscope, with the support of researchers from the biology department of the Universidad Pedagógica y Tecnológica de Colombia. Tracks of *D. pernigra* were identified according to Navarro and Muños (2000). The identification of the species was confirmed through photographic records (Lemos and Cerqueira 2002).

On average, the feces were 4.5 cm long and had a dry mass of 5.0 g. We identified the remains of red crayfish, a pair of wings of an odonate and Poaceae seeds. We found 4 pairs of claws, an exoskeleton with claws attached and 3 rostrum structures with antennae corresponding to at least 5 individuals of *P. clarkii* (Figure 1).

The tracks, of both front and rear paws, had 5 digits with sole and palm pads. The front tracks were 3.9 ± 0.05 cm long and 4.4 ± 0.65 cm wide. The rear tracks were 7.3 ± 0.4 cm long and 4.6 ± 0.3 cm wide, and the rear limbs' first digits, or hallux, were opposable and did not have claws. The tracks and photographed specimens match the description of the species *D. pernigra* (Figure 2), which is the only species of *Didelphis* previously recorded in the zone and therefore the most likely source of the feces found during the study.

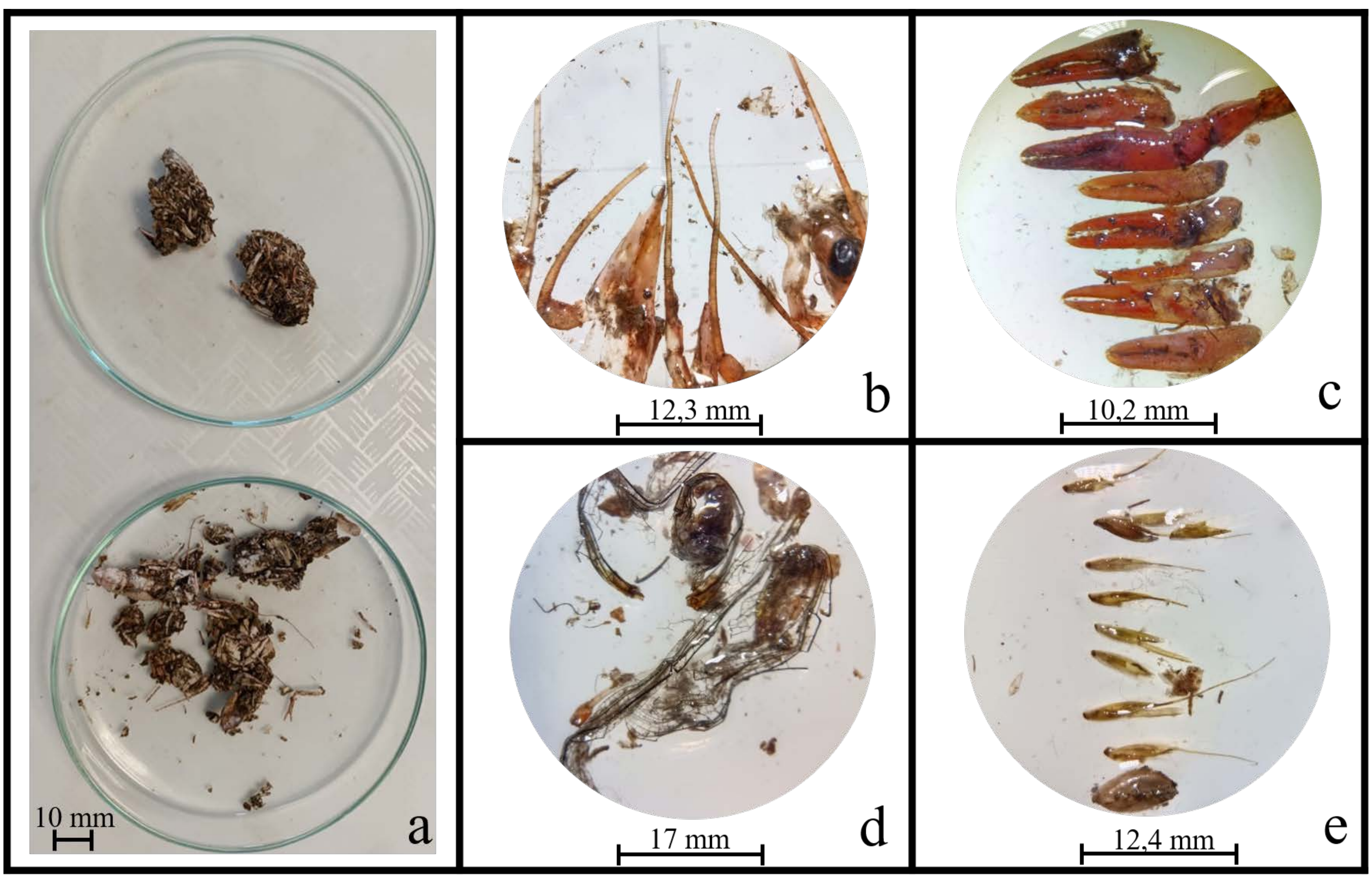

Figure 1. Identification of the remains found in feces of *Didelphis pernigra* in the Chicamocha River, Colombia: a) feces, b) rostrum remains, c) cheliform appendages, d) odonate wings, e) gramineae seeds (Poaceae).

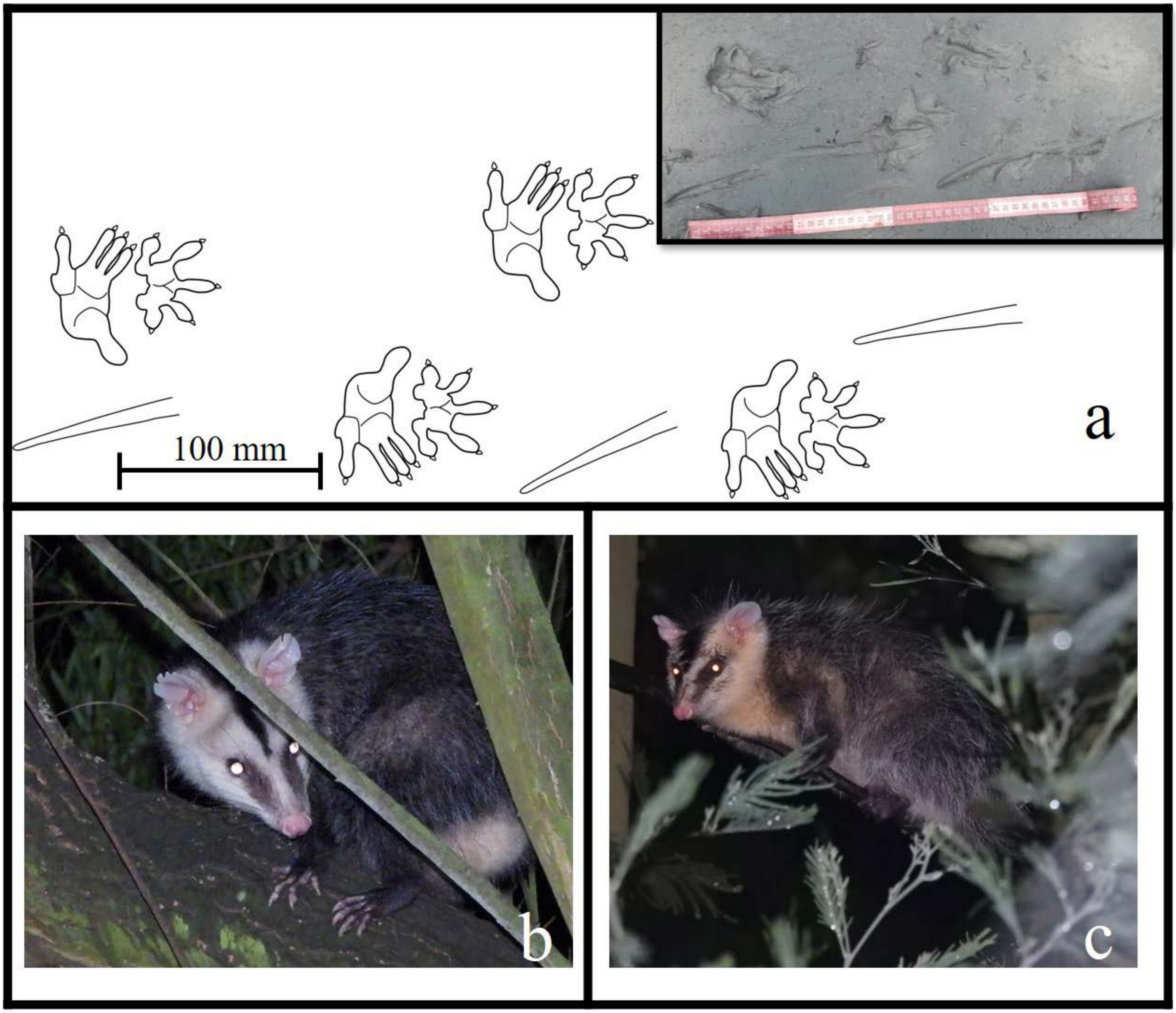

Figure 2. Records obtained: a) tracks, b) and c) individuals of the species *Didelphis pernigra* photographed in the Chicamocha River, Colombia. Photographs by L. Arias.

This is the first record of the red swamp crayfish in the diet of the Andean white-eared opossum in South America. The genus *Didelphis* is an omnivorous group that consumes resources according to their availability in the environment. However, it is uncommon for them to include native decapod crustaceans in their diet. Evidence of *D. albiventris* eating native decapods has only been reported in the south of Brazil (Cáceres 2002). However, in North America, *D. virginiana* includes crayfish in its diet (Knudsen and Hale 1970; Hobbs 1993).

The introduction of exotic species creates the opportunity of new food sources for omnivorous and opportunistic mammal species. This is the case of *P. clarkii* in South America, where it has apparently become a new permanent food supply for *D. pernigra*, which will cause modifications to their diet.

Encounters and predation by *D. pernigra* on *P. clarkii* most likely occur during the night, given that both species have nocturnal habits, and the red swamp crayfish has greater locomotive exploratory activity out of the water at night (Rodríguez-Sosa *et al.* 2017). Birds have also been observed in predating nocturnally on *P. clarkii* in the swamps of Portugal and the Guadalquivir Marshes in Spain (Correia 2001; Montesinos *et al.* 2008).

This paper leads us to suppose that this South American species of the *Didelphis* genus may rapidly adapt to changes in the food supply in its ecosystems, due to the introduction of exotic species such as *P. clarkii*. It appears that *P. clarkii* is advancing their distribution from the north to the south of South America (Palaoro *et al.* 2013; Camacho-Portocarrero *et al.* 2021) and possibly other species of the *Didelphis* genus might integrate it into their diet.

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