Therya

Volumen 13 Número 2 Mayo 2022

AMMAC

www.mastozoologiamexicana.org

La portada

La zorra gris (*Urocyon cinereoargenteus*) es uno de los cánidos de más amplia distribución en Norteamérica, se encuentra en prácticamente todos los Estados Unidos de Norte América y México. Solamente existen dos especies de este género y la segunda especie está restringida a las islas frente al estado de California. Aunque las zorras son del orden de los carnívoros, su alimentación es prácticamente omnívora, siendo común que un alto porcentaje de su dieta sea plantas e insectos (foto tomada por Sergio Ticul Álvarez Castañeda).

Nuestro logo "Ozomatli"

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

Therya

Volumen 13, número 2 mayo 2022

Contenido

Articles

Abundance, microhabitat and feeding of *Peromyscus yucatanicus* and *Peromyscus mexicanus* in the Mexican tropics

Elisa Paulina Zaragoza-Quintana, Nallely Verónica Rodríguez-Santiago, Silvia Filomena Hernández-Betancourt, Livia Socorro León-Paniagua, and María Cristina MacSwiney González 129

Analysis of the male annual antler cycle, reproductive behavior and spotted fawn presence in the tropical white-tailed deer

Miguel Rodríguez-Ramírez, and José Manuel Mora 143

Daytime diet of the lesser sac-winged bat (*Saccopteryx leptura*) in a Colombian Pacific Island

Isabela Vivas-Toro, and Julián Alexander Mendivil-Nieto 153

In search of bachelorettes: Observations of male *Leptonycteris yerbabuenae* with dorsal patches across its range

Theresa M. Laverty, and Kathryn E. Stoner 163

Habitat use and activity patterns of ungulates in a tropical rainforest of southern México

Fredy A. Falconi-Briones, Eduardo J. Naranjo, Rafael Reyna-Hurtado, Manuel Spínola, Paula Enríquez-Rocha, and Rodrigo A. Medellín 171

Effect of an urban area in the distribution pattern and diversity of Neotropical rodents

Gloria Tapia-Ramírez, Consuelo Lorenzo, Arturo Carrillo-Reyes, Darío Navarrete, and Óscar Retana 183

Spatial and temporal distribution of bat mortality on a highway in southeast Brazil

Marcione Brito de Oliveira, and Cecília Bueno 195

Evidence of the genetic and spatial structure of *Nasua narica* in Central America and northern South America from mitogenomic analysis

María Fernanda Jaramillo, and Manuel Ruiz-García 205

Potential Distribution of the Neotropical Otter (*Lontra longicaudis annectens*) in the State of Yucatán, México

Alicia Andrea Ortega-Padilla, Juan Pablo Gallo-Reynoso, Verónica Farías-González Javier Enrique Sosa- Escalante, Silvia Hernandez-Betancourt, Gloria Ponce-García, and Tania Elizabeth Quintana-Salvador 225

Effect of anthropogenic noise on the echolocation pulses of the bats *Molossus sinaloae* and *Mormoops megalophylla*

Ana Cristel Lara-Nuñez, José Antonio Guerrero, and Areli Rizo-Aguilar 235

Evaluation of the distribution pattern on a Neotropical microcarvivora

Rodolfo Rodríguez-Ruiz, Alejandro Juárez-Agis, Silberio García Sánchez, Branly Oliver Salome, and Víctor Hugo Reza Galicia 245

fascículo 38 http://www.revistas-conacyt.unam.mx/therya/index.php/THERYA/issue/view/42

DERECHOS DE AUTOR Y DERECHOS CONEXOS, año 13, No. 2, Mayo-agosto del 2022, es una publicación cuatrimestral editada por la Asociación Mexicana de Mastozoología A. C. Hacienda Vista Hermosa 107, Colonia Villa Quietud, Coyoacan 04960. Distrito Federal, México. Telefono (612) 123-8486, www.mastozoologiamexicana.org, therya@cibnor.mx. Editor responsable: Dr. Sergio Ticul Álvarez Castañeda. Reservas de Derechos al Uso Exclusivo No. 04-2009-112812171700-102, ISSN: 2007-3364 ambos otorgados por el Instituto Nacional de Derechos de Autor. Responsable de la última actualización de este número, Unidad de informática de la Asociación Mexicana de Mastozoología A. C. Dr. Sergio Ticul Álvarez Castañeda. Instituto Politécnico Nacional 195. La Paz, Baja California Sur, C. P. 23096. Tel. (612) 123-8486, fecha de la última modificación 30 mayo 2022.

Therya

Consejo Editorial

THERYA

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

Sergio Ticul Álvarez Castañeda. Editor general. Centro de Investigaciones Biológicas del Noroeste. Av. Instituto Politécnico Nacional 195. La Paz 23096, Baja California Sur. México. E-mail: sticul@cibnor.mx.

Cintya A. Segura Trujillo. Editor asistente. Universidad de Guadalajara, CUCSUR, Autlán 48900, Jalisco, México. E-mail: c.a.biolsegura@gmail.com.

Rafael Ávila Flores. Editor asociado. División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco. Carr. Villahermosa-Cárdenas Km. 0.5 S/N, Entronque a Bosques de Saloya CP. 86150. Villahermosa, Tabasco , México. E-mail: rafaelavilaf@yahoo.com.mx.

Lázaro Guevara. Editor asociado. Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, 04510, Mexico City, Mexico. E-mail: llg@ib.unam.mx

Guillermo D'Elía. Editor asociado. Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Valdivia, Chile. E-mail: guille.delia@gmail.com.

Monica Díaz. Editor Asociado. CONICET, PIDBA (Programa de Investigaciones de Biodiversidad Argentina), PCMA (Programa de Conservación de los Murciélagos de la Argentina. Facultad de Ciencias Naturales e Instituto Miguel Lillo - Universidad Nacional de Tucumán. Fundación Miguel Lillo, Miguel Lillo 251, (4000) San Miguel de Tucumán, Argentina. E-mail: mmonicadiaz@yahoo.com.ar.

Jesús A. Fernández. Editor asociado. Departamento de Recursos Naturales, Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, México. E-mail: jaff1789@gmail.com.

Mariana Freitas Nery. Editor asociado. Departamento de Genética, Evolução e Bioagentes, Instituto de Biologia,Universidade Estadual de Campinas. Rua Bertrand Russel, s/n. Caixa Postal 6109 – CEP 13083---970 Campinas/SP. Brasil. E-mail: mariananery@gmail.com.

Juan Pablo Gallo Reynoso. Editor asociado. Centro de Investigación en Alimentos y Desarrollo. Laboratorio de Ecofisiología. Carretera a Varadero Nacional km 6.6. Col. Las Playitas 85480. Guaymas, Sonora. México. E-mail: jpgallo@ciad.mx.

Consuelo Lorenzo Monterrubio. Editor asociado. El Colegio de la Frontera Sur. Área Conservación de la Biodiversidad. Carretera Panamericana y Periférico Sur s/n. San Cristóbal de Las Casas 29290, Chiapas. México. E-mail: clorenzo@ecosur.mx.

Lia Celina Méndez Rodriguez. Editor asociado. Centro de Investigaciones Biológicas del Noroeste. Av. Instituto Politécnico Nacional 195. La Paz 23096, Baja California Sur. México. E-mail: lmendez04@cibnor.mx.

Eduardo Mendoza. Editor asociado. Instituto de Investigaciones sobre los Recursos Naturales (INIRENA) Universidad Michoacana de San Nicolás de Hidalgo. Av. San Juanito Itzicuaro s/n. Col. Nueva Esperanza C.P. 58337. Morelia, Michoacán, México. E-mail: mendoza.mere@gmail.com.

Robert D. Owen. Editor asociado. Departament of Biology. Texas Tech University. Lubbock, Texas 79409. Estados Unidos de Norte América. Dr. Raúl Casal 2230 (ex Martín Barrios) c/Pizarro. C.P. 1371. Barrio Republicano. Asunción, Paraguay. E-mail: rowen@tigo.com.py.

Rafael Reyna Hurtado. Editor asociado. El Colegio de la Frontera Sur, unidad Campeche. Avenida Rancho s/n, Lerma Campeche, 24500. México. E-mail: rafaelcalakmul@gmail.com.

Jorge Servin. Editor asociado. Universidad Autónoma Metropolitana, Unidad Xochimilco. Calz. Del Hueso #1100, Col. Villa Quietud, 14910, Ciudad de México, México. E-mail: Jorge.servin170@gmail.com.

Sergio Solari. Editor asociado. Instituto de Biología. Universidad de Antioquia. Calle 67 No53-108 / AA 1226. Medellín, Colombia. E-mail: solari.udea@gmail.com.

THERYA, 2022, Vol. 13(2):129-142 DOI:10.12933/therya-22-1189 ISSN 2007-3364

Abundance, microhabitat and feeding of *Peromyscus yucatanicus* and *Peromyscus mexicanus* in the Mexican tropics

Elisa Paulina Zaragoza-Quintana[1], Nallely Verónica Rodríguez-Santiago[1], Silvia Filomena Hernández-Betancourt[2],
Livia Socorro León-Paniagua[3], and María Cristina MacSwiney González[1]*

[1] Centro de Investigaciones Tropicales, Universidad Veracruzana. José María Morelos y Pavón 44 y 46, Centro, C. P. 91000. Xalapa, Veracruz, México. Email: nasuanarik@yahoo.com.mx (EPZ-Q), nayver82@gmail.com (NVR-S), cmacswiney@uv.mx (MCMG).
[2] Departamento de Zoología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán. Km 15.5 Carretera Mérida Xmatkuil, C.P. 97000. Mérida, Yucatán, México. Email: hbetanc@uady.mx (SFH-B).
[3] Museo de Zoología, Departamento de Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México. Circuito exterior s/n, Ciudad Universitaria, Coyoacán, C. P. 04510. Ciudad de México, México. Email: llp@ciencias.unam.mx (LSL-P).
*Corresponding author

Rodents influence the processes of succession and regeneration in tropical forests, functioning as important dispersers and predators of seeds and plants. In this study, we describe aspects of the population structure and dynamics, and characterize the microhabitat and composition of the feces of the semi-tree-dwelling rodents *Peromyscus yucatanicus* and *Peromyscus mexicanus* in tropical forests of Quintana Roo and Veracruz, in Mexico. We trapped *Peromyscus yucatanicus* with 122 Sherman traps between 2.00 to 10.00 m of height in 62 trees. *Peromyscus mexicanus* was trapped with 105 Sherman traps between 0 to 11.00 m of height on 57 trees. We characterized the microhabitat, identified the trees, and calculated the canopy openness in the sites where individuals were trapped. We collected the feces of each species from inside the traps and were analyzed to know their composition. From September 2014 to February 2015, we captured 48 individuals of *Peromyscus yucatanicus* at an average height of 2.58 m above ground level. Its population density was 26.60 ind/ha, with reproductive activity in the dry season. The average canopy openness was 7.75 %. Their feces mainly contained seeds, fruit pulp and starch. From March to September of 2015, we recorded 54 individuals of *Peromyscus mexicanus* at an average height of 1.15 m. Its population density was 20.83 ind/ha, with reproductive activity in both dry and rainy seasons. The canopy openness was 4.78 % (dry season) and 4.50 % (rainy season). Their feces mainly contained fruit pulp, starch and chitin remains in both seasons. The captured of rodents were higher on trees with small diameters and low leaf litter percentage; the rainy season favored the captures. Both species were mainly captured in sites with a covering of organic matter on the soil and in sites with higher vegetal cover and reduced canopy openness. Conservation of a diverse tropical forest is fundamental for the conservation of both semiarboreal rodents.

Los roedores influyen en los procesos de sucesión y regeneración de las selvas, siendo importantes dispersores y depredadores de semillas y plantas. En este estudio describimos aspectos sobre la estructura y dinámica poblacional, caracterización del microhábitat y composición de los excrementos de los roedores semiarborícolas *Peromyscus yucatanicus* y *Peromyscus mexicanus*, en selvas de Quintana Roo y Veracruz, México. Se realizaron capturas de *Peromyscus yucatanicus* colocando 122 trampas Sherman entre los 2.00 y 10.00 m de altura sobre 62 árboles. *Peromyscus mexicanus* se capturó disponiendo 105 trampas entre 0 y 11.00 m de altura sobre 57 árboles. Se caracterizó el microhábitat, se identificaron los árboles y se calculó la apertura de dosel de los sitios donde ocurrieron las capturas. De ambas especies se colectaron excrementos del interior de las trampas y se analizaron para conocer su composición. De septiembre 2014 a febrero de 2015, capturamos 48 individuos de *Peromyscus yucatanicus* a una altura promedio de 2.58 m. Su densidad poblacional fue de 26.60 ind/ha y actividad reproductiva en temporada de secas. La apertura de dosel fue de 7.75 %. Sus excrementos estuvieron mayormente integrados por semillas, pulpa de frutos y almidón. De marzo a septiembre de 2015 registramos 54 individuos de *Peromyscus mexicanus* a una altura promedio de 1.15 m. Su densidad poblacional fue de 20.83 ind/ha, con actividad reproductiva en ambas temporadas. La apertura de dosel fue de 4.78 % (secas) y 4.50 % (lluvias). Sus excrementos estuvieron mayormente compuestos por pulpa de frutos, almidón y restos de quitina en ambas temporadas. Las capturas de los roedores fueron mayores sobre árboles con diámetros pequeños y bajos porcentajes de hojarasca, además, la temporada de lluvia favoreció el número de capturas. Las dos especies se capturaron principalmente en sitios con una cobertura de materia orgánica en el suelo y en sitios con mayor cobertura vegetal y dosel cerrado. La conservación de las selvas tropicales y su diversidad biológica, es clave para la conservación de ambos roedores semiarborícolas.

Keywords: Density; feeding habits; microhabitat; Quintana Roo; Rodentia; Veracruz.

Introduction

Rodents influence the processes of tropical forest regeneration and the structure and function of plant communities, since they prey on and disperse seeds (Sánchez-Cordero and Martínez-Gallardo 1998; Shiels and Drake 2011; Fleury *et al.* 2014), as well as consuming other plant parts (Meyer and Butaud 2009; Shiels *et al.* 2013). Likewise, they influence the populations of other groups through their role as predators of invertebrates and small vertebrates (Trujano-Alvarez and Álvarez-Castañeda 2010; Witmer and Pitt 2012). They use a wide range of ecological niches, and changes in their abundance and diversity reflect modifications in their habitat (Cimé-Pool *et al.* 2010; Whitehead *et al.* 2014).

In Mexico, few studies have described the biology, structure and population size of the small tropical rodents, especially those of tree-dwelling and semi-tree-dwelling habits (Schnell *et al.* 2010; Hernández-Betancourt *et al.* 2008, 2012; Panti-May *et al.* 2014). Equally, patterns relating to the use or selection of habitat have rarely been described in this group (Domínguez-Castellanos *et al.* 2007; Poindexter *et al.* 2013). For some rodents, this differential site selection depends on the structural and vegetal characteristics that comprise their specific microhabitat and that, in turn, affect their distribution and behavior. For example, *Heteromys irroratus* is associated with high proportions of dead plant material and most heteromids are generally recorded in certain combinations of stony sites with a cover typical of scrublands (Tapia-Ramírez *et al.* 2012). Likewise, microhabitat use can vary considerably according to the habitat structure, species population density and the inter-specific interactions (Bellows *et al.* 2001; Rojas-Martínez *et al.* 2012; Villanueva-Hernández *et al.* 2017). In this sense, it has been reported that the females of *Peromyscus leucopus*, unlike the males, present an association with different microhabitats that include the canopy, since they make greater use of the vertical space of the habitat in the search for resources, or as a response to competition for food due to high population density (Klein and Cameron 2012). Studies addressing the feeding habits of small rodents are scarce; however, these mammals play an important ecological role in the ecosystems they inhabit (Montenegro-Díaz *et al.* 1991; San-José *et al.* 2014). Some studies of small rodents have used methods of analysis of feces and stomach contents, since these techniques can provide reliable information regarding the material ingested by the species (*e. g.*, López-Cortés *et al.* 2007; Peralta 2015; Panti-May *et al.* 2019).

The group of small rodents includes the genus *Peromyscus*, which is the most diverse in Mexico. Some of its species have been studied from an ecology perspective (Morris *et al.* 2011; Dallas *et al.* 2012; Kalkvik *et al.* 2012), behavior (Weber and Hoekstra 2009; Williams *et al.* 2013), physiology (Schmidt and Hood 2014; Sun *et al.* 2014), systematics and evolution (Kenney-Hunt *et al.* 2014; Harris *et al.* 2015). However, little has been reported about the biology of the species, such as the Yucatecan deer mouse *Peromyscus yucatanicus*, which is semi-tree-dwelling and endemic to the Yucatan peninsula, with one record in each of Guatemala and Belize (MacSwiney *et al.* 2012). The same is true for the Mexican deer mouse *Peromyscus mexicanus*, which is also semi-tree-dwelling in habit and presents the widest geographic distribution within the genus *Peromyscus*, stretching from San Luis Potosí, Mexico to Panama, except for the Yucatan peninsula (Trujano-Alvarez and Álvarez-Castañeda 2010). Its abundance is highest in conserved remnants of tropical medium and high forest present in states such as Veracruz, Oaxaca and Chiapas, Mexico (Sánchez-Hernández *et al.* 2001; Cruz-Lara *et al.* 2004; Cruz-Lara *et al.* 2010).

Considering the ecological importance of the rodents and the paucity of biological information regarding these two species *P. yucatanicus* and *P. mexicanus*, this paper studies these two species to evaluate aspects of their population structure and dynamics, characterization and microhabitat and feeding preferences in sites of tropical medium forest in the states of Quintana Roo and Veracruz, Mexico, in order to obtain basic information regarding their biology that could contribute to the conservation of both the rodents and the ecosystems they inhabit.

Materials and Methods

Study areas. The study was conducted in two localities. The first site was located within a tropical medium subevergreen forest (21° 12´ 35" N, -87° 12´ 26" W and 21° 12´ 38" N, -87° 12´ 28" W) of El Edén Ecological Reserve (EEER), in the municipality of Lázaro Cárdenas in Quintana Roo, Mexico (Figure 1). The EEER covers an area of 3,077 ha and presents an elevation range of 5 to10 masl. The vegetation types present are tropical medium subevergreen forest, wetlands with savannah, palm stands, tintales and areas of acahuales of tropical medium forest in recovery (Allen and Rincón 2003). The tropical medium subevergreen forest has an average canopy height of 10 to 12 m, with emergent trees that reach up to 15 m in height (Schultz 2003). Its most abundant species are *Metopium brownei*, *Manilkara zapota*, *Lysiloma latisiliquum*, *Thrinax radiata*, *Sabal yapa*, *Bursera simaruba*, *Brosimum alicastrum* and *Vitex gaumeri* (Schultz 2003). The climate is warm subhumid, with a dry season in winter and spring and a rainy season from June to October. Mean annual temperature is 26 ºC and mean annual precipitation is 1,200 mm (Allen and Rincón 2003).

The second site was located in a tropical medium subevergreen forest (19° 50´ 54" N, -96° 35´ 49" W and 19° 50´ 55" N, -96° 35´ 46" W) of the Zona de Protección Forestal y Faúnica Santa Gertrudis (ZPSG), in the municipality of Vega de Alatorre in Veracruz, Mexico (Figure 1). This reserve covers an area of 925 ha and has an elevation range of 400 to 900 masl (Bojorges and López-Mata 2005). The climate is semi-warm, with a mean annual temperature of 22 ºC and mean annual precipitation of 1,845 mm (Godínez-Ibarra and López-Mata 2002), with a rainy season from June to November (García 2004). The trees of the tropical forest in this study site reach heights exceeding 20 m and the species most prominent for their structural importance are

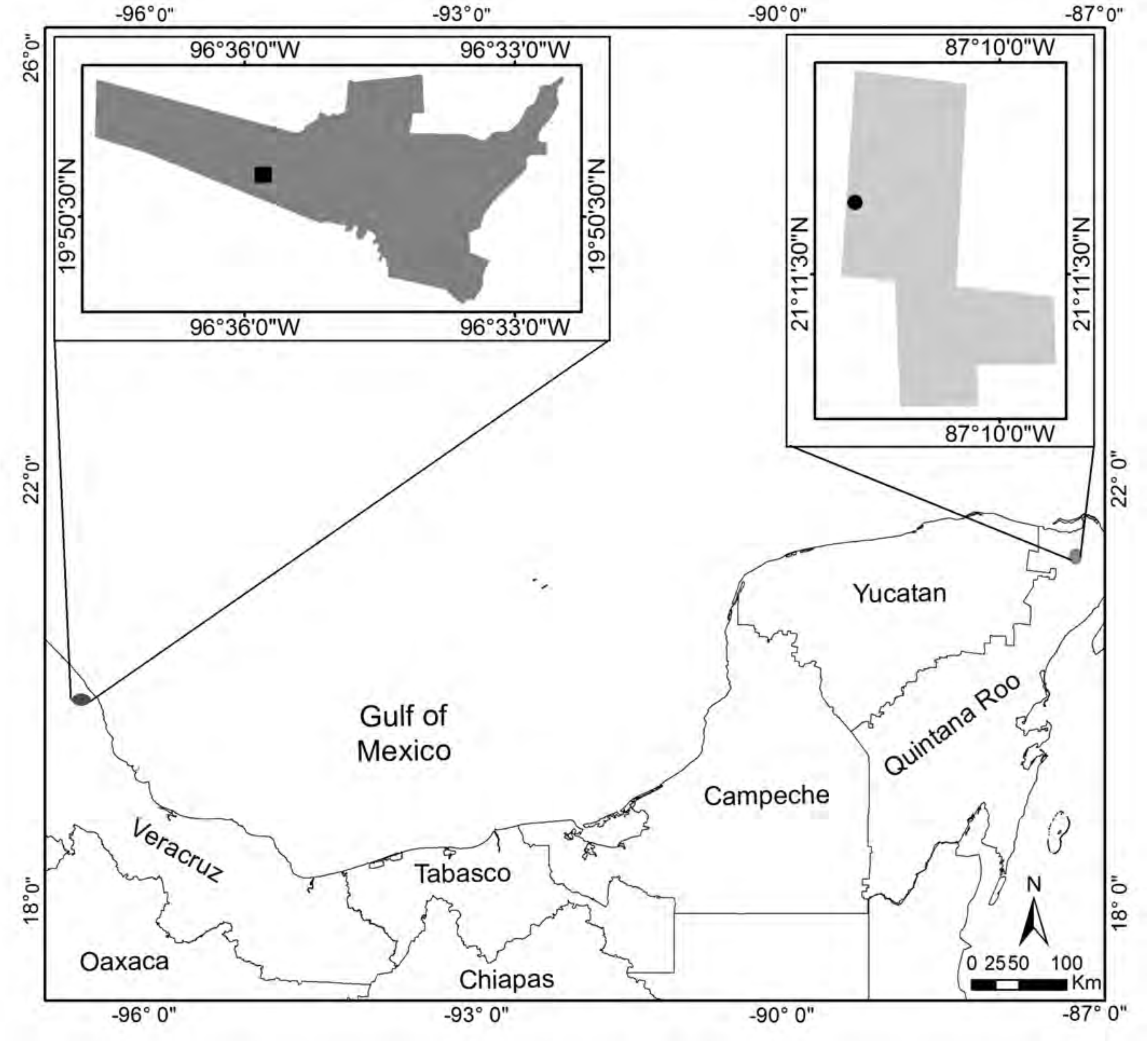

Figure 1. Geographic location of the study site (black square) in Zona de Protección Forestal y Faúnica Santa Gertrudis, Veracruz (dark gray polygon) and study site (black circle) in El Edén Ecological Reserve, Quintana Roo (light gray polygon), México.

Aphananthe monoica, *Brosimum alicastrum*, *Bursera simaruba*, *Dendropanax arboreus*, *Faramea occidentalis*, *Protium copal*, *Sapindus saponaria* and *Tabernaemontana alba* (Godínez-Ibarra and López-Mata 2002).

Data collection. To capture *P. yucatanicus* in the EEER, one field visit was conducted during the rainy season (September 2014) and two in the dry season (November 2014 and February 2015). A total of 122 Sherman traps (23 x 8 x 9 cm) were set at between 2.00 and 10.00 m above ground level, in 62 trees spaced equidistantly at 10.00 m apart, forming a grid of 11 columns by six rows. To capture *P. mexicanus* in the ZPSG, three samplings were conducted in the dry season (March, May and June 2015) and three in the rainy season (July, August and September 2015). A total of 48 trees were selected at 9.00 m apart, forming a grid of eight columns and six rows, over which 96 traps (23 x 8 x 9 cm) were distributed at between 0 and 4.50 m in height above ground level. In addition, to record captures of *P. mexicanus* at greater height, a trap was set at between 6.60 and 11.20 m above ground level on each of nine trees selected from the interior of the grid.

In each site, the traps remained open for five consecutive nights per sampling, and used sunflower seeds and oats mixed with vanilla as bait. Then trees selected for the traps were characterized by presenting branches and lianas that interconnected them with other individuals. The traps were fixed to the trunks and branches using metal mesh wire and an elevator system with wooden platforms, nylon strings and carabiners (techniques modified from Vieira 1998 and Graipel 2003). The capture and recapture method (Krebs 1966) was used, and the captured individuals were marked with perforations on the ears (Sikes and The American Society of Mammalogists 2016). For each captured individual, the weight, sex, age (juvenile or adult), reproductive condition (whether the males presented scrotated testicles and the females presented an open vagina, were pregnant or in lactation) and the following morphometric measurements were recorded with a digital vernier: total length (TL), tail length (TaL), right hind-foot length (HFL) and right ear length (EL). The feces of both captured species were collected from inside the traps and stored in Eppendorf tubes at -20 ºC (Vázquez *et al.* 2004).

Analysis of the population information. The capture effort for each species was calculated from the number of traps located in each site (122 in the EEER and 105 in the ZPSG), multiplied by the number of nights of each sampling, expressed as night/traps. The percentage of sampling success was calculated with the total number of captures in each month divided by the number of night/traps in the month. The population structure of each species was obtained considering the sex and age data of the captured individuals. From the total number of reproductive individuals, it was possible to determine the variation of the reproductive activity in each site (Rojas-Martínez *et al.* 2012). Mann-Whitney U tests were used to determine whether differences existed in the heights of capture between sexes per sampling period. Population density was calculated using the method of Minimum Number Alive (MNA, Krebs 1966). Contingency tables and Pearson $\chi 2$ tests were used to determine the probable dependence of the frequency of sexes, ages and reproductive condition (with the latter used only for *P. mexicanus*), with respect to the months and seasons of sampling. Given that, in both species, only the proportions of ages showed dependence, *post-hoc* Analysis of Standardized Residuals was conducted to determine which age classes were dependent. In order to evaluate whether significant differences existed in the somatic measurements between sexes, a one-way analysis of variance (ANOVA) was used for the variables with normal distribution, while the Mann-Whitney U test was used for those without normal distribution.

Characterization and preference of microhabitat. The trees in which the rodents were captured were identified taxonomically using keys (Castillo-Campos and Medina-Abreo 2005) and the help of botanical experts. Tree diameter at breast height (DBH, in cm) was recorded and the percentage of canopy openness calculated (CO, Frazer *et al.* 1999). Around the base of each tree, four plots of 1.00 m^2 were established, each oriented towards a cardinal point. These were used to characterize the microhabitat at ground level through the percentage of vegetal coverage, organic matter (composed of leaf litter and dead wood deposited on the soil), rocky and bare soil surfaces (Poindexter *et al.* 2013). Characterization was conducted in February 2015 for *P. yucatanicus*, and in June and September 2015 for *P. mexicanus*. For *P. mexicanus*, one-way ANOVA and Mann-Whitney U tests were used to determine whether significant differences existed in the percentages of the variables between seasons. The variables coverage of grasses and cacti were not subjected to these statistical tests, since the data were insufficient to make the comparisons. Principal components analysis (PCA) with matrices of variance-covariance and generalized linear models (GLM) were used to define which variables of the microhabitat influenced the frequency of capture of both species (Dobson and Barnett 2008). For *P. mexicanus*, GLM were developed between seasons and sexes.

Composition of the feces. The fecal material of each species was analyzed using preparations on slides, with a 70.00 % alcohol solution and crystalline mucilage adhesive (Stafford de México, S. A. de C. V.) for mounting (Baltazar 2014). Each preparation was placed on millimetric paper under a stereoscopic microscope (Modelo CSM2-Labomed, Labomed, Inc.) and elements of vegetal (seeds, epidermis, fruit pulp and starch, fibers, roots and bark) and animal (larvae, complete insects, arthropod appendages and chitin remains) origin identified in five randomly selected fields of area 25.00 mm^2 (Montenegro-Díaz *et al.* 1991; Peña-Ramos *et al.* 2009; Lanzone *et al.* 2012). The percentages of appearance were estimated based on the number of fields occupied by each element (López-Cortés *et al.* 2007). Remains of animal origin were identified through consultation of specialized literature (Triplehorn and Johnson 2005; AntWeb 2017) and specialist taxonomists. The remains of some insects, apparently consumed by *P. mexicanus* and found within the traps in which the rodent was captured, were collected and identified.

Contingency tables and calculations of Pearson χ^2 were used to determine whether the frequencies of the elements were dependent on the months of sampling, in the case of *P. yucatanicus*, and between seasons for *P. mexicanus*. To determine which elements were dependent, *post-hoc* Analysis of Standardized Residuals was performed. One-way ANOVA, Kruskal-Wallis and Mann-Whitney U tests were used to determine whether differences existed in the frequencies of the elements between sexes and ages for *P. yucatanicus* and between seasons, sexes and reproductive conditions for *P. mexicanus*. Only those elements with sufficient data to make the comparisons were subjected to these statistical tests. The seeds found were assigned to different morphotypes based on their shape, color, surface texture and width (Wells *et al.* 2009) for which reason their lengths and widths were measured (in mm) with the software ImageJ Version 1.48 (Ferreira and Rasband 2012). The contingency tables and calculations of Pearson χ^2 were used to determine whether the frequencies of the morphotypes were dependent on the sampling periods. To determine which morphotypes were dependent, *post-hoc* Analysis of Standardized Residuals was performed. Statistical analysis was conducted using the software STATISTICA Version 7 (Stat Soft, Inc.) and the graphic options of the statistical package VCD (Meyer et al. 2006; Zeileis et al. 2007;

Meyer *et al.* 2015) for the software R (R Core Team 2014; RStudio, Inc.).

Results

Peromyscus yucatanicus: *Population aspects.* A total of 48 individuals of *P. yucatanicus* were captured and marked. This group comprised 26 males (54.00 %) and 22 females (46.00 %). Of these, 85 recaptures were achieved in 1,205 night/traps, with a capture success of 11.00 %. The average population density was 26.60 ind/ha (± 23.00 *SD*), with the highest density found in February (50.00 ind/ha). Over the entire sampling, 48 males (56.50 %) and 37 females (43.50 %) were recaptured. The greatest number of individuals of both sexes was obtained in November (21 males and 14 females, Figure 2a). The frequency of sexes was not dependent on the sampling month (contingency table of 2 x 3, $\chi^2 = 0.875$, *d. f.* = 2, $P = 0.646$). Twenty-two juvenile (52.40 %) and 20 adult (47.60 %) males, and 17 juvenile (48.60 %) and 18 adult (51.40 %) females were recorded. More juveniles of both sexes were recorded in November (15 males and eight females), while the highest number of adults was obtained in February (13 males and 10 females, Figure 2b). Only the frequencies of juvenile and adult males depended on the month of February (contingency table of 4 x 3, $\chi^2 = 24.39$, *d. f.* = 6, $P < 0.001$) (Supplementary material 1, Figure A1). Of the total number of individuals captured, 10 presented reproductive activity (seven males and three receptive females); these were present only in February. None of the somatic measurements of adult males and females (Table 1) presented significant differences between sexes: TL ($F_{1,26} = 0.12$, $P = 0.73$), TaL ($F_{1,26} = 1.43$, $P = 0.24$), HFL ($U = 75$, $P = 0.13$), EL ($F_{1,28} = 1.52$, $P = 0.22$).

Characterization and preference of the microhabitat. The rodents were captured on 41 trees with mean DBH of 19.10 cm (± 12.53 *SD*, range: 3.80 to 55.40 cm) and a CO of 7.75 % (± 3.71 *SD*). The identified tree species were: *Lysiloma latisiliquum*, *Brosimum alicastrum*, *Pouteria campechiana*, *Bursera simaruba*, *Ceiba pentandra*, *Chrysophyllum cainito*, *Guettarda combsii* and one further unidentified species of the family Sapotacea. The captures occurred at between 2.00 and 5.00 m in height above ground level, with an average height of 2.58 m (± 0.82 *SD*). Table 2 presents the mean heights per month, sex and age. In general, the population of males presented a mean capture height of 2.45 m (± 0.74 *SD*, height: 2.00 to 4.50 m) and the females 2.73 m (± 0.90 *SD*, height: 2.00 to 5.00 m), values that differed significantly ($U = 1502.5$, $P = 0.006$).

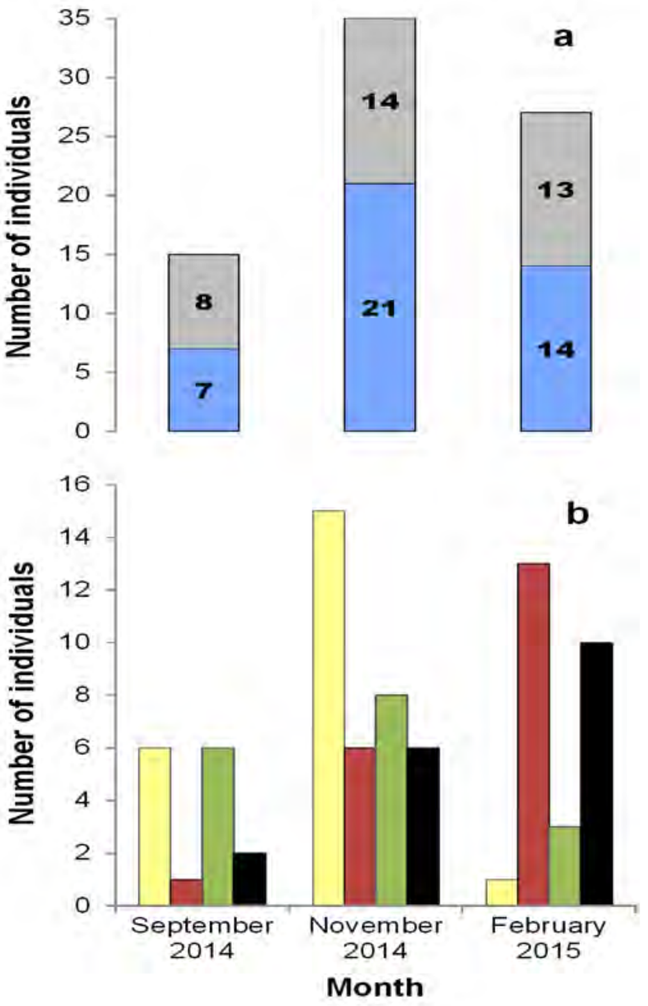

Figure 2. Structure according to: a) sex (males in blue, females in gray) and b) age (juvenile males in yellow, adult males in red, juvenile females in green, adult females in black) of the mouse *Peromyscus yucatanicus* in the El Edén Ecological Reserve in Quintana Roo, Mexico.

The dominant element of the microhabitat consisted of organic matter, with a proportion of 65.71 % (± 14.62 *SD*), followed by vegetal cover with 26.83 % (± 14.47 *SD*), which comprised climbing and herbaceous plants, as well as trees (Table 3). According to the PCA, the variables coverage of trees and leaf litter (Component 1) and DBH (Component 2) presented the greatest variations. Both components explained 86.67 % of the variation (Supplementary material 2, Table B1). The GLM conducted with the variables selected from the PCA showed that the vegetal cover of the trees positively influenced the frequency of capture ($D_{1,39} = 6.910$, $P = 0.02$) (Supplementary material 1, Figure A2). In

Table 1. Mean values of somatic measurements (in mm), ± standard deviations, of adult individuals of *Peromyscus yucatanicus* and *Peromyscus mexicanus.*

Species	Sex	TL	TaL	HFL	EL
P. yucatanicus	Males (*n* = 15)	194.86 ± 8.23	100.80 ± 5.99	21.05 ± 0.82	16.58 ± 0.70
P. yucatanicus	Females (*n* = 13)	195.92 ± 7.97	103.69 ± 6.81	20.53 ± 0.87	16.24 ± 0.81
P. mexicanus	Males (*n* = 17)	215.23 ± 28.16	124.70 ± 21.21	23.64 ± 0.93	18.10 ± 1.07
P. mexicanus	Females (*n* = 22)	211.22 ± 31.33	126.09 ± 13.44	23.68 ± 0.89	17.60 ± 0.75

n (number of individuals), TL (total length), TaL (tail length), HFL (right hind-foot length) and EL (right ear length) contrast, leaf litter had a negative influence on the number of rodents captured of the rodent ($D_{1, 39} = 9.497$, $P = 0.008$) (Supplementary material 1, Figure A3).

Composition of the feces. A total of 80 fecal samples of *P. yucatanicus* were analyzed (47 males and 33 females) for a total of 800 fields of 25.00 mm^2. The composition of the feces was dominated by elements of vegetal origin: seeds (85.00 %), fruit pulp and starch (34.60 %), fibers (2.50 %) and different types of epidermis (0.75 %). The components of animal origin were chitin remains (0.50 %) and different types of arthropod appendages (0.10 %), including some parts of legs of the Orders Orthoptera and Coleoptera, scales of Lepidoptera and complete specimens of ants of the family Formicidae. The material that could not be identified represented 3.10 % of the total.

In both November and February, seeds were the element that presented the greatest proportion, and remains of animal origin were present only in November (Table 4). The frequencies of the elements seeds and fruit pulp and starch were dependent on the sampling month (Supplementary material 1, Figure A4). Likewise, the element of fibers was found to be dependent on the month of February (contingency table of 6 x 2, $\chi^2 = 108.887$, *d. f.* = 5, $P < 0.001$). The frequencies of the elements seeds and fruit pulp and starch, did not present significant differences between sexes (seeds: $U = 657$, $P = 0.24$; fruit pulp and starch: $U = 646.5$, $P = 0.20$) or ages (seeds: $H = 3.60$, *d. f.* = 3, $n = 80$, $P = 0.30$; fruit pulp and starch: $H = 2.27$, *d. f.* = 3, $n = 80$, $P = 0.51$). The seeds were grouped into nine morphotypes; the morphotype V was the largest in size (Length = 0.26 mm, Width = 0.26 mm), while the morphotype VIII was the smallest (Length = 0.04 mm, Width = 0.03 mm). The morphotypes I and II were mainly present in both sampling months (Supplementary material 2, Table B2). Only the frequencies of the morphotypes III, IV, V and VIII were dependent on the month of sampling (contingency table of 9 x 2, $\chi^2 = 211.010$, *d. f.* = 8, $P < 0.001$) (Supplementary material 1, Figure A5).

Peromyscus mexicanus: *Population aspects.* A total of 54 individuals were marked (27 males and 27 females), with 196 recaptures in 2,429 night/traps. Capture success was 10.30 %. The average population density was 20.66 ind/ha (± 8.70 *SD*), with a mean value of 14.30 ind/ha (± 5.50 *SD*) in the dry season and 27.00 ind/ha (± 6.24 *SD*) in the rainy season. Moreover, the highest record occurred in August with 32.00 ind/ha (Figure 3). A total of 95 recaptures of males was obtained: 27 in the dry season (28.40 %) and 68 in the rainy season (71.60 %). The females presented 101 recaptures: 32 in the dry season (32.00 %) and 69 in the rainy season (68.00 %). During the dry season, the month of March presented the highest number of individuals (12 males and 16 females), while in the rainy season, the highest numbers of both sexes were obtained in July (10 and 8, respectively, Figure 4a). The frequency of sexes was not dependent on the season (contingency table of 2 x 6, $\chi^2 = 1.0$, *d. f.* = 1, $P = 0.317$).

Eleven juvenile (33.30 %) and 17 adult (60.70 %) males were captured, while the total numbers of females were seven juveniles (23.30 %) and 23 adults (76.70 %). The highest number of juveniles was presented in the dry season (nine males and six females) and no juveniles were captured in September (Figure 4b). The frequencies of juvenile and adult males were dependent on the month of March in the dry season, while those of females of both ages were dependent on the month of July in the rainy season (contingency table of 4 x 6, $\chi^2 = 41.32$, *d. f.* = 15, $P < 0.001$) (Supplementary material 1, Figure A6).

Twenty-three reproductive individuals were obtained: 11 males (47.80 %) and 12 females (52.20 %). Pregnant females were the most abundant, with 11 records (48.00 %), and these were present in all samplings (Figure 4c). The frequencies of reproductive males and females were not dependent on the season of sampling (contingency table of 5 x 6, $\chi^2 = 3.299$, *d. f.* = 1, $P = 0.069$). The mean values of the somatic measurements are shown in Table 1. None of these measurements presented significant differences between sexes, for which reason sexual dimorphism did not exist: TL ($U = 183.50$, $P = 0.92$), TaL ($U = 140.50$, $P = 0.18$), HFL ($U = 185.50$, $P = 0.96$), EL ($F_{1, 37} = 2.79$, $P = 0.10$).

Characterization and preference of the microhabitat. In the dry season, the mice were captured in 25 trees with an average DBH of 38.62 cm (± 41.60 *SD*, range: 8.11 to 149.60 cm) and a CO of 4.78 % (± 2.20 *SD*). In the rainy season, the captures occurred in 38 trees with an average DBH of 29.98 cm (± 29.60 *SD*, range: 6.36 to 132.73 cm) and a CO of 4.50 % (± 2.37 *SD*). The tree species identified were *Pimenta dioica*, *Psychotria* sp., *Pouteria* sp., *Spondias* sp., *Ficus* sp., *Bursera simaruba* and *Forchhammeria trifoliata*. The captures and recaptures occurred at between 0 and 11.00 m in height above ground level, and consisted of 173 captures on the ground (14 juvenile males, 69 adult males, 15 juvenile females and 75 adult females) and 77 in the trees (3 juvenile males and 36 adult males, 3 juvenile

Table 2. Mean height above ground level (in meters) ± standard deviations, at which male and female individuals of different ages and of both study species were collected.

Species	Sampling/Season	Male juveniles	Male adults	Female juveniles	Female adults
Peromyscus yucatanicus	Sep-2014	2.12 ± 0.21	2.10	2.44 ± 0.33	2.0
	Nov-2014	2.35 ± 0.12	2.80 ± 0.24	2.89 ± 0.25	2.95 ± 0.28
	Feb-2015	2.20	2.57 ± 0.18	3.40 ± 0.36	2.48 ± 0.18
Peromyscus mexicanus	Dry	-	1.18 ± 1.55	0	1.42 ± 1.41
	Rainy	2.92 ± 2.77	0.97 ± 1.36	0.89 ± 1.35	1.28 ± 2.28

(-) no data

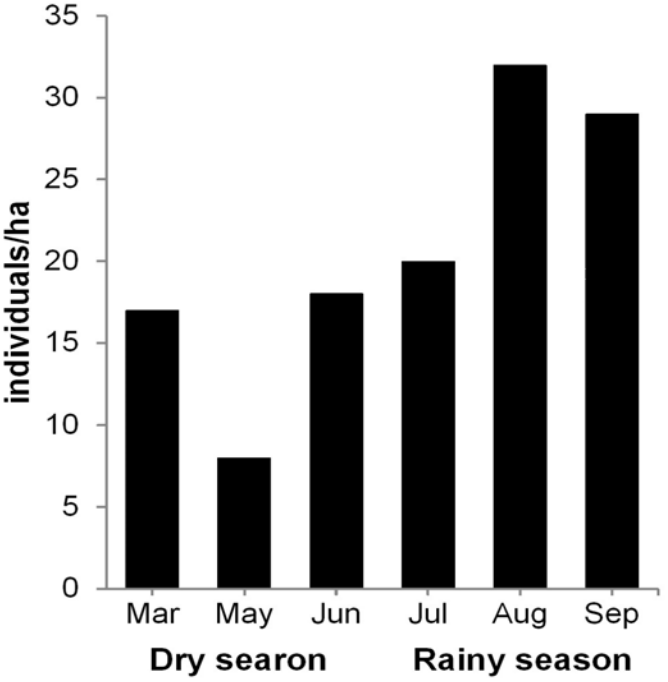

Figure 3. Population density of *Peromyscus mexicanus* in a tropical medium sub-evergreen forest of Zona de Protección Forestal y Faúnica Santa Gertrudis, in Veracruz, Mexico.

females and 35 adult females), at an average height of 1.15 m (± 1.75 *SD*). In the rainy season, the juvenile males presented the greatest average capture height of 2.92 m (± 2.70 *SD*, Table 2), while in the dry season, the adult females presented an average capture height of 1.42 m (± 1.41 *SD*). In general, the population of males was captured at an average height of 1.09 m (± 1.50 *SD*, height: 0 to 6.70 m), while that of the females was 1.21 m (± 1.98 *SD*, height: 0 to 11.00 m). These values did not differ significantly ($U = 5085$, $P = 0.97$). The mean heights of capture between sexes in the dry (males = 1.18 m ± 1.55 *SD*, height: 0 to 4.30 m; females = 1.17 m ± 1.39 *SD*, height: 0 to 4.50 m) and rainy (males = 1.07 m ±1.49 *SD*, height: 0 to 6.70 m, females = 1.23 m ± 2.18 *SD*, height: 0 to 11.00 m) seasons presented no significant differences (dry: $U = 360.50$, $P = 0.97$; rainy: $U = 2713$, $P = 0.92$).

In both seasons, the organic matter (mainly leaf litter) was mainly present in the microhabitat, comprising 61.45 % (± 13.45 *SD*), followed by the vegetal cover at 29.25 % (± 13.33 *SD*), bare soil at 7.52 % (± 9.90 *SD*) and rocky surface at 1.76 % (± 3.37 *SD;* Table 3). The CO did not differ significantly between seasons ($U = 427$, $P = 0.50$). The percentage of climbing plants was significantly greater in the dry season ($U = 212$, $P < 0.001$), while the herbaceous plants and ferns did not differ between seasons (herbaceous plants: $U = 345.50$, $P = 0.06$; ferns: $U = 471$, $P = 0.95$). The percentage of shrubs was significantly greater in the dry season ($U = 316$, $P = 0.02$) and the coverage of trees was similar between the two seasons ($U = 390.50$, $P = 0.23$). The percentages of remains of wood ($U = 460$, $P = 0.83$) and leaf litter ($F_{1, 61} = 0.207$, $P = 0.65$) did not differ significantly between seasons. The rocky surface was also similar between seasons ($U = 427.50$, $P = 0.50$) and the proportion of bare soil was greater in the rainy season ($U = 198.50$, $P < 0.001$; Table 3).

The three first components of the PCA of the rainy and dry seasons were selected. These explained 89.36 % of the accumulated variation. The variables DBH (Component 1), leaf litter (Component 2) and herbaceous plants (Component 3) presented the greatest variation (Supplementary material 2, Table B3). The GLM were conducted with the variables selected from the PCA, which showed that the interaction

Table 3. Mean values ± standard deviations, of the variables used for characterization of the microhabitat of both study species.

	Peromyscus yucatanicus		***Peromyscus mexicanus***	
Variable	**February-2015 (*n* = 41)**	**Dry season (*n* = 25)**	**Rainy season (*n* = 38)**	**General (*n* = 63)**
DBH	19.11 ± 12.53	38.62 ± 41.60	29.98 ± 29.60	33.41 ± 34.80
CO	7.75 ± 3.71	4.78 ± 2.20	4.50 ± 2.37	4.61 ± 2.29
Plant cover (%)				
Climbing plants	3.21 ± 3.07	1.06 ± 0.91	0.30 ± 0.36	0.60 ± 0.73
Grasses	0	0.15 ± 0.48	0	0.06 ± 0.31
Herbaceous plants	0.30 ± 1.49	17.10 ± 13.65	11.48 ± 10.94	13.70 ± 12.30
Cacti	0	0.15 ± 0.48	0.14 ± 0.49	0.14 ± 0.48
Ferns	0	5.41 ± 7.59	4.16 ± 4.74	4.65 ± 6.01
Shrubs	0	2.48 ± 4.60	0.26 ± 0.68	1.14 ± 3.11
Trees	23.31 ± 13.65	9.40 ± 15.77	8.65 ± 5.69	8.94 ± 10.76
Organic matter (%)				
Dead wood	5.41 ± 3.42	4.13 ± 2.17	4.37 ± 2.78	4.27 ± 2.54
Leaf litter	60.29 ± 14.83	56.21 ± 13.81	57.81 ± 13.60	57.17 ± 13.59
Rocky surface (%)	3.87 ± 4.74	1.23 ± 2.44	2.11 ± 3.85	1.76 ± 3.37
Bare soil (%)	3.58 ± 3.55	2.70 ± 2.17	10.70 ± 11.63	7.52 ± 9.90

n (number of trees), DBH (diameter at breast height in cm), CO (canopy openness: %)

Table 4. Proportion (%) of the elements found in the feces of *Peromyscus yucatanicus* during the months of sampling. The percentages of appearance were estimated based on the number of fields occupied by each element in five randomly selected fields of area 25 mm^2 (López-Cortés *et al.* 2007).

Month/Season	Seeds	Fruit pulp and starch	Fibers	Epidermis	Chitin remains	Arthropod appendages
P. yucatanicus						
November-2014	78.50	48.50	3.80	1.10	0.75	0.20
February-2015	98.0	7.40	-	-	-	-
P. mexicanus						
Dry	1.72	74.0	0.17	21.20	30.30	0.51
Rainy	6.48	60.70	0.13	10.13	68.20	2.70

(-) no data

with the variables DBH and leaf litter had a negative effect on the frequency of capture of the rodents ($D_{1,58} = 5.726$, $P = 0.04$) (Supplementary material 1, Figure A7). Moreover, the rainy season had a positive influence on the number of captures ($D_{1,61} = 14.907$, $P = 0.001$; Supplementary material 1, Figure A8). The three first components of the PCA of males and females were selected; these individually presented the variables DBH, leaf litter and herbaceous plants as those of greatest explanatory variation. The accumulated variation of the three components was 88.76% (Supplementary material 2, Table B4). The GLM produced with the variables selected from the PCA showed the interaction of DBH and leaf litter to be significant, negatively influencing the number of mice captured ($D_{1,88} = 9.567$, $P = 0.001$; Supplementary material 1, Figure A9).

Composition of the feces. A total of 132 fecal samples of the Mexican deer mouse (62 males and 70 females) were collected, of which 1320 fields of area 25.00 mm^2 were analyzed. The feces contained fruit pulp and starch (66.51 %), chitin remains (51.59 %), different types of epidermis (15.00 %), seeds (4.40 %), arthropod appendages (1.70 %) and fibers (0.15 %), and 5.70 % of the material fecal could not be identified. In both seasons, the elements fruit pulp and starch as well as chitin remains were present in greater proportion. Seeds and remains of arthropod appendages were more frequent in the rainy season, while the element epidermis was more frequent in the dry season (Table 4).

The frequencies of all of the elements of the diet were dependent on both seasons (Supplementary material 1, Figure A10), apart from fibers in both seasons and arthropod appendages in the rainy season only (contingency table of 6 x 2, $\chi^2 = 145.049$, $d.f. = 5$, $P < 0.001$). The element chitin remains presented significant differences between seasons ($U = 714.5$, $P < 0.001$) but did not differ between sexes ($U = 1981.5$, $P = 0.39$) or with the reproductive condition of the males ($F_{1,54} = 0.164$, $P = 0.68$) and females ($H = 1.11$, $d.f. = 3$, $n = 25$, $P = 0.77$). Likewise, fruit pulp and starch differed between seasons ($U = 1460$, $P = 0.001$), but not between sexes ($U = 2147$, $P = 0.91$), or male ($U = 361$, $P = 0.91$) and female ($H = 1.96$, $d.f. = 3$, $n = 25$, $P = 0.57$) reproductive conditions. The element epidermis presented similar proportions in both seasons ($U = 1846.5$, $P = 0.16$), as with between sexes ($U = 2084$, $P = 0.69$), reproductively active and inactive males ($U = 337.5$, $P = 0.61$) and among females in a reproductive state ($H = 1.15$, $d.f. = 3$, $n = 25$, $P = 0.76$).

The seeds were assigned to 24 morphotypes; morphotype number XI was that of greatest size (length = 1.31 mm, width = 0.92 mm), while number VII was the smallest (length = 0.03 mm, width = 0.04 mm; Supplementary material 2, Table B5). Remains of seeds belonging to *Ficus* sp. were identified in the samples from the months of May and June. The frequencies of the morphotypes I, II, III, VI and XII were the only ones that depended on the dry season (contingency table of 8 x 2, $\chi^2 = 53.858$, $d.f. = 7$, $P < 0.001$; Supplementary material 1, Figure A11).

Three seedlings of less than 2.00 mm in length were found in the feces, possibly belonging to the genus *Selaginella*. Some arthropod appendages corresponded to the Orders Coleoptera and Hymenoptera (some to the family Formicidae), and unidentifiable immature individuals were observed (pupae and larvae). The remains of insects collected from within the traps belonged to four individuals of the family Blattidae (Blattodea), three beetles (Coleoptera) of the family Scarabaeidae and the head of an ant (Hymenoptera) of the genus *Pachycondyla*.

Discussion

Population structure and dynamics. The population density of a species can be affected by different factors such as climatic fluctuations, site conservation state and the availability and diversity of resources (Santos-Moreno *et al.* 2007; Briones-Salas and González 2016). In this study, the population density of *P. yucatanicus,* particularly in February (50.00 ind/ha), was similar to that observed in other localities of the Yucatan peninsula during the dry season (33 to 36 ind/ha, Cimé-Pool *et al.* 2007; 23 ind/ha, Hernández-Betancourt *et al.* 2012). The abundance of species of legumes and gramineae that produce large quantities of seeds during the dry season seems to explain the greater density of *P. yucatanicus* in different habitats such as agroecosystems of pasture and of the tropical forests of Yucatan (Cimé-Pool *et al.* 2007; Hernández-Betancourt *et al.* 2012). In contrast, for *P. mexicanus*, the estimated population density was lower (26.60 ind/ha) than that recorded in a cloud forest (34 ind/ha), but greater than that reported in an abandoned pasture (11 ind/ha) in Costa Rica (Rojas and Barboza 2007). Our results show that the rainy season presents a population increase in *P. mexicanus*, similar to that found in other studies (Cruz-Lara *et al.* 2004; Rojas and Barboza 2007; Rodríguez-Macedo *et al.* 2014).

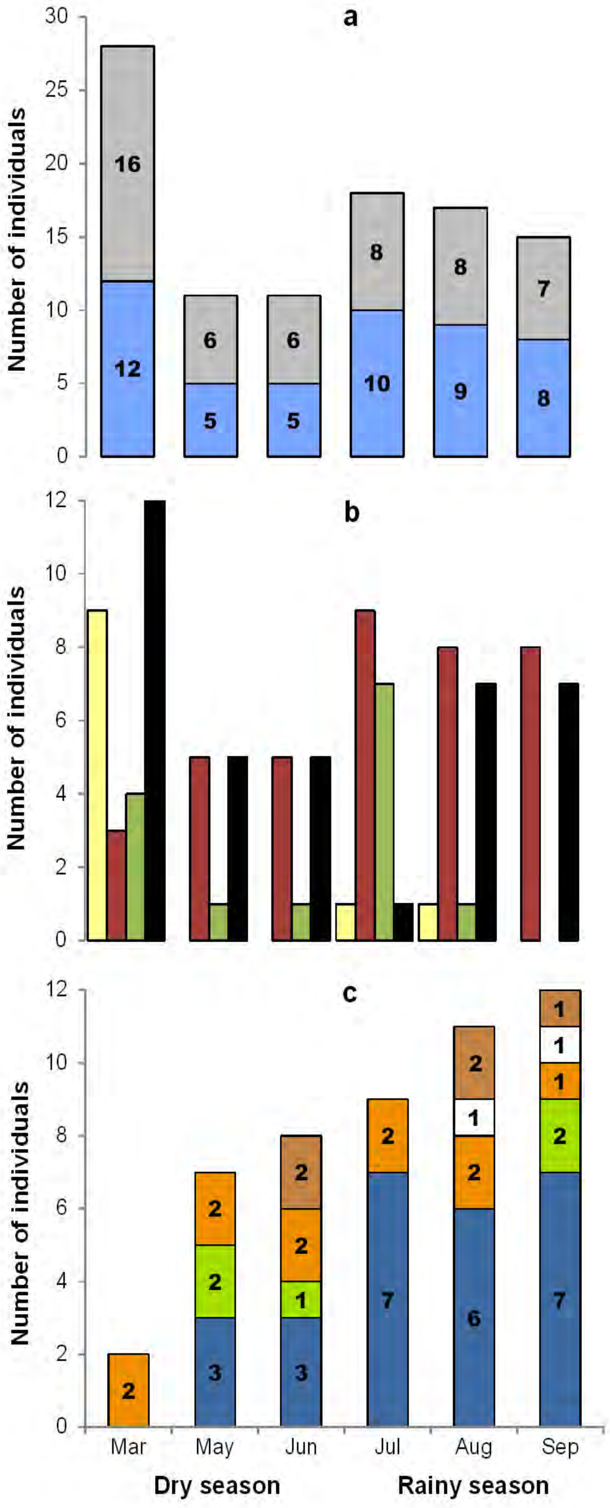

Figure 4. Structure according to: a) sex (males in blue, females in gray), b) age (juvenile males in yellow, adult males in red, juvenile females in green, adult females in black) and c) reproductive state (active males in blue, receptive females in green, pregnant females in orange, lactating female in white, postlactating females in brown) of the mouse *Peromyscus mexicanus* in Zona de Protección Forestal y Faúnica Santa Gertrudis, Veracruz, México.

Some environmental factors can influence the proportion of sexes in some species of small rodents, as is the case in *Peromyscus maniculatus borealis*, the proportion of which seems to be affected by precipitation causing a biased mortality among the young (Havelka and Millar 1997). For *Sigmodon hirsutus*, a greater mortality among juvenile females could be the cause of the existence of a greater quantity of adult males (Monge 2008). In other rodents, such as *Oryzomys chapmani*, it is proposed that some of their populations of tropical montane cloud forest present a greater proportion of males as a response to disturbances in their environment (Santos-Moreno *et al.* 2007). In this study, despite the fact that were no significant differences in the proportion of sexes of *P. yucatanicus*, the capture of males was slightly greater, as has been reported in other environments of tropical low forest and pasture in Yucatan (Hernández-Betancourt *et al.* 2012). It should be noted that the presence of fires and hurricanes is common in the state of Yucatán, for which reason future studies should evaluate the effect of these phenomena on the populations and sexual proportions of *P. yucatanicus*, as have been reported for other rodent species (Santos-Moreno *et al.* 2007). Likewise, we identified that the frequency of sexes of *P. mexicanus* did not depend on the season, which could indicate that neither species has suffered a selective pressure that influences the proportion of the sexes in their populations.

Some species present the birth of their young in favorable periods such as the rainy season, since this is a strategy that maximizes the availability of food for the young and increases their probability of survival and maturation (Crespin and Lima 2006; Santos-Moreno *et al.* 2007). More juveniles of the Yucatecan deer mouse were captured in November, possibly due to the fact that most births occur at the end of the dry season and, by the rainy season, the juveniles have separated from their mothers (MacSwiney *et al.* 2012). We also recorded the highest capture of adults and reproductive individuals in the dry season, which coincides with another study conducted in the same area, in which receptive females were observed in the month of February (MacSwiney *et al.* 2012). This leads us to suggest that the reproductive activity of *P. yucatanicus* occurs with greater frequency at the end of the dry season and beginning of the rainy season (Hernández-Betancourt *et al.* 2012; MacSwiney *et al.* 2012), as is the case with *Heteromys gaumeri* in the tropical forests of Yucatan, where the number of pregnant and post-lactating females is higher during the dry season (Hernández-Betancourt *et al.* 2003).

In general, the number of adult *P. mexicanus* was usually greater than that of the juveniles. However, in March, mainly juvenile males were caught, while a greater quantity of juvenile females was captured in July. Under experimental conditions, *P. mexicanus* can reach adulthood 35 days after birth, regardless of sex, and reach sexual maturity at 46 to 66 days (Duquette and Millar 1995; Trujano-Alvarez and Álvarez-Castañeda 2010). This rapid maturation of the juvenile rodents could explain the difference in ages observed in our study. Moreover, the availability of vegetation with high productivity could allow the juveniles to reach adulthood in a short time (Duquette and Millar 1995; Rojas and Barboza 2007).

During the dry and rainy seasons, we observed that *P. mexicanus* maintained its reproductive activity, since we captured reproductive males in each month of sampling (except March), with a slightly greater abundance in the rainy season. Receptive females were also observed in both seasons and pregnant females were present in almost all samplings, with lactating and post-lactating females mainly present in the rainy season. This leads us to assume that *P. mexicanus*, as with other species of the same genus, is an opportunist in terms of its reproduction since in favorable years or environments, it can reproduce throughout the year (Heideman and Bronson 1993; Duquette and Millar 1995; Rojas and Barboza 2007; Ventura 2014).

Characterization and preference of microhabitat. Different species of semi-tree-dwelling rodents, such as *P. leucopus, P. maniculatus* and *P. perfulvus*, demonstrate the recurring use of the arboreal stratum in search of food, refuge in the high parts of the trees or for establishment of their nests (Graves *et al.* 1988; Sánchez-Hernández *et al.* 2009; Klein and Cameron 2012). In the case of *P. yucatanicus*, the present study recorded the maximum height at which it was captured at 5.00 m above ground level. It had previously been recorded at 1.65 m in a tropical medium subevergreen forest of Quintana Roo (MacSwiney *et al.* 2012). Our results indicate that the females can move to a greater height towards the canopy and both juveniles and adults make use of the arboreal stratum, a situation that contrasts with some records of other species, such as *Ototylomys phyllotis*, in which only the adults were found to use this stratum (Hernández-Betancourt *et al.* 2008).

With respect to *P. mexicanus*, one third of the total number of captures and recaptures were made on the trees, reaching a maximum capture height of 11.00 m, confirming this species as semi-tree-dwelling. The Mexican deer mouse generally establishes its burrows beneath fallen tree trunks, among the tree roots or under the ground (Trujano-Alvarez and Álvarez-Castañeda 2010), which could explain why we captured them more frequently at ground level. The average heights at which we recorded this rodent indicate that the species does not present a marked response according to sex or season in terms of climbing to the arboreal stratum. This contrast with other species of *Peromyscus*, such as *P. perfulvus*, which increase their movements on the trees during the rainy season (Domínguez-Castellanos *et al.* 2007).

The trees on which *P. yucatanicus* was captured had an average DBH of 19.11 cm, *i. e.*, a low value if we consider that some individuals were captured in trees of up to 55.00 cm in diameter. Prominent among the tree species on which the species was captured were *L. latisiliquum*, *B. alicastrum* and *B. simaruba*, which are common species in the tropical medium subevergreen forest of the EEER (Schultz 2003). In contrast, *Peromyscus mexicanus* was captured on trees with a DBH of almost 150.00 cm; however, the average diameter of the trees on which it was captured was 33.41 cm. *Bursera simaruba* was a species on which *P. mexicanus* was captured at ground level, and one that is included among those of greatest importance for the vegetal structure of the tropical medium subevergreen forest of the ZPSG (Godínez-Ibarra and López-Mata 2002). Most of the trees on which both species were captured presented rough bark and were covered with climbing plants, which can facilitate the ascent of the rodents on the trunk (Lambert *et al.* 2006; Kilgore *et al.* 2010). Tree-dwelling and semi-tree-dwelling rodents prefer to move on arboreal substrates (trunks and branches) of small and medium diameters. This is due to morphological adaptations of the cranium and feet (in addition to the claws that help them climb vertical strata), their specialized locomotion and corporal posture, which allow them unlimited movement on narrow substrates (Graves *et al.* 1988; Hyams *et al.* 2012; Youlatos *et al.* 2015; Camargo *et al.* 2019).

The specific sites of capture for both species displayed a closed canopy since low percentages of canopy openness (7.75 % for *P. yucatanicus* and 4.61 % for *P. mexicanus*). Different authors state that tree-dwelling and semi-tree-dwelling rodents often prefer habitats with dense vegetation in both understory and canopy (Schnell *et al.* 2008; Pérez-Lustre and Santos-Moreno 2010; Briones-Salas *et al.* 2012), since these characteristics offer the advantage of avoiding detection by predators (Villanueva-Hernández *et al.* 2017).

Captures of both *P. yucatanicus* and *P. mexicanus* diminished in sites with a higher percentage of leaf litter. In this regard, some studies document that rodents of the same genus prefer to inhabit sites with less leaf litter and organic material, or at least to limit their movements in areas in which these are abundant (preferring to move along the length of fallen trunks and branches), with the aim of avoiding being more visually or aurally detectable by predators (Roche *et al.* 1999; Klein and Cameron 2012).

Composition of the feces. The dominant element in the feces of *P. yucatanicus* was the seeds, which comprised nine microscopic morphotypes. This indicated the likelihood that the fruits that produced these seeds are important in its diet, particularly in the middle of the dry season (February). Fruit pulp and starch was abundant at the beginning of the dry season (November), but diminished considerably by the middle of this period (February), possibly due to the lack of fruits. However, some tropical forests of the Yucatan peninsula can maintain this particular food supply throughout the year, due to succession in the production of fruits, such as those of the species *Diospyros anisandra*, *Diospyros tetrasperma* and *Diospyros acapulcensis* subsp. *verae-crucis* (Hernández-Betancourt *et al.* 2003; MacSwiney *et al.* 2012).

The elements of vegetal origin that dominated the feces of *P. mexicanus* were different types of epidermis, as well as fruit pulp and starch. The latter element is the most recurring during the dry season. This coincides with that reported in other studies that consider this rodent to be a consumer of a great variety of fruits, stems and leaves (Trujano-Alvarez and Álvarez-Castañeda 2010). Species of the same genus, such as *P. aztecus* and *Peromyscus difficilis*, also present a diet dominated by vegetal elements, with the consumption of fruits and stems prominent in these two species, respectively (Vázquez *et al.* 2004; Peralta 2015). Although seeds had a low abundance in the feces, 24 morphotypes were recorded, indicating the wide variety of resources available in the tropical forest, particularly in the dry season.

Studies of diet in small rodents rarely analyze the diversity and quantity of seeds that are ingested and defecated by these mammals (Montenegro-Díaz *et al.* 1991; Wells *et al.* 2009; Shiels and Drake 2011; Yang *et al.* 2018). These analyses could influence the knowledge regarding the ecological role of each rodent species as a predator and potential disperser of seeds (Yang *et al.* 2018), as well as providing information relating to the distribution of resources and mechanisms of coexistence among rodent species of similar sizes (Vieira *et al.* 2006; Wells *et al.* 2009). In this study, the types of microscopic seeds that pass through the digestive tract of the study species could not be identified; however, their sizes and frequencies of appearance were recorded. Of the total number of morphotypes, 70.00 % were smaller than those reported in other rodents of similar length and corporal weight to *P. yucatanicus* and *P. mexicanus* (Vieira *et al.* 2006).

In its feces, the Yucatecan deer mouse presented low proportions of elements of animal origin, and even then only in the month at the beginning of the dry season. The arthropods found in the feces included ants and acari of less than 1.00 mm^2 in size, which could have been the result of accidental ingestion and association with the vegetal elements consumed by the rodent (López-Cortés *et al.* 2007; Peña-Ramos *et al.* 2009). In contrast, the feces of the Mexican deer mouse presented a high proportion of chitin remains, with a higher presence in the rainy season, which could be related to the fact that some arthropods are often more abundant during this season (Zavala-León *et al.* 2016). In this sense, it has been determined that the presence of arthropods in the diet can indicate a dietary flexibility that is advantageous in disturbed environments, or in situations where trophic resources are scarce (Vázquez *et al.* 2004; Lanzone *et al.* 2012). These arthropods also act as protein and hydric supplements that can increase the nutritional quality of the diet (Vázquez *et al.* 2004; Orr *et al.* 2015).

Acknowledgments

The first author thanks the Consejo Nacional de Ciencia y Tecnología (CONACyT) for the support of grant No. 297705 awarded for doctorate studies. Thanks to A. Gómez-Pompa, M. Lazcano and M. E. Martínez for support with sampling in the El Edén Ecological Reserve, and to B. Vega-Hernández and K. Vega-Hernández, property owners of the Zona de Protección Forestal y Faúnica Santa Gertrudis, for their hospitality and for facilities provided for conducting this study. The field assistance of M. Avendaño, J. L. Libreros, C. Martínez, E. Priego, F. D. Ruz, R. Rodríguez and T. Santiago is gratefully acknowledged. We thank Juan de la C. Tun-Garrido and G. Castillo-Campos for their help with the taxonomic identification of the plants. We also thank C. A. Martínez-Adriano for advice regarding certain statistical tests and help with the identification of insect remains.

Literature cited

Allen, M. F., and E. Rincón. 2003. The changing Global environment and the lowland Maya: past, patterns and current dynamics. Pp. 13-29, *in* The lowland Maya area: three millennia at the human-wildland interface (Gómez-Pompa, A., M. Allen, S. L. Feddick, and J. J. Jiménez-Osornio, eds.). Food Products Press. Binghamton, U.S.A.

AntWeb. 2017. Genus: Pachycondyla Smith, 1858. https://www.antweb.org/description.do?subfamily=ponerinae&genus=pachycondyla&rank=genus&bioregionName=Neotropical. Accessed 4 December 2017.

Baltazar, S. 2014. Importancia de la dispersión de semillas en la recuperación del Bosque Mesófilo de Montaña del centro de Veracruz, México. M. Sc. Thesis. Instituto de Ecología, A. C. Xalapa, México.

Bellows, A. S., J. F. Pagels, and J. C. Mitchell. 2001. Macrohabitat and microhabitat affinities of small mammals in a fragmented landscape on the upper coastal plain of Virginia. The American Midland Naturalist 146:345-360.

Bojorges, J. C., and L. López-Mata. 2005. Riqueza y diversidad de especies de aves en una selva mediana subperennifolia en el centro de Veracruz, México. Acta Zoológica Mexicana (n. s.) 21:1-20.

Briones-Salas, M., and G. González. 2016. Ecología poblacional de *Heteromys pictus* (Rodentia: Heteromyidae), en un bosque tropical caducifolio con perturbación humana, en la costa de Oaxaca, México. Revista de Biología Tropical 64:1415-1429.

Briones-Salas, M., A. Hernández-Allende, M. Martínez, and G. González. 2012. New records of the endemic chinanteco deermouse *Habromys chinanteco* (Rodentia: Cricetida) in the Sierra Madre de Oaxaca, Mexico. The Southwestern Naturalist 57:222-223.

Camargo, N. F., L. F. Machado, A. F. Mendonça, and E. M. Vieira. 2019. Cranial shape predicts arboreal activity of Sigmodontinae rodents. Journal of Zoology. https://doi.org/10.1111/jzo.12659.

Castillo-Campos, G., and M. E. Medina-Abreo. 2005. Árboles y arbustos de la Reserva Natural de La Mancha, Veracruz: manual para la identificación de las especies. Instituto de Ecología, A. C. Xalapa, México.

Cimé-Pool, J. A., S. F. Hernández-Betancourt, R. Barrientos, and A. Castro-Luna. 2010. Diversidad de pequeños roedores en una selva baja caducifolia espinosa del noreste de Yucatán, México. Therya 1:23-40.

Cimé-Pool, J. A., S. F. Hernández-Betancourt, and J. B. Chablé-Santos. 2007. Comunidad de pequeños roedores en dos agroecosistemas de Yucatán, México. Revista Mexicana de Mastozoología 11:57-68.

Crespin, L., and M. Lima. 2006. Supervivencia adulta y dinámica poblacional del lauchón orejudo *Phyllotis darwini* en Chile central. Revista Chilena de Historia Natural 79:295-308.

Cruz-Lara, L. E., C. Lorenzo, O. G. Retana, and E. C. Sántiz. 2010. Interspecific variability in the abundande of small rodents in the highlands of Chiapas, Mexico. Therya 1:129-136.

Cruz-Lara, L. E., *et al*. 2004. Diversidad de mamíferos en cafetales y selva mediana de las cañadas de la Selva Lacandona, Chiapas, México. Acta Zoológica Mexicana (n. s.) 20:63-81.

Dallas, T. A., S. Foré, and H. J. Kim. 2012. Modeling the influence of *Peromyscus leucopus* body mass, sex, and habitat on immature *Dermacentor variabilis* burden. Journal of Vector Ecology 37:338-341.

Dobson, A. J., and A. G. Barnett. 2008. An introduction to generalized linear models. Chapman & Hall/CRC. Boca Raton, U.S.A.

Domínguez-Castellanos, Y., F. Pimentel, and G. Ceballos. 2007. Uso de hábitat de roedores arborícolas en la selva seca de la Reserva de la Biósfera Chamela-Cuixmala, Jalisco. Revista Mexicana de Mastozoología 11:21-40.

Duquette, L. S., and J. S. Millar. 1995. The effect of supplemental food on life-history traits and demography of a tropical mouse *Peromyscus mexicanus*. Journal of Animal Ecology 64:348-360.

Ferreira, T., and W. Rasband. 2012. ImageJ User Guide, IJ 1.46r. https://imagej.nih.gov/ij/docs/guide/user-guide.pdf. Accessed 16 May 2016.

Fleury, M., R. R. Rodrigues, H. T. Z. do Couto, and M. Galetti. 2014. Seasonal variation in the fate of seeds under contrasting logging regimes. Plos One 9: e90060.

Frazer, G. W., C. D. Canham, and K. P. Lertzman. 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies. New York, U.S.A.

García, E. 2004. Modificaciones al Sistema de Clasificación Climática de Köppen, Serie Libros, Núm. 6. Instituto de Geografía, Universidad Nacional Autónoma de México. Ciudad de México, México.

Godínez-Ibarra, O., and L. López-Mata. 2002. Estructura, composición, riqueza y diversidad de árboles en tres muestras de selva mediana subperennifolia. Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Botánica 73:283-314.

Graipel, M. E. 2003. A simple ground-based method for trapping small mammals in the forest canopy. Mastozoología Neotropical 10:177-181.

Graves, S., J. Maldonado, and J. O. Wolff. 1988. Use of ground and arboreal microhabitats by *Peromyscus leucopus* and *Peromyscus maniculatus*. Canadian Journal of Zoology 66:277-2178.

Harris, S. E., R. J. O´Neill, and J. Munshi-South. 2015. Transcriptome resource for the white-footed mouse (*Peromyscus leucopus*): new genomic tools for investigating ecologically divergent urban and rural populations. Molecular Ecology 15:382-394.

Havelka, M. A., and J. S. Millar. 1997. Sex ratio of offspring in *Peromyscus maniculatus borealis*. Journal of Mammalogy 78:626-637.

Heideman, P. D., and F. H. Bronson. 1993. Potential and realized reproduction in a tropical population of *Peromyscus* (Rodentia). Journal of Mammalogy 74:261-269

Hernández-Betancourt, S. F., J. A. Cimé-Pool, S. Medina, and C. M. Durán. 2012. Parámetros poblacionales del ratón yucateco *Peromyscus yucatanicus* de una selva baja caducifolia del norte de Yucatán, México. Pp. 151-163 *in* Estudios sobre la biología de roedores silvestres mexicanos (Cervantes, F. A., and C. Ballesteros-Barrera, eds.). Instituto de Biología, UNAM y UAM-Unidad Iztapalapa. Ciudad de México, México.

Hernández-Betancourt, S. F., J. A. Cimé-Pool, S. Medina, and M. L. González-Villanueva. 2008. Fluctuaciones poblacionales de *Ototylomys phyllotis* Merriam, 1901 (Rodentia: Muridae) en una selva mediana subcaducifolia del sur de Yucatán, México. Acta Zoológica Mexicana (n. s.) 24:161-177.

Hernández-Betancourt, S. F., R. López-Wilchis, J. A. Cimé-Pool, and S. Medina. 2003. Área de actividad, movimiento y organización social de *Heteromys gaumeri* Allen y Chapman, 1897 (Rodentia: Heteromyidae) en una selva mediana subcaducifolia de Yucatán, México. Acta Zoológica Mexicana (n. s.) 90:77-91.

Hyams, S. E., B. C. Jayne, and G. N. Cameron. 2012. Arboreal habitat structure affects locomotor speed and perch choice of white-footed mice (*Peromyscus leucopus*). Journal of Experimental Zoology 317:540-551.

Kalkvik, H. M., I. J. Stout, T. J. Doonan, and C.L.Parkinson. 2012. Investigating niche and lineage diversification in widely distributed taxa: phylogeography and ecological niche modeling of the *Peromyscus maniculatus* species group. Ecography 35:54-64.

Kenney-Hunt, J., *et al*. 2014. A genetic map of *Peromyscus* with chromosomal assignment of linkage groups (a *Peromyscus* genetic map). Mammalian Genome 25:160-179.

Kilgore, A., T. D. Lambert, and G. H. Adler. 2010. Lianas influence fruit and seed use by rodents in a tropical forest. Tropical Ecology 51:265-271

Klein, G. P., and G. N. Cameron. 2012. Effect of habitat gradients on space use by White-footed mice (*Peromyscus leucopus*). Journal of Mammalogy 93:706-715.

Krebs, C. J. 1966. Demographic changes in fluctuating populations in *Microtus californicus*. Ecological Monographs 36:239-273.

Lambert, T. D., J. R. Malcolm, and B. L. Zimmerman. 2006. Amazonian small mammal abundance in relation to habitat structure and resource abundance. Journal of Mammalogy 87:766-776.

Lanzone, C., *et al*. 2012. Dry season diet composition of *Eligmoontia moreni* (Rodentia, Cricetidae, Sigmodontinae) in a hyper-arid region of the Monte desert (Mendoza, Argentina). Multequina 21:25-30.

López-Cortés, F., A. Cortés, E. Miranda, and J. R. Rau. 2007. Dietas de *Abrothrix andinus*, *Phyllotis xanthopygus* (Rodentia) y *Lepus europaeus* (Lagomorpha) en un ambiente altoandino de Chile. Revista Chilena de Historia Natural 80:3-12.

MacSwiney, M. C., S. F. Hernández-Betancourt, J. A. Panti-May, and J. M. Pech-Canché. 2012. Ecología poblacional del ratón yucateco *Peromyscus yucatanicus* (Rodentia: Cricetidae) en las selvas de Quintana Roo, México. Pp. 237-246 *in* Estudios sobre la biología de roedores silvestres mexicanos (Cervantes, F. A., and C. Ballesteros-Barrera, eds.). Instituto de Biología, UNAM y UAM-Unidad Iztapalapa. Ciudad de México, México.

Meyer, D., A. Zeileis, and K. Hornik. 2006. The strucplot framework: visualizing multi-way contingency tables with vcd. Journal of Statistical Software 17:1-48.

Meyer, D., A. Zeileis, and K. Hornik. 2015. vcd: visualizing categorical data. R package version 1.4-1. Journal of Statistical Software 17:1-48.

Meyer, J. Y., and J. F. Butaud. 2009. The impacts of rats on the endangered native flora of French Polynesia (Pacific Islands): drivers of plant extinction or *coup de grâce* species? Biological Invasions 11:1569-1585.

Monge, J. 2008. Estructura poblacional y actividad reproductiva de la rata de campo (*Sigmodon hirsutus*) durante un ciclo de producción de maní (*Arachis hypogaea*) en Costa Rica. Agronomía Costarricense 32:161-167.

Montenegro-Díaz, O., H. López-Arévalo, and A. Cadena. 1991. Aspectos ecológicos del roedor arborícola *Rhipidomys latimanus* Tomes, 1860, (Rodentia: Cricetidae) en el oriente de Cundinamarca, Colombia. Caldasia 16:565-572.

Morris, G., J. A. Hostetler, M. K. Oli, and L. M. Conner. 2011. Effects of predation, fire, and supplemental feeding on populations of two species of *Peromyscus* mice. Journal of Mammalogy 92:934-944.

Orr, T. J., S. D. Newsome, and B. O. Wolf. 2015. Cacti supply limited nutrients to a desert rodent community. Oecologia 178:1045-1062.

Panti-May, J. A., *et al*. 2019. Diet of two invasive rodent species in two Mayan communities in Mexico. Mammalia 83:567-573.

Panti-May, J. A., M. C. MacSwiney, S. F. Hernández-Betancourt, and O. A. Valdes-Rodríguez. 2014. Reproduction and postnatal development in the Yucatan vesper mouse. Mammalia 79:169-176.

Peña-Ramos, J. L., *et al*. 2009. Diet composition of *Sigmodon hispidus* (Rodentia: Cricetidae) in sugar-cane. Mastozoología Neotropical 16:365-378

Peralta, C. M. 2015. Dieta de *Peromyscus difficilis* (Rodentia: Muridae) en un bosque templado en el Parque Nacional Desierto de los Leones, D. F. M. Sc. Thesis. Universidad Autónoma Metropolitana. Ciudad de México, México.

Pérez-Lustre, M., and A. Santos-Moreno. 2010. Movements and capture-recapture data analysis of the vesper rat (*Nyctomys sumichrasti*: Rodentia, Muridae) in a tropical forest in northeastern Oaxaca, México. Acta Zoológica Mexicana (n. s.) 26:627-638.

Poindexter, C. J., *et al*. 2013. Demographic features and habitat preferences of *Osgoodomys banderanus* (osgood´s deermouse) in Colima, México. The Southwestern Naturalist 58:8-19.

R Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. URL: http://www.R-project.org/.

Roche, B. E., A. I. Schulte-Hostedde, and R. J. Brooks. 1999. Route choice by deer mice (*Peromyscus maniculatus*): reducing the risk of auditory detection by predators. American Midland Naturalist 142:194-197.

Rodríguez-Macedo, M., A. González-Christen, and L. S. León-Paniagua. 2014. Diversidad de los mamíferos silvestres de Misantla, Veracruz, México. Revista Mexicana de Biodiversidad 85: 262-275.

Rojas, L., and M. Barboza. 2007. Ecología poblacional del ratón *Peromyscus mexicanus* (Rodentia: Muridae) en el Parque Nacional Volcán Poás, Costa Rica. Revista de Biología Tropical 55:1037-1050.

Rojas-Martínez, A., M. Aguilar-López, C. Sánchez-Hernández, and O. Noguera-Cobos. 2012. Uso del espacio y dinámica poblacional del ratón de los volcanes (*Neotomodon alstoni*) en el Cerro del Ajusco, Distrito Federal, México. Pp. 211-223 *in* Estudios sobre la biología de roedores silvestres mexicanos (Cervantes, F. A., and C. Ballesteros-Barrera, eds.). Instituto de Biología, UNAM y UAM-Unidad Iztapalapa. Ciudad de México, México.

San-José, M., V. Arroyo-Rodríguez, and V. Sánchez-Cordero. 2014. Association between small rodents and forest patch and landscape structure in the fragmented Lacandona rainforest, Mexico. Tropical Conservation Science 7:403-422.

Sánchez-Cordero, V., and R. Martínez-Gallardo. 1998. Postdispersal fruit and seed removal by forest-dwelling rodents in a lowland rainforest in Mexico. Journal of Tropical Ecology 14:139-151.

Sánchez-Hernández, C., M. L. Romero-Almaraz, H. Colín-Martínez, and C. García-Estrada. 2001. Mamíferos de cuatro áreas con diferente grado de alteración en el sureste de México. Acta Zoológica Mexicana (n. s.) 84:35-48.

Sánchez-Hernández, C., G. D. Schnell, and M. L. Romero-Almaraz. 2009. *Peromyscus perfulvus* (Rodentia: Cricetidae). Mammalian Species 833:1-8.

Santos-Moreno, A., M. A. Briones-Salas, and R. López-Wilchis. 2007. Diferencias en algunos parámetros demográficos de *Oryzomys chapmani* (Rodentia: Muridae) asociados a tres estados sucesionales de bosque mesófilo de montaña en Oaxaca, México. Acta Zoológica Mexicana (n. s.) 23:123-137.

Schmidt, C. M., and W. R. Hood. 2014. Bone loss is a physiological cost of reproduction in white-footed mice (*Peromyscus leucopus*). Mammalian Biology 79:96-100.

Schnell, G. D., *et al*. 2010. Microhabitat preferences and spatial distribution of the vesper rat (*Nyctomys sumichrasti*) in Colima, Mexico. Mammalian Biology 75:482-495.

Schnell, G. D., *et al*. 2008. Habitat preference of the endemic tawny deermouse (*Peromyscus perfulvus*), a species of conservation concern. The Southwestern Naturalist 53:9-20.

Schultz, G. P. 2003. Structure and diversity of the forests at the El Edén Ecological Reserve. Pp. 91-114 *in* The lowland Maya area: three millennia at the human-wildland interface (Gómez-Pompa, A., M. Allen, S. Feddick, and J. Jiménez-Osornio, eds.). Food Products Press. Binghamton, U.S.A.

Shiels, A. B., and D. R. Drake. 2011. Are introduced rats (*Rattus rattus*) both seed predators and dispersers in Hawaii? Biological Invasions 13:883-894.

Shiels, A. B., *et al*. 2013. Dietary niche differentiation among three species of invasive rodents (*Rattus rattus*, *R. exulans*, *Mus musculus*). Biological Invasions 15:1037-1048.

Sikes, R. S., and The Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663-688.

Sun, Y., *et al*. 2014. *Peromyscus leucopus* mice: a potential animal model for haematological studies. International Journal of Experimental Pathology 95:342-350.

Tapia-Ramírez, G., C. López-González, A. González-Romero, and S. F. Hernández-Betancourt. 2012. Diversidad de roedores y su relación con la heterogeneidad ambiental en la cuenca del río Nazas, Durango, México. Pp. 59-70 *in* Estudios sobre la biología de roedores silvestres mexicanos (Cervantes, F. A., and C. Ballesteros-Barrera, eds.). Instituto de Biología, UNAM y UAM-Unidad Iztapalapa. Ciudad de México, México.

Triplehorn, C. A., and N. F. Johnson. 2005. Borror and delong´s introduction to the study of insects. Thomson Brooks/Cole. California, U.S.A

Trujano-Alvarez, A. L., and S. T. Álvarez-Castañeda. 2010. *Peromyscus mexicanus* (Rodentia: Cricetidae). Mammalian Species 42:111-118.

Vázquez, L. B., G. N. Cameron, and R. A. Medellín. 2004. Characteristics of diet of *Peromyscus aztecus* and *Reithrodontomys fulvescens* in montane western Mexico. Journal of Mammalogy 85:196-205.

Ventura, P. D. 2014. Estructura poblacional, parámetros demográficos y reproducción de *Peromyscus furvus* Allen & Champman, 1897 en Coacoatzintla, Veracruz. B. Sc. Thesis, Universidad Veracruzana. Xalapa, México.

Vieira, E. M. 1998. A technique for trapping small mammals in the forest canopy. Mammalia 62:306-310.

Vieira, E. M., G. Paise, and P. H. D. Machado. 2006. Feeding of small rodents on seeds and fruits: a comparative analysis of three species of rodents of the *Araucaria* forest, southern Brazil. Acta Theriologica 51:311-318.

Villanueva-Hernández, A. I., *et al*. 2017. Habitat selection by rodents at the transition between the Sierra Madre Occidental and the Mexican Plateau, México. Journal of Mammalogy 98:293-301.

Weber, J. N., and H. E. Hoekstra. 2009. The evolution of burrowing behavior in deer mice (genus *Peromyscus*). Animal Behaviour 77:603-609.

Wells, K., *et al*. 2009. Seed composition by small mammals from Borneo. Journal of Tropical Ecology 25:555-558.

Whitehead, T., M. Goosem, and N. D. Preece. 2014. Use by small mammals of a chronosequence of tropical rainforest revegetation. Wildlife Research 41:233-242.

Williams, S. A., *et al*. 2013. Effects of developmental bisphenol A exposure on reproductive-related behaviors in California mice (*Peromyscus californicus*): a monogamous animal model. PLoS ONE 8(2): e55698. https://doi.org/10.1371/journal.pone.0055698.

Witmer, G. W., and W. C. Pitt. 2012. Invasive rodents in the United States: ecology, impacts, and management. Pp. 45-75 *in* Invasive species: threats, ecological impacts and control methods (Blanco, J. J., and A. T. Fernandes, eds.). Nova Science Publishers, Inc. New York, U.S.A.

Yang, Y., Y. Zhang, Y. Deng, and X. Yi. 2018. Endozoochory by granivorous rodents in seed dispersal of green fruits. Canadian Journal of Zoology 97:42-49.

Youlatos, D., N. E. Karantanis, C. D. Byron, and A. Panyutina. 2015. Pedal grasping in an arboreal rodent relates to above-branch behavior on slender substrates. Journal of Zoology 296:239-248.

Zavala-León, E. A., *et al*. 2016. Distribución temporal de los insectos del suelo en el bosque tropical caducifolio de Santiago Dominguillo, Oaxaca. Entomología Mexicana 3:543-548.

Zeileis, A., D. Meyer, and K. Hornik. 2007. Residual-based shadings for visualizing (conditional) independence. Journal of Computational and Graphical Statistics 16:507-525.

Associated editor: Mariana Freitas Nery
Submitted: June 10, 2021; Reviewed: June 29, 2021
Accepted: September 13, 2021; Published on line: November 13, 2021

THERYA, 2022, Vol. 13(2):143-151 DOI:10.12933/therya-22-1158 ISSN 2007-3364

Analysis of the male annual antler cycle, reproductive behavior and spotted fawn presence in the tropical white-tailed deer

Miguel Rodríguez-Ramírez[1], and José Manuel Mora[2, 3]*

[1] WANATURA, No. 36. Calle Jiménez, Rincón Oeste. Zaragoza, Palmares. Alajuela, Costa Rica. Email: marr.2010@outlook.com (MR-R).
[2] Carrera de Gestión Ecoturística, Sede Central, Universidad Técnica Nacional, Villa Bonita de Alajuela 20101. Alajuela, Costa Rica. Email: jomora@pdx.edu (JMM).
[3] Department of Biology and Museum of Vertebrate Biology, Portland State University, 97207. Portland, Oregon, U.S.A.
*Corresponding author

The rutting season of the white-tailed deer (*Odocoileus virginianus*) is seasonal in North America, but in tropical latitudes it occurs year-round with peaks locally variable. Reproductive cycle of this species in Costa Rica is variable, clearly seasonal in San Lucas Island but continuous with two birth peaks in Palo Verde and Santa Rosa national parks in northwestern Costa Rica. In Hacienda y Refugio de Vida Silvestre Curú in the Nicoya Peninsula, a site south of these parks but in a different life zone, we observed males with hard antlers in 2015 but only from July to November. We therefore hypothesized that white-tailed deer have a continuous reproductive cycle during the year in Curú because the rainfall pattern is the same as in Palo Verde and Santa Rosa. We visited Curú every month and recorded the status of white-tailed deer antler growth: nubs, velvet, and hard, from January 2016 to December 2019. We also recorded other rut behaviors and the presence of spotted fawns. We recorded 1,134 observations of the status of antler growth, 13 observations on reproductive behavior and 133 observations of spotted fawns. The antler cycle was seasonal in the four years of study. Male antler casting took place from mid-November to mid-February and the growth of new antlers began in mid-December and lasted until mid-March. Velvet shedding occurred in April, May and June, and by July males had hard antlers. The rutting season lasted three months from mid-June to mid-August. Small spotted fawns were observed from the middle of the dry season to the beginning of the rainy season. All this highly contrasts with the reproduction pattern observed in Santa Rosa. Lack of seasonal variation in the photoperiod is likely an important factor that allows deer to reproduce throughout the year in these protected areas, but this variation does not exist in Curú either, where the species is seasonal. The reproductive patterns of white-tailed deer in Central and South America may have evolved in response to seasonal fluctuations in specific food availability, competition, or predation, all of which may be directly related to rainfall patterns. Greater knowledge of the link between rainfall patterns and food availability for deer would be of great help to further our understanding of factors driving the reproduction cycle of the White-tailed deer.

La temporada de reproducción o brama del venado cola blanca (*Odocoileus virginianus*) es estacional en América del Norte, pero en latitudes tropicales ocurre durante todo el año con picos variables localmente. El ciclo reproductivo de esta especie en Costa Rica es variable, claramente estacional en la isla San Lucas, pero continuo con dos picos de nacimientos en los parques nacionales Palo Verde y Santa Rosa en el noroeste de Costa Rica. En Hacienda y Refugio de Vida Silvestre Curú en la Península de Nicoya, un sitio al sur de estos parques, pero en una zona de vida diferente, observamos machos con astas duras en 2015, pero solo de julio a noviembre. Por lo tanto, planteamos la hipótesis de que el venado cola blanca tiene un ciclo reproductivo continuo durante el año en Curú porque el patrón de lluvia es igual al de Palo Verde y Santa Rosa. Visitamos Curú todos los meses y registramos el estado del crecimiento de las astas de los venados de enero de 2016 a diciembre de 2019. También registramos otros comportamientos de celo y la presencia de cervatillos manchados. Registramos 1,134 observaciones del estado del crecimiento de las astas, 13 observaciones sobre el comportamiento reproductivo y 133 observaciones de cervatillos manchados. El ciclo de las astas fue estacional en los cuatro años de estudio. Los machos botaron las astas de mediados de noviembre a mediados de febrero y el crecimiento de las nuevas astas inició a mediados de diciembre y se prolongó hasta mediados de marzo. La caída del terciopelo ocurrió en abril, mayo y junio y a partir de julio los machos tenían astas duras. La brama duró tres meses, desde mediados de junio hasta mediados de agosto. Observamos cervatillos manchados pequeños desde la mitad de la estación seca hasta el inicio de la temporada de lluvias. Todo esto contrasta con el patrón de reproducción observado en Palo Verde y Santa Rosa. La falta de variación estacional en el fotoperiodo es probablemente un factor importante que permite que los venados se reproduzcan durante todo el año en estas áreas protegidas, pero esta variación tampoco existe en Curú, donde la especie es estacional. Los patrones reproductivos del venado cola blanca en América Central y del Sur pueden haber evolucionado en respuesta a fluctuaciones estacionales en la disponibilidad de alimentos específicos, la competencia o la depredación, todo lo cual puede estar directamente relacionado con los patrones de lluvia. Un mayor conocimiento del vínculo entre los patrones de lluvia y la disponibilidad de alimentos para los venados sería de gran ayuda para comprender mejor los factores que impulsan los ciclos de reproducción del venado cola blanca.

Keywords: Antler status; dry forest; humid forest; nubs; reproduction; rut; velvet.

Introduction

White-tailed deer (*Odocoileus virginianus*) has the widest distribution and is the most abundant of all New World cervids (Teer 1994). Its distribution extends from southeastern Alaska in North America to northern South America with up to 38 recognized subspecies (Mattioli 2011). There is controversy regarding whether there is only one species or several species (Molinari 2007). In the latter case, the species found in Central America corresponds to *Odocoileus cariacou* of wide distribution on the Neotropical region (Molinari 2007).

Endogenous recognition of photoperiod changes drives seasonal reproductive cycles (Bronson 2009). A decrease in the daylight-darkness ratio triggers the onset of the reproductive cycle. Since the photoperiod is related to latitude (Ditchkoff 2011), the rutting season of white-tailed deer progresses along a continuum from November in most of North America, to January to March in northern México (Mattioli 2011), and from January to April in central and southern México (Weber *et al.* 1994; Contreras-Moreno *et al.* 2019). The annual cycle of antlers is related to the reproductive cycle with variation in the reproductive chronology linked to environmental variables mainly associated with latitude, especially photoperiods and food availability (Goss 1983; Price *et al.* 2005a; Hanon *et al.* 2008; Contreras-Moreno *et al.* 2019).

The annual phases of primary development of the testes, the complete production of sperm, and the rest period (Robinson *et al.* 1965) are associated with the onset of antler growth, shed of velvet, and antlers fall off, respectively (Verme and Ullrey 1984). The photoperiod acts through melatonin to modulate the secretion of reproductive hormones, mainly testosterone (Goldman 2001; Hanon *et al.* 2008) and regulates antlers cycle in white-tailed deer (Price *et al.* 2005a; Price *et al.* 2005b). However, because there is only a change in photoperiod in the tropics with a weak temporal association with seasonal climatic changes, it results that this environmental factor could have a reduced influence in controlling antler development in Southern México (Contreras-Moreno *et al.* 2019).

In temperate and cold regions of North America, severe weather and food availability are the critical environmental factors that dictate the timing of the birth season (Lincoln 1992; Price *et al.* 2005b). The reproductive cycle of white-tailed deer in these regions of North America is seasonal (Verme and Ullrey 1984; Mattioli 2011). The status of the antlers is associated with the growth and secretion cycle of testosterone by the testicles (Vasantha 2016). The testosterone cycle is governed largely by photoperiod (Loudon and Curlewis 1988; Tomás 1995). The decrease of daylight and the increase in testosterone cause antlers to mineralize and shed their velvet (Tomás 1995). In contrast, increased daylight and decreased testosterone cause antler casting (Vasantha 2016). On the opposite, there is little information about the reproductive aspects of white-tailed deer in the Neotropics (Contreras-Moreno *et al.* 2019). The birth of fawns and the development and casting of male antlers attracted the interest of the first naturalists working in the region (Rodríguez and Solís 1994). They speculated that fawns are born throughout the year and that there is no annual cycle in the growth and shedding of antlers due to the lack of seasonality in tropical and subtropical regions (Rodríguez and Solís 1994). However, studies conducted in the 1980s concluded that there may be some degree of synchrony in the growth of antlers and the birth of fawns in some places (Klein 1982; Branan and Marchinton 1987; Rodríguez and Solís 1994). Nonetheless, even in sites located nearby, the results seem contradictory because births occur in any season and the time of the year in which males shed their antlers is a function of individual age (Webb and Nellis 1981; Brokx 1984; Rodríguez 1994; Fuller *et al.* 2020).

Studies on the reproductive cycle of white-tailed deer in Costa Rica have yielded contrasting results with clearly seasonal cycles at San Lucas Island (Rodríguez and Solís 1994) and continuous cycles with two birth peaks in Palo Verde and Santa Rosa national parks (Rodríguez 1994; Fuller *et al.* 2020). In the Nicoya Peninsula in northwestern Costa Rica, a peninsular site south of these areas and a different life zone, we observed males with hard antlers in 2015 but only from July to November. As a result, we made extensive, regular field observations from 2016 to 2019 to analyze the antler cycle in a white-tailed deer population in this area. We also recorded fawn birth periods in these four years. We hypothesize that white-tailed deer have a continuous reproductive cycle during the year in Curú because the rainfall pattern in this area is the same as in Palo Verde and Santa Rosa National Parks, where this species has an almost continuous annual reproductive pattern.

Material and methods

Study site. The Hacienda y Refugio de Vida Silvestre Curú (Curú) combines private and public lands totalizing 1,496 ha: 312 ha dedicated to cattle ranching and 1,184 ha dedicated to conservation and ecotourism (Schutt and Vaughan 1995). It is located on the Nicoya Peninsula in northwestern Costa Rica (9° 47′ 23″ N, -84° 55′ 28″ W; Figure 1). Curú vegetation belongs to the Tropical Humid Forest Life Zone (Holdridge 1967; Bolaños *et al.* 2005).

Although the refuge is relatively small it is part of a continuum mosaic of cattle ranching operations and forest remnants along most of the south of the peninsula. Adjacent to Curú there is a secondary forest of over 5,000 ha located at the Peninsula de Nicoya Protected Zone. Curú is known to contain a high level of biodiversity due to the presence of several habitat types and its protection status (Schutt and Vaughan 1995). Seventy-eight species of mammals, 232 species of birds, 87 species of reptiles, and at least 500 species of plants have been identified in the refuge (McKinney 2014). Annual precipitation averaged 1,957 mm for the period 1970-2005, but it was 2,177 mm during our study (MN 2020a; IMN 2020b).

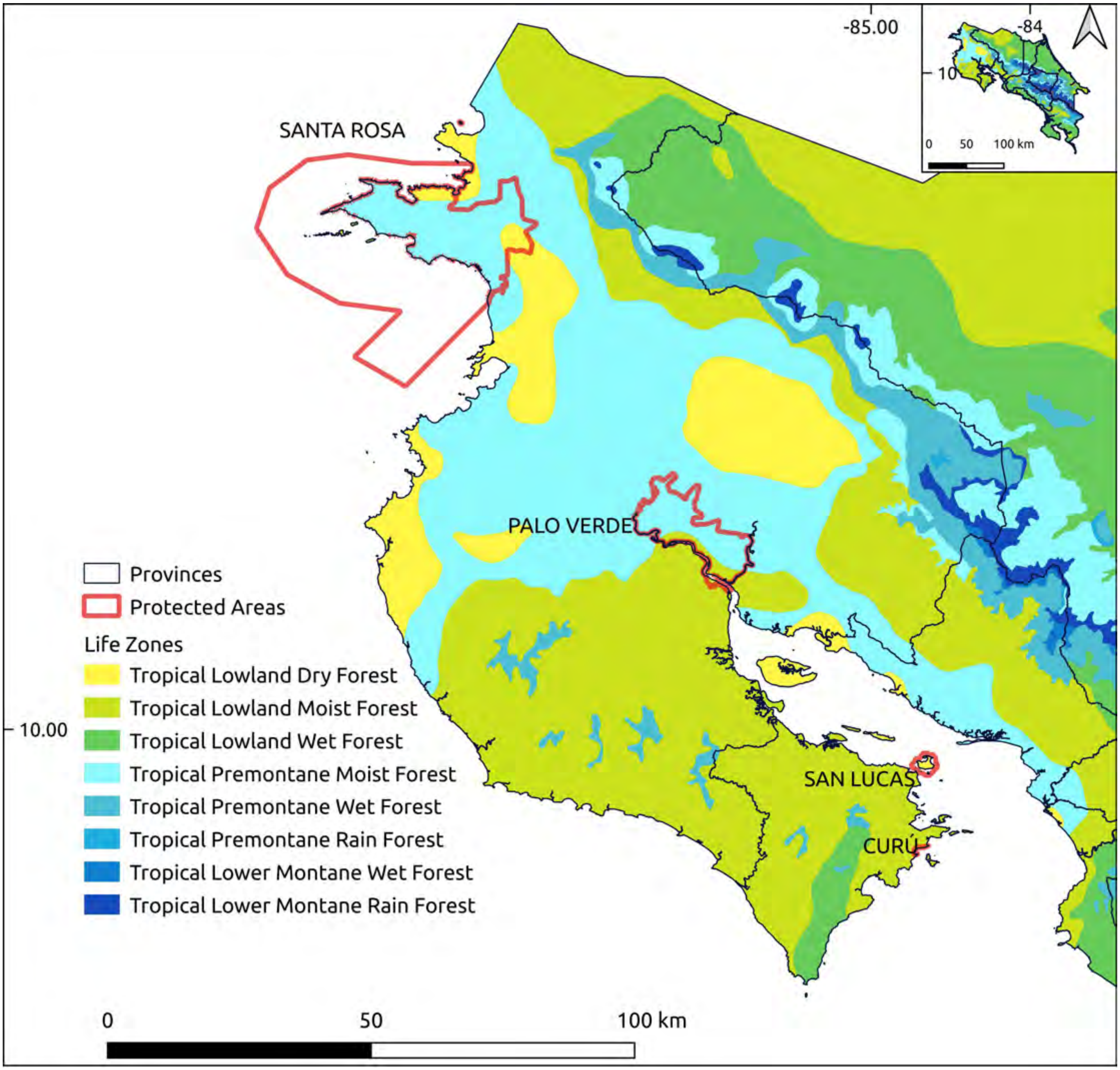

Figure 1. Life zones in Guanacaste province at Northwestern Costa Rica, and the four study sites mentioned in the text. The Hacienda y Refugio de Vida Silvestre Curú is located at the extreme southeastern portion of the Nicoya Peninsula in the Tropical Lowland Moist Forest. Map by G. Chaves (Cachí).

The dry season occurs from December to March when only 4 % of total annual rainfall accumulates. The transition to the rainy season is in April and by the third week in May, the rainy season is typically well established (IMN 2020). The first rainy period occurs between May and August when 50 % of the annual total precipitation accumulates. The first maximum of rains occurs in June as the southeastern trade winds intensify causing local storms and heavy downpours. The northeast trade winds intensify again causing a brief dry season between July and August called the "Pacific summer" or "canicula". A second rainy season extends from September to November. The greatest amount of rain, 46 % of the annual total, falls during September and October due to the greater influence of Caribbean cyclonic events. The transition to the dry season occurs in November (IMN 2020).

Data collection. We visited Curú once a month between January 2016 and December 2019, except in September 2016 and September 2019 when it was impossible to enter the area due to flooding and landslides on the access road. However, this did not affect our study since September is not of high significance for our purposes. We looked for deer on two transects: the main road (2,090 m) and a secondary road (1,958 m). We walked each transect in the morning (6:00 h to 9:00h) and the afternoon (15:00h to 17:00h) every month. We recorded deer data using 10 x 50 binoculars and a digital camera.

Every time we observed a deer we recorded the sex and age group (fawn, yearling, adult) of each individual. We classified adult males by the presence of antlers and testicles and adult females by the absence of antlers and testicles, as well as by the presence of one or two fawns. Juvenile deer were classified as males or females based on body size and by the presence or absence of pedicels and testicles, respectively. Fawns were identified by the presence of spots on their fur. Both researchers have vast experience identifying and classifying deer by age.

Each time we observed an adult male we took a digital photograph for a database on the monthly growth status of deer antlers. For individual recognition of males and to avoid double-counting, we considered: 1) antlers size, 2) antler shape (number of tips, malformations) and 3) physical characteristics of each animal (presence of scars and wounds). Later we classified adult males as 1) nubs (no antlers or just one antler, pedicels in velvet), 2) velvet (growing antlers covered in velvet with rounded tine ends), and 3) hard (fully formed antlers, velvet-less; Figure 2). Additionally, we recorded rubs, odor marks, nonaggressive sparring, fights matching dominant bucks, chasing, and tending bucks, and bucks scraping the ground and leaving odor marks locally known as "rascaderos" (Rodríguez 2015). Although we also recorded the presence of adult females in the field, we made no effort to identify them individually. At each visit, we also counted spotted fawns and recorded them as small and large spotted fawns (see below).

Data interpretation and assumptions. All data were analyzed by month and year, however, for this report, the data were grouped for the whole study period: 2016 to 2019. To identify the reproductive seasonality of deer, we made the following assumptions concerning deer morphology and development based on a literature review. Antlers are shed annually (Sauer 1984). Antler's growth lasts six months, and velvet dries and falls. Adult males keep hard antlers for about six months and then shed them (Sauer 1984). Testis's cycle has three phases: 1) primary development, 2) full sperm production, and 3) resting period (Verme and Ullrey 1984). These phases are associated with the onset of antler growth, velvet shedding, and antler casting respectively (Verme and Ullrey 1984). Adult males are fertile only during the hard antler period. Female gestation varies among subspecies and ranges from 187 to 222 days, with a mean of 202 days (Smith 1991). Fawns spend most of their time hidden away from their mothers for three to four weeks (Marchinton and Hirth 1984), but gradually fawns spend more time with their mothers.

It is not possible to know the date of birth of the fawns in the field. Therefore, we classified spotted fawns into two groups: 1) small spotted fawns were two weeks to two months old, they do not reach the height of the mother's

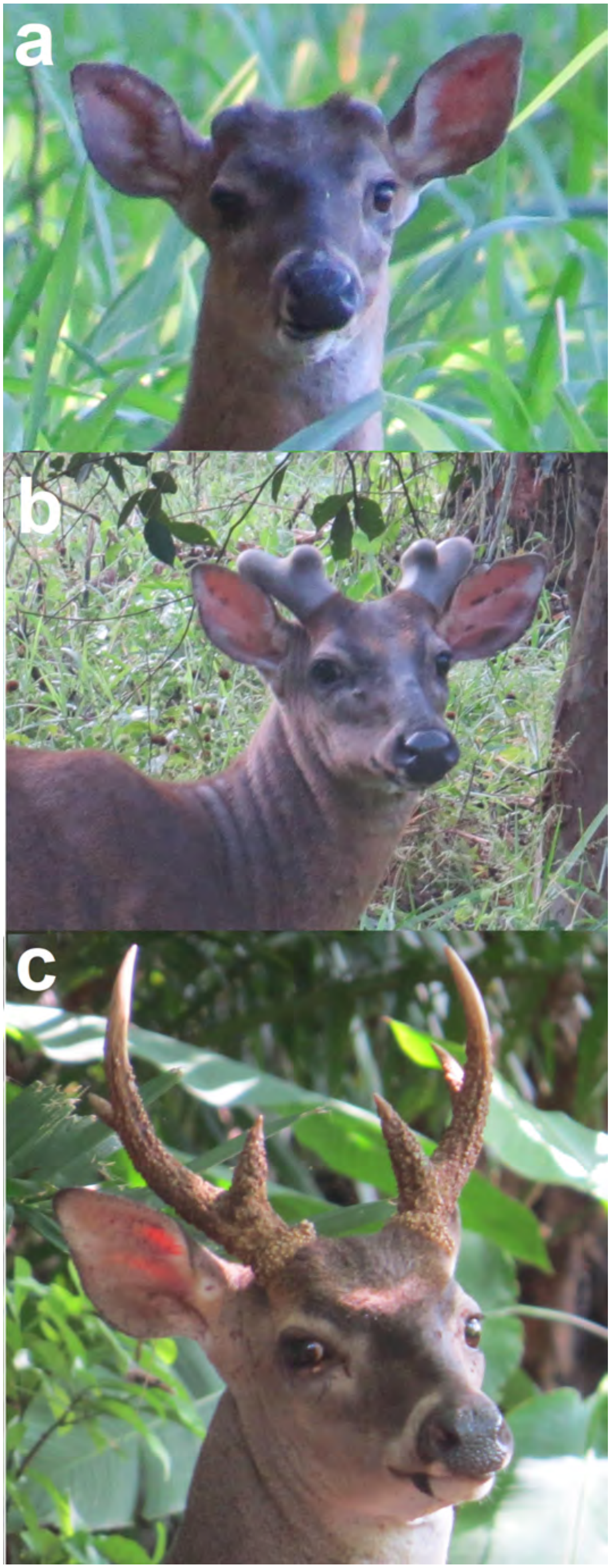

Figure 2. Antler growth stages of white-tailed deer (*Odocoileus virginianus*) at Hacienda y Refugio de Vida Silvestre Curú, Northwestern Costa Rica: a) nubs, b) velvet, c) hard.

belly, and they are generally observed alone in the forest or accompanied by the mother, and 2) large spotted fawns were three to four months old, they exceed the height of the mother's belly, and they are usually accompanied by the mother and other deer.

To test for the independence between the status of antler growth and the month of the year (which are related to the amount of rain) we analyzed the data with a Chi Square Test. We compared the absolute number of white-tailed deer (1,134) according to antler status per month. We used the adjusted residuals of the Chi Square Test as a posteriori test to evaluate in which cells of the contingency table it is observed more or less frequently than is expected at random. The critical value is the range from -1.96 to 1.96, so the values outside this range differ from the frequency expected by pure chance (Table 2).

Results

We totalized 1,134 observations of the status of antler growth of male white-tailed deer at Curú (n = 233 in 2016, n = 367 in 2017, n = 294 in 2018 and n = 240 in 2019; Table 1). The way we walked the transects and our knowledge of the area reduced the probability to count any male more than once. Additionally, we recorded 13 observations on reproductive behavior and 133 observations of spotted fawns.

Antlers. The antler cycle of the white-tailed deer at Curú was seasonal in the four years of study ($\chi^2 = 970$, d.f. = 22, $P < 0.001$). Antlers casting occurred over a three-month period that began in mid-November and ended in mid-February, during the first part of the dry season. The earliest antlerless or single-antlered deer were observed on 19 November 2017. The last single-antlered deer was observed on 5 February 2017. Antlerless or single-antlered males were not observed in any other month. Antlers grew during the dry season from mid-December to mid-March. A hundred percent of the males had velvet antlers in March in the four years of study (Figure 3a, Table 1).

Velvet shedding started in April and lasted through May and June, just at the beginning of the rainy season. In July less than 5 % of the males presented velvet antlers. We did not observe males with velvet antlers from August to October. We observed males with hard antlers from April until January or February of the following year. From July to October, 100 % of the males had hard antlers (Figure 3a, Table 1).

There was a significantly lower frequency of hard antlers than expected by chance in the first five months of the year, and significantly more frequency from June to December (Table 2). On the opposite, there was significantly higher frequency of antlers in velvet than expected from February to May and a lower frequency from July to January of the following year. Nubs were less frequent than expected in general during the year except in January and December, although differences were not significant in February, September, and November (Table 2).

Table 1. The absolute number of white-tailed deer (*Odocoileus virginianus*) according to antler status per month in the Hacienda y Refugio de Vida Silvestre Curú between January 2016 and December 2019. Nicoya Peninsula, Costa Rica.

	Antler status			
Month	**Hard**	**Velvet**	**Nubs**	**n**
Jan	33	37	47	117
Feb	4	106	9	119
Mar	0	96	0	96
Apr	7	93	0	100
May	25	78	0	103
Jun	75	42	0	117
Jul	88	2	0	90
Aug	82	0	0	82
Set	42	0	0	42
Oct	70	0	1	71
Nov	69	1	8	78
Dec	80	15	24	119
			Total	1,134

$\chi^2 = 970$, d.f. = 22, $P < 0.001$.

Rutting season. The rutting season of white-tailed deer in Curú Refuge lasted three months from mid-June to mid-August. Rutting began when adult males were ready to reproduce, indicated by their hard antlers and reproductive

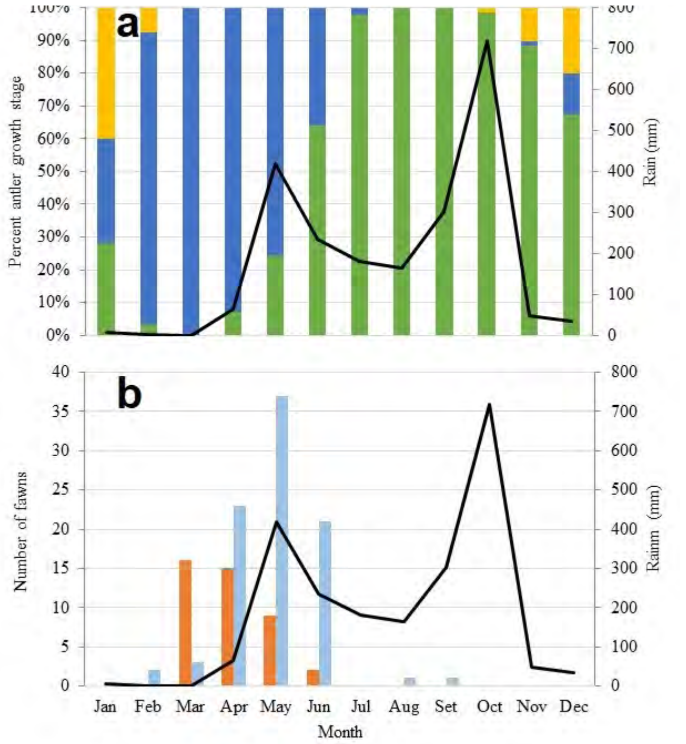

Figure 3. a) Monthly relative frequency (%) of antler status (n = 1134, monthly range 42-119). Hard (green), velvet (blue) and nubs (yellow). b) number of monthly observations of small spotted fawns (orange) and large spotted fawns (light blue) of white-tailed deer (*Odocoileus virginianus*) in Hacienda y Refugio de Vida Silvestre Curú between January 2016 and December 2019. Black lines indicate the average monthly precipitation (mm) in the same period at the Paquera Station: 2016 = 1,473.4 mm; 2017 = 2,475.8 mm; 2018 = 2,436.4 mm; 2019 = 2,327.8 mm (IMN 2020a), Nicoya Peninsula, Costa Rica.

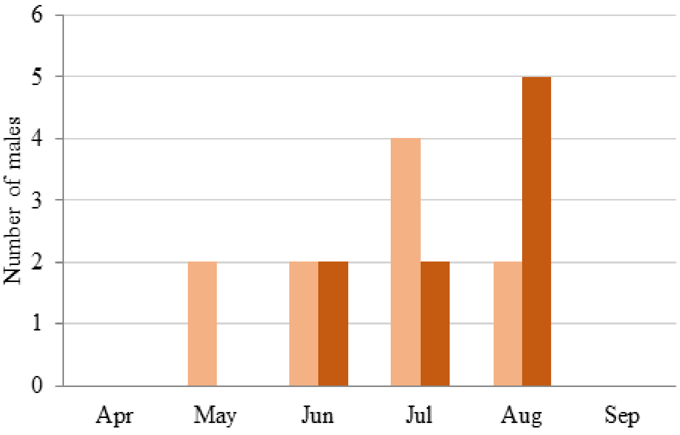

Figure 4. The number of males white-tailed deer (*Odocoileus virginianus*) per month that chased females, and the number of males per month that accompanied females, between January 2016 and December 2019 (other months have cero males engage in these activities). Hacienda y Refugio de Vida Silvestre Curú, Nicoya Peninsula, Costa Rica. Chasing deer males (pink) and tending deer males (brown).

behavior. In mid-June, males started rubbing antlers, scraping, and sparring. This behavior lasted for the first part of the rainy season. They adopted tending bond mating systems, courtship and copulating receptive does in August and September when males had hard antlers (Figures 3a, 4). The first males observed chasing does entering estrus were in August. We observed does accompanied by bucks in August and September, and by October neither chasing nor courtships were observed (Figure 4).

Fawning season. Small spotted fawns (Figure 5) were observed between March and June (Figure 3a); this is from the middle of the dry season to the beginning of the rainy season. During March most of the observations were of small spotted fawns (16 out of 19), while only three observations corresponded to large spotted fawns (Figure 3a). On the opposite, observations of large spotted fawns (Figure 5) predominated in April (23 out of 38).

A peak of spotted fawns was reached in May, although most of them were large-bodied spotted fawns (37 out of 46). Large-bodied spotted fawns (21 out of 23) also predominated in June when only two small spotted fawns were observed (Figure 3a). Fawns were five months old by July and had no spots. We did not see any spotted fawns after June, except by two large spotted fawns in August and September 2017, that were born probably by April-May given their sizes.

Discussion

Because there are comparatively few studies of white-tailed deer in the Neotropical region, a general conclusion emerged over time that white-tailed deer would be less seasonal in antler growth and casting in regions without strong seasonal changes in weather (Marchinton and Hirth 1984). The assumption was that the same would happen with other reproductive processes such as rut and fawn births. However, some studies challenge this generalization. White-tailed deer reproduction seemed seasonal, lasting four to six months, as reported from limited observations in the Virgin Islands (Webb and Nellis 1981), Honduras (Klein 1982), Venezuela (Brokx 1984), Colombia (Blouch 1987) and Surinam (Branan and Marchinton 1987). A recent study in Campeche wetlands in Southeastern México, a tropical site, showed that the antler cycle of the white-tailed deer is seasonal (Contreras-Moreno *et al.* 2019).

Studies of white-tailed deer reproduction in Costa Rica have shown considerable variation in reproductive processes among relatively nearby populations. Rodríguez (1994) found no seasonality in antlers casting and growth or in fawn births in Palo Verde National Park in the mid- 1990s and recently Fuller *et al.* (2020) obtained similar results in a study in Santa Rosa National Park. However, on San Lucas Island, situated approximately 78 km in a straight line from Santa Rosa, the white-tailed deer life cycle had a marked seasonality (Rodríguez and Solís 1994). Antler growth occurred from March to June, velvet shedding in June and July, hard antlers from July to December, and antler casting in January and February (Rodríguez and Solís 1994). Antler status pattern, as well as fawn births, were even more seasonal in Curú than in San Lucas Island, the only real dry forest in Costa Rica included in this comparison (Figure 1). These processes took three months to complete in Curú. Some minor variations in the onset of antler casting and velvet shedding appear to be responses to variations in precipitation patterns between consecutive years. The first signs of the start of the rut appeared in June in Curú when antler velvet had dried and shed, and bucks had hard antlers (Figure 2c). Antler cycle as well all other processes were significantly seasonal (Table 2). Antler cycle seasonality in Curú coincides with the antler cycle of the white-tailed deer in a tropical site in southeastern México (Contreras-Moreno *et al.* 2019). In Curú, fawns were five months old by July and

Table 2. Value of adjusted residuals of the χ^2 test for antler status of white-tailed deer (*Odocoileus virginianus*) per month in the Hacienda y Refugio de Vida Silvestre Curú Nicoya Peninsula, Costa Rica, between January 2016 and December 2019. Residuals in bold indicate significant differences. The value of the sign indicates if the frequency is higher (+) or lower (-) than expected by chance.

	Antler status		
Month	**Hard**	**Velvet**	**Nubs**
Jan	**-5.140**	**-2.277**	**13.728**
Feb	**-10.919**	**11.148**	-0.122
Mar	**-10.387**	**12.172**	**-2.988**
Apr	**-9.155**	**10.959**	**-3.056**
May	**-5.628**	**7.407**	**-3.106**
Jun	**3.061**	-1.286	**-3.333**
Jul	**9.309**	**-7.872**	**-2.885**
Aug	**9.270**	**-7.909**	**-2.744**
Set	**6.512**	**-5.556**	-1.927
Oct	**8.336**	**-7.322**	**-2.084**
Nov	**6.912**	**-7.462**	0.819
Dec	**3.810**	**-6.750**	**5.282**

had no spots, which means they were born in February but were not observed sooner due to their hiding behavior during the first weeks of life (Rodríguez 2015).

Rain seasonality at Santa Rosa National Park (Fuller *et al.* 2020) is similar to that of Curú. Rainfall peaks in May - June, decrease in July – August, and peaks again with greater precipitation around October. That could explain why the phenology of Curú vegetation demonstrates similar patterns to those of other sectors in Northwestern Costa Rica where many dry forest species are deciduous and recover much of their foliage at the beginning of the rainy season in May and June (Castro *et al.* 2018). However, several tree species maintain foliage throughout the dry season, as do half of the shrubs, saplings, and seedlings (Frankie *et al.* 1974; Opler *et al.* 1980), and many animal species reflect these patterns (Rockwood 1975; Janzen 1993; Orkin *et al.* 2019).

Curú is located in a more humid life zone than Santa Rosa (Figure 1), but this seems to not affect the white-tailed deer reproductive cycle. Although the rainy season determines the availability of food, which is a proximate cause of high variation of the reproductive cycle in Santa Rosa during the year (Fuller *et al.* 2020), it does not seem to have a strong influence on the deer cycle in Curú. However, there was a coincidence between the peak of precipitation occurring in May and the highest number of fawns with spots (36, Figure 3a).

Lack of seasonal variation in the photoperiod is the decisive factor that likely favor deer reproduction throughout the year in Santa Rosa (Fuller *et al.* 2020).Yet this variation is identical in Curú, where the reproduction of the species is seasonal. The peak of births in February and March ensures the survival of the fawns, which will be weaned at the beginning of the rainy season when there is plenty of food. The high amount of rain in September and October, including flooding, will not affect the fawns because they are already grown up by this time of the year (Figure 3a). However, these severe rains and flooding could be an impacting seasonal factor on the fawning season at Curú. The association of flooding with changes in the fawning season has been reported in white-tailed deer populations that occur in areas affected by severe flooding such as the Everglades in Florida (MacDonald-Beyers and Labisky 2005).

The reproduction pattern we observed in Curú is similar to the pattern on San Lucas Island, located in a dry forest at a distance less than 20 km in a straight line (Rodríguez and Solís 1984). What is different among the sites? The traditional or more commonly used system of ecosystem classification in Costa Rica is Holdridge´s Life Zones (Bolaños *et al.* 2005). In this classification, Santa Rosa and Palo Verde are in the Pre-montane humid Forest Life Zone and Curú in the Tropical humid Forest zone, leaving San Lucas Island as the only one of the sites included in our comparison that is located in a tropical dry forest. This explains to some degree the highly seasonal reproductive process of the white-tailed deer in San Lucas, but it does not explain the seasonality pattern we found in Curú. However, Curú is even more humid than Santa Rosa and Palo Verde, even though all three of these sites as well as San Lucas have a marked dry season. Rainfall in Curú is higher than in Santa Rosa, and it may make a difference in year-round food availability, but severe rains and flooding by September and October can have a more determining impact on fawning seasonality.

Figure 5. a) Small spotted fawn and b) large spotted fawn of white-tailed deer (*Odocoileus virginianus*) at Hacienda y Refugio de Vida Silvestre Curú, Northwestern Costa Rica.

The reproductive patterns of white-tailed deer in Central and South America may have evolved in response to seasonal fluctuations in specific food availability, competition, or predation, all of which may be related to rainfall patterns (Asher 2011). However, these factors do not explain why white-tailed deer reproduction is seasonal in some places, but not in others that are very close. Is there a direct relationship to weather or is some other factor that differs between the sites responsible for the differences in white-tailed deer reproduction? Are white-tailed deer more genetically related to North American forms that are more seasonal, or does another factor make them different from non-seasonal deer? It appears that white-tailed deer exhibit a very flexible and therefore variable reproductive pattern (Fuller *et al.* 2020). More research is needed to understand what factors more directly explain the observed pattern in the white-tailed deer reproduction.

Acknowledgements

Our deep appreciation to the Schutt Valle family for allowing us to conduct the research and for their hospitality at Curú. We greatly appreciate the language review and input by Mickie Swisher. We also thank Eduardo Mendoza and three anonymous reviewers for their corrections and suggestions. Lucía López helped us with the preparation of the figures. JMM acknowledges Emilce Rivera, GEC department head, Sede Central, Universidad Técnica Nacional, Costa Rica, for her continuous academic support.

Literature cited

Asher, G. W. 2011. Reproductive cycles of deer. Animal Reproduction Science 124:170–175.

Blouch, R. 1987. Reproductive seasonality of white-tailed deer on the Colombian Llanos. Pp. 339–343, *in* Biology and management of the Cervidae (Wemmer, C., ed.). Smithsonian Institution Press. Washington, U.S.A.

Bolaños, R., V. Watson, and J. Tosi. 2005. Mapa ecológico de Costa Rica (Zonas de Vida), según el sistema de clasificación de zonas de vida del mundo de L. R. Holdridge, Scale 1:750 000. Centro Científico Tropical. San José, Costa Rica.

Branan, W., and R. Marchinton. 1987. Reproductive ecology in white-tailed deer and red Brocket deer in Suriname. Pp. 344–351, *in* Biology and management of the Cervidae (Wemmer, C., ed.). Smithsonian Institution Press, Washington, U.S.A.

Brokx, P. 1984. South America. Pp. 525–546, *in* White-tailed deer: ecology and management (Halls, L. K., ed.). Stackpole Press. Harrisburg, U.S.A.

Bronson, F. H. 2009. Climate change and seasonal reproduction in mammals. Philosophical Transactions of the Royal Society of London, B. Biological Sciences 364:3331–3340.

Castro, S. M., G. A. Sánchez-Azofeifa, and H. Sato. 2018. Effect of drought on productivity in a Costa Rican tropical dry forest. Environmental Research Letters 13:045001.

Contreras-Moreno, F. M., *et al*. 2019. Seasonal antler cycle in white-tailed deer in Campeche wetlands in Southeastern Mexico. European Journal of Wildlife Research 65:53.

Ditchkoff, S. S. 2011. Anatomy and physiology. Pp. 43–73, *in* Biology and management of white-tailed deer (Hewitt, D. G. , ed.). CRC Press/Taylor and Francis Group. Boca Raton, U.S.A.

Frankie, G. W., H. G. Baker, and P. A. Opler. 1974. Comparative phenological studies of trees in tropical wet and dry forests in the lowlands of Costa Rica. Journal of Ecology 62:881–919.

Fuller, T. K., A. M. Silva, V. H. Montalvo, C. Sáenz-Bolaños, and E. Carrillo. 2020. Reproduction of white-tailed deer in a seasonally dry tropical forest of Costa Rica: a test of aseasonality. Journal of Mammalogy 101:241–247.

Goldman, B. D. 2001. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. Journal of Biological Rhythms 16:283–301.

Goss, R. J. 1983. Deer antlers – regeneration, function, and evolution. Academic Press. New York, U.S.A.

Hanon, E. A., *et al*. 2008. Ancestral TSH mechanism signals summer in a photoperiodic mammal. Current Biology 18:1147–1152.

Holdridge, L. R. 1967. Life Zone Ecology. Tropical Science Center. San José, Costa Rica.

IMN (Instituto Meteorológico Nacional). **2020a**. Boletín Meteorológico Mensual. San José, Costa Rica. https://www.imn.ac.cr/boletin-meteorologico. Accessed on 3 May 2020.

IMN (Instituto Meteorológico Nacional). 2020b. Clima en Costa Rica. https://www.imn.ac.cr. Accessed on 03 May 2020.

Janzen, D. H. 1993. Caterpillar seasonality in a Costa Rican dry forest. Pp. 448–477, *in* Caterpillars – ecology and evolutionary constraints on foraging (Stamp, N. E., and T. M. Casey, eds.). Chapman and Hall. New York, U.S.A.

Klein, E. 1982. Phenology of breeding and antler growth in white-tailed deer in Honduras. Journal of Wildlife Management 46:826–829.

Lincoln, G. A. 1992. Biology of seasonal breeding in deer. Pp. 565–574, *in* The biology of deer (Brown, R. D., ed.). Springer Verlag. New York, U.S.A.

Loudon, A. S. I., and J. D. Curlewis. 1988. Cycles of antler and testicular growth in an aseasonal tropical deer (*Axis axis*). Journal of Reproduction and Fertility 83:729–738.

MacDonald-Beyers, K., and R. F. Labisky. 2005. Influence of flood waters on survival, reproduction, and habitat use of white-tailed deer in the Florida Everglades. Wetlands 25:659–666.

Marchinton, R., and D. Hirth. 1984. Behavior. Pp. 129–168, *in* White-tailed deer: ecology and management (Halls, L. K., ed.). Stackpole Press. Harrisburg, U.S.A.

Mattioli, S. 2011. Family Cervidae (deer). Pp. 350-443, *in* Handbook of the mammals of the world, volume 2: Hoofed Mammals (Wilson, D. E., and R. A. Mittermeier, eds.). Lynx Edicions. Barcelona, Spain.

McKinney, T. 2014. Species-specific responses to tourist interactions by white-faced capuchins (*Cebus imitator*) and mantled howlers (*Alouatta palliata*) in a Costa Rican wildlife refuge. International Journal of Primatology 35:573–589.

Molinari, J. 2007. Variación geográfica en los venados de cola blanca (Cervidae, *Odocoileus*) de Venezuela, con énfasis en *O. margaritae*, la especie enana de la Isla de Margarita. Memoria de la Fundación La Salle de Ciencias Naturales 167:29–72.

Opler, P. A., G. W. Frankie, and H. G. Baker. 1980. Comparative phenological studies of treelet and shrub species in tropical wet and dry forests in the lowlands of Costa Rica. Journal of Ecology 68:167–188.

Orkin, J. D., *et al*. 2019. Seasonality of the gut microbiota of free-ranging white-faced capuchins in a tropical dry forest. The ISME Journal 13:183–196.

Price, J., C. Faucheux, and S. Allen. 2005a. Deer antlers as a model of mammalian regeneration. Current Topics in Developmental Biology 67:1-48.

Price, J., S. Allen, C. Faucheux, T. Althnaian, and J. C. Mount. 2005b. Deer antlers: a zoological curiosity or the key to understanding organ regeneration in mammals? Journal of Anatomy 207:603–618.

Robinson, M. R., J. W. Thomas, and R. G. Marburguer. 1965. The reproductive cycle of white-tailed deer in Central Texas. Journal of Wildlife Management 29:53–59.

Rockwood, L. L. 1975. The effects of seasonality on foraging in two species of leaf-cutting ants (*Atta*) in Guanacaste Province, Costa Rica. Biotropica 7:176–193.

Rodríguez, M., and V. Solís. 1994. Ciclo de vida del venado cola blanca en la Isla San Lucas, Costa Rica. Pp. 63–71, *in* Ecología y Manejo del Venado Cola Blanca en México y Costa Rica (Vaughan, C., and M. A. Rodríguez, eds.). EUNA. Heredia, Costa Rica.

Rodríguez, M. 1994. Ciclo de las astas del venado cola blanca en el Refugio Nacional de Vida Silvestre Palo Verde, Guanacaste, Costa Rica. Pp. 103–109, *in* Ecología y Manejo del Venado Cola Blanca en México y Costa Rica (Vaughan, C., and M. A. Rodríguez, eds.). EUNA. Heredia, Costa Rica.

Rodríguez. M. A. 2015. El venado cola blanca en el bosque seco tropical de Costa Rica. Windmills Editions, California, U.S.A.

Sauer, P. R. 1984. Physical Characteristics. Pp. 73–90, *in* White-tailed deer: ecology and management (Halls, L. K., ed.). Stackpole Press. Harrisburg, U.S.A.

Schutt, A., and C. Vaughan-Dickhaut. 1995. Incorporating wildlife into development: the case of the Curú Wildlife Refuge and Farm, Costa Rica. Pp 250–254, *in* International Wildlife Management Congress (Bissonette, J. A., and P. R. Krausman, eds.). San José, Costa Rica.

Smith, W. P. 1991. *Odocoileus virginianus*. Mammalian Species 388:1–13.

Teer, J. 1994. El Venado Cola Blanca: Historia Natural y Principios de Manejo. Pp. 33–47, *in* Ecología y Manejo del Venado Cola Blanca en México y Costa Rica (Vaughan, C., and M. A. Rodríguez, eds.). EUNA. Heredia, Costa Rica.

Tomás, W. M. 1995. Seasonality of the antler cycle of pampas deer (*Ozotoceros bezoarticus leucogaster*) from the Pantanal Wetland, Brazil. Studies on Neotropical Fauna and Environment 30:221–227.

Vasantha, I. 2016. Physiology of seasonal breeding: A review. Journal of Veterinary Science and Technology 7:331.

Verme J. L., and D. E. Ullrey. 1984. Physiology and Nutrition. Pp. 91–118, *in* White-tailed deer: ecology and management (Halls, L. K., ed.). Stackpole Press. Harrisburg, U.S.A.

Webb, J., and D. Nellis. 1981. Reproductive cycle of white-tailed deer at St. Croix, Virgin Islands, USA. Journal of Wildlife Management 45:253–258.

Weber, M., P. Rosas-Becemil, A. Morales-García, and C. Galindo-Leal. 1994. Biología reproductiva del venado cola blanca en Durango, México. Pp. 111–127, *in* Ecología y Manejo del Venado Cola Blanca en México y Costa Rica (Vaughan, C., and M. A. Rodríguez, eds.). EUNA. Heredia, Costa Rica.

Associated editor: Eduardo Mendoza
Submitted: March 24, 2021; Reviewed: April 17, 2021
Accepted: November 15, 2021; Published on line: March 23, 2022

THERYA, 2022, Vol. 13(2):153-161 DOI:10.12933/therya-22-2099 ISSN 2007-3364

Daytime diet of the lesser sac-winged bat (*Saccopteryx leptura*) in a Colombian Pacific Island

Isabela Vivas-Toro[1,2]*, and Julián Alexander Mendivil-Nieto[3]

[1] Museo de Zoología "Alfonso L. Herrera", Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior s/n, CP. 04510. Ciudad Universitaria. Ciudad de México, México. Email: isavivas94@gmail.com (IV-T).
[2] Departamento de Biología, Facultad de Ciencias Naturales y Exactas, Universidad del Valle, Colombia.
[3] Grupo de Investigaciones Entomológicas, Departamento de Biología, Facultad de Ciencias Naturales y Exactas, Universidad del Valle, Colombia. Email: chalcidoidea88@gmail.com (JAM-N).
* Corresponding author

Insectivorous bats represent more than half of all the Chiropterans of the world. Although they are important stabilizers of insect populations within their habitat due to their feeding habits, just few studies have been focused on the diet of insular bat species. The lesser sac-winged bat, *Saccopteryx leptura,* is widely distributed in the new world tropics, but little is known about its prey selection. In this study we determined the diet composition of the population of *S. leptura* from Gorgona Island, Colombia, using stomach and intestinal content samples. We focused our research on their atypical daytime feeding behavior to evaluate differences in prey selection considering two main factors: 1) plant canopy cover and 2) bat sex. We found prey representatives of nine orders of insects, with Hymenoptera, Coleoptera and Hemiptera as the most consumed according to their abundance and volume percentage. We identified two new records at genus level in *S. leptura´s diet, Camponotus* (Formicidae) and *Trigona* (Apidae). In general terms, we did not find differences in the diet between canopy covers nor sexes. However, when analyzing the consumed percentage volume by order, there were significant differences in consumption of Psocoptera in both factors. Our results suggest that *S. leptura* has an opportunistic diet, as they tend to feed on prey of a wide range of sizes, usually the most abundant and available in the environment.

Los murciélagos insectívoros representan más de la mitad de la diversidad de quirópteros del mundo. Aunque son importantes estabilizadores de las poblaciones de insectos dentro de su hábitat debido a sus hábitos de alimentación, pocos estudios se han centrado en la dieta de las especies de murciélagos insulares. El murciélago de sacos pequeño, *Saccopteryx leptura,* es una especie ampliamente distribuida en el trópico del nuevo mundo, pero con poca información sobre su dieta. En este estudio se determinó la composición de la dieta de una población de *S. leptura* en la isla Gorgona, a partir de muestras de contenido estomacal e intestinal. Esta investigación se centró en su patrón atípico de alimentación diurna para evaluar las diferencias en la selección de presas considerando dos factores: (1) la cobertura de dosel y (2) el sexo. Se encontraron representantes de nueve órdenes de insectos, siendo Hymenoptera, Coleoptera y Hemiptera los más consumidos, según la abundancia y porcentaje de volumen. Se identificaron dos nuevos registros a nivel de género dentro de la dieta de *S. leptura*, *Camponotus* (Formicidae) y *Trigona* (Apidae). En términos generales, no se encontraron diferencias de la dieta entre coberturas de dosel o entre sexos. Sin embargo, al analizar el porcentaje de volumen consumido a nivel de orden, se encontraron diferencias significativas en el consumo del orden Psocoptera para ambos factores. Los resultados sugieren que *S. leptura* adopta una alimentación oportunista, ya que consume presas de una amplia gama tamaños, usualmente las más abundantes y disponibles en el medio.

Keywords: Daytime activity; diet; Gorgona island; insectivorous bat; intestinal content; stomach content.

Introduction

Insectivorous and arthropodophagous bats represent about 70 % of the world's Chiropteran diversity, and more than half of its Neotropical diversity (Fenton and Simmons 2014). Their eating habits highlight their importance as crop pest controllers and stabilizers of arthropod populations (Boyles *et al.* 2011; Boyles *et al.* 2013). However, little is known about the feeding habits of insular arthropodophagous and insectivorous bat species, which, unlike their mainland conspecifics, have limited resources that depend on the availability and population dynamics of the island's exclusive invertebrates, as well as on the competition with sympatric species (Sedlock *et al.* 2014). This means that island bats must adapt to the restrictions imposed by their environment to survive (McNab 2010). Some bat trophic strategies reported on islands are: 1) opting for a generalist diet (Razakarivony *et al.* 2005; Rakotoarivelo *et al.* 2007; Racey *et al.* 2010), 2) resource partitioning to reduce competition (Zhang *et al.* 2005; Fukui *et al.* 2009; Rolfe *et al.* 2014), and 3) modifying their foraging periods according to the activity patterns of their prey (Bradbury and Vehrencamp 1976; Pavey *et al.* 2001).

The lesser sac-winged bat, *Saccopteryx leptura*, is a small insectivorous bat that is widely distributed in the new world tropics, mainly in the intertropical zone. This species can live in a wide range of habitats, from preserved tropical forests to urban areas, and there are even populations on islands (Bradbury and Emmons 1974; Cadena *et al.* 1990; Vivas-Toro and Murillo-García 2019). Knowledge about *S. leptura* is scarce, including that concerning its feeding habits. Bradbury and Vehrencamp (1976) made an approximation of *S. leptura*'s diet composition based on analyses of other emballonurid sympatric species, which suggested they have a diet mainly consisting of Coleoptera and Diptera, with lower consumption of Lepidoptera. However, there are two studies (albeit consisting of few individuals or samples) that propose Hymenoptera as the most consumed order (Nogueira *et al.* 2002; Cruz-Parrado *et al.* 2018). Although these studies provide valuable contributions, they still represent first data regarding the diet of *S. leptura*.

Interestingly, *S. leptura* has shown a positive response to moonlight intensity, implying higher activity at brighter nights (Appel *et al.* 2017, 2019). Furthermore, this species has also been reported foraging during the day on islands such as Trinidad (Bradbury and Emmons 1974) and Gorgona (Vivas-Toro and Murillo-García 2020), generating more questions concerning *S. leptura* feeding habits and what they eat during those time frames. Frequent daytime activity of insectivorous bats is unusual (Russo *et al.* 2011a). Except for the early forager *Pipistrellus pygmaeus* from central Italy (Russo *et al.* 2011a), to date, all species reported exhibiting this behavior reside on islands (Bradbury and Emmons 1974; Moore 1975; Russo *et al.* 2011a; Russo *et al.* 2011b; Chua and Aziz 2019; Vivas-Toro and Murillo-García 2020). Among the common patterns displayed by these bats during the day are the tendency to feed mainly in closed canopy sites with abundant insect prey, and a decreasing activity with increasing light intensity (Speakman 1995; Russo *et al.* 2011a; Russo *et al.* 2011b; Chua and Aziz 2019; Vivas-Toro and Murillo-García 2020). There is no detailed information on the selection of prey during the day in most species of bats that exhibit these habits, however, *P. pygmaeus* tends to feed on insect swarms when available, particularly nematocerans flies, and on the most abundant taxa, such as brachycerans (Russo *et al.* 2011b).

Various studies have shown that bats foraging emergence times are closely related to canopy cover and forest closure (Russo *et al.* 2007; Marques *et al.* 2015), being common the occurrence of earlier emergencies in cluttered areas compared with open areas (Jones *et al.* 1995; Russo *et al.* 2007; Thomas and Jacobs 2013). This has been associated to strategies to evade the detection of predators or the risk of hyperthermia (Speakman 1995) and to maximize foraging time (Jones and Rydell 1994; Duvergé *et al.* 2000). Furthermore, the early emergence and late return of pregnant and lactating females in some species has been observed, which has been considered a strategy to feed for longer to meet energy demands during reproduction (Shiel and Fairley 1999; Duvergé *et al.* 2000; Lee and McCracken 2001).

There are currently 15 bat species reported for Gorgona Island, 9 of them are insectivorous (Murillo *et al.* 2014). Since *S. leptura* is abundant on Gorgona and exhibits an extended active period (Vivas-Toro and Murillo-García 2020), its insular population is suitable for studying diet composition and feeding habits of the species. Therefore, the aim of this study is to describe the diet composition of *S. leptura*, based on the daytime foraging of an insular resident population. Particularly, we seek to answer to the following questions: 1) are there differences in *S. leptura* prey consumption according to the canopy cover? 2) Are there differences in prey selection between males and females? 3) Is daytime feeding a good representation of the diet of *S. leptura*?

Materials and Methods

This study was conducted in Gorgona National Natural Park (GNNP; 2° 58′ 4″ N, -78° 11′ 3″ W), a continental island of volcanic origin located 35 km from the Pacific coast of Colombia (Giraldo 2012). The island has an equatorial climate with super-humid rainforest vegetation, that is part of the Chocó biogeographic province, and is mainly constituted by secondary forest (Vásquez-Vélez 2014). We determined *S. leptura*´*s* diet by analyzing stomach content and fecal samples collected in roosts only occupied by *S. leptura*, or from bats captured in flight. Sampling was carried out over 10 days between July and November 2017, five days during each month, in areas with high (≥ 70 %) and low (≤ 35 %) plant canopy cover. We collected fecal samples from roosts between 8:00 and 15:00 h. We collected fresh scats which were no more than four hours old; that is, of a soft consistency; a moist, dark color; and recognizable odor. We consider the foraging territory of bats in roosts, who was recorded in a previous study (Vivas-Toro and Murillo-García 2020), to define what type of canopy cover the samples belonged to. We captured bats between 17:00 and 19:00 h using a hand net and kept them in individual cloth bags until they defecated. All individuals were released after collecting the droppings. We carried out all captures and management of bats following the protocol for obtaining data on mammals on GNNP (Murillo *et al.* 2011). We stored the samples individually in plastic vials filled with 80 % alcohol. We also analyzed the stomach content of a recently dead individual (not collected), found around 11:00 h in November. Since in most cases several taxonomic groups were detected within a single scat, for a better standardization of the samples, and to avoid possible biases in the subsequent analyzes (according to Whitaker *et al.* (1996) for differences in size or volume of the samples), each scat (of approximately 6 x 2 mm) was considered as an independent unit. We identified prey at the most specific taxonomic level possible, in most cases to family, based on all recognizable fragments such as legs, wings, antennae, heads, tegument, scales, and/or structures with diagnostic characteristics (Whitaker *et al.* 2009). We estimated prey size by reconstructing them by grouping structures and fragments found using a Nikon SMZ745 stereomicroscope. All the material was identified, size estimated and quantified according to the taxonomic criteria and knowledge of an expert taxonomist.

We calculated the frequency of occurrence (FO) as the number of scats including the taxa divided by the total number of scats; the percentage composition (C) as the number of individuals of each taxon divided by the total number of individuals from all taxa, multiplied by 100 (Deagle *et al.* 2018; Vallejo *et al.* 2019); and the percentage volume (V) as the total volume of each taxon in the fecal-stomach samples divided by the total volume of all samples, multiplied by 100 for each taxon within the samples. We used the spheroid volume formula to approximate the volume value for each prey (Toshiaki 2004). We performed an arcsine transformation of all percentage data to correct non-normality before the analyses (Zar 1984). We used V to determine whether there were significant differences in diet between canopy covers and sexes through independent non parametrical ANOVAs, since the data was not normally distributed. Additionally, we tested differences in prey consumption by order relating the percentage volume of each order with each independent variable (canopy cover and sex) trough simple lineal regressions (Moosman *et al.* 2012) or fitted for quasi-Poisson distributions according to prior examination of the distribution and dispersion of each data set. All the analyses were conducted in R v.4.0.2 (R Development Core Team 2021).

Results

Intestinal content. We collected 63 fecal samples, 24 pellets from 7 bats captured in flight and 39 pellets from roosts; 29 pellets in low canopy cover and 34 in high canopy cover; 29 pellets were from males, 13 from females, and 21 mixed samples from a roost occupied by 2 males and 2 females. We identified 493 prey items belonging to 9 orders and 23 families of insects (Table 1). We also found mites (Arachnida), which are not intentionally ingested, but are present due to their phoretic relationship with the consumed insects; thus, they were not considered in the analyses.

The average size of prey eaten by *S. leptura* on Gorgona island was 5.1 ± *2.1 mm,* ranging from 2 to 13 mm. The most abundant orders found were Hymenoptera (C = 32.25 %), Coleoptera (C = 30.83 %) and Hemiptera (C = 20.08 %; Figure 1), with Formicidae as the most consumed family (C = 18.46 %), followed by Chrysomelidae (C = 7.91 %) and Miridae (C = 7.71 %). The order Hymenoptera also comprised the highest percent volume (V = 36.25 %), followed by Hemiptera (V = 32.08 %) and Coleoptera (V = 21.18 %; Figure 1); with Formicidae as the most consumed family (V = 26.65 %), followed by Miridae (V = 15.46 %) and Chrysomelidae (V = 13.62 %) respectively. We identified the genus *Trigona* (stingless bees) and *Camponotus* (carpenter ants, two winged-morphospecies; Hymenoptera), representing new records in the diet of these bats. We also found two cephalic capsules of lepidopteran larvae.

We found no significant differences in the diet of *S. leptura* between between plant canopy covers ($X^2 = 0.004$, *df* = 1, $P > 0.05$) or between sexes ($X^2 = 0.370$, *df* = 1, $P > 0.05$). When analyzing by order, we only found significant differ-

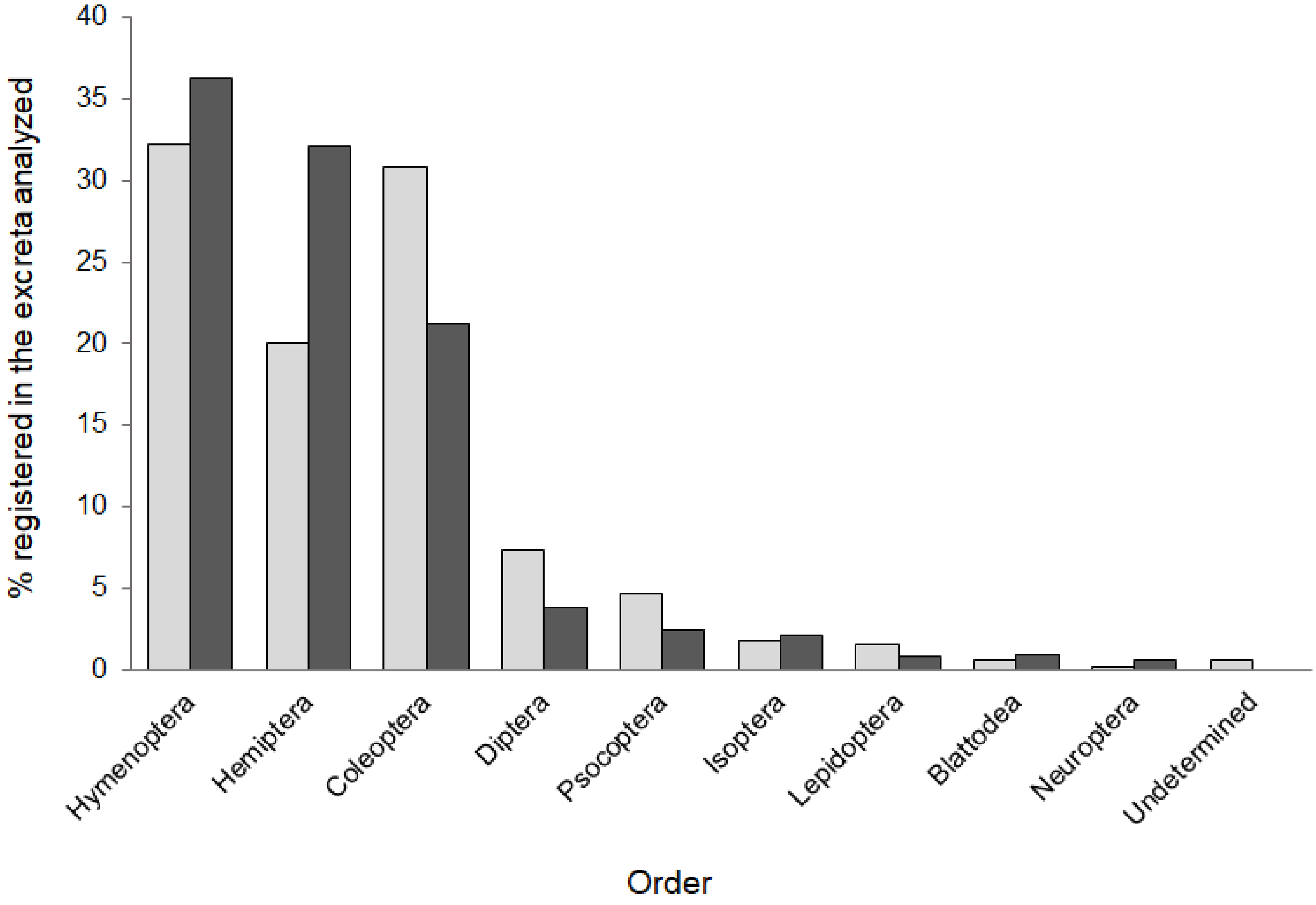

Figure 1. Percentage composition (light gray) and percentage volume (dark gray) of insect orders consumed by *Saccopteryx leptura* on Gorgona Island.

ences for Psocoptera, which were consumed more often in low canopy cover ($F_{1,61}$ = 9.38, $P < 0.01$; Figure 2a) and by males ($F_{1,40}$ = 6.62, $P < 0.05$; Figure 2b). That is, a volume of 74.40 % in low canopy cover vs. 25.60 % in high cover, and 90.27 % in males vs. 9.73 % in females from the total volume of Psocoptera consumed.

Stomach content. The stomach content of the *S. leptura* male we found dead during the study consisted of insects almost entirely of the order Hymenoptera (C = 94.40 %), with remains from the families Braconidae and Formicidae, and the subfamilies Myrmicinae and Formicinae (*Camponotus* sp.1 and *Camponotus* sp.2; Table 2); and a bupres-

Table 1. Diet of *Saccopteryx leptura* in Gorgona Island. Frequency of occurrence (FO), percentage composition (C) and percentage volume (V) of prey found in *S. leptura* feces. N = number of individuals.

Order	Suborder/Family	Minimum taxa level	N	FO	C	V
Blattodea			**3**	**0.05**	**0.61**	**0.88**
	Ectobiidae		1	0.02	0.20	0.72
	Undetermined		2	0.03	0.41	0.15
Isoptera	Termitidae		**9**	**0.14**	**1.83**	**2.05**
Hemiptera			**99**	**0.76**	**20.08**	**32.08**
	Delphacidae		3	0.06	0.61	0.97
	Cicadellidae		24	0.29	4.87	4.66
	Reduviidae		2	0.03	0.41	1.16
	Cixiidae		1	0.02	0.20	0.18
	Rhyparochromidae		3	0.05	0.61	0.37
	Miridae		38	0.38	7.71	15.46
	Auchenorrhyncha		6	0.11	1.22	1.27
	Fulgoromorpha		6	0.08	1.22	2.25
	Undetermined		16	0.25	3.25	6.18
Psocoptera			**23**	**0.35**	**4.67**	**2.40**
	Psocidae		6	0.10	1.22	0.77
	Epipsocidae		3	0.05	0.61	0.64
	Undetermined		14	0.21	2.84	0.98
Coleoptera			**151**	**0.86**	**30.83**	**21.18**
	Chrysomelidae		39	0.54	7.91	13.62
	Curculionidae		30	0.37	6.09	4.47
		Scolytinae	28	0.35	5.68	4.35
		Platypodinae	2	0.03	0.41	0.12
	Endomychidae		1	0.02	0.20	0.35
	Staphylinidae		6	0.11	1.22	1.23
	Undetermined		76	0.59	15.42	1.64
Neuroptera	Undetermined		**1**	**0.02**	**0.20**	**0.55**
Hymenoptera			**159**	**0.79**	**32.25**	**36.25**
	Formicidae		91	0.70	18.46	26.65
		Myrmicinae	18	0.21	3.65	0.87
		Formicinae	20	0.19	4.06	5.49
		Camponotus sp. 1	28	0.30	4.46	13.08
		Camponotus sp. 2	14	0.19	2.84	5.47
		Undetermined	17	0.19	3.41	1.75
	Apocrita (parasitica)		1	0.02	0.20	0.01
	Braconidae		16	0.17	3.25	1.26
	Ichneumonidae		2	0.03	0.41	0.32
	Halictidae		30	0.38	6.09	4.66
	Apoidea		1	0.03	0.20	0.26
	Apidae		15	0.17	3.04	2.49
		Trigona	10	0.11	2.03	1.04
		Undetermined	5	0.08	1.01	1.45
	Vespoidea		2	0.02	0.41	0.34

Table 1. Continuation...

Lepidoptera		**8**	**0.08**	**1.62**	**0.80**
	Noctuidae	4	0.03	0.81	0.37
	Undetermined[1]	2	0.02	0.41	0.06
	Undetermined	2	0.03	0.41	0.25
Diptera		**36**	**0.44**	**7.30**	**3.81**
	Muscomorpha	2	0.03	0.41	0.30
	Nematocera	3	0.05	0.61	0.21
	Ulidiidae	1	0.02	0.20	0.12
	Dolichopodidae	1	0.02	0.20	0.62
	Sciaridae	1	0.02	0.20	0.02
	Phoridae	1	0.02	0.20	0.06
	Undetermined	27	0.37	5.48	2.47
Undetermined		**3**	**0.03**	**0.61**	-

[1] Undetermined Lepidoptera family represented by larvae cephalic capsules.

tid beetle (C = 5.60 %). The largest prey found was a 13 mm *Camponotus* sp.2 ant; while the smallest was a 3 mm braconid wasp.

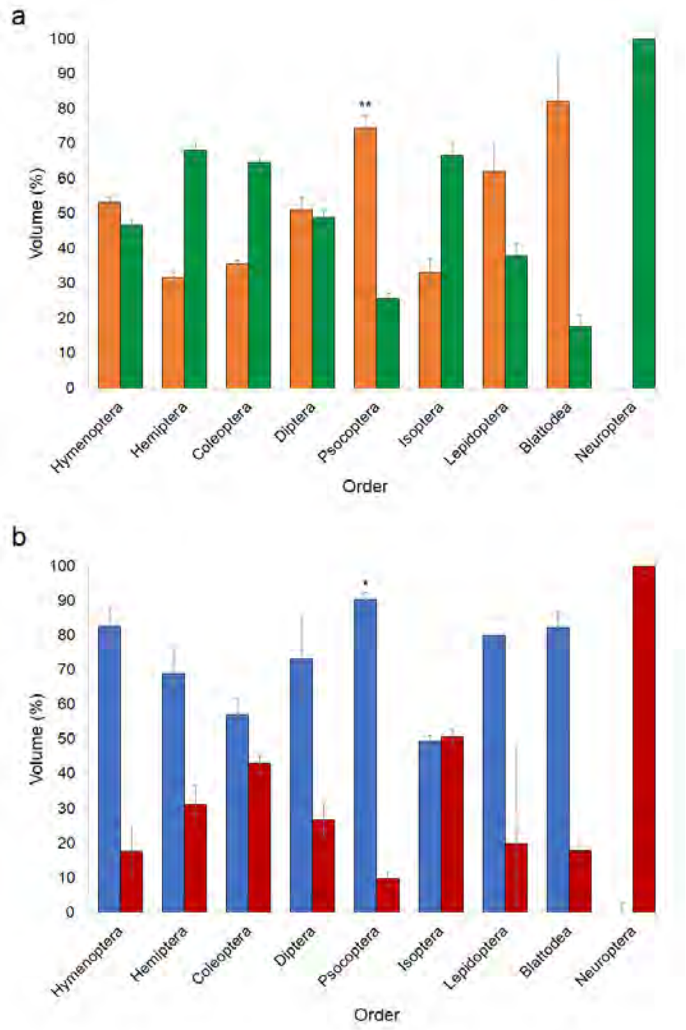

Figure 2. Variation in the diet of *Saccopteryx leptura* in Gorgona island in relation to (A) canopy cover and (B) sex. Percent volume and standard error are illustrated for each insect order. Asterisks represent significant differences in the diet of *S. leptura* according to individual regression tests for each order (* = $P < 0.05$; ** = $P < 0.01$). Colors code: orange, low canopy cover; green, high canopy cover; blue, males; red, females.

Discussion

Insectivorous bats tend to be selective with their food, their preferences may depend on features such as hunting methods and echolocation, prey characteristics such as size and texture (Barclay and Brigham 1994; Jung *et al.* 2007; Sedlock *et al.* 2014), the bat's cranial morphology, among others (Freeman 1979; Van Cakenberghe *et al.* 2002). Also, several studies have demonstrated that they can be flexible in terms of food selectivity according to the availability and seasonality of their prey (Whitaker 1994; Whitaker *et al.* 1996; Agosta *et al.* 2003), by inter or intraspecific competition (Whitaker 2004; Novella-Fernandez *et al.* 2020) and even due to their reproductive and/or developmental states (Verts *et al.* 1999; Agosta and Morton 2003). According to the stomach and fecal samples content found in this study, the insular population of *S. leptura* on Gorgona had a considerable variety of prey in its diet compared with what was previously reported in continental populations (Nogueira *et al.* 2002; Cruz-Parrado *et al.* 2018), and the approximations for *S. leptura* populations in Trinidad Island (Bradbury and Vehrencamp 1976). In general, we found that *Saccopteryx leptura* feeds on a wide variety of prey, from soft insects such as Psocoptera, Diptera and Lepidoptera; to harder insects such as Coleoptera and Hemiptera. For example, the relative consumption of Hymenoptera, in terms of composition and volume, was mainly determined by the FO of winged formicids in the samples (70 %). The latter can be explained by the abundance of these insects in the island since they are social insects whose populations and colonies are very numerous (Universidad del Valle 2014). Additionally, the caste of reproductive (winged) individuals within their colonies perform daytime nuptial flights consisting of hundreds of individuals, (Quirán and Corró 1998; Lara-Juárez *et al.* 2015). The consumption of winged ants has been previously reported in *S. leptura*´s diet, representing one of its most frequent prey (Nogueira *et al.* 2002; Cruz-Parrado *et al.* 2018). Many tropical bats feed on seasonal nuptial swarms of termites or ants, since they involve an important food resource, clustered in one single

Table 2. Percentage composition (C) and percentage volume (V) of prey found in the stomach content of a dead male specimen of *Saccopteryx leptura* found in Gorgona island. N = number of individuals.

Order	Family	Subfamily	Minimum taxa level	N	C	V
Coleoptera				**1**	**5.60**	**0.40**
	Buprestidae			1	5.60	0.40
Hymenoptera				**17**	**94.4**	**99.6**
	Formicidae	Myrmicinae		7	38.9	3.80
		Formicinae	*Camponotus* sp. 1	4	22.2	49.60
			Camponotus sp. 2	5	27.8	45.10
	Braconidae			1	5.60	1.20

area (Kunz *et al.* 1995; Pavey *et al.* 2001). There are even species such as *Rhinopoma microphyllum* that can feed almost exclusively on *Camponotus* alates during their summer massive nuptial flights (Levin *et al.* 2009). Therefore, since *S. leptura* also feeds during the day, it makes perfect sense that it takes advantage of the activity patterns of these formicidae swarms (mainly *Camponotus*) as an abundant food resource to complement its diet. It is worth noting that, as far as we know, the only prey identified at genus level in *S. leptura´*s diet is *Pheidole* (big-headed winged ant; Nogueira *et al.* 2002), therefore, both *Camponotus* and *Trigona* (Apidae) represent new records within the diet of these bats.

Similarly, the high consumption of beetles may be because Coleoptera is a very abundant order of flying insects on the island (Universidad del Valle 2014), and very accessible to the bats even during the daytime, which is demonstrated by the highest FO (86 %) among the insect orders in the samples. This coincides with the study conducted by Bradbury and Vehrencamp (1976), which demonstrated that the foraging cycles of *S. leptura* are spatially and temporally correlated with patterns of insect abundance in riparian forests in Costa Rica and Trinidad Island. On the latter, daytime flight was also reported for the species under the canopy. The average size of prey eaten by these bats in Gorgona (5.1 ± 2.1 mm) was almost twice the expected size of 2.6 mm suggested for bats of similar sizes (Bradbury and Vehrencamp 1976*)*. Also, the smallest prey captured (2 mm) was 1.3 times smaller than the expected average suggested by Bradbury and Vehrencamp (1976), and the largest (13 mm), was 2.6 times larger than that reported by Nogueira *et al.* (2002). Therefore, our results demonstrate the plasticity of *S. leptura* prey selection, compared with many insectivorous species that are very strict with their prey selection (Burles *et al.* 2008; Kolkert *et al.* 2019). This could be considered a strategy of the species to take advantage of as many resources as possible on the island through opportunism. Opportunistic feeding behavior has been recorded in many bats under a variety of environmental and competitive pressures (Brigham *et al.* 1992; Heim *et al.* 2017), showing a tendency to consume locally abundant taxa (Whitaker 2004; Krauel *et al.* 2018). Note that the representativity of other soft insects, such as Diptera, which is a very abundant group on Gorgona, and which have sizes within the range of prey consumed by *S. leptura*, could be underestimated, since they are easily destroyed during digestion. It is also worth noting the presence of two cephalic capsules of lepidoptera larvae within the food remains as an atypical finding, since, from what is known, *S. leptura* only hunt airborne prey; gleaning or trawling behaviors have not been reported to date (Kalko 1995; Jakobsen *et al.* 2015). However, this could be explained by the fact that there are larvae of Noctuidae and Geometridae (both present in the island) that, in their first development stages, hang on silk threads to evade parasitoids and predators (Hagstrum and Subramanyam 2010), thus, facilitating its detection and predation by *S. leptura*.

The significant differences in prey consumption, which were only found in order Psocoptera, can be attributed to the distribution of these insects in different microhabitats of Gorgona, since they are more abundant in low canopy coverage areas (Sarria *et al.* 2014), and because in tropical moist forests they are usually found in the middle to upper part of the vertical strata, which just overlaps with the daytime feeding territory of these bats. *Saccopteryx leptura* usually chooses specific feeding territories that they defend constantly (even for generations), below the canopy during the day, and in open areas above the canopy at night (Bradbury and Emmons 1974; Bradbury and Vehrencamp 1976; Kalko 1995). Something similar could be happening between males and females, since we observed that it is common for individuals that roost together to share their feeding territories both simultaneously and asynchronously. If we consider the assumption that *S. leptura* feeds on the insects available in its territory, then the chances of differentiation in prey consumed between the sexes would decrease considerably. Thus, it is possible that the heterogeneity in the diet of this species is mostly determined by the richness, composition, and abundance of prey in its hunting areas as well as its ability to locate and capture them, rather than some type of prey preference or specialization.

Although we only limited our sampling to *S. leptura* daytime activity, we consider our data to be a valid approach to trophic habits of the species. However, considering the type of food consumed and the activity of some insects found in our samples, we can infer that their diet composition during the day and during the night is not completely homogenous. For example, the presence of bees such as *Trigona* sp., winged ants and diurnal chrysomelid beetles in the samples analyzed are irrefutable proof that there is some diet differentiation with respect to their conspecifics and other nocturnal insectivorous bats species residing on the island. Although there is no previous research in insectivorous bats with diurnal habits that explore the differences between the diurnal and nocturnal diet, differentiation has been previously suggested (Russo *et al.* 2011a). However, when comparing the results of diet surveys for *Pipistrellus pygmaeus* during the night (Bartonička *et al.* 2008) and the day (Russo *et al.* 2011b) to provide an example, we did not observe noticeable differences in the main food resources, but in insects whose frequency of occurrence seems to be related to the bats' foraging activity and the characteristics of the foraging sites. Thus, more detailed studies focused on this topic are required for a better understanding of the general diet of these species. In the same way, more studies providing a better understanding of the night-time prey selection *S. leptura* are still required.

Our data is sufficient to suggest that the population of *S. leptura* in GNNP has a heterogenous and opportunistic diet, without an apparent preference for a particular type of insect, presumably to take advantage of the most abundant and available seasonal resources. It is important to increase the research on the prey selection of this species on the mainland in order to determine whether the diet composition of *S. leptura* on the island is locally exclusive or is generalized for the species. Studying insular species allows us to inquire about the strategies and adjustments that their populations use to survive, given the resources that the environment provides. Thus, studying the diet of other resident species would increase our understanding of the similarities and differences between them, and would allow us to determine how these species contribute to the balance of the ecosystem on the island.

Acknowledgements

We thank to National Natural Parks of Colombia, specifically Gorgona National Natural Park for letting us work on the island and for the authorization to manipulate the bats. To the Departamento de Biologia of the Universidad del Valle for supporting our research, and to the Departamento de Entomologia for lending us the necessary equipment to analyze the samples. We also thank O. E. Murillo-García for his advice during the groundwork from which this research was derived. Finally, we would like to thank the anonymous reviewers for their valuable suggestions that helped us improve the manuscript.

Literature cited

Agosta, S. J., and D. Morton. 2003. Diet of the big brown bat *Eptesicus fuscus* from Pennsylvania and western Maryland. Northeastern Naturalist 10:89–104.

Agosta, S. J., D. Morton, and K. M. Kuhn. 2003. Feeding ecology of the bat *Eptesicus fuscus*: "preferred" prey abundance as one factor influencing prey selection and diet breadth. Journal of Zoology 260:169–177.

Aguiar, L. M. S., and Y. Antonini. 2008 . Diet of two sympatric insectivorores (Chiropteroptera: Vespertilionidae) in the Cerrado of Central Brazil. Revista Brasileira de Zoologia 25:28–31.

Appel, G., *et al.* 2017. Aerial insectivorous bat activity in relation to moonlight intensity. Mammalian Biology 85:37–46.

Appel, G., *et al.* 2019. Temperature, rainfall, and moonlight intensity effects on activity of tropical insectivorous bats. Journal of Mammalogy 100:1-12.

Barclay, R. M. R., and R. M. Brigham. 1994. Constraints on optimal foraging: a field test of prey discrimination by echolocating insectivorous bats. Animal Behaviour 48:1013–1021.

Bartonička, T., Z. Řehák, and M. Andreas. 2008. Diet composition and foraging activity of *Pipistrellus pygmaeus* in a floodplain forest. Biologia 63:266-272.

Boyles, J. G., *et al.* 2011. Economic importance of bats in agriculture. Science 332:41–42.

Boyles, J. G., *et al.* 2013. On estimating the economic value of insectivorous bats: prospects and priorities for biologists. Pp. 501–515, *in* Bat evolution, ecology, and conservation (Adams, R. A., and C. S. Pedersen, eds.). Springer. New York, U.S.A.

Bradbury, J. W., and L. H. Emmons. 1974. Social organization of some Trinidad bats I. Emballonuridae. Zeitschrift für Tierpsychologie 36:137–183.

Bradbury, J. W., and S. L. Vehrencamp. 1976. Social organization and foraging in emballonurid bats: I. Field studies. Behavioral ecology and sociobiology 1:337–381.

Brigham, R. M., H. D. J. N. Aldridge, and R. L. Mackey. 1992. Variation in habitat use and prey selection by yuma bats, *Myotis yumanensis*. Journal of Mammalogy 73:640–645.

Burles, D. W., *et al.* 2008. Diet of two insectivorous bats, *Myotis lucifugus* and *Myotis keenii*, in relation to arthropod abundance in a temperate Pacific Northwest rainforest environment. Canadian Journal of Zoology 86:1367–1375.

Cadena, A., *et al.* 1990. Notas sobre la fauna de murciélagos de Gorgona. Pp. 236–243, *in* Biota y ecosistemas de Gorgona (Aguirre, J., and J. O. Rangel, eds.). Fondo para la protección del medio ambiente FEN. Bogotá, Colombia.

Chua, M. A. H., and S. A. Aziz. 2019. Into the light: atypical diurnal foraging activity of Blyth's horseshoe bat, *Rhinolophus lepidus* (Chiroptera: Rhinolophidae) on Tioman Island, Malaysia. Mammalia 83:78–83.

Cruz-Parrado, K., G. Moreno, and F. Sánchez. 2018. Dieta de *Saccopteryx leptura* (Chiroptera: Emballonuridae) en un área exurbana del piedemonte llanero colombiano. Revista Facultad de Ciencias Básicas 1:1–5.

Deagle, B. E., *et al.* 2018. Counting with DNA in metabarcoding studies: how should we convert sequence reads to dietary data? Molecular Ecology 28:391–406.

Duverge, P. L., *et al.* 2000. Functional significance of emergence timing in bats. Ecography 23:32–40.

Fenton, M., and N. Simmons. 2014. Bats: a world of science and mystery. University of Chicago Press. Chicago, U.S.A.

Freeman, P. W. 1979. Specialized insectivory: beetle-eating and moth-eating molossid bats. Journal of Mammalogy 60:467–479.

Fukui, D., O. Katsunori, and K. Maeda. 2009. Diet of three sympatric insectivorous bat species on Ishigaki Island, Japan. Endangered Species Research 8:117–128.

Giraldo, A. 2012. Geomorfología e hidroclimatología de la isla Gorgona. Pp. 17–23, *in* Isla Gorgona: Paraíso de biodiversidad y ciencia (Giraldo A., and B. Valencia, eds.). Programa editorial Universidad del Valle. Santiago de Cali, Colombia.

Hagstrum, D. W., and B, Subramanyam. 2010. Immature insects: ecological roles of mobility. American Entomologist 56:230–241.

Heim, O., *et al*. 2017. Landscape and scale-dependent spatial niches of bats foraging above intensively used arable fields. Ecological Processes 6:24.

Jakobsen, L., M. N. Olsen, and A. Surlykke. 2015. Dynamics of the echolocation beam during prey pursuit in aerial hawking bats. Proceedings of the National Academy of Sciences 112:8118–8123.

Jones, G., and J. Rydell. 1994. Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. Philosophical Transactions of the Royal Society B 346:445–455.

Jones, G., P. L. Duvergé, and R. D. Ransome. 1995. Conservation biology of an endangered species: field studies of greater horseshoe bats. Symposia of the Zoological Society of London 67:309–324.

Jung, K., E. K. V. Kalko, and O. Von Helversen. 2007. Echolocation calls in Central American emballonurid bats: signal design and call frequency alternation. Journal of Zoology 272:125–137.

Kalka, M. B., A. R. Smith, and E. K. V. Kalko. 2008. Bats limit arthropods and herbivory in a tropical forest. Science 320:71–71.

Kalko, E. K. V. 1995. Echolocation signal design, foraging habitats and guild structure in six Neotropical sheath-tailed bats (Emballonuridae). Symposia of the Zoological Society of London 67:259–273.

Kolkert, H., *et al*. 2019. Insectivorous bats selectively source moths and eat mostly pest insects on dryland and irrigated cotton farms. Ecology and Evolution 10:371–388.

Krauel, J., *et al*. 2018. Brazilian free tailed bats (*Tadarida brasiliensis*) adjust foraging behaviour in response to migratory moths. Canadian Journal of Zoology 96:513–520.

Kunz, T. H., J. O. Whitaker, and M. D. Wadanoli. 1995. Dietary energetics of the insectivorous Mexican free-tailed bat (*Tadarida brasiliensis*) during pregnancy and lactation. Oecologia 101:407–415.

Lara-Juárez, *et al*. 2015. Biología y aprovechamiento de la hormiga de escamoles, *Liometopum apiculatum* Mayr (Hymenoptera: Formicidae). Acta Zoológica Mexicana 31:251–264.

Lee, Y., and G. F. McCracken. 2001. Timing and variation in the emergence and return of Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. Zoological Studies 40:309–316.

Levin, E., Y. Yom-Tov, and A. Barnea. 2009. Frequent summer nuptial flights of ants provide a primary food source for bats. Naturwissenschaften 96:477–483.

Marques, J. T., M. J. Ramos-Pereira, and J. M. Palmeirim. 2015. Patterns in the use of rainforest vertical space by Neotropical aerial insectivorous bats: all the action is up in the canopy. Ecography 39:476–486.

McNab, B. 2010. Physiological Adaptation of Bats and Birds to Island Life. Pp. 153–175, *in* Island Bats: Evolution, Ecology, and Conservation (Fleming, H., and P. Racey, eds.). University of Chicago Press. Chicago, U.S.A.

Moore, N. W. 1975. The diurnal flight of the Azorean bat (*Nyctalus azoreum*) and the avifauna of the Azores. Journal of Zoological Society of London 77:483–506.

Moosman Jr, P. R., H. H. Thomas, and J. P. Veilleux. 2012. Diet of the widespread insectivorous bats *Eptesicus fuscus* and *Myotis lucifugus* relative to climate and richness of bat communities. Journal of Mammalogy 93:491–496.

Murillo, O. E., *et al*. 2014. Riqueza de especies, nuevos registros y actualización del listado taxonómico de la comunidad de murciélagos del Parque Nacional Natural Gorgona, Colombia. Revista de Biología Tropical 62:407–417.

Murillo, O. E., *et al*. 2011. Protocolo para la obtención de datos de mamíferos. Pp. 55–83, *in* Protocolos de investigación en ecosistemas terrestres, intermareales, submareales y pelágicos para el Parque Nacional Natural Gorgona. Departamento de Biología, Facultad de Ciencias Naturales y Exactas, Universidad del Valle. Santiago de Cali, Colombia.

Nogueira, M. R., A. L. Peracchi, and A. Pol. 2002. Notes on the lesser white-lined bat, *Saccopteryx leptura* (Schreber) (Chiroptera, Emballonuridae), from southeastern Brazil. Revista Brasileira de Zoologia 19:1123–1130.

Novella-Fernandez, R., *et al*. 2020. Trophic resource partitioning drives fine-scale coexistence in cryptic bat species. Ecology and evolution 10:14122–14136.

Pavey, C., *et al*. 2001. Dietary benefits of twilight foraging by the insectivorous bat *Hipposideros speoris.* Biotropica 33:670–681.

Quirán, E. M., and M. B. M. Corró. 1998. Vuelo nupcial y fundación de colonias de *Acromyrmex lobicornis* (Hymenoptera: Formicidae) en laboratorio, en la provincia de La Pampa, Argentina. Revista de la Sociedad Entomológica Argentina 57:67–70.

R Development Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Racey, P., S. Goodman, and R. Jenkins. 2010. The ecology and conservation of Malagasy bats. Pp. 369–404, *in* Island bats: evolution, ecology, and conservation (Fleming, H., and P. Racey, eds.). University of Chicago Press. Chicago, U.S.A.

Rakotoarivelo, A., *et al*. 2007. Seasonal food habits of five sympatric forest microchiropterans in western Madagascar. Journal of Mammalogy 88:959–966.

Razakarivony, V., B. Rajemison, and S. M. Goodman. 2005. The diet of Malagasy microchiroptera based on stomach contents. Mammalian Biology 70:312–316.

Rolfe, A., A. Kurta, and D. Clemans. 2014. Species-level analysis of diets of two mormoopid bats from Puerto Rico. Journal of Mammalogy 95:587–596.

Russo, D., L. Cistrone, and G. Jones. 2007. Emergence time in forest bats: the influence of canopy closure. Acta Oecologica 31:119–126.

Russo, D., *et al*. 2011a. Out of the dark: diurnal activity in the bat *Hipposideros ruber* on São Tomé Island (West Africa). Mammalian Biology 76:701–708.

Russo, D., *et al*. 2011b. The early bat catches the fly: daylight foraging in soprano pipistrelles. Mammalian Biology 76: 87–89.

Sarria, F., A. N. Garcia-Aldrete and R. González. 2014. Diversidad de Psocoptera (Insecta: Psocodea) en el Parque Nacional Natural Gorgona (Océano Pacífico, Colombia). Revista de Biología Tropical 62:257–264.

Schnitzler, H. U., and E. K. V. Kalko. 2001. Echolocation by insect-eating bats. BioScience 51:557–569.

Sedlock, J. L., F. Krüger, and E. L. Clare. 2014. Island bat diets: does it matter more who you are or where you live? Molecular Ecology 23:3684–3694.

Shiel, C. B., and J. S. Fairley. 1999. Evening emergence of two nursery colonies of Leisler's bat (*Nyctalus leisleri*) in Ireland. Journal of Zoology 247:439–447.

Speakman, J. R. 1995. Chiropteran nocturnality. Symposia of the Zoological Society of London 67:187–201.

Srinivasulu, B., and C. Srinivasulu. 2005. Diet of the black-bearded tomb bat *Taphozous melanopogon* Temminck, 1841 (Chiroptera: Emballonuridae) in India. Zoos Print Journal 20:1935–1938.

Thomas, A. J., and D. S. Jacobs. 2013. Factors Influencing the Emergence Times of sympatric insectivorous bat species. Acta Chiropterologica 15:121–132.

Toshiaki, H. 2004. Diet composition of introduced bullfrog, *Rana catesbeiana*, in the Mizorogaike Pond of Kyoto, Japan. Ecological Research 19:375–380.

Universidad del Valle. 2014. Colección de artrópodos del PNN Gorgona - Museo de Entomología de la Universidad del Valle (MUSENUV). Available from https://ipt.biodiversidad.co/sib/resource?r=entomofaunagorgona. 10 October 2020.

Vallejo, N., *et al*. 2019. The diet of the notch-eared bat (*Myotis emarginatus*) across the Iberian Peninsula analysed by amplicon metabarcoding. Hystrix 30:59–64.

Vásquez-Vélez, A. I. 2014. Estructura y diversidad de la vegetación del Parque Nacional Natural de la Isla Gorgona, Colombia. Revista de Biología Tropical 62:13–26.

Van Cakenberghe, V., A. Herrel, and L. F. Aguirre. 2002. Evolutionary relationships between cranial shape and diet in bats (Mammalia: Chiroptera). Pp. 205–236, *in* Topics in Functional and Ecological Vertebrate Morphology (Aerts, P., *et al*., eds.). Shaker publishing. Germany.

Vivas-Toro, I., and O. Murillo-García. 2019. Taxonomic identity of an insular population of sac-winged bat *Saccopteryx* (Chiroptera: Emballonuridae). Revista de Biología Tropical 67:306–405.

Vivas-Toro, I., and O. Murillo-García. 2020. Diurnal flying activity of a Neotropical bat (*Saccopteryx leptura*): effect of light intensity, temperature, and canopy cover. Acta Chiropterologica 22:87–94.

Verts, B. J., L. N. Carraway, and J. O. Whitaker. 1999. Temporal variation in prey consumed by big brown bats (*Eptesicus fuscus*) in a maternity colony. Northwest Science 73:114–120.

Whitaker, J. O. 1994. Food availability and opportunistic versus selective feeding in insectivorous bats. Bat Research News 35:75–77.

Whitaker, J. O. 2004. Prey selection in a temperate zone insectivorous bat community. Journal of Mammalogy 85:460–469.

Whitaker, J. O., C. Neefus, and T. H. Kunz. 1996. Dietary variation in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). Journal of Mammalogy 77:716–724.

Whitaker, J. O., G. F. McCracken., and B. M. Siemers. 2009. Food habits analysis of insectivorous bats. Pp. 567–592, *in* Ecological and behavioral methods for the study of bats (Kunz, T. H., and S. Parsons, eds.). 2nd edition. The Johns Hopkins University Press. Baltimore, U.S.A.

Zar, J.H. 1984. Biostatistical analysis. 2nd edition. Prentice-Hall. New Jersey, U.S.A.

Zhang, L. B., *et al*. 2005. Diet of flat-headed bats, *Tylonycteris pachypus* and *T. robustula*, in Guangxi, South China. Journal of Mammalogy 86:61–6.

Associated editor: Sergio Solari

Submitted: January 8, 2022; Reviewed: February 19, 2022

Accepted: March 6, 2022; Published on line: March 24, 2022

THERYA, 2022, Vol. 13(2):163-170 DOI:10.12933/therya-22-1146 ISSN 2007-3364

In search of bachelorettes: Observations of male *Leptonycteris yerbabuenae* with dorsal patches across its range

Theresa M. Laverty[1,2]*, and Kathryn E. Stoner[1]

[1] Department of Fish, Wildlife, and Conservation Biology, Colorado State University. 1474 Campus Delivery, 80523. Fort Collins, Colorado, U.S.A. Email: theresalaverty@gmail.com (TML), kathryn.stoner@colostate.edu (KES).
[2] Department of Ecology and Evolution, University of Chicago. 1101 East 57th Street, 60637. Chicago, Illinois, U.S.A.
*Corresponding author

The lesser long-nosed bat (*Leptonycteris yerbabuenae*) is a partially migratory, nectarivorous species that exhibits reproductive asynchrony across its range. Both migratory and resident populations of sexually active males of *L. yerbabuenae* may form an odoriferous dorsal patch during their mating season. This is created by smearing saliva, urogenital fluids, and anal secretions over the interscapular dorsal region with their feet. Dorsal patches are believed to influence female mate choice. We synthesized the sightings of male dorsal patches across the species' range, including gathering new observations of male *L. yerbabuenae* with dorsal patches captured at the northern extent of their range and citing previously unreported observations that we obtained by contacting other researchers. We also conducted a literature review to include all previously documented records of male *L. yerbabuenae* presenting dorsal patches. We document the first observations of male *L. yerbabuenae* with dorsal patches in the southwestern United States. In the Big Hatchet Mountains in New Mexico, we captured 1 male with a developed dorsal patch (*i. e.,* bare dorsal skin with sticky fur) on 25 July 2019 and two others on the night of 13 August 2019. New observations of males with developed dorsal patches were obtained from Hilltop Mine in Arizona (June 2006 or 2007) and at a hummingbird feeder at a residence near the Hilltop Mine (July 2013). A male with a recovering dorsal patch (*i. e.,* bare dorsal skin with regenerating fur) was also captured in a roost near Patagonia, Arizona, in late August 2008 and at a hummingbird feeder at a residence near Silver City, New Mexico (September 2021). All previously published records of males with dorsal patches occurred in roosts in Mexico during known mating seasons. These new observations suggest that *L. yerbabuenae* may breed in New Mexico and Arizona between June and August, but follow-up studies are needed to confirm this behavior. Much of the reproductive biology of this important pollinator remains unknown. Therefore, identifying regions where males present dorsal patches may not only assist in locating and protecting mating roosts, but would also further our understanding of the population ecology of this migratory species.

El murciélago magueyero menor (*Leptonycteris yerbabuenae*) es una especie nectarívora parcialmente migratoria que exhibe asincronía reproductiva en toda su área de distribución. Tanto las poblaciones migratorias como las residentes de machos sexualmente activos de *L. yerbabuenae* pueden formar un parche dorsal odorífero durante la temporada de apareamiento. Esto se crea por el comportamiento de frotar la saliva con sus patas, los fluidos urogenitales y las secreciones anales en la región dorsal interescapular. Se cree que los parches dorsales influyen en la elección de pareja. Sintetizamos los avistamientos de parches dorsales masculinos en el área de distribución de la especie, e incluimos la recopilación de nuevas observaciones de machos de *L. yerbabuenae* capturados con parches dorsales en el norte de su distribución, citando observaciones no reportadas previamente que obtuvimos al contactar a otros investigadores. También realizamos una revisión de la literatura para incluir todos los registros previamente documentados de machos de *L. yerbabuenae* que presentan parches dorsales. Documentamos las primeras observaciones de machos de *L. yerbabuenae* con parches dorsales en el suroeste de los Estados Unidos. En las montañas Big Hatchet en Nuevo México, capturamos un macho con un parche dorsal desarrollado (*i. e.,* piel dorsal desnuda con pelaje pegajoso) el 25 de julio de 2019 y otros dos en la noche del 13 de agosto de 2019. Se obtuvieron nuevas observaciones de machos con parches dorsales desarrollados de la mina Hilltop en Arizona (junio de 2006 o 2007) y en un comedero para colibríes en una residencia cerca de la mina Hilltop (julio de 2013). También fue capturado un macho con un parche dorsal en recuperación (*i. e.,* piel dorsal desnuda con pelaje en regeneración) cerca de Patagonia, Arizona, a finales de agosto de 2008 y en un comedero para colibríes en una residencia cerca de Silver City, Nuevo México (septiembre de 2021). Todos los registros anteriores de machos con parches dorsales, provienen hasta ahora de refugios de murciélagos en México durante las temporadas de reproducción conocidas. Estas nuevas observaciones sugieren que *L. yerbabuenae* puede reproducirse en Nuevo México y Arizona entre junio y agosto, pero se necesitan más estudios para confirmar este comportamiento. Gran parte de la biología reproductiva de esta importante especie de murciélago polinizador aún es desconocida. Por lo tanto, identificar las regiones donde los machos presentan parches dorsales, no solo puede ayudar a localizar y proteger los refugios de apareamiento, sino que también mejoraría nuestra comprensión de la ecología de la población de esta especie migratoria.

Keywords: Dorsal patch; Glossophaginae; lesser long-nosed bat; México; migratory; reproductive status; southwestern United States.

Introduction

Migration, an important life history trait for many species, allows individuals to spatially and temporally exploit changing environments, including ephemeral food sources. Long-range movements are exhibited by several mammals, including some ungulates, pinnipeds, cetaceans, and bats (Dingle 2014). With over 1,400 extant species (Simmons and Cirranello 2020), bats are one of the most diverse groups of mammals in the world. Despite their recognized importance in community ecology as seed dispersers, pollinators, and suppressors of insect populations, many aspects of their basic biology remain unknown due to the difficulties of studying nocturnal, flying organisms (Frick *et al.* 2020). Adding to their complexity, reproductive cycles of bats can vary within the same family and even within the same genus at similar latitudes making generalizations difficult (Racey 1982). To assist with bat conservation strategies and planning, studies should aim to increase our understanding of bat reproductive ecology (Racey and Entwistle 2000), including the effect of mating opportunities on the distributions of migratory bats.

Among North American bats, migratory species include temperate bats (many vespertilionids) that move seasonally between hibernation roosts and breeding habitats, temperate species (mostly tree-roosting bats) that migrate south for the winter, and tropical bats that move north to follow food resources and/or give birth (McNab 1982). Long-nosed bats, *Leptonycteris* spp. (Chiroptera: Phyllostomidae: Glossophaginae), are tropical migrants that rely on the availability of flowering plants. Their migration follows nectar corridors of flowering columnar cacti and paniculate *Agave* spp. between seasonal roosts (Fleming *et al.* 1993; Burke *et al.* 2019), and *Leptonycteris* spp. are among the most important nocturnal pollinators for many of these species (Valiente-Banuet *et al.* 1996; Peñalba *et al.* 2006; Rocha *et al.* 2006). Observations of low *Leptonycteris* densities may be caused by population declines due to habitat destruction or natural variation in flowering plant density (Moreno-Valdez *et al.* 2000), thus requiring a spatial and temporal understanding of bat and plant populations across their range.

Due to current technology limitations, most long-nosed bat movements are inferred from direct observations, passive integrated transponder (PIT) tag detections (Frick *et al.* 2018), and genetic studies (Wilkinson and Fleming 1996; Menchaca *et al.* 2020), which led to the discovery that only some populations of the lesser long-nosed bat (*Leptonycteris yerbabuenae*) migrate. Of the populations dwelling in Mexico, some females are resident to central and southern Mexico, where they form maternity roosts in the winter. Other females migrate between winter roosts in central Mexico and summer maternity roosts in northern Mexico and the southwestern United States (Nassar *et al.* 2016). For both migratory and resident populations, sexually active males of *L. yerbabuenae* may form an odoriferous dorsal patch during the mating season, created by a smearing behavior in which saliva, urogenital fluids, and anal secretions are spread over the interscapular dorsal region, similar to the behavior displayed by reproductive males of the Curaçaoan long-nosed bat (*Leptonycteris curasoae*) in Venezuela (Muñoz-Romo *et al.* 2011a). Indeed, one observation of a *L. yerbabuenae* male displaying a similar behavior supports this hypothesis (Laverty and Stoner 2022). Males with developed dorsal patches (*i. e.,* bare dorsal skin with sticky fur; Frick *et al.* 2018) have larger testes (Rincón-Vargas *et al.* 2013), and may have lower ectoparasite loads as noted by studies of *L. curasoae* (Muñoz-Romo and Kunz 2009; Muñoz-Romo *et al.* 2011b). Together, this suggests that dorsal patches may influence female mate choice; however, other potential explanations have not yet been evaluated.

Due to the differences in migratory behavior among *L. yerbabuenae* populations combined with reproductive asynchrony, we asked how does the phenology of male reproductive status, specifically the presence of dorsal patches, vary temporally across the species' range? By identifying where and when mating may occur based on distributions of males with dorsal patches, this study furthers our understanding of the reproductive ecology of *L. yerbabuenae* and highlights potential mating roosts or regions. We also document the first observations of dorsal patches at the northern extent of *L. yerbabuenae*'s migratory range and discuss the conservation implications of these findings.

Materials and methods

Study species. The pattern of reproduction of *Leptonycteris yerbabuenae* has been described as either bimodal polyestrous and monoestrous, but individuals are thought to mate only once per year (Ceballos *et al.* 1997; Rojas-Martínez *et al.* 1999; Stoner *et al.* 2003; Cole and Wilson 2006). The timing of reproduction varies across the species' range such that females either give birth in the winter or in the spring (Rojas-Martínez *et al.* 1999; Menchaca *et al.* 2020). Those that give birth in the winter are residents of central and southern Mexico, and may remain in a single roost year-round (Galindo *et al.* 2004), or move between roosts seasonally (*e. g.,* altitudinal movements due to food availability; Ceballos *et al.* 1997; Herrera 1997; Stoner *et al.* 2003). Females that give birth in the spring are believed to mate in the fall or winter in central Mexico, and migrate in the spring along the Pacific Coast to maternity roosts in northern Mexico and southern Arizona to give birth (Cockrum 1991; Ceballos *et al.* 1997; Rojas-Martínez *et al.* 1999; Stoner *et al.* 2003). Those arriving in the southwestern United States later in the summer fly along the foothills of the Sierra Madre Mountains and may travel from as far south as Jalisco (Wilkinson and Fleming 1996). Occupancy of *L. yerbabuenae* in southeastern Arizona and southwestern New Mexico generally peaks from mid-August to mid-September and then dwindles by early October when most individuals are thought to have returned south to Mexico

(Cockrum 1991; Bogan *et al.* 2017). Occasionally, small groups of *L. yerbabuenae* are found beyond October in Arizona (US Fish and Wildlife Service 2016; Menchaca *et al.* 2020), but it is not known if these individuals survive the winter (S. Wolf, pers. comm.).

While *L. yerbabuenae* was originally classified as endangered at the northern extent of its range in the late 1980s (Cole and Wilson 2006), the species was removed from the Endangered Species List in the United States in 2018, following its removal from threatened status in México in 2013 (Frick *et al.* 2018). It is one of three species of nectar-feeding bats—along with the Mexican long-nosed bat (*Leptonycteris nivalis*) and the Mexican long-tongued bat (*Choeronycteris mexicana*)—that migrate seasonally from Mexico to the southwestern United States along corridors of ephemeral flowers of cacti and *Agave* spp. (Bogan *et al.* 2017; Burke *et al.* 2019). These bat species are thought to benefit the tequila and mezcal industries by enhancing the genetic diversity of *Agave* spp. through cross-pollination (Trejo-Salazar *et al.* 2016).

Study site. Our fieldwork focuses on the migratory populations of *L. yerbabuenae* at the northern extent of its range. We specifically study those bats inhabiting a cave in the Big Hatchet Mountains in New Mexico, which harbors the easternmost distribution of *L. yerbabuenae* in the United States (Bogan *et al.* 2017). *Leptonycteris nivalis* and *L. yerbabuenae* in this region are believed to mostly feed on nectar and pollen in the Animas Mountains, requiring bats in the Big Hatchet Mountains roost to commute >20 km in each direction across the Playas Valley to reach this foraging area (Bogan *et al.* 2017). While the diet of *L. yerbabuenae* includes flowering cacti in Arizona, the main food source in southwestern New Mexico appears to be limited to *Agave* spp. (mostly *Agave palmeri*; Ober and Steidl 2004; Scott 2004). This region is comprised of semidesert grasslands interspersed at higher elevations with patches of Madrean evergreen woodland (alligator bark juniper, piñon, Chihuahua pine, and species of oak), lower interior chaparral (manzanita, mountain mahogany), and interior southwest riparian deciduous forest (sycamore, cottonwood, and rabbitbrush–Apache plume; Brown 1994).

Bat sampling. We captured individuals of *L. yerbabuenae* in the Big Hatchet Mountains during their seasonal occupancy in 2019, for a total of six nights of sampling between 13 July and 12 September 2019. To capture bats, we placed a 12 m long by 2.6 m high mist net (38-mm mesh; Avinet Inc., Dryden, NY) just downhill of the main entrance of the cave opening. Mist nets were opened at sunset (*i. e.,* at 2030 h in July or as early as 1940 h in September), but the amount of time the net remained open each night was dependent on bat activity and varied across our sampling period (median = 3.84 h/night, range = 3.28 – 6.47 h/night, total = 26.62 h). We scanned for bats in the net every five min, untangling captured individuals that were then temporarily stored in cotton bags until they could be processed. For each individual, we recorded age, sex, reproductive condition, forearm length, and body mass. We determined the age of individuals (*i. e.,* juveniles or adults) based on the relative ossification of the metacarpal-phalangeal joint in the wings (Brunet-Rossinni and Wilkinson 2009). Females were classified as non-pregnant, pregnant, lactating, or post-lactating by abdominal palpation and nipple examination (Racey 2009). Reproductive condition of adult males was determined by examining if the testes were swollen and distended, and if an individual's interscapular dorsal region presented a dorsal patch (Figure 1). For those individuals without a dorsal patch or wing damage, we inserted a PIT tag subcutaneously between the shoulder blades as part of an on-going migration study. All bats were released at the capture location within 30 min of capture. Field protocols were approved prior to implementation by Colorado State University's Institutional Animal Care and Use Committee (Protocol #19-8891A), the New Mexico Department of Game & Fish (Permit #3611), and the U.S. Fish & Wildlife Service (Permit #TE63195B-0).

Literature review. In addition to our field study, we searched for peer-reviewed publications that included information on the dorsal patch of male *Leptonycteris* spp. On 27 August 2020, we used Google Scholar to search for articles published through August 2020 including the keywords "*Leptonycteris*" and "male" that also included the term "dorsal patch" or "sebaceous patch." We similarly conducted a search on Web of Science on 27 August 2020 using the topic search TS = *Leptonycteris* AND male AND ("dorsal patch" OR "sebaceous patch").

To supplement data at the northern extent of *L. yerbabuenae*'s range, we also searched through grey literature materials and contacted various researchers in New Mexico and Arizona for observations of reproductive males with dorsal patches.

Results

New observations of dorsal patches in New Mexico and Arizona. Between July and September 2019, we captured a total of 55 *L. yerbabuenae*, which were comprised of 33 adults (9 males and 24 females) and 22 juveniles (5 males and 17 females) in the Big Hatchet Mountains. Three (33 %) of the adult males were identified as reproductively active due to the presence of swollen testes and developed dorsal patches (*i. e.,* bare dorsal skin with sticky fur; Figure 1 and Table 1). A reproductively active male with a forearm length of 55.4 mm and a weight of 29.0 g was captured at 2158 h on 25 July 2019 and a wing punch was taken. Two different males were captured at 2339 h on 13 August 2019 and 0042 h on 14 August 2019. While no marking techniques were used on these latter individuals, they differed in forearm length (54.8 and 56.0 mm), weight (23.5 and 27.5 g), and the appearance of the dorsal patch (*i. e.,* one individual had more bare skin exposed). Since neither of these individuals presented a scar on their wings where the wing punch was taken from the first male and they all differed in forearm lengths and body mass, we could confidently assume these were 3 different adult males (Figure 2).

Three separate studies on *L. yerbabuenae* in Arizona and one recent study in New Mexico also reported observations of dorsal patches (Figure 2 and Table 1), and three of those observations were confirmed through photographs (Figure 1). Males with developed dorsal patches were captured at the Hilltop Mine (June 2006 or 2007; D. Dalton, pers. comm.) and at a hummingbird feeder located at a residence near the mine (July 2013; J. Danielson and K. Ekholm, pers. comm.). In late August 2008, a male with a recovering dorsal patch (*i. e.,* bare dorsal skin with regenerating fur) was captured at a roost near Patagonia, Arizona (D. Buecher and J. Ramirez, pers. comm.). More recently, another male with a recovering dorsal patch was captured in mid-September 2021 at a hummingbird feeder near Silver City, New Mexico (M. Davies and R. Burke, pers. comm.).

Published observations of dorsal patches from other roosts. Our literature review using Google Scholar resulted in 29 publications: 20 peer-reviewed articles, 3 book chapters, 2 independent study reports, 1 Ph.D. dissertation, 1 M.Sc. thesis, and 1 preprint. We found 9 peer-reviewed articles using Web of Science, all of which were also identified by Google Scholar. Of the 29 publications, only 14 were primary literature studies that sampled *Leptonycteris* spp. in the field. Reproductive males of *L. nivalis* do not develop dorsal patches as documented by a study that included observations from several mating seasons at the only known mating roost for the species, Cueva del Diablo near Tepoztlan, Morelos, central Mexico (Nassar *et al.* 2016). Dorsal patches do occur, however, in both *L. curasoae* and *L. yerbabuenae* and were first described for both species by Nassar *et al.* (2008). For *L. curasoae*, males with dorsal patches have been observed during November and December in Guano Cave and Piedra Honda Cave, Paraguaná Peninsula, Falcón State, Venezuela (Nassar *et al.* 2008; Muñoz-Romo and Kunz 2009; Muñoz-Romo *et al.* 2011b; Muñoz-Romo *et al.* 2011a; Muñoz-Romo *et al.* 2012). The 8 remaining publications in addition to Nassar *et al.* (2008) described dorsal patches of male *L. yerbabuenae* in Mexican roosts (Table 1), where the phenology of dorsal patches—like mating seasons—appears to be asynchronous (Figure 2). Dorsal patches were documented between May and September at southern roosts, between September and January in Chamela, Jalisco, and between September and October at roosts on the southern Baja peninsula.

Figure 1. Photographs of new observations of male *Leptonycteris yerbabuenae* individuals with dorsal patches in the southwestern United States. Images include (a) a male with a developed dorsal patch at the Big Hatchet Mountains roost, New Mexico in July 2019, (b) a male with a developed dorsal patch at a hummingbird feeder near Hilltop Mine, Arizona in July 2013, and (c) a male with a recovering dorsal patch at the Patagonia bat cave, Arizona in August 2008. Photographs by (a) Theresa Laverty, (b) Joseph Danielson, and (c) Debbie Buecher.

Table 1. The location, timing, and prevalence of male dorsal patches in *Leptonycteris yerbabuenae*.

Location	Timing of dorsal patch	Percentage of males with dorsal patch	Reference
Big Hatchet Mountains, New Mexico, USA	July – August 2019	33.3 % (*n* = 3/9)	This study
Hummingbird feeder near Silver City, New Mexico, USA	mid-September 2021	20.0 % (*n* = 1/5) with a recovering dorsal patch[a]	M. L. Davies & R.A. Burke, pers. comm.
Hilltop Mine, Arizona, USA	June 2006 or 2007	20 to 30 males, most with dorsal patches[b]	D. C. Dalton, pers. comm.
Hummingbird feeder near Hilltop Mine, Arizona, USA	July 2013	1 male with dorsal patch[c]	J. R. Danielson & K. L. Ekholm, pers. comm.
Patagonia, Arizona, USA	late August 2008	1 male with recovering dorsal patch[d]	J. Ramirez & D. C. Buecher, pers. comm.
Chivato, southern Baja California penísula, México	September 2017	61.1 % (*n* = 11/18)	(Frick *et al.* 2018)
Las Cuevas, southern Baja California penísula, México	early October 2013	90.0 % (*n* = 9/10)	(Frick *et al.* 2018)
Chamela Bay, Jalisco, Mexico	November 2002	24.2 % (*n* = 16/66)	(Nassar *et al.* 2008)
	December 2002	35.1 % (*n* = 27/77)	(Nassar *et al.* 2008)
	September 2003	35.5 % (*n* = 22/62)	(Nassar *et al.* 2008)
	October 2003	50.0 % (*n* = 24/48)	(Nassar *et al.* 2008)
	October 2008 –January 2009	22.2 % (*n* = 26/117)	(Rincón-Vargas *et al.* 2013)
	January 2019	20 of 33 males were reproductive[e]	(Zamora-Mejías *et al.* 2020)
Tlilapan, Veracruz, México	June – September 2011 and 2012	38.9 % (*n* = 7/18)	(Ramírez Hernández and Herrera 2016)
El Salitre, Morelos, México	June 2019	15 of 28 males were reproductive with incipient patches[e]	(Zamora-Mejías *et al.* 2020)
Colotlipa, Guerrero, México	July 2017	18 of 25 males were reproductive[e]	(Zamora-Mejías *et al.* 2020)
San Juan Noxchitlán, Oaxaca, México	June 2015	17 males captured, but only 11 used for DNA extraction[f]	(Gaona *et al.* 2016; Gaona *et al.* 2019a; Gaona *et al.* 2019b)
Los Laguitos, Chiapas, México	May – August 2009	61.9 % (*n* = 13/21) in May diminished to 11.7 % (n = 2/17) in August	(Martínez-Coronel *et al.* 2017)

[a] Correspondence including photographs with researchers Mallory L. Davies and Rachel A. Burke confirmed that a male was captured with a recovering dorsal patch (*i. e.,* new hair growth present) at a hummingbird feeder at a private residence in mid-September 2021. There was a suspected second male with a recovering dorsal patch, but unfortunately no photograph was taken.

[b] Correspondence with researcher David C. Dalton indicated that all of the 20-30 individuals captured were adult males. Most, if not all, had a dorsal patch. In some cases, the dorsal patches were mostly healed and were partially re-furred. In other cases, the dorsal patches were still open wounds that looked very fresh.

[c] Correspondence including photographs with researchers Joseph R. Danielson and Kelsey L. Ekholm confirmed that a male was captured with a dorsal patch at a hummingbird feeder at a private residence in July 2013.

[d] Correspondence including photographs with researchers Judith Ramirez and Debbie C. Buecher confirmed that a male was captured with a recovering dorsal patch (*i. e.,* new hair growth was present) in late August 2008.

[e] Authors did not state in the publication how many reproductively active males had a dorsal patch. Correspondence with Daniel Zamora-Mejías indicated the presence of males with dorsal patches as well as observed copulations during these time periods with the exception of El Salitre (*i. e.,* where incipient dorsal patches were observed, and it was assumed mating would begin in the upcoming weeks).

[f] Authors did not state in these publications how many males they captured without a dorsal patch. Correspondence with Osiris Gaona indicated that at least 50 % of the males captures in the Obispos cave presented a dorsal patch.

Discussion

Much of what is already known about the reproductive ecology of *L. yerbabuenae* is derived from research in Mexico. Reproductive males with dorsal patches have been documented at several mating roosts in Mexico since first being documented in the literature by Nassar *et al.* (2008). Due to the individual variation in the timing of dorsal patches throughout the species' range, more longitudinal studies are needed to identify periods in which dorsal patches are present and when mating occurs (*e. g.,* Rincón-Vargas *et al.* 2013; Frick *et al.* 2018). Thus far, these odoriferous patches have only been found in males of *L. curasoae* and *L. yerbabuenae* during their mating seasons. Although dorsal patches do not form in *L. nivalis* (Nassar *et al.* 2016), they likely play an important role in female mate choice for *L. curasoae* (Muñoz-Romo and Kunz 2009; Muñoz-Romo *et al.* 2011a). Dorsal patches could have a similar function in *L. yerbabuenae* since copulations observed in western central Mexico (Chamela, Jalisco) have only included male individuals with developed dorsal patches (*i. e.,* bare dorsal skin with sticky fur; Laverty and Stoner 2022).

Given the important functions that mating and maternity roosts play in bat population ecology, these roosts are often the focus of conservation efforts. Prior to this study, roosts of *L. yerbabuenae* in the southwestern United States were thought to function as maternity roosts and transient roosts (*i. e.,* where females and volant young feed before migrating south; Bogan *et al.* 2017). Through our fieldwork and communication with other researchers in New Mexico and Arizona, we report occasional sightings of developed or recovering dorsal patches (*i. e.,* bare dorsal skin with sticky or regenerating fur, respectively) at the northern extent of *L. yerbabuenae*'s range, suggesting that males may be seeking out mating opportunities in the southwestern United States between June and September. If so, one or more transient roosts may also function as mating roosts

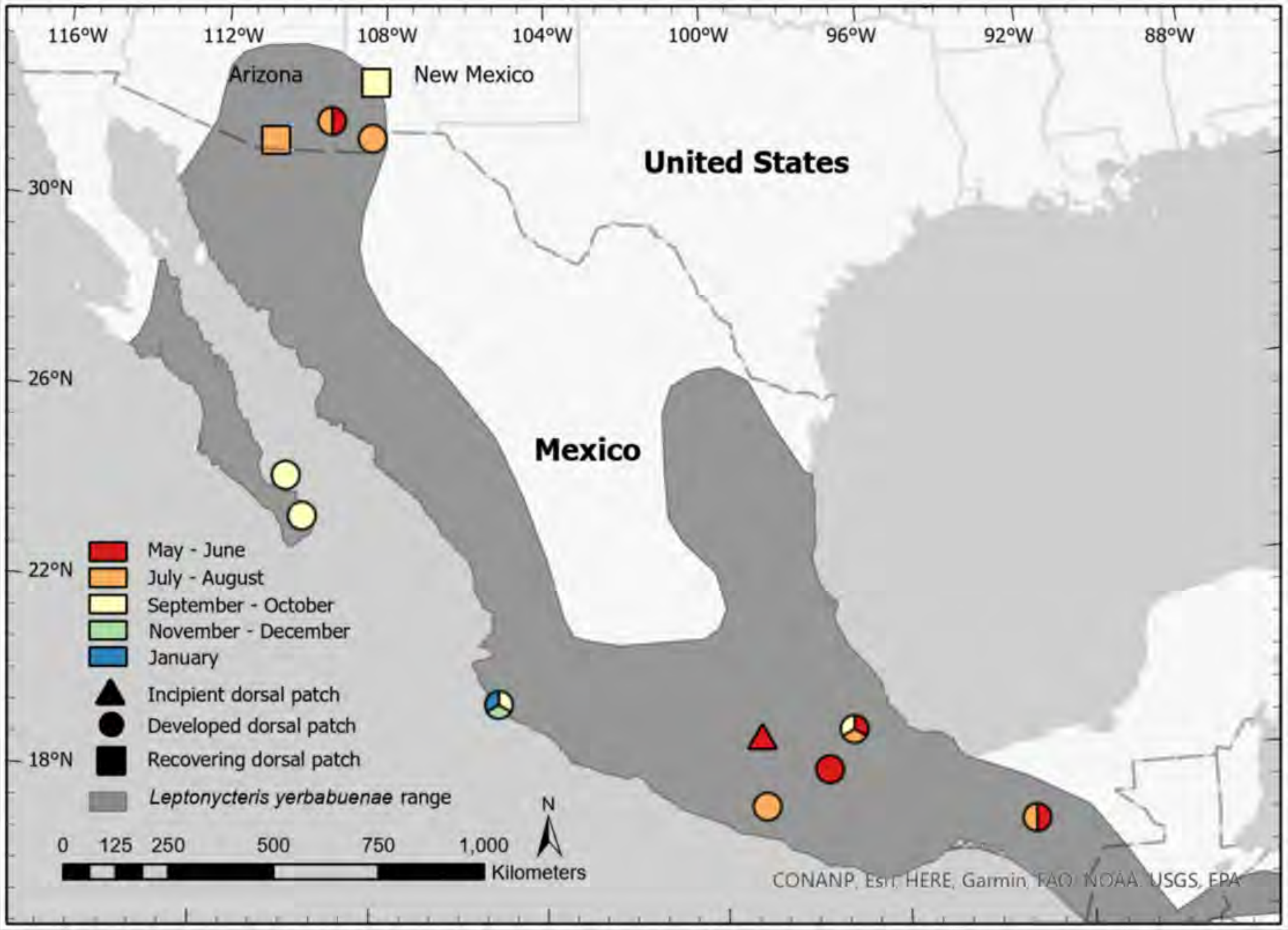

Figure 2. Map displaying locations and timing of observations of male *Leptonycteris yerbabuenae* with incipient (triangle), developed (circle), and recovering (square) dorsal patches. Sites with observations spanning multiple months are represented by multiple colors. Polygon data obtained from http://www.iucnredlist.org/

and harbor additional conservation value. Future studies should confirm if *L. yerbabuenae* breeds in the southwestern United States. Ideally, this would involve researchers briefly visiting roosts during their periods of occupancy to scan for copulating *L. yerbabuenae*. While netting bats, reproductive males have historically been identified by observing swollen and distended testes, but we recommend that all future studies of *L. yerbabuenae* measure the external testes with calipers to confirm they meet a minimum size corresponding to complete spermatogenesis (*e. g.*, 48.2 mm^2 for *L. yerbabuenae* in Chamela; Rincón-Vargas *et al.* 2013) to relate dorsal patch presence with reproductive activity. Prior to the discovery of dorsal patches, Cockrum and Ordway (1959) reported gravid female *L. yerbabuenae* and males with enlarged testes in mid-August 1955 at a mine near Paradise, Arizona, which suggests that reproductively active males may not be a new occurrence in the United States. While further research is needed exploring the functional role of dorsal patches, all known mating roosts of *L. yerbabuenae* contain males presenting dorsal patches (*e. g.*, Rincón-Vargas *et al.* 2013; Frick *et al.* 2018; Laverty and Stoner 2022). Therefore, our study highlights how the reproductive status and presence of dorsal patches in male *L. yerbabuenae* may inform research priorities and aid in the identification of mating roosts.

The role of migration in determining the reproductive status of *L. yerbabuenae* remains largely unknown. Research in Chamela, Jalisco, found spermatogenesis (*i. e.*, the process of sperm cell development) and the presence of dorsal patches in *L. yerbabuenae* to occur during peak food availability and the return of migratory females to the region (Rincón-Vargas *et al.* 2013). However, males can be captured year-round at this study site (Stoner *et al.* 2003). Roosts in the southwestern United States, on the other hand, are largely unoccupied and food is not available for nectarivorous bats from roughly November to May (Cockrum 1991; Bogan *et al.* 2017). Given the presumed energetic costs of developing a dorsal patch and mating (Muñoz-Romo and Kunz 2009; Rincón-Vargas *et al.* 2013), particularly after migrating from and before migrating to unknown roosts in Mexico, we did not expect to find males with dorsal patches that could potentially be seeking out mating opportunities in the southwestern United States. Therefore, our research echoes other studies citing the need for more information on the movements of male *L. yerbabuenae* (Cockrum 1991; Menchaca *et al.* 2020). While we do not encourage PIT tagging males with dorsal patches, we recommend future studies mark these males (*e. g.,* with a wing punch or banding) to ensure accurate counts of individuals with dorsal patches at a roost. Males without dorsal patches should continue to be PIT tagged. Until more information is known on the movements of males and until roosts are thoroughly searched for copulatory behaviors, we cannot confirm the presence of a mating roost in the southwestern United States, but the presence of dorsal patches at transient roosts at the northern extent of the species' range demands further investigation into this matter.

Acknowledgements

We are grateful to D. Buecher, R. Burke, D. Dalton, J. Danielson, M. Davies, K. Ekholm, W. Frick, O. Gaona, J. Ramirez, and D. Zamora-Mejías for providing additional information or observations of male dorsal patches. In New Mexico, we thank A. Pagels, A. Ibarra, I. Tanshi, B. Obitte, and R. Burke for assistance in the field, as well as W. Hurt for private land access. We are also grateful to M. Ramsey and J. Barnitz, Jr. for their support of this study. Kathryn E. Stoner received funding for this project from the U.S. Bureau of Land Management (grant # L17AS00186).

Literature Cited

Bogan, M. A., P. M. Cryan, C. D. Weise, and E. W. Valdez. 2017. Landscape movements by two species of migratory nectar-feeding bats (*Leptonycteris*) in a northern area of seasonal sympatry. Western North American Naturalist 77:317–330.

Brown, D. E. 1994. Chihuahuan desertscrub. Pp. 169–179, *in* Biotic communities: southwestern United States and northwestern Mexico (Brown, D. E., ed.). University of Utah Press. Salt Lake City, U.S.A.

Brunet-Rossinni, A. K., and G. S. Wilkinson. 2009. Methods for age estimation and the study of senescence in bats. Pp. 315–325, *in* Ecological and behavioral methods for the study of bats (Kunz, T. H., and S. Parsons, eds.). Johns Hopkins University Press. Baltimore, U.S.A.

Burke, R. A., J. K. Frey, A. Ganguli, and K. E. Stoner. 2019. Species distribution modelling supports "nectar corridor" hypothesis for migratory nectarivorous bats and conservation of tropical dry forest. Diversity and Distributions 25:1399–1415.

Ceballos, G., T. H. Fleming, C. Chávez, and J. Nassar. 1997. Population dynamics of *Leptonycteris curasoae* (Chiroptera: Phyllostomidae) in Jalisco, Mexico. Journal of Mammalogy 78:1220–1230.

Cockrum, E. L. 1991. Seasonal distribution of northwestern populations of the long-nosed bats, *Leptonycteris sanborni* family Phyllostomidae. Anales del Instituto de Biología. Serie Zoología 62:181–202.

Cockrum, E. L., and E. Ordway. 1959. Bats of the Chiricahua Mountains, Cochise County, Arizona. American Museum Novitates 1938:1–35.

Cole, F. R., and D. E. Wilson. 2006. *Leptonycteris yerbabuenae*. Mammalian Species 797:1–7.

Dingle, H. 2014. Migration: The biology of life on the move, 2nd ed. Oxford University Press. New York, USA.

Fleming, T. H., R. A. Nuñez, and L. da S. L. Sternberg. 1993. Seasonal changes in the diets of migrant and non-migrant nectarivorous bats as revealed by carbon stable isotope analysis. Oecologia 94:72–75.

Frick, W. F., *et al.* 2018. Seasonal ecology of a migratory nectar-feeding bat at the edge of its range. Journal of Mammalogy 99:1072–1081.

Frick, W. F., T. Kingston, and J. Flanders. 2020. A review of the major threats and challenges to global bat conservation. Annals of the New York Academy of Sciences 1469:5–25.

Galindo G., C., A. Sánchez Q., R. H. Quijano, and L. G. Herrera M. 2004. Population dynamics of a resident colony of *Leptonycteris curasoae* (Chiroptera: Phyllostomidae) in central Mexico. Biotropica 36:382–391.

Gaona, O., *et al.* 2019a. Microbiota composition of the dorsal patch of reproductive male *Leptonycteris yerbabuenae*. Plos One 14:e0226239.

Gaona, O., D. Cerqueda-García, D. Martínez-Martínez, A. Moya, and L. I. Falcón. 2016. Microbiome of the sexual scent organ of *Leptonycteris yerbabuenae*. PeerJ Preprints 4:e2627v1.

Gaona, O., E. S. Gómez-Acata, D. Cerqueda-García, C. X. Neri-Barrios, and L. I. Falcón. 2019b. Fecal microbiota of different reproductive stages of the central population of the lesser-long nosed bat, *Leptonycteris yerbabuenae*. Plos One 14:e0219982.

Herrera M., L. G. 1997. Evidence of altitudinal movements of *Leptonycteris curasoae* (Chiroptera: Phyllostomidae) in Central Mexico. Revista Mexicana de Mastozoología (Nueva Época) 2:116–118.

Laverty, T. M., and K. E. Stoner. 2022. Media files for "In search of bachelorettes: Observations of male *Leptonycteris yerbabuenae* with dorsal patches across its range." figshare. Media. https://doi.org/10.6084/m9.figshare.15138702

Martínez-Coronel, M., Y. Hortelano-Moncada, V. Corral, and L. R. Cuevas. 2017. Relationship between subcutaneous fat and reproductive activity in males of *Leptonycteris yerbabuenae* in Los Laguitos Cave, Chiapas, Mexico. Pp. 36–48, *in* Frontiers in reproductive science, vol. 1; Reproductive biology, physiology and biochemistry of male bats (Arenas-Ríos, E. ed.). Bentham Science Publishers. Sharjah, United Arab Emirates.

McNab, B. K. 1982. Evolutionary alternatives in the physiological ecology of bats. Pp. 151–200 in Ecology of bats (Kunz, T. H., ed.). Plenum Publishing Corporation. New York, U.S.A.

Menchaca, A., M. C. Arteaga, R. A. Medellín, and G. Jones. 2020. Conservation units and historical matrilineal structure in the tequila bat (*Leptonycteris yerbabuenae*). Global Ecology and Conservation 23:e01164.

Moreno-Valdez, A., W. E. Grant, and R. L. Honeycutt. 2000. A simulation model of Mexican long-nosed bat (*Leptonycteris nivalis*) migration. Ecological Modelling 134:117–127.

Muñoz-Romo, M., J. F. Burgos, and T. H. Kunz. 2011a. Smearing behaviour of male *Leptonycteris curasoae* (Chiroptera) and female responses to the odour of dorsal patches. Behaviour 148:461–483.

Muñoz-Romo, M., J. F. Burgos, and T. H. Kunz. 2011b. The dorsal patch of males of the Curaçaoan long-nosed bat, *Leptonycteris curasoae* (Phyllostomidae: Glossophaginae) as a visual signal. Acta Chiropterologica 13:207–215. https://doi.org/10.3161/150811011X578769

Muñoz-Romo, M., and T. H. Kunz. 2009. Dorsal patch and chemical signaling in males of the long-nosed bat, *Leptonycteris curasoae* (Chiroptera: Phyllostomidae). Journal of Mammalogy 90:1139–1147.

Muñoz-Romo, M., L. T. Nielsen, J. M. Nassar, and T. H. Kunz. 2012. Chemical composition of the substances from dorsal patches of males of the Curaçaoan long-nosed bat, *Leptonycteris curasoae* (Phyllostomidae: Glossophaginae). Acta Chiropterologica 14:213–224.

Nassar, J. M., R. Galicia, A. Ibarra, and R. A. Medellín. 2016. Tracking the origin of the smearing behavior in long-nosed bats (*Leptonycteris* spp.). Mammalian Biology 81:623–627.

Nassar, J. M., *et al*. 2008. Seasonal sebaceous patch in the nectar-feeding bats *Leptonycteris curasoae* and *L. yerbabuenae* (Phyllostomidae: Glossophaginae): phenological, histological, and preliminary chemical characterization. Zoology 111:363–376.

Ober, H. K., and R. J. Steidl. 2004. Foraging rates of *Leptonycteris curasoae* vary with characteristics of *Agave palmeri*. The Southwestern Naturalist 49:68–74.

Peñalba, M. C., F. Molina-Freaner, and L. L. Rodríguez. 2006. Resource availability, population dynamics and diet of the nectar-feeding bat *Leptonycteris curasoae* in Guaymas, Sonora, Mexico. Biodiversity and Conservation 15:3017.

Racey, P. A. 1982. Ecology of bat reproduction. Pp. 57–104, *in* Ecology of bats (Kunz, T. H., ed.). Plenum Publishing Corporation. New York, U.SA..

Racey, P. A. 2009. Reproductive assessment of bats. Pp. 249–263, *in* Ecological and behavioral methods for the study of bats (Kunz, T. H., and S. Parsons, eds.). Johns Hopkins University Press. Baltimore, U.S.A.

Racey, P. A., and A. C. Entwistle. 2000. Life-history and reproductive strategies of bats. Pp. 363–414, *in* Reproductive biology of bats (Crichton, E. G., and P. H. Krutzsch, eds.). Academic Press. London, UK.

Ramírez Hernández, G., and L. G. Herrera M. 2016. Allocation of endogenous nutrients for reproduction in the lesser long-nosed bat (*Leptonycteris yerbabuenae*) in central Mexico. Journal of Mammalogy 97:23–31.

Rincón-Vargas, F., *et al*. 2013. Internal and external indicators of male reproduction in the lesser long-nosed bat *Leptonycteris yerbabuenae*. Journal of Mammalogy 94:488–496.

Rocha, M., *et al*. 2006. Pollination biology and adaptive radiation of Agavaceae, with special emphasis on the genus *Agave*. Aliso: A Journal of Systematic and Evolutionary Botany 22:329–344.

Rojas-Martínez, A., *et al*. 1999. Seasonal distribution of the long-nosed bat (*Leptonycteris curasoae*) in North America: does a generalized migration pattern really exist? Journal of Biogeography 26:1065–1077.

Scott, P. E. 2004. Timing of *Agave palmeri* flowering and nectar-feeding bat visitation in the Peloncillos and Chiricahua Mountains. The Southwestern Naturalist 49:425–434.

Simmons, N. B., and A. L. Cirranello. 2020. Bat species of the world: A taxonomic and geographic database. http://batnames.org. Accessed on 7 January 2020.

Stoner, K. E., K. A. O.-Salazar, R. C. R.-Fernandez, and M. Quesada. 2003. Population dynamics, reproduction, and diet of the lesser long-nosed bat (*Leptonycteris curasoae*) in Jalisco, Mexico: implications for conservation. Biodiversity and Conservation 12:357–373.

Trejo-Salazar, R.-E., L. E. Eguiarte, D. Suro-Piñera, and R. A. Medellín. 2016. Save our bats, save our tequila: industry and science join forces to help bats and agaves. Natural Areas Journal 36:523–530.

US Fish and Wildlife Service. 2016. Species status assessment for the lesser long-nosed bat. Southwest Region. Albuquerque, U.S.A.

Valiente-Banuet, A., M. D. C. Arizmendi, A. Rojas-Martínez, and L. Domínguez-Canseco. 1996. Ecological relationships between columnar cacti and nectar-feeding bats in Mexico. Journal of Tropical Ecology 12:103–119.

Wilkinson, G. S., and T. H. Fleming. 1996. Migration and evolution of lesser long-nosed bats *Leptonycteris curasoae*, inferred from mitochondrial DNA. Molecular Ecology 5:329–339.

Zamora-Mejías, D., *et al*. 2020. Does latitudinal migration represent an advantage in the decrease of ectoparasitic loads in *Leptonycteris yerbabuenae* (Chiroptera)? Journal of Mammalogy 101:979–989.

Associated editor: Rafael Avila Flores
Submitted: March 9, 2021; Reviewed: March 24, 2021
Accepted: February 3, 2022; Published on line: March 30, 2022

THERYA, 2022, Vol. 13(2):171-182 DOI:10.12933/therya-22-1167 ISSN 2007-3364

Habitat use and activity patterns of ungulates in a tropical rainforest of southern México

Fredy A. Falconi-Briones[1], Eduardo J. Naranjo[1*], Rafael Reyna-Hurtado[2], Manuel Spínola[3], Paula Enríquez-Rocha[1], and Rodrigo A. Medellín[4]

[1] Departamento de Conservación de la Biodiversidad, El Colegio de la Frontera Sur, Carretera Panamericana y Periférico Sur s/n, C. P. 29290, San Cristóbal de Las Casas. Chiapas, México. Email: falconi.fab@hotmail.com (FAF), enaranjo@ecosur.mx (EJN), penrique@ecosur.mx (PE-R).
[2] Departamento de Conservación de la Biodiversidad, El Colegio de la Frontera Sur, Av. Rancho Polígono 2-A, C. P. 24500. Ciudad Industrial Lerma. Campeche, México. Email: rreyna@ecosur.mx (RR-H).
[3] Instituto Internacional en Conservación y Manejo de Vida Silvestre, Universidad Nacional, C. P. 1350-3000. Heredia, Costa Rica. Email: mspinola@una.cr (MSP).
[4] Instituto de Ecología, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria C. P. 04510. Ciudad de México. Email: medellin@iecologia.unam.mx (RAM).
*Corresponding author

Baird's tapir (*Tapirella bairdii*), white-lipped peccary (*Tayassu pecari*), and collared peccary (*Dicotyles tajacu*) sympatrically occur in the Lacandon Forest of Chiapas, México. These species contribute to maintain ecosystem dynamics through herbivory, seed dispersal, and seed predation, constituting important prey for large carnivores and hunters. We analyzed activity patterns and habitat use of the three focal species to assess the degree of temporal and spatial habitat segregation among them in Montes Azules Biosphere Reserve (REBIMA) and surrounding communities. Between February and October 2015 we deployed camera-traps during 8,463 camera-trap days to estimate the presence and activity of tapirs and peccaries in two habitat types: "conserved" (REBIMA), and "transformed" (community forests; AFC). Habitat use and activity patterns of tapirs and peccaries were assessed through logistic regression models. We found that Baird's tapir was almost exclusively nocturnal with a trend towards crepuscular activity, while both peccary species were diurnal, therefore showing a high daily temporal segregation from the tapir. Both peccary species were similarly active in the two study sites, while tapirs were more active in continuous forest within the protected area. The occurrence of tapirs and white-lipped peccaries depended on the presence and proximity of water sources and roads. Our results suggest that spatial segregation of the habitat allows coexistence of both peccary species in the study area. AFC have potential for maintaining populations of tapirs and peccaries in the Lacandon Forest. Conserving these forests by local communities is essential to ensure the persistence of these mammals.

El tapir centroamericano (*Tapirella bairdii*), el pecarí de labios blancos (*Tayassu pecari*) y el pecarí de collar (*Dicotyles tajacu*) habitan en la Selva Lacandona, Chiapas, México, los cuales contribuyen a mantener la dinámica de los ecosistemas a través de la herbivoría, la dispersión y la depredación de semillas, además de constituir presas importantes para grandes carnívoros y cazadores locales. Analizamos el uso de hábitat y los patrones de actividad de las tres especies para evaluar su grado de segregación espacio-temporal en un paisaje con selva continua y en un paisaje modificado en el área de estudio. Entre febrero y octubre de 2015 utilizamos cámaras-trampa durante 8,463 días- cámara para estimar la presencia y actividad de las especies focales analizando dos tipos de hábitat: "conservado" (Reserva de la Biosfera Montes Azules; REBIMA), y "transformado" (bosques comunitarios; AFC). Evaluamos el uso de hábitat de las especies mediante modelos de regresión logística. Encontramos que el tapir fue casi exclusivamente nocturno con una tendencia hacia la actividad crepuscular, mientras que ambas especies de pecaríes fueron diurnas, mostrando una alta segregación temporal diaria, respecto al tapir. Ambas especies de pecaríes tuvieron una actividad similar en los dos sitios de estudio, mientras que los tapires fueron más activos en el bosque continuo dentro del área protegida. La presencia del tapir y el pecarí de labios blancos dependió de la presencia y cercanía del agua, caminos y carreteras. Nuestros resultados sugieren que la segregación espacial del hábitat permite la coexistencia de ambas especies de pecaríes en el área de estudio. AFC tiene potencial para mantener poblaciones de estas especies; su conservación por las comunidades locales es esencial para asegurar la persistencia de estos mamíferos.

Keywords: Coexistence; habitat use; Maya forest; resource partitioning; ungulates.

Introduction

The Lacandon Forest of Chiapas hosts one of the largest remnants of humid tropical forest in Mexico, inhabited by numerous species of wild flora and fauna (Carabias *et al.* 2015; Carrara *et al.* 2015), many of which are also natural resources for the inhabitants of rural communities (Naranjo *et al.* 2010). However, it has been shown that habitat deterioration, fragmentation, and loss in these tropical forests have resulted in the local and regional disappearance and isolation of populations of white-lipped peccary (*Tayassu pecari*) and tapir (*Tapirella bairdii*; Tejeda-Cruz *et al.* 2009; Schank *et al.* 2017; Meyer *et al.* 2019; Thornton *et al.* 2020). These two species, along with the collared peccary (*Dicotyles tajacu*), live in sympatry in southern Mexico (Naranjo *et al.* 2015). Tapir and white-lipped peccary are threatened of extinction in Mexico (SEMARNAT 2010). Both species currently face reductions in their population sizes and distribution ranges in southern Mexico, Central America, and

South America, as a consequence of changes in plant cover and land use, poaching, and disease transmissions (Naranjo *et al.* 2015; Moreira-Ramírez *et al.* 2019).

Tapir and peccary populations (orders Perissodactyla and Cetartiodactyla, respectively; hereafter referred to as ungulates) play important ecological roles (Eisenberg 1989; Bodmer 1991; Reyna-Hurtado *et al.* 2014; Naranjo 2018). As primary consumers, they contribute to the dynamics and structuring of the ecosystems where they thrive through herbivory, seed dispersal or seed predation, and are also important prey of large Neotropical carnivores (Naranjo 2009; Reyna-Hurtado *et al.* 2014; Malhi *et al.* 2016). Conservation actions for these threatened species require reliable information on the ecology and spatio-temporal dynamics of their populations. For this reason, the need to accurately estimate variables related to habitat use and the relationships between these large herbivores is now greater than ever. Considering the current effects of various environmental stressors such as climate change and habitat loss (Frey *et al.* 2017) and the pressures from human activities (Naranjo 2009), it is important to understand the relationships between the local fauna and its habitat, how they use the habitat (Pianka 1983; Dirzo *et al.* 2014), and their responses to these emerging effects (*e.g.*, loss of biodiversity and changes in abundance and distribution; Schaefer *et al.* 2008).

Closely related sympatric species tend to differ ecologically to coexist, so that they become segregated in some niche dimensions (Gause 1934; Schoener 1974). The tapir and peccary species inhabiting the Lacandon region live in sympatry and, although differing in how they use the local habitat (Tejeda-Cruz *et al.* 2009), they consume similar food resources such as fruits, stems, and leaves (Reyna-Hurtado *et al.* 2014; Naranjo 2019). This leads to assuming that there is clear segregation in terms of hours of activity and sites used most frequently.

A broad variety of methods have been used for analyzing how wild animals use the resources available in the local habitat (Johnson 1980; Burnham and Anderson 2004). The present study considered the use of the habitat and the analysis of activity patterns of tapir and peccaries as a proxy to assess the existence of spatio-temporal segregation in the areas inhabited by these species at the landscape scale in the Lacandon Forest. We sought to understand the relationships of the tapir and peccaries with their environment, especially to assess the influence of the landscape on the probability of habitat use of the species studied. To this end, we considered the effects of anthropic influence (i.e., expansion of the farming frontier) as *a priori* variables (i.e., occurrence of wildlife in relation to towns, water bodies, roads, and vegetation types; MacKenzie and Royle 2005; Kéry and Royle 2016).

This work addressed the following research questions: 1) Is there segregation in the activity patterns of the tapir the two peccary species that inhabit the Lacandon Forest? 2) Which of the *a priori* variables considered in this study are drivers of habitat use by each species in the study area? Our overall objective was to assess the habitat use and activity patterns of the ungulates under study in the Montes Azules Biosphere Reserve and adjacent areas. In particular: 1) we compared the occurrence, distribution, and patterns of activity of these species at sites with and without frequent human presence in the study area; 2) we analyzed habitat use for the three species studied.

Materials and methods

Study area. This work was conducted from February to October 2015, in the southern portion of the Lacandon Forest (16°08'56"-16°11'58.3" N and 90°53'57"-91°18'45" W), Chiapas, Mexico. The sampling was carried out in an effective sampling area (ESA) of 150 km^2 within the southern sector of the Montes Azules Biosphere Reserve (REBIMA, 3,312 km^2) and in community forest areas (ACF; ESA = 162.5 km^2) of *ejidos* (communal land) adjacent to the REBIMA in the municipality of Marques de Comillas (Figure 1). The altitude of the study area ranges from 150 to 250 meters a.s.l. (Naranjo 2008), with approximately 50 % of the natural forest cover still present (Tejeda-Cruz *et al.* 2009; Garmendia *et al.* 2013, Muench and Martinez-Ramos 2016).

The prevailing climate in the region is warm humid with abundant summer rainfall (Am (i') gw"; García 2004). The mean annual precipitation oscillates between 2,500 and 3,500 mm, with 80 % of rains between June and November (García-Alaniz *et al.* 2010; Arce-Peña *et al.* 2019). Currently, the Lacandon region, except for the REBIMA, is dominated by heterogeneous landscapes composed of fragments of evergreen tropical forest of different sizes and successional states (Rzedowski 1978; Challenger and Soberón 2008) within a matrix of crop fields (mainly maize and oil palm), livestock ranches, and scattered human settlements (Carrara *et al.* 2015; Muench and Martinez-Ramos 2016; De La Torre and Rivero 2019).

We analyzed two types of habitats, namely "conserved areas" (the REBIMA) and a transformed landscape ("community forest areas"; ACF) as this anthropic condition is one of the key drivers of the abundance and distribution of the tapir and peccaries (Naranjo 2019). Fieldwork took place over 250 days between February and October 2015, comprising the rainy season (which peaked in September) and the dry season of the year (*i.e.,* March), as well as the maximum mean temperature (28°C) recorded in May (García and Lugo 1992). The sampling effort was 8,463 camera trap-days. Cuddeback® Black Flash E3 digital cameras were installed in 64 sampling points (hereafter camera trap stations) with a detection zone of 134 m^2 per camera trap, approximately. Of these, 32 camera trap stations were located in the southern REBIMA sector and 32 in ACFs. These traps were used for recording data on occurrence and frequency, sex (tapir only), and hours of activity of the ungulates of interest in the study area. Using a geographic information system (GIS), the study area was spatially delimited and the proportion of different types of coverage (for-

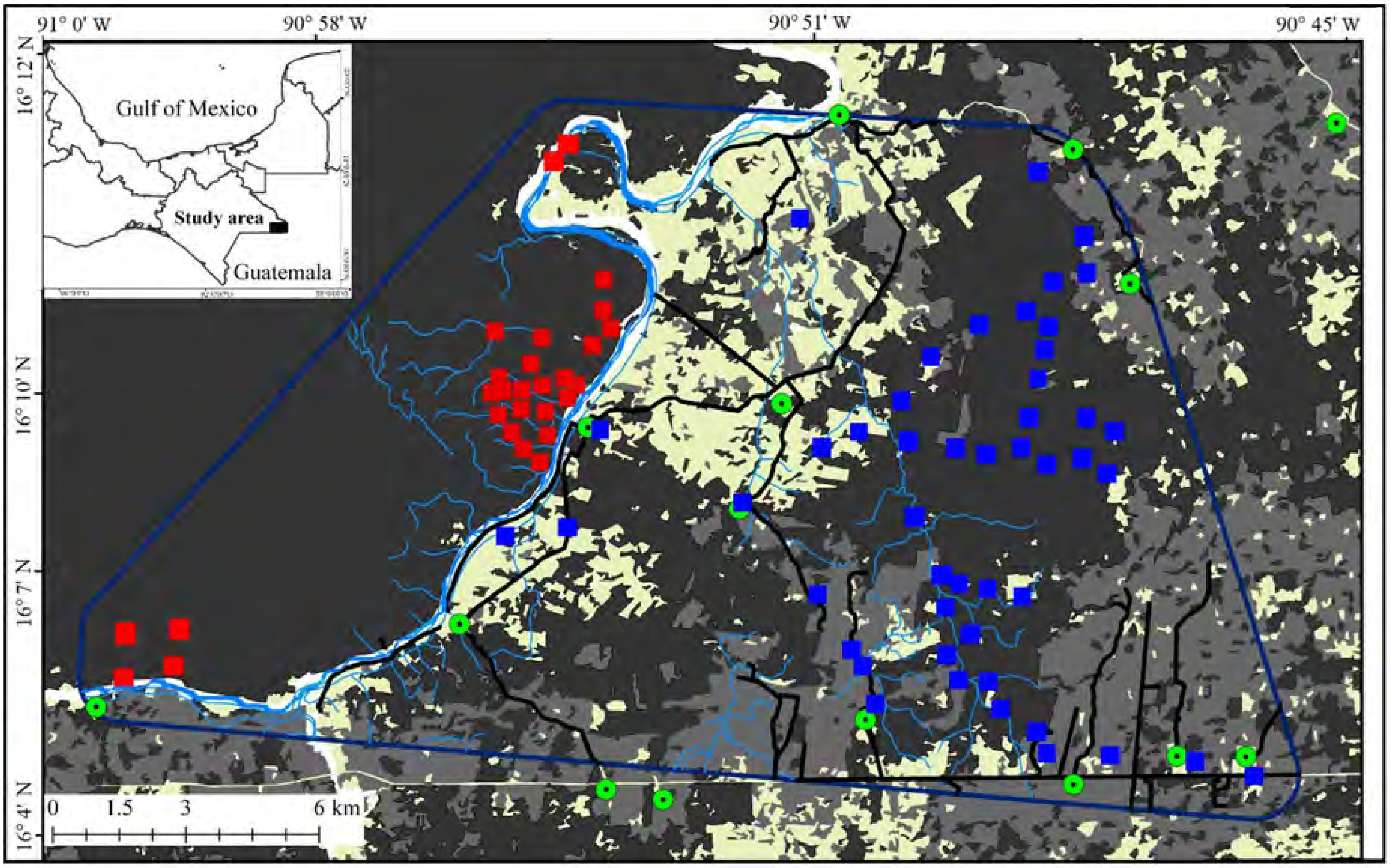

Figure 1. Study Area. Location of sampling sites, which include a portion of the southern sector of the Montes Azules Biosphere Reserve (REBIMA, red squares), and community forest areas (ACF, blue squares). Lacandon Forest, Chiapas, Mexico (February–October 2015).

est cover, land uses, and water bodies) and the proximity to anthropic elements (*e.g.*, roads and towns) in the study area were estimated.

The spatial distribution of camera trap stations followed a grid layout in each of the study sites (REBIMA and ACF), with a minimum separation of 1 km between them to ensure the independence of records, considering the monitoring protocols applied to the study of other big mammals (modified from Silver 2004 and Chávez *et al.* 2013; Figure 1). Trap cameras were set to operate 24 hours a day and capture sequences of three photos per minute in case of motion detection. For each capture, the date, time, and geographic location of each camera trap station (sampling unit) were verified, as well as the number of individuals recorded. To consider a record as an independent capture and thus minimize any potential autocorrelation (Moreira-Ramírez *et al.* 2019), only photographs captured with a separation of more than 24 h between them were considered for each species.

Sampling was carried out on areas of dense evergreen tropical forest of various successional stages, as well as patches of riparian vegetation, which were randomly selected to record tapir and peccary individuals and their traces, and for use as sites for installing camera traps. Although pastures and meadows were excluded because they have not been mentioned in the literature as suitable for tapir or white-lipped peccary in Mexico (March and Naranjo 2005; Carrillo-Reyna *et al.* 2015; Reyna-Hurtado *et al.* 2009), some camera-trap stations were placed in areas adjacent (~10 to 100 m) to these types of land use, considering that the collared peccary is listed as a generalist species (Sowls 1997; Reyna-Hurtado *et al.* 2014). Photographic records were used to construct binomial matrices ("1" to "0", where 1 = presence and 0 = absence) for each camera-trap station. The matrix for each species was processed with the *Tidyverse* (Wickham 2017) and *SjPlot* (Lüdecke 2018) libraries in R v.4.0.2 (R Core Team 2019).

Habitat use. The use of the habitat by each species was analyzed using logistic regression models with the *Glmulti* package (Calcagno 2019); these models are suggested for their suitability to work with binomial variables (Manly *et al.* 2002). In addition, covariates were used to identify some of the environmental conditions (Table 1, Supplementary material) that influenced habitat use by ungulates in the study area (Manly *et al.* 2002; Gaillard *et al.* 2010). To this end, a high-resolution multi-spectral satellite image for the year 2014 (Landsat 8 Oli Tars) was used, along with the classification of plant cover and land uses previously generated in the Geographic Information Analysis Laboratory (LAIGE, in Spanish) at Colegio de la Frontera Sur (ECOSUR). This monitored classification was modified in the ArcMap program to produce a map of the study area with four layers of different types of classification of the landscape elements studied: 1) water bodies, 2) human settlements, 3) roads, and 4) vegetation and land use (evergreen tropical forest, secondary tropical forest, crops, pastures and meadows, water bodies, and roads). The logistic regression models were compared using the Akaike information criterion corrected for small sample size (AICc) and weight of each variable, to determine the relative evidence in favor of each model (Burnham and Anderson 2004; Fletcher and Fortin 2018) and the influence of each covariate on the probability of occurrence (interpreted as the probability that a given species is present or absent, and thus, that sites with certain characteristics are used). The models with the best support were selected considering those that yielded the minimum AIC score and ΔAICc ≤ 2 as proposed by Burnham and Anderson (2004), in addition to the weight (Wi). The models with ΔAICc ≤ 2 were selected and averaged to evaluate the relative importance of the variables and the significance of their values (Barton 2019).

Spatio-temporal comparisons of occurrence records for each species were performed using parametric (Student's *t*) and non-parametric (Mann-Whitney *U*) tests to evaluate the differences between frequencies of photographic records, occurrences, and relative abundances of species within and between types of conditions across sites in the study area (Sokal and Rohlf 1995).

Activity patterns. We consider the total number of photographs captured and pooled the number of capture events into one-hour intervals for the 24 hours of the day to search for patterns in the actual hours of activity of each focal species (Tobler *et al.* 2008). We used the "Overlap" package in R by Meredith and Ridout (2017), which allowed plotting the hours of activity of each species using the kernel density estimation, in addition to estimating the overlap coefficient (Dhat) between the patterns of one or two species in different sites.

Results

Frequency of records. Of the 64 camera-trap stations, 24 recorded tapir individuals (11 in REBIMA and 13 in ACF). Collared peccaries were recorded in 11 REBIMA and 16 ACF stations, respectively, while white-lipped peccaries were recorded in 18 stations (6 in REBIMA and 12 in ACF). According to independent photographic records, the tapir was captured more frequently in the protected area (REBIMA; $n = 59$) than in community forest areas (ACF, $n = 28$; $U = 89.5$, $P = 0.01$). The collared peccary was most frequently recorded at the ACF site, attaining statistical significance ($n = 47$; $U = 386.5$, $P = 0.04$). However, this trend was not observed for the white-lipped peccary; although it had more records in the ACF site (48 records) than in REBIMA (33), this difference between sites was non-significant ($U = 127$; $P = 0.60$). In the case of the tapir, a higher proportion of males was recorded in REBIMA versus ACF ($U = 30$; $P = 0.01$), while females showed a similar proportion between sites ($U = 66.5$, $P = 0.78$; Table 2, Supplementary material). The sex of peccary individuals captured could not be determined based on the photographs recorded.

Habitat-use models. For each of the models generated for the species, the number of variables included, AICc, difference between each model and the model with the lowest delta AICc (Δ_i), and Akaike weight (*Wi*) are reported (Tables 2-4, Supplementary material). According to the AICc, the best models to explain the occurrence of the tapir in the study area (Table 2a, Supplementary material) included the variables *distance to permanent water bodies* and *roads* (*e.g.,* dirt roads and roads; with weight [$W_i = 0.63$]) and the vegetation types *evergreen tropical forest* (BTP) and *secondary tropical forest* (BTS; Table 2b, Supplementary material). The best model indicated that the probability of site use by the tapir increases as the distance to water bodies decreases, especially when considering the tropical forest (BTP and BTS; Tables 2a, 2b, Supplementary material; Figure 2).

In the case of the collared peccary, the probability of habitat use increased with the presence and proximity of roads and human settlements, and with the distance from water bodies ($W_i = 0.~20$; Table 3a, Supplementary material; Figure 2). However, the presence of different types of vegetation in the study landscape was relatively unimportant (Table 3b, Supplementary material). On the other hand, the probability of habitat use by the white-lipped peccary increased in sites with little anthropic intervention. The response in the probability of use was directly related to the distance to variables associated with anthropic disturbance, such as human settlements, roads and dirt roads, and inversely related to the distance to water bodies ($W_i =$ 42; Tables 4 and 5, Supplementary material; Figure 2).

Activity patterns. A total of 235 independent photographic records were captured for the ungulates under study (tapir: $n = 87$; white-lipped peccary: 81; collared peccary: 67), including date and time. For the tapir, the photographic evidence revealed a clear preference for nighttime activity (mostly between 2:00 h and 5:00 h; Figure 3). In contrast, photographic records of collared peccary and white-lipped peccary suggested that both species are mostly diurnal, with a slight tendency to be active in the afternoon (after 16:00 h), and with virtually no activity at night (Figure 3).

The overlap coefficients allowed distinguishing the high degree of segregation between the ungulates in the study area across the time dimension. The two peccary species showed an overlap of more than 80 % (Dhat = 0.82; Figure 3). Each of these two species showed a high temporal segregation relative to the tapir. The indicators of temporal overlap between each of the peccary species and the tapir showed little overlap in their hours of activity (*i.e.,* white-lipped peccary *vs.* tapir: Dhat = 0.34; collared peccary *vs.* tapir: Dhat = 0.42; Figure 3).

Discussion

Our results show trends in habitat use for the three ungulates studied and high levels of temporal segregation between the peccaries and the tapir in the study area. Considering

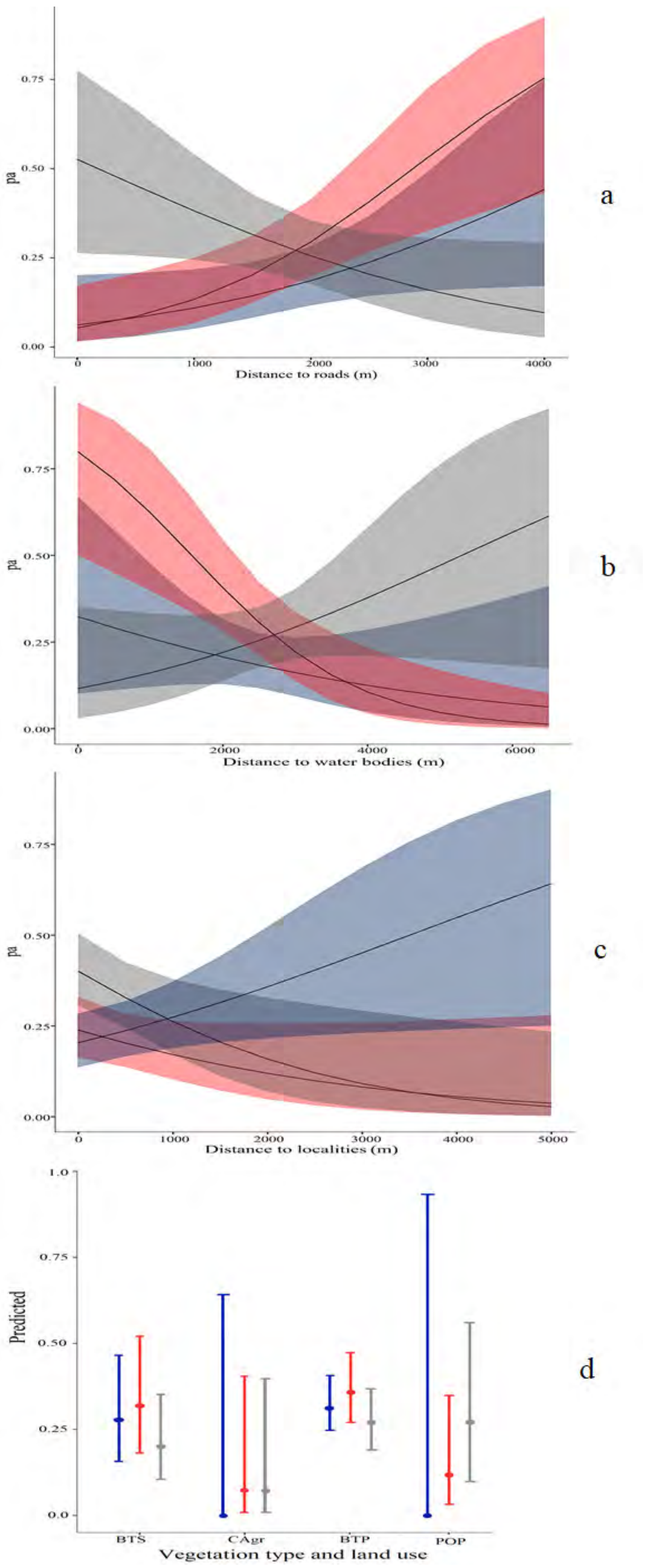

Figure 2. Logistic regression graphs considering variables predictive of habitat use by *Tapirella bairdii* (red), *Dicotyles tajacu* (gray), and *Tayassu pecari* (blue) with respect to distance to roads (a), distance to water bodies (b), distance to human settlements (c), and types of vegetation and land use (d) in community forest areas (ACF) and the protected area (Montes Azules Biosphere Reserve; REBIMA) in the Lacandon Forest, Mexico. pa: Presence-absence predictive values Aca: Mature secondary forest, BTP Evergreen tropical forest, BTS: Secondary tropical forest, CAgr: Agricultural crops, POP: Pastures and meadows.

time as a resource (*i.e.,* something that is 'consumed', similar other resources; Kronfeld-Schor and Dayan 2003; Frey *et al.* 2017), the tapir and peccaries have partitioned niches. It is important to highlight the difference between the habitat use by the two peccary species, as they exhibit opposite response patterns to anthropic variables, such as the presence of roads and proximity to human settlements, and to resources such as water (Figure 2). The habitat variables considered *a priori* in this study were limited; however, their assessment could be key for continuing the study of the interactions between the spatial and temporal dimensions of the ecological niches of these species (Frey *et al.* 2017).

Habitat use. Given the frequencies and occurrences observed in collared peccary records at both sites, our null hypothesis was validated since this species indiscriminately used both habitat types. This result has been reported in other studies conducted in America for this species, which have documented the presence of collared peccary in a wide range of habitats and environmental conditions, as well as its high tolerance to anthropic pressures (Kiltie 1982; Reyna *et al.* 2014; Briceño-Méndez *et al.* 2016). The similar use intensity of both habitat types by the white-lipped peccary despite the anthropic disturbance factors in the ACF is probably because the ecological conditions meet the resource requirements (*i.e*., water, food, and shelter, among others) of this species in this site. Muench and Martínez-Ramos (2016) reported that the ACF preserved all species of medium- and large-sized mammals in the region. Thus, in spite of the anthropic pressures, this area still functions as a suitable habitat for peccary populations (Fryxell *et al.* 2014; Krausman and Morrison 2016). This area also contains numerous water bodies (Figure 1), a key factor for the movement of white-lipped peccaries (Reyna-Hurtado *et al.* 2009). However, to determine the viability of the local populations of these ungulates in ACF, studies on age structure and population movement ecology are required, which may indicate that the species is reproducing in the area and that there is structural and functional connectivity (Morrison *et al.* 2006; Garrido-Garduño and Vázquez-Domínguez 2013).

The fact that a higher number of tapir records were recorded in REBIMA (*n* = 59) than in ACF (*n* = 28), i.e., a higher relative abundance of tapir in the former (20.9 ind/1000 camera trap-days in REBIMA *vs* 10.8 ind/1000 camera trap-days in ACF; Tables 3 to 5, Supplementary material) may be due to the greater surface area covered by mature forest in the protected site. Besides, the tapir tends to move away or become more elusive in areas with human presence and hunting (Naranjo and Cruz 1998; Zapata-Ríos and Branch 2015). It has also been documented that the presence of domestic or feral canids can lead to changes in the habitat use of native species, thus affecting their movement and hours of activity (Vanak and Gompper 2009; Silva-Rodríguez *et al.* 2010).

The ACF contains a large fragment of tropical forest (~5800 ha) in different successional stages that maintains some connectivity with other adjacent forest patches (Fig-

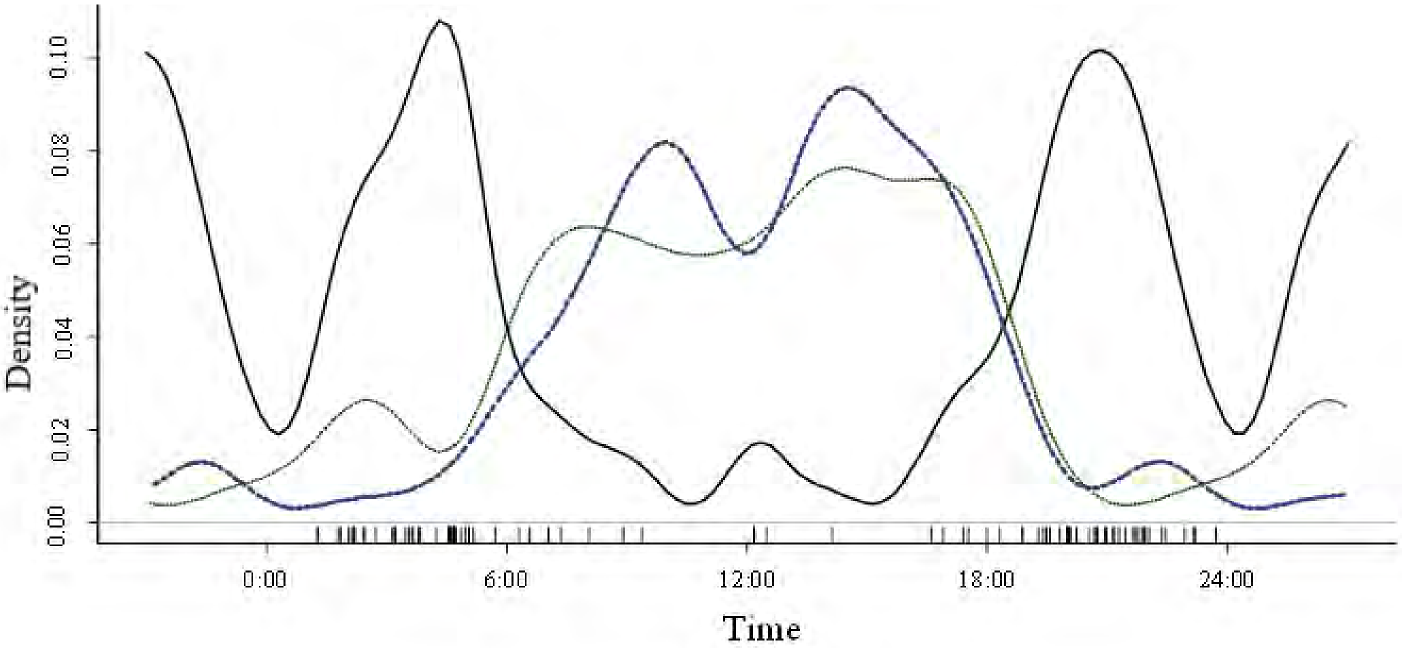

Figure 3. Overlap of activity between ungulate species in community forest areas (ACF) and the protected area (Montes Azules Biosphere Reserve; REBIMA) in the Lacandon Forest, Mexico. Black line: *Tapirella bairdii*; blue line: *Tayassu pecari*; green line: *Dicotyles tajacu* (overlap coefficient), values of the confidence interval. *D. tajacu. T. bairdii–T.pecari* = 0.34 (0.17–0.37); *T. pecari–D. tajacu* = 0.82 (0.84–1.03), and *D. tajacu–T. bairdii* = 0.42 (0.22–0.44).

ure 1; Gil-Fernández *et al.* 2017). The white-lipped peccary was recorded more frequently in ACF than in REBIMA (Table 5, Supplementary material), likely due to migratory or episodic movements that favor their presence even in non-protected areas (Mendes-Pontes and Chivers 2007; Reyna-Hurtado *et al.* 2009).

Habitat use models. The models constructed to evaluate habitat use by the species confirm previous reports, which describe low tolerance to anthropic effects (*i.e.*, presence of roads and infrastructure) and preference for areas with connectivity to water bodies by the tapir and white-lipped peccary (O'Farrill *et al.* 2014; Naranjo 2019; Reyna-Hurtado *et al.* 2014; Moreira-Ramírez *et al.* 2019). In contrast, our results show that the collared peccary uses both types of sites (protected area and community forest areas) in a similar way. Consistent results have been reported for this species in similar Neotropical environments where food is not a limiting factor (Terborgh 1986; Peres 19946; Galetti *et al.* 1999). This helps to explain the occasional records of this species crossing roads and in agricultural areas in the present study. To note, the use of agricultural systems by *T. pecari* has been documented in dry savannas of Venezuela, the dry Chaco in Paraguay (Mayer and Wetzel 1987; Redford and Eisenberg 1992), and grasslands of central and central-western Brazil, where they are even considered a pest for the damage and losses caused to farmers (Jacomo *et al.* 2013; Lima *et al.* 2019). This is not the case in southeastern Mexico, since the populations of *T. pecari* rarely cause damages to agricultural systems, to the best of our knowledge (Naranjo 2002; Romero-Balderas *et al.* 2006). This discrepancy may be related to the regional context of the areas where this species thrives, since the great Chaco and the Amazon are characterized by large extensions of habitat (> 1.5 million km^2; Cartes *et al.* 2015; Camino *et al.* 2017) and low human densities, contrary to our study area, which shows smaller areas of suitable habitat and high human density and disturbance (Naranjo 2019).

Activity patterns. For the tapir, previous studies in Mexico and elsewhere report activity patterns within a specific time span (nocturnal or diurnal; Eisenberg 1989; Sanderson and Trolle 2005), or with hours of activity split into two periods of the day (typically daytime and nighttime; Matola *et al.* 1997; Foerster and Vaughan 2002). Besides, these ungulates tend to move away or become more elusive in areas with human presence and hunting (Naranjo and Cruz 1998; Zapata-Ríos and Branch 2015). It has also been documented that the presence of domestic or feral canids can lead to changes in the habitat use of native mammals, affecting their movement and hours of activity (Vanak and Gompper 2009; Silva-Rodríguez *et al.* 2010). In the study area, intrusion events of domestic dogs and hunters were recorded in the ACF; however, the current hunting rate or frequency has not been documented (Figures 2 and 4).

This study found that the tapir displays activity patterns with two marked peaks of nighttime activity (20:00 to 23:00 h and 2:00 to 5:00 h; Figure 3). This finding is consistent with reports for other tapir populations in Mexico, such as those inhabiting Los Chimalapas, Oaxaca (Lira-Torres *et al.* 2014) and the Calakmul Biosphere Reserve, Campeche (unimodal period of activity: 20:00 to 3:00 h; Sánchez-Pinzón *et al.* 2020). The bimodal pattern reported in this study has also been observed for *T. pinchaque* in the central Andes of Colombia (Lizcano and Cavelier 2000) and *T. terrestris* in the Ecuadorian Amazon (Espinosa and Salvador 2017). In contrast, the unimodal pattern of activity of the tapir in Calakmul may be the result of monitoring associated with water bodies (open canopy areas), a vegetation structure allowing greater moonlight permeability (*i .e*, subdeciduous tropical forest; Lizcano and Cavelier 2000), and anthropic pressures such as hunting (Oliveira-Santos *et al.* 2010; Sánchez-Pinzón *et al.* 2020). In addition, environmental heterogeneity in different habitats can influence wildlife activity patterns (Liu *et al.* 2017). The evergreen tropical forest of the Lacandon Forest is more exuberant and rich in food resources and moisture than other vegetation types (Rzedowski 1978; Hubbel 2001; Pennington and Sarukhan 2005), which may influence the activity pattern of this species.

The activity patterns recorded for both peccary species show that they were mainly diurnal, with peaks of activity between 14:00 and 18:00 h and sporadic activity in the evenings or nights. Our results are consistent with Briceño-Méndez *et al.* (2016), who reported for these peccary species diurnal patterns with peak activity between 08:00 h and 14:00 h in sites with persistent hunting in Calakmul, Mexico. However, they differ slightly from the findings in a similar ecosystem in Brazil (Galetti *et al.* 2015), where peaks of activity were recorded between 5:00 and 10:00 h for the white-lipped peccary and from 17:00 to 22:00 h for the collared peccary. Other studies have shown activity patterns different from those exhibited by both peccary species in the present study, showing that they can be active throughout the day (Mayer and Wetzel 1987; Arroyo-Arce *et al.* 2017). Our results differ from those of Keuroghlian *et al.* (2004), who did not observe a temporal overlap between these species in a landscape of southeast Brazil. The differences in the hours of activity just mentioned may be due to factors associated with particular habitat characteristics, community structure, and local (hunting) or regional disturbance variables that influence the activity patterns of species (Frey *et al.* 2017). The hours of activity of peccaries recorded in this study suggest a mechanism to avoid predation since these are opposite to the activity peaks of their main predator in the study area, the jaguar *Panthera onca* (De la Torre and Medellín 2011), as well as to avoid hunting (Briceño-Méndez *et al.* 2016).


[redacted]

Figure 4. Evidence of firearm hunting and presence of domestic canids within the ACF, Lacandon Forest, Chiapas, Mexico (2015).

This suggests that habitat segregation (spatial dimension) rather than temporal segregation, may be one of the main mechanisms allowing the sympatric coexistence of both species of peccaries in the Lacandon Forest. Niche partitioning between these two tayassuids in the study area could be based on: 1) the differential use of food resources, with minor variations in the diet, as reported in several studies throughout its distribution range (Reyna-Hurtado *et al.* 2014); and 2) differences in the movement ecology of the white-lipped peccary, which, given the amplitude of its displacement, allows gaining an advantage over collared peccaries (Reyna-Hurtado and Tanner 2005; Reyna-Hurtado *et al.* 2009). This means that while *T. pecari* is able of travelling long distances of up to 120 km in preserved forests in search of food, forced by its metabolism, the collared peccary can meet its metabolic and ecological requirements even in disturbed areas (Reyna-Hurtado *et al.* 2014) and in smaller areas of suitable habitat, according to the home range of 7.3 km^2 reported by Naranjo (2002) in the Lacandon Forest.

The similarities and differences in the parameters evaluated in this study versus other studies were due to different causes, considering that animals can modify their spatio-temporal activity behavior, either to reduce the risk of natural predation (Oliveira-Santos *et al.* 2010; Lone *et al.* 2017) or as an effect of anthropic alterations such as habitat fragmentation and hunting (Tejeda-Cruz *et al.* 2009; Carreira *et al.* 2020). This has cascading effects on the rest of the community (Carreira *et al.* 2020), a potential situation that should be monitored by managers of protected and non-protected areas under the current laws and regulations seeking to preserve the viability of populations through management plans and conservation actions (Robinson *et al.* 2016; Dellinger *et al.* 2019).

Although the low tolerance of the white-lipped peccary to the effects of human activities has been documented (Reyna-Hurtado and Tanner 2007; Altrichter *et al.* 2012; Keuroghlian *et al.* 2013; Mandujano and Reyna-Hurtado

2019), the fitness of logistic regression models showed an unexpected positive projection for the use of the ACF forest area, similar to that of areas within the REBIMA (see Figure 2). The fact that white-lipped peccaries have been observed in areas with human settlements is encouraging for their conservation. We hope these results will contribute to convince decision-makers and the inhabitants of the Lacandon Forest that the coexistence between human and wildlife populations in the region is feasible.

Our results indicate that: 1) both peccary species display different habitat preferences in the study area, while tapir preferred using the protected area, as significant evidence of niche partitioning; and 2) the ACF site is an important relict of tropical forest with the potential to maintain populations of these species; therefore, the conservation of these forests by local communities is essential to ensure the persistence of these ungulates. The conservation of these populations and other natural resources in the Lacandon Forest has become urgent given the unsustainable production practices that promote deforestation (*i.e.*, extensive livestock ranching and commercial agriculture) in areas adjacent to federal and community protected natural areas. In our view, more environmentally friendly productive activity schemes such as agroforestry systems should be implemented in the region to mitigate unsustainable practices and their effects. Also, the protection and management of the remaining habitat in managed areas (ACF) are relevant and necessary, as well as the protection of wildlife from poaching.

We suggest addressing this information gap in future studies by assessing the movements of wild ungulates to better know their habitat occupation, distribution, and potential metapopulation dynamics, which may explain their presence in fragments of habitat surrounding the REBIMA (Naranjo and Bodmer 2007). Another aspect worth investigating is the influence of structural and functional connectivity, as well as habitat suitability for these species at the landscape level in the study area.

It is both convenient and relevant to generate information with spatially explicit variables that ensure greater fidelity and independence of captures of the movements of ungulates across the study area, such as the estimation of their home ranges (Frey *et al.* 2017; Mandujano and Reyna-Hurtado 2019) and approximations with occupation models, habitat preferences, and other resource selection models (*e.g.*, resource selection functions; Manly *et al.* 2002; Lele *et al.* 2013). Future research should contribute to reversing these processes, for instance, by supporting the generation of conservation strategies and strengthening management plans for the populations of wild ungulates and their habitats in the Lacandon Forest. One approach to this end would be through Wildlife Conservation Management Units with species that tolerate the sustainable use of their populations, such as the collared peccary and the lowland paca (*Cuniculus paca*).

Acknowledgments

The Consejo Nacional de Ciencia y Tecnología (CONACYT) awarded a fellowship for the first author's postgraduate studies. The project "Abundancia y uso de fauna silvestre en la Selva Lacandona Chiapas" (Abundance and use of wild fauna in the Lacandon Forest, Chiapas; (CONACYT Basic Science Fund 105896) financed part of the field work of this research. The inhabitants from the rural communities visited provided invaluable assistance and guidance during field work. A. de la Torre, Robert Owen, S. T. Álvarez-Castañeda, and two anonymous reviewers provided valuable suggestions and comments to the manuscript. María Elena Sánchez-Salazar translated the manuscript into English.

Literature Cited

Altrichter, M., *et al.* 2012. Range-wide declines of a key Neotropical ecosystem architect, the Near Threatened White-lipped peccary *Tayassu pecari*. Oryx 46:87–98.

Arce-Peña, N. P., *et al.* 2019. Landscape predictors of rodent dynamics in fragmented rainforests. Biodiversity and Conservation 28:655–669.

Arroyo-Arce, S., I. Thomson, C. Fernández, and R. Salom-Pérez. 2017. Relative abundance and activity patterns of terrestrial mammals in Pacuare Nature Reserve, Costa Rica. Cuadernos de Investigación UNED 9:15–21.

Barton, K. 2019. MuMIn: Multi-Model Inference. https://CRAN.R-project.org/package = MuMIn. Downloaded on July 20, 2021.

Bodmer, R. E. 1991. Strategies of seed dispersal and seed predation in Amazonian ungulates. Biotropica 23:255–261.

Briceño-Méndez, M., *et al.* 2016. Responses of two sympatric species of peccaries (*Tayassu pecari* and *Pecari tajacu*) to hunting in Calakmul, Mexico. Tropical Conservation Science 9:1–11.

Burnham, K. P., and D. R. Anderson. 2004. Model Selection and Multimodel Inference: A Practical Information-theoretic Approach. 2nd ed. Springer-Verlag. New York, U.S.A.

Calcagno, V. 2019. Glmulti: Model Selection and Multimodel Inference Made Easy. R package version 1.0.7.1. https://CRAN.R-project.org/package=glmulti . Downloaded on July 20, 2021 .

Camino, M., S. Cortez, S. D. Matteucci, and M. Altrichter. 2017. Experiencia de monitoreo participativo de fauna en el Chaco seco argentino. Maztozoología Neotropical 24:31–46.

Carabias, J. J. de la Maza, and R. Cadena (eds.). 2015. Conservación y Desarrollo Sustentable en la Selva Lacandona: 25 Años de Actividades y Experiencias. Natura y Ecosistemas Mexicanos. Ciudad de México, México.

Carrara, E., *et al.* 2015. Impact of landscape composition and configuration on forest specialist and generalist bird species in the fragmented Lacandona rainforest, Mexico. Biological Conservation 184:117–126.

Carreira, D. C., *et al.* 2020. A question of size and fear: Competition and predation risk perception among frugivores and predators. Journal of Mammalogy 101:648–657.

Carrillo-Reyna. N., R. Reyna-Hurtado, and B. Schmook. 2015. Abundancia relativa y selección de hábitat de *Tapirus bairdii* en las reservas de Calakmul y Balam kú, Campeche, México. Revista Mexicana de Biodiversidad 86:202–207.

Cartes, J. L., J. Thompson, and A. Yanosky. 2015. El Chaco paraguayo como uno de los últimos refugios para los mamíferos amenazados del Cono Sur. Paraquaria Natural 3:37–47.

Challenger, A., and J. Soberón. 2008. Los ecosistemas terrestres. Pp. 87–108, *in* Capital Natural de México (CONABIO, ed.). Vol. I: Conocimiento Actual de la Biodiversidad. Cmisión Nacional para el Conocimiento y Uso de la Biodiversidad. Ciudad de México, México.

Chávez, C., *et al.* (eds.). 2013. Manual de Fototrampeo para Estudio de Fauna Silvestre: El Jaguar en México como Estudio de Caso. Alianza WWF-Telcel, Universidad Nacional Autónoma de México. Ciudad de México, México.

De la Torre, J. A., and R. A. Medellín. 2011. Jaguars *Panthera onca* in the greater Lacandona ecosystem, Chiapas, Mexico: Population estimates and future prospects. Oryx 45:546–553.

De la Torre, J. A., and M. Rivero. 2019. Insights on the movements of the jaguar in the tropical forests of southern Mexico. Pp. 217–241, *in* Movement Ecology of Neotropical Forest Mammals (Reyna-Hurtado, R., and C. A. Chapman, eds.). Springer. Gewerbestasse, Switzerland.

Dellinger, J. A., *et al.* 2020. Using Mountain lion habitat selection in management. The Journal of Wildlife Management 84:359–371.

Dirzo, R., *et al.* 2014. Defaunation in the Anthropocene. Science 345:401–406.

Eisenberg, J. F. 1989. Mammals of the Neotropics. Vol 1: The Northern Neotropics. University of Chicago Press. Chicago, U.S.A.

Espinosa, S., and J. Salvador. 2017. Hunters' landscape accessibility and daily activity of ungulates in Yasuní Biosphere Reserve, Ecuador. Therya 8:45–52.

Fletcher, R., and M. J Fortin. 2018. Spatial ecology and conservation modeling. Springer International Publishing. Gewerbestasse, Switzerland.

Foerster, C. R., and C. Vaughan. 2002. Home range, habitat use and activity of Baird's tapir in Costa Rica. Biotropica 34:423–437

Frey, S., J. T. Fisher, A. C. Burton, and J. P. Volpe. 2017. Investigating animal activity patterns and temporal niche partitioning using camera-trap data: Challenges and opportunities. Remote Sensing in Ecology and Conservation 3:123–132.

Fryxell, J. M., A. R. E. Sinclair, and G. Caughley. 2014. Wildlife Ecology, Conservation, and Management. 3rd ed. Wiley-Blackwell, Oxford, United Kingdom.

Gaillard, J., *et al.* 2010. Habitat – performance relationships: Finding the right metric at a given spatial scale. Philosophical Transactions of the Royal Society of London 365:2255–2265.

Galetti, M., R. S. Bovendorp, and R. Guevara. 2015. Defaunation of large mammals leads to an increase in seed predation in the Atlantic forests. Global Ecology and Conservation 3:824–830.

Galetti, M., V. B. Zipparro, and L. P. C. Morellato. 1999. Fruiting phenology and frugivory on the palm *Euterpe edulis* in a lowland Atlantic forest of Brazil. Ecotropica 5:115–122.

García, E. 2004. Modificaciones al Sistema de Clasificación Climática de Köppen. Serie Libros Núm 6. Instituto de Geografía, Universidad Nacional Autónoma de México. Ciudad de México, México.

García, M., *et al.* 2016. *Tapirus bairdii*. In: IUCN 2016. The IUCN Red List of Threatened Species. https://www.iucnredlist.org. Downloaded on April 5, 2021.

Garcia-Alaniz, N., E. J. Naranjo, and F. F. Mallory. 2010. Human-felid interactions in three mestizo communities of the Selva Lacandona, Chiapas, Mexico: Benefits, conflicts and traditional uses of species. Human Ecology 38:451–457.

García-Gil, J.G., and J. Lugo-Hupb. 1992. Las formas de relieve y los tipos de vegetación en la Selva Lacandona. Pp. 39–49, *in* Reserva de la Biósfera Montes Azules, Selva Lacandona: Investigación para su Conservación (Vásquez-Sánchez, M. A., and M. A. Ramos-Olmos, eds.). Centro de Estudios para la Conservación de los Recursos Naturales, A. C. Publicaciones Especiales de Ecosfera 1. San Cristóbal de Las Casas, México.

Garmendia, A., *et al.* 2013. Landscape and patch attributes impacting medium- and large-sized terrestrial mammals in a fragmented rain forest. Journal of Tropical Ecology 29:331–344.

Garrido-Garduño, T., and E. Vázquez-Domínguez. 2013. Métodos de análisis genéticos, espaciales y de conectividad en genética del paisaje. Revista Mexicana de Biodiversidad 84:1031–1054.

Gause, G. F. (ed.). 1934. The Struggle for Existence in Natural Conditions. Williams and Wilkins. Baltimore, U.S.A.

Gil–Fernández, M., *et al.* 2017. Wild felid species richness affected by a corridor in the Lacandona forest, Mexico. Animal Biodiversity and Conservation 40:115–120.

Hubbell, S. P. 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press. New Jersey, U.S.A.

Jacomo, A. T. A., *et al.* 2013. White-lipped peccary home-range size in a protected area and farmland in the central Brazilian grasslands. Journal of Mammalogy 94:137–145.

Johnson, D. H. 1980. The comparison of usage and availability measurements for evaluating resources preference. Ecology 61:65–71.

Kéry, M., and J. A. Royle. 2016. Applied Hierarchical Modelling in Ecology: Analysis of Distribution, Abundance and Species Richness in R and BUGS. Vol. 1: Prelude and static models. Academic Press. London, United Kingdom.

Keuroghlian, A., *et al.* 2013. *Tayassu pecari*. In: The IUCN Red List of Threatened Species 2013. https://www.iucnredlist.org. Downloaded on April 5, 2021.

Keuroghlian, A., D. P. Eaton, and W. S. Longland. 2004. Area use by White-lipped and Collared peccaries (*Tayassu pecari* and *Tayassu tajacu*) in a tropical forest fragment. Biological Conservation 120:411–425.

Kiltie, R. A. 1982. Bite force as a basis for niche differentiation between rain forest peccaries (*Tayassu tajacu* and *T. pecari*). Biotropica 14:188–195.

Krausman, P. R., and M. L. Morrison. 2016. Another plea for standard terminology. The Journal of Wildlife Management 80:1143–1144.

Kronfeld-Schor, N., and T. Dayan. 2003. Partitioning of time as an ecological resource. Annual Review of Ecology, Evolution, and Systematics 34:153–181.

Lele, S. R., E. H. Merrill, J. Keim, and M. S. Boyce. 2013. Selection, use, choice and occupancy: clarifying concepts in resource selection studies. Journal of Animal Ecology 82:1183–1191.

Lima, M., *et al.* 2019. The paradoxical situation of the White-lipped peccary (*Tayassu pecari*) in the state of Mato Grosso, Brazil. Perspectives in Ecology and Conservation 17:36–39.

Lira-Torres, I., M. Briones-Salas, and G. Sánchez-Rojas. 2014. Abundancia relativa, estructura poblacional, preferencia de hábitat y patrones de actividad del tapir centroamericano

Tapirus bairdii (Perissodactyla: Tapiridae), en la selva de Los Chimalapas, Oaxaca, México. Revista de Biología Tropical 62:1407–1419.

Liu, X., *et al.* 2017. Diversity and activity patterns of sympatric animals among four types of forest habitat in Guanyinshan Nature Reserve in the Qinling Mountains, China. Environmental Science and Pollution Research 24:16465–16477.

Lizcano, D. L., and J. Cavelier. 2000. Daily and seasonal activity of the mountain tapir (*Tapirus pinchaque*) in the Central Andes of Colombia. Journal of Zoology 252:429–435.

Lone, K., *et al.* 2017. Temporal variation in habitat selection breaks the catch-22 of spatially contrasting predation risk from multiple predators. Oikos 126:624–632.

Lüdecke, D. 2018. SjPlot: Data Visualization for Statistics in Social Science. R package version 2(1). https://cran.r-project.org/web/packages/sjPlot/index.html. Downloaded on October 12, 2019.

MacKenzie, D. L., and J. A. Royle. 2005. Designing occupancy studies: general advice and allocating survey effort. Journal of Applied Ecology 42:1105–1114.

Malhi, Y., *et al.* 2016. Megafauna and ecosystem function from the Pleistocene to the Anthropocene. Proceedings of the National Academy of Sciences 113:838–846.

Mandujano, S., and R. Reyna-Hurtado. 2019. Recent studies of peccaries in the Neotropics. Pp. 415–438, *in* Ecology and Conservation of Tropical Ungulates in Latin America (Gallina, S. ed.). Springer. Cham, Switzerland.

Manly, B. F., *et al.* 2002. Resource Selection by Animals. Springer. The Netherlands.

March, I.J., and E.J. Naranjo. 2005. Tapir (*Tapirus bairdii*). Pp. 496–497, *in* Los Mamíferos Silvestres de México (Ceballos, G., and G. Oliva. eds.). CONABIO and Fondo de Cultura Económica. Ciudad de México, México.

Matola, S., A. D. Cuarón, and H. Rubio-Torgler. 1997. Conservation status and action plan of Baird's tapir (*Tapirus bairdii*). Pp. 29–45, *in* Tapirs: Status Survey and Conservation Action Plan (Brooks, D., R. Bodmer, and S. Matola, eds.). Gland, Switzerland and Cambridge, United Kingdom.

Mayer, J. J., and R. M. Wetzel. 1987. *Tayassu pecari*. Mammalian Species 293:1–7.

Mendes-Pontes, A. R., and D. J. Chivers. 2007. Peccary movements as determinants of the movements of large cats in Brazilian Amazonia. Journal of Zoology 273:257–265.

Meredith, M., and M. Ridout. 2017. Overlap: Estimates of Coefficient of Overlapping for Animal Activity Patterns. R package version 0.3.0. Downloaded on October 12, 2019.

Meyer, N. F., *et al.* 2019. Effectiveness of Panama as an intercontinental land bridge for large mammals. Conservation Biology 34:207–219.

Moreira-Ramírez, J., *et al.* 2019. Estado de conservación de dos ungulados sociales en Guatemala: Pecarí de labios blancos y pecarí de collar. Pp. 75–96, *in* Perspectivas de Investigación sobre los Mamíferos Silvestres de Guatemala (Kraker-Castañeda, C., A., P. Calderón, y A. A. Cabrera, eds.). Asociación Guatemalteca de Mastozoólogos. Ciudad de Guatemala, Guatemala.

Morrison, M. L, B. G. Marcot, and R. W. Mannan. 2006. Wildlife-habitat relationships: concepts and applications. Island Press. Washington D. C., U.S.A.

Muench, C., and M. M. Ramos. 2016. Can community protected areas conserve biodiversity in human modified tropical landscapes? The case of terrestrial mammals in southern Mexico. Tropical Conservation Science 9:178–202.

Naranjo, E. J. 2002. Population Ecology and Conservation of Ungulates in the Lacandon Forest, Mexico. PhD Dissertation. University of Florida. Gainesville, U.S.A.

Naranjo, E. J. 2008. Uso y conservación de mamíferos en la Selva Lacandona, Chiapas, México. Pp. 675–691, *in* Avances en el Estudio de los Mamíferos de México II (Lorenzo, C., E. Espinoza, and J. Ortega, eds.). Publicaciones Especiales Volumen II. Asociación Mexicana de Mastozoología, A. C. Ciudad de México, México.

Naranjo, E. J. 2009. Ecology and conservation of Baird's tapir in Mexico. Tropical Conservation Science 2:140–158.

Naranjo, E. J. 2018. Baird's tapir ecology and conservation in Mexico revisited. Tropical Conservation Science 11:1–4.

Naranjo, E. J. 2019. Ecology and conservation of ungulates in the Lacandon Forest, Mexico. Pp. 105–115, *in* Ecology and Conservation of Tropical Ungulates in Latin America (Gallina-Tessaro S, ed.). Springer. Cham, Switzerland.

Naranjo, E. J, S. A. Amador, F. A. Falconi, and R. A. Reyna. 2015. Distribución, abundancia y amenazas a las poblaciones de tapir centroamericano (*Tapirus bairdii*) y pecarí de labios blancos (*Tayassu pecari*) en México. Therya 6:227–249.

Naranjo, E. J., J. López-Acosta, and R. Dirzo. 2010. La cacería en México. Biodiversitas 91:6–10.

Naranjo, E. J., and E. Cruz. 1998. Ecología del tapir en la Reserva de la Biósfera La Sepultura. Acta Zoológica Mexicana 73:111–125.

Naranjo, E. J., and R. E. Bodmer. 2007. Source-sink systems of hunted ungulates in the Lacandon Forest, Mexico. Biological Conservation 138:412-420.

O' Farrill, G., *et al.* 2014. The potential connectivity of waterhole networks and the effectiveness of a protected area under various drought scenarios. PloS One 9:1–10.

Oliveira-Santos, L. G. R., *et al.* 2010. Influence of extrinsic variables on activity and habitat selection of lowland tapirs (*Tapirus terrestris*) in the coastal sand plain shrub, southern Brazil. Mammalian Biology 75:219–226.

Pennington, T. D., and J. Sarukhán. 2005. Árboles Tropicales de México: Manual para la Identificación de las Principales Especies. 3rd ed. Universidad Nacional Autónoma de México and Fondo de Cultura Económica. Ciudad de México, México.

Pianka, E. 1983. Evolutionary Ecology. Omega. Barcelona, Spain.

Peres, C. A. 1994. Composition, density, and fruiting phenology of arborescent palms in an Amazonian terra firme forest. Biotropica 26:285–294.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. www.R-project.org. Downloaded on October 25, 2019.

Redford, K. H., and J. F. Eisenberg. 1992. Mammals of the Neotropics. Vol. II: The Southern Cone. University of Chicago Press. Chicago, U.S.A.

Reyna-Hurtado, R., E. Rojas-Flores, and G. W. Tanner. 2009. Home range and habitat preferences of White-lipped peccaries (*Tayassu pecari*) in Calakmul, Campeche, Mexico. Journal of Mammalogy 90:1199–1209.

Reyna-Hurtado, R., I. March, E. Naranjo, and S. Mandujano. 2014. Pecaríes en México. Pp. 377–387, *in* Ecología y Manejo de

Fauna Silvestre en México (Valdez, R., and J. A. Ortega, eds.). Colegio de Postgraduados and New Mexico State University. Texcoco, México.

Reyna-Hurtado, R., and G. W. Tanner. 2005. Habitat preferences of ungulates in hunted and non-hunted areas in the Calakmul Forest, Campeche, Mexico. Biotropica 37:676–685.

Reyna-Hurtado, R., and G. W. Tanner. 2007. Ungulate relative abundance in hunted and non-hunted sites in Calakmul Forest (southern Mexico). Biodiversity and Conservation 16:743–756.

Robinson, K. F., *et al.* 2016. Structured decision-making as a framework for large-scale wildlife harvest management decisions. Ecosphere 7:12.

Romero-Balderas, K. G., E. J. Naranjo, H. H. Morales, and R. B. Nigh. 2006. Daños ocasionados por vertebrados silvestres al cultivo de maíz en la Selva Lacandona, Chiapas, México. Interciencia 31:276–283.

Rzedowski, J. 1978. Vegetación de México. Limusa. Ciudad de México, México.

Sánchez-Pinzón, K., R. Reyna-Hurtado, and N. F. V. Meyer. 2020. Moon light and the activity patterns of Baird's tapir in the Calakmul region, Southern Mexico. Therya 11:137–142.

Sanderson, J. G., and M. Trolle. 2005. Monitoring elusive mammals: unattended cameras reveal secrets of some of the world's wildest places. American Scientist 93:148–155.

Schaefer, H. C., W. Jetz, and K. Böhning-Gaese. 2008. Impact of climate change on migratory birds: community reassembly versus adaptation. Global Ecology and Biogeography 17:38–49.

Schoener, T. W. 1974. Resource partitioning in ecological communities. Science 185:27–39.

Schank, C. J., *et al.* 2017. Using a novel model approach to assess the distribution and conservation status of the endangered Baird's tapir. Diversity and Distributions 23:1459–1471.

Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). 2010. Norma Oficial Mexicana NOM-059-ECOL-2010. Protección ambiental, especies de flora y fauna silvestres de México, categorías de riesgo y especificaciones para su inclusión, exclusión o cambio, y lista de especies en riesgo. Diario Oficial de la Federación. 30 de Diciembre 2010.

Silva-Rodríguez, E. A., G. R. Ortega-Solís, and J. E. Jiménez. 2010. Conservation and ecological implications of the use of space by chilla foxes and free-ranging dogs in a human-dominated landscape in southern Chile. Austral Ecology 35:765–777.

Silver, S. 2004. Assessing Jaguar Abundance using Remotely Triggered Cameras. Jaguar Conservation Program, Wildlife Conservation Society. New York, U.S.A.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. 3rd ed. WH Freeman. New York, U.S.A.

Sowls, L. K. 1997. Javelinas and Other Peccaries: Their Biology, Management, and Use. Texas A&M University Press. College Station, U.S.A.

Tejeda-Cruz, C., *et al.* 2009. Habitat use of wild ungulates in fragmented landscapes of the Lacandon Forest, southern Mexico. Mammalia 73:211–219.

Terborgh, J. 1986. Community aspects of frugivory in tropical forest. Pp. 371–384, *in* Frugivores and Seed Dispersal (Estrada, A., and T. H. Fleming, eds.). W. Junk. Dordrecht, The Netherlands.

Thornton, D., *et al.* 2020. Precipitous decline of White-lipped peccary populations in Mesoamerica. Biological Conservation 242:1–12.

Tobler, M. W., *et al.* 2008. An evaluation of camera traps for inventorying large- and medium-sized terrestrial rainforest mammals. Animal Conservation 11:169–178.

Vanak, A. T., and M. E. Gompper. 2009. Dogs *Canis familiaris* as carnivores: their role and function in intraguild competition. Mammal Review 39:265–283.

Wickham, H. 2017. Tidyverse: Easily Install and Load. https://CRAN.R-project.org/package=tidyverse. Downloaded on November 12, 2017.

Zapata-Ríos, G., and L. C. Branch. 2016. Altered activity patterns and reduced abundance of native mammals in sites with feral dogs in the high Andes. Biological Conservation 193:9–16.

Associated editor: Robert Owen
Submitted: April 13, 2021; Reviewed: July 8, 2021
Accepted: September 28, 2021; Published on line: March 30, 2022

Supplementary material

https://www.revistas-conacyt.unam.mx/therya/index.php/THERYA/article/view/1167/1167_Supplementary%20material

THERYA, 2022, Vol. 13(2):183-193 DOI:10.12933/therya-22-2100 ISSN 2007-3364

Effect of an urban area in the distribution pattern and diversity of Neotropical rodents

Gloria Tapia-Ramírez[1], Consuelo Lorenzo[1]*, Arturo Carrillo-Reyes[2], Darío Navarrete[3], and Óscar Retana[4]

[1] Departamento de Conservación de la Biodiversidad, El Colegio de La Frontera Sur, C. P. 29290. San Cristóbal de Las Casas, Chiapas, México. Email: tapiaramglo@gmail.com (GT-R); clorenzo@ecosur.mx (CL).
[2] Facultad de Ingeniería, Universidad de Ciencias y Artes de Chiapas, C. P. 29000. Tuxtla Gutiérrez, Chiapas, México. Email: arturocarrilloreyes@gmail.com (AC-R).
[3] Departamento de Observación de la Tierra Atmósfera y Océano, El Colegio de La Frontera Sur, C. P. 29290. San Cristóbal de Las Casas, Chiapas, México. Email: dnavarre@ecosur.mx (DN).
[4] Centro de estudios en Desarrollo Sustentable, Universidad Autónoma de Campeche, C. P. 24095. Campeche, México. Email: ogretana@uacam.mx (OR).
*Corresponding author

Urbanization implies the loss of biodiversity and promotes the extirpation of local flora and fauna. It favors habitat fragmentation and the establishment and increase of non-native species and, eventually, the homogenization of the landscape. This study aims to evaluate the urbanization process in a medium-sized city in the Mexican southeast, San Cristóbal de Las Casas (SCLC) in the Neotropics, and the response of the rodent community to such process. The study area was categorized according to its structural characteristics into four coverage classes: urban, forest, agricultural and mountain wetlands. Rodents were sampled over urbanization gradients. In each gradient, nine sampling sites were established. The responses of rodent alpha and beta diversities to landscape structure (landscape metrics) were evaluated. The larger diversity of rodents was found in the mosaic and transition landscape categories and the least diverse were the conserved and urban ones. In SCLC, rodent richness responds to the number of fragments and the diversity of the landscape. *Mus musculus* and *Rattus rattus* were more abundant in landscapes with a high percentage of urban cover, while *Peromycus mexicanus* and *P. beatae*, in landscapes with higher forest cover. San Cristóbal de Las Casas is a city that grows over areas with native vegetation, forests and mountain wetlands; of which fragments remain embedded in the urban matrix. Mosaic and transition landscapes favor areas with high diversity and richness of rodent species (intermediate disturbance hypothesis). This study suggests that both native and non-native rodent species are abundant in areas with natural vegetation and also in urban sites. Sanitary measures are granted, since reservoir species of pathogens with zoonotic potential can be found in the area.

La urbanización representa la pérdida de biodiversidad y promueve la extirpación de la flora y fauna locales. Favorece la fragmentación y el establecimiento e incremento de especies no nativas, y eventualmente, la homogenización del paisaje. Este estudio tiene como objetivo evaluar el proceso de urbanización en una ciudad mediana del sureste mexicano, San Cristóbal de Las Casas (SCLC) en el Neotrópico, y la respuesta de la comunidad de roedores a ésta. Se caracterizó el área de estudio en 4 clases de cobertura: urbana, boscosa, agropecuaria y humedal de montaña. Se muestrearon roedores en gradientes de urbanización. En cada gradiente se establecieron 9 sitios de muestreo. El área de estudio se categorizó según sus características estructurales. Se evalúo la diversidad alfa y beta, y la respuesta de la diversidad de roedores a la estructura del paisaje (métricas de paisaje). La mayor diversidad de roedores, se encontró en las categorías de paisaje tipo mosaico y de transición y las menos diversas fueron las conservadas y urbanas. En SCLC la riqueza de roedores responde al número de fragmentos y a la diversidad del paisaje. *Mus musculus* y *Rattus rattus* fueron más abundantes en paisajes con alto porcentaje de cobertura urbana y *Peromycus mexicanus* y *P. beatae*, en paisajes con mayor cobertura de bosque. San Cristóbal de Las Casas es una ciudad que avanza sobre las áreas con vegetación nativa, bosques y humedales de montaña, de las cuales, quedan fragmentos embebidos en la matriz urbana. Los paisajes de mosaico y transición propician áreas con altas diversidades y riqueza de especies de roedores (hipótesis del disturbio intermedio). Este estudio sugiere que las especies de roedores nativos y no nativos son abundantes en áreas con vegetación natural y también en sitios urbanos. Es importante considerar algunas medidas sanitarias, pues existen especies reservorios de patógenos con potencial zoonótico.

Keywords: Cities; Cricetidae; fragmentation; landscape; Muridae.

Introduction

Nearly fifty-six percent of the global human population lives in medium-sized cities harboring between 100,000 to 500,000 inhabitants. It has been predicted that, by the year 2035, 62.5 % of the human population will live in a city (UN-HABITAT 2020). Such an increase in the world's urban population implies: 1) The establishment of new cities or the growth of those already established to the detriment of natural landscapes; since, this entails the extirpation of native flora and fauna at rates even higher than those produced by agriculture or forestry (Marzluff and Ewing 2001; McKinney 2006). 2) The establishment and increase of non-native wildlife species adapted to urban conditions and, eventually, the homogenization of the landscape (McKinney 2006).

The expansion of the urban territory onto the natural landscape alters its structure in terms of composition (cover classes) and configuration (spatial arrangement of cover classes; Liu *et al.* 2016), reconfiguring the landscape.

Additionally, fragmented and disorderly growth (coupled with the lack of associated regulations) favors urban configurations where limits (or peripheries) are unclear. Since poorly urbanized neighborhoods are constantly added to the peripheries, they represent points of growth blurring the limits of cities (Terradas *et al.* 2011). This gives rise to cities with a broad variety of landscapes, with a varying degree of disturbance, sometimes mixed with fragments of conserved vegetation (Doygun 2009).

The reconfiguration of urban space alters the communities of native flora and fauna, in terms of species composition, abundance, richness and equitability (Aronson *et al.* 2014; Lättman *et al.* 2014). Wildlife has been reported to present different types of response to urbanization, according to the taxonomic group in question (Magle *et al.* 2019); for example, large predatory mammals are poorly adapted to survive in large cities (Ordiz *et al.* 2013). In contrast, other medium and large mammals, such as raccoons (*Procyon lotor*) or capybaras (*Hydrochoerys hydrochaeris*), are well adapted to urban conditions in some regions of the continent (Dybas 2017; Serra-Medeiros *et al.* 2021).

In the case of small mammals such as rodents, there are species that respond positively to urbanization, increasing their abundances (Santini *et al.* 2019); the so-called "urban dwellers", which live permanently in the urban environment. The abundance increase of these species represents serious economic and public health problems for humans, similar those generated from the non-native rodent species like *Mus musculus* and *Rattus* species in the World (Meerburg *et al.* 2009; McFarlane *et al.* 2012). Other species exist that make occasional visits: "urban visitors" (Baker *et al.* 2003). Finally, some species respond negatively, reducing their abundances (Lopucki and Kitowski 2017), and are known as "urban avoiders" (McKinney 2006).

Previous studies have shown that urbanization influences the diversity of native rodent species, regardless of city size, reducing native species richness while increasing richness of non-native species. At the same time, these studies recognize the importance of preserving fragments of the landscape with natural coverage, in order to maintain high diversity of native rodents in urban areas (Rizkalla and Swihart 2009; Fernández and Simonetti 2013; Johnson and Karels 2016; Lopucki and Kitowski 2017). It is also important to control stray dogs and cats, common in many cities throughout the continent, which can be predators of native species and endanger their survival (Coronel-Arellano *et al.* 2021; Mella-Méndez *et al.* 2022).

The main objective of this work was to evaluate the response of the rodent community to the urbanization process in San Cristóbal de Las Casas, Chiapas, México (SCLC). For this purpose: 1) The alpha and beta diversity of the rodent community were recorded and compared against urbanization gradients; 2) the relationships between landscape metrics (in terms of configuration and composition) with rodent species richness and abundance were evaluated; and 3) based on the landscape-site approach, the study area was categorized into different categories of landscapes. We explain the rodent species distribution and suggest areas for conservation based on landscape categories and rodent diversity.

Materials and Methods

Study area. The city of San Cristóbal de Las Casas, Chiapas (SCLC; 16° 44′ 12″ N, -92° 38′ 18″ W) is in the region of The Highlands of Chiapas. The main ecosystem types in the city are: mountain wetlands (Ramsar sites; The Ramsar Convention Secretariat 2013), coniferous forests (pine, oak and pine-oak), and cloud forests (Trucíos-Caciano *et al.* 2013). The original layout of SCLC was made up of 18 blocks and 12 streets (Aubry 2008; Appendix 1). The city continued to expand towards the mountains, and towards the south of the valley, where the wetlands are located (Cantú Luna and Fenner Sánchez 2020). Today, it is a medium-sized city of 202,459 inhabitants (INEGI 2021). The urban area has increased by 12.5 % between 2001 and 2018 (Camacho-Valdez *et al.* 2019). Natural areas embedded in the city have reduced their size, and the current fragments have been designated for conservation in private, federal, and municipal reserves: Huitepec Ecological Reserve, Moxviquil Ecological Reserve, El Encuentro Ecological Park, Montetik Natural Park, La Kisst Wetland, and María Eugenia Wetland (Supplementary material 1).

Delimiting and characterizing the study area. The study area was delimited by 6 km radius circumference (Figure 1a). The characterization was conducted through a supervised classification of a Sentinel 2 image, of 10 m resolution. The image, from 2020, was analyzed using QGIS 3.16 (QGIS Development Team 2021) and the SCP (Semiautomatic Classification Plugin) plug-in 6.4 (Congedo 2020). The classification was performed with the method Maximum Similarity Algorithm. The accuracy of the classification was established by the Kappa coefficient at 80 % confidence value per land cover class. The area, was classified into four land cover classes, served for the subsequent categorization of the sampling sites: urban (soil covered by concrete, devoid of vegetation, houses, roads), forests (altered coniferous forest, oak forest, pine-oak forest), agriculture (agricultural plots, sown agricultural, agricultural in preparation, pastures), and wetlands (grasslands, areas covered by water).

Rodent species sampling. Seven bimonthly rodent samplings were performed, three in 2019 (June, August and October), one in 2020 (January) and three in 2021 (March, May and July), along seven urbanization gradients, established through the study area in straight line, and directed to the cardinal points (see Figure 1a). Along each of the seven urbanization gradients, nine sampling sites were established for each one: at 0 (1), 1.5 (2), 3.5 (3) and 6 km (3; Figure 1a). The minimum distance between sampling sites was 500 m. At each sampling site, 20 Sherman traps were placed simultaneously, for a total of 180 traps per gradient, per sampling month. The sampling sites had the following characteristics: at 0 km, the urban sites included houses,

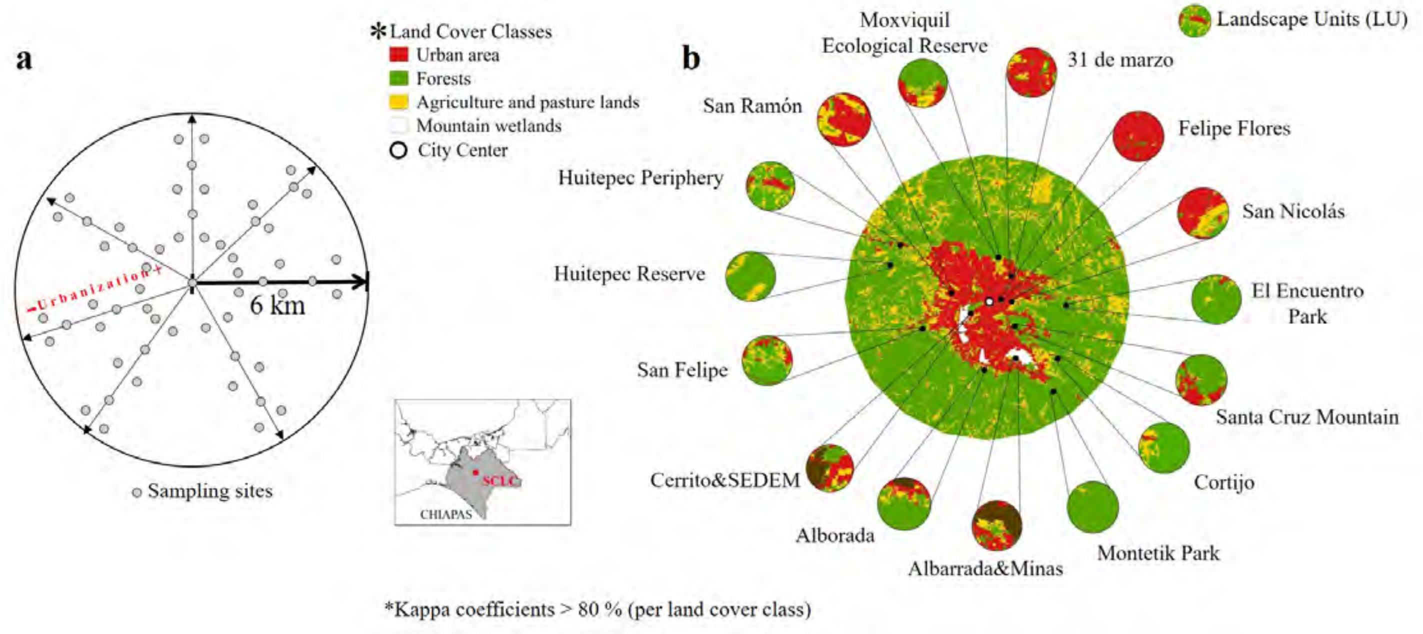

Figure 1. a) Sampling design for rodents in San Cristóbal de Las Casas, Chiapas, México. Arrows = representing urbanization gradients performed in this study; b) classified land use classes and landscape units (LU) in San Cristóbal de Las Casas, Chiapas, México.

parking lots, hotels or hostels. Then, at 1.5 km, two contrasting sites were chosen, forests and wetlands or agricultural plots or urban areas. At 3.5 km, three contrasting sites were chosen, forests and wetlands and agricultural plots or pastures or urban areas. Finally, at 6 km, the selection of three contrasting sites was repeated.

In each sampling site, the traps were placed in two lines of 10 traps each one, with a separation of 10 meters between each trap and 10 meters between lines. However, in the case of traps placed in houses, five traps were located per house (totaling four houses). Traps were placed inside rooms, kitchens, grain storage warehouses, corrals, or patios. All traps were baited with a mixture of oats, peanut butter, and sunflower seeds. The traps were checked every morning at 7:00 hrs. Each specimen sampled was identified to species level with specialized guides and taxonomic keys (Álvarez-Castañeda *et al.* 2017); sex, reproductive condition and weight were recorded, in addition to conventional somatic measurements.

Rodents were marked with ear notches (Sántiz-Vázquez 2018) and released *in situ*. Each ear sample was stored in 70 % alcohol, for future genetic analysis. Two specimens of each native species by location were collected and deposited in the Mammal Collection of El Colegio de la Frontera Sur (ECOSUR). All non-native rodents were sacrificed and conserved in 70 % alcohol. The scientific collection license FAUT-0143 provided to CL from SEMARNAT was used. Rodents were handled according to the guidelines of the American Society of Mammalogists (Sikes and The Animal Care and Use Committee 2016).

Categorizing the study area. To categorize the study area into different landscape categories, we used a site-landscape approach (Fahrig 2013). This approach consisted of establishing a buffer of 500 m ratio in each effective sampling site, considering landscape units (LU; Figure 1b). The buffer was set to the home range of the rodent species previously collected in the region (San-José *et al.* 2014; Rodríguez Macedo 2016; Arce-Peña *et al.* 2019).

Each LU was characterized in terms of its composition and configuration, using eight landscape metrics obtained with FRAGSTATS 4.2.1 software (McGarigal and Ene 2015). We chose two metrics for composition: PLAND (percentage of landscape / land cover class), and Shannon's diversity index (SHDI). The land cover classes considered were: urban (URB), forest (FOR), agricultural (AGRO), and wetland coverage (WET). Other six metrics for configuration were, number of patches (NP), contagion index (CONTAG), percentage of like adjacencies index (PLADJ), division index (DIVISION), patch richness (PR), and aggregation index (AI). The definition of each landscape metric is in Supplementary material 2.

Through a Principal Component Analysis (PCA), the spatial relationship between the LUs, the landscape metrics, and the relative abundance were explored via correlations. With the resultant ordination, landscape categories were assigned according to the structural characteristics with the highest loadings. The threshold to recognize those components best explaining variation was the asymptote of the distribution of the variance. This analysis was performed using the FactoMineR package (Le *et al.* 2020) in the R 4.10 programming language (R Core Team 2018).

Data Analysis. Alpha diversity was calculated using Hill numbers ^0D (species richness), ^1D (exponential of the Shannon diversity index), and ^2D (inverse Simpson index; Moreno *et al.* 2011). Diversity analyzes were performed using the Vegan 1.8 package (Oksanen *et al.* 2007). Range-abundance graphs were built to simultaneously visualize abundance and diversity in the categories of LU (Magurran 1988). Differences in abundance and richness were estimated with a Hutcheson t-test (Zar 2010) between landscape categories. Additionally, beta diversity (*i. e.*, species turnover) was calculated using the multiplicative-partition-of-diversity method. Furthermore, the Sorensen dissimilarity index was used to determine the dissimilarity between the LUs categories, as well as the partition of beta diversity into its components $\beta_{SOR} = \beta_{SIM} + \beta_{SNE}$; for which we used the betapart 1.5.4 package (Baselga and Orme 2012). Finally, the hypothesis of a relationship between rodent diversity and landscape metrics was tested, using a series of generalized linear models with the Glmulti 1.0.8 package (Calcagno and de Mazancourt 2010).

Results

Characterization of the study area. The study area covered 15,143 ha; forests of oak, pine, pine-oak or oak-pine forests, in some state of conservation covered the 66 % of the area, urban 14 %, agricultural crops and pastures 18 %, and conserved mountain wetlands less than 2 % (Figure 1b).

Categorization of the study area. Rodents were captured only in 15 out of 63 sampling sites, such sites were established as landscape units (LUs; Figure 1b). Landscape metrics are presented in Table 1. The study area was categorized into four categories of landscape: urban, of transition, of mosaic and conserved. This categorization was conducted using principal component analysis (PCA). The first principal component (PC1) explained 53.1 % of the variation and was interpreted as a proxy for habitat heterogeneity (Figure 2), since it showed higher weights for the number of patches (NP), landscape division index (DIVISION), contagion index (CONTAG), and percentage of like adjacencies (PLADJ; Table 2). Consequentially, the LUs positively related to PC1 were units diverse in composition, with a mixture in land cover classes, subdivided into fragments, mainly with urban and forest coverage. Principal component two (PC2), explained 22 % of the variation and was interpreted as a proxy for conservation (Figure 2). This component represents the urban gradient, due to the weight presented by the percentage of forest and urban coverage (Table 2).

Describing the landscape categories. The urban category was represented only by one unit (FFL; Figure 2). This category had the highest urban coverage and the highest aggregation index. The unit is located at the center of SCLC. In turn, the transition category was represented by heterogeneous units, with the highest richness of fragments (types of land cover). This category grouped units in process of urban transformation, with urban coverage between 28 % and 65 %; even though they retained proportions of other land cover classes; units are located between 1.5 and 3.5 km (Figure 2). The mosaic category gathered the units with the highest number of fragments; separated one from another, describing more than a continuum, a mosaic of forest, houses, agricultural plots and wetlands (Figure 2). Mosaic units were placed between 3.5 and 6 km from the city center. Conserved category was formed by units with the highest percentages of forest cover, and the lowest coverage of urban, and all are located at 6 km from the city center (Figure 2). A more detailed description of each category is presented in Supplementary material 3.

Rodent diversity, richness, and abundance. A sampling effort of 3,780 trap nights was carried out, during which 10 rodent species and 147 individuals were recorded (Supplementary material 4). Seven of these species, *Oligoryzomys fulvescens, Peromyscus beatae, P. mexicanus, Reithrodonto-*

Table 1. Landscape metrics per landscape units (LU) in San Cristóbal de Las Casas, Chiapas, México.

		Landscape metrics of composition					Landscape metrics of configuration						Distance (m)
LU name	ID	%URB	%FOR	%AGRO	%WET	SHDI	PR	NP	DIVISION	AI	CONTAG	PLADJ	DIS
Felipe Flores	FFL	95.44	3.72	0.84	0.00	0.21	3.00	16.00	0.09	96.80	85.01	95.49	0
C.31 de marzo	C31	78.84	9.77	11.39	0.00	0.66	3.00	27.00	0.37	92.12	56.04	90.59	1500
San Nicolas	SNI	60.01	13.67	28.91	0.00	0.93	3.00	17.00	0.60	90.84	41.93	89.17	1500
Santa Cruz	SCR	26.60	67.00	6.39	0.00	0.80	3.00	31.00	0.57	91.00	48.06	89.41	3500
Cerrito SEDEM	CSE	32.50	22.91	13.46	31.13	1.34	4.00	34.00	0.80	89.88	35.60	87.89	3500
Reserva Moxviquil	MOX	20.58	63.17	16.25	0.00	0.91	3.00	16.00	0.60	93.26	45.36	91.54	3500
San Ramón	SRA	65.26	5.83	28.91	0.00	0.80	3.00	18.00	0.55	92.35	49.63	90.73	3500
San Felipe	SFE	14.03	65.86	20.12	0.00	0.87	3.00	29.00	0.57	84.43	37.37	82.90	3500
Albarrada Minas	ALM	28.75	12.94	11.54	46.77	1.23	4.00	28.00	0.71	89.84	40.81	87.93	3500
Alborada	ALB	14.65	65.55	11.96	7.84	1.01	4.00	21.00	0.56	91.46	51.51	89.61	3500
Parque Encuentro	ENC	2.37	91.24	6.39	0.00	0.35	3.00	15.00	0.17	95.55	76.40	94.14	6000
Cortijo	COR	2.26	83.12	14.61	0.00	0.52	3.00	17.00	0.32	95.33	67.36	93.81	6000
Parque Montetik	MON	0.00	98.57	1.43	0.00	0.08	2.00	2.00	0.03	99.06	91.85	97.84	6000
Periferia Huitepec	PHU	12.69	63.75	23.56	0.00	0.89	3.00	41.00	0.59	83.44	36.38	81.91	6000
Reserva Huitepec	RHU	0.00	84.32	15.68	0.00	0.43	2.00	3.00	0.28	95.94	57.02	94.53	6000

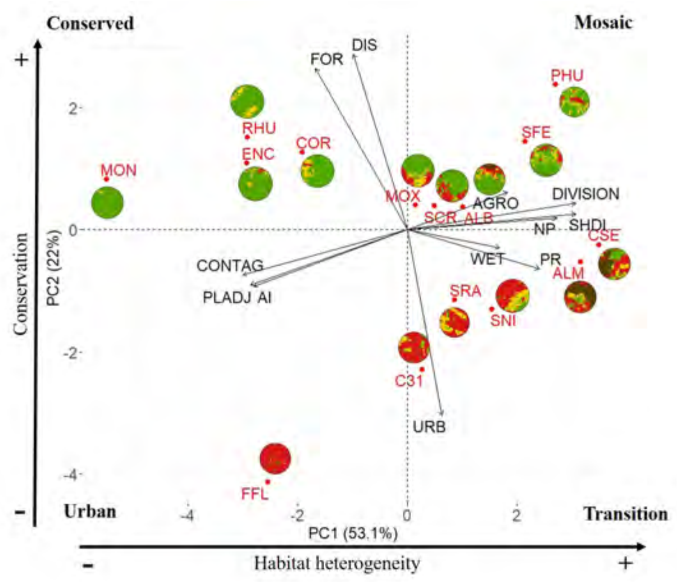

Figure 2. Plot loadings of PCA model, for landscape units by landscape metrics. Arrows = loadings per landscape metrics. Color poligons = landscape units.

Table 2. Scores of Principal Component Analysis (PCA) of landscape metrics and abundance of rodents in Landscapes Units (LU) in San Cristóbal de Las Casas, Chiapas, México.

PCA Landscapes Unites (LU)			**PCA Landscapes Unites (LU) and Rodent species**		
Loadings	**PC1**	**PC2**	**Loadings**	**PC1**	**PC2**
URB	0.193	-0.935	URB	0.275	-0.918
FOR	-0.518	0.812	FOR	-0.591	0.759
AGRO	0.562	0.191	AGRO	0.563	0.102
WET	0.516	-0.090	WET	0.519	0.056
PR	0.738	-0.195	SHDI	0.915	0.184
NP	0.835	0.061	PR	0.719	-0.033
DIVISION	0.943	0.128	NP	0.833	0.182
AI	-0.862	-0.287	DIVISION	0.915	0.202
CONTAG	-0.924	-0.229	AI	-0.846	-0.325
PLADJ	-0.881	-0.280	CONTAG	-0.895	-0.270
Eigenvalue	6.379	2.639	Eigenvalue	7.713	3.782
% of variance	53.161	21.991	% of variance	35.060	17.191
Cumulative % of variance	53.161	75.152	Cumulative % of variance	35.060	52.251

mys fulvescens, R. microdon, R. sumichrasti, and *Sigmodon toltecus*, belonged to the Cricetidae family and were considered native species; the remaining three species belong to the Muridae family and were considered non-native: *Mus musculus, Rattus rattus* and *R. norvegicus*. Regarding landscape categories, the most abundant species in the transition was *S. toltecus* and *M. musculus* was the most abundant in the mosaic (Figure 3). *Rattus rattus* was present throughout the study area, except in conserved units. In the conserved units, the most abundant species was *P. mexicanus*, which was also abundant in the mosaic units (Figure 3). The results of alpha diversity for each of the 15 LUs and for category of landscape are in Supplementary material 5.

According to the diversity of order 1 (^{1}D), the units in the transition category have a diversity of 4.56 abundant species. In contrast to the 5.77 of the mosaic units

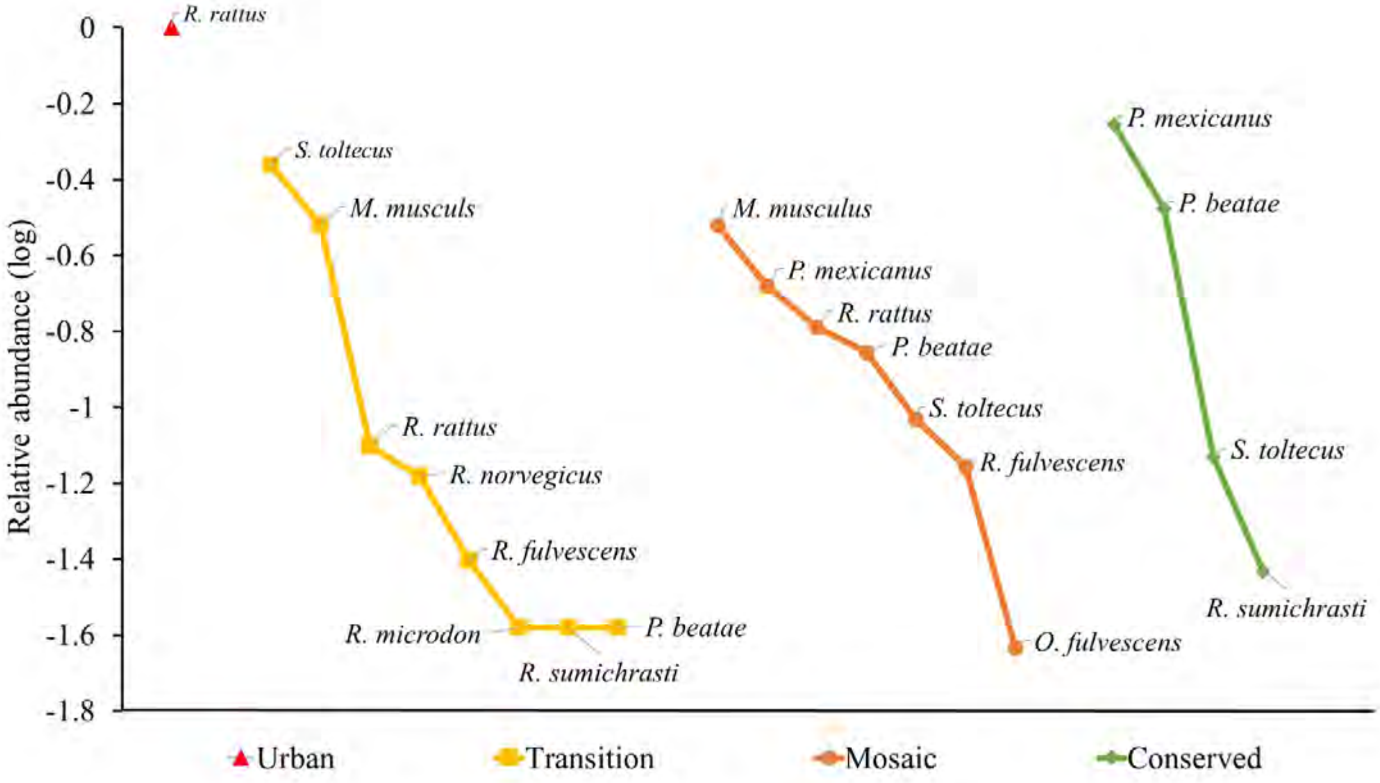

Figure 3. Range-abundance graphs by landscape categories in San Cristóbal de Las Casas, Chiapas, México.

(Supplementary material 5). This means that, among mosaic units, there are 1.21-fold more abundant species, in comparison to transition units, and 3-fold more than among conserved units (2.73). On the other hand, the highest-richness landscape categories were the transition (8 species) and the mosaic (7 species) units, while the lowest were the urban units (1 species; Figure 3). There are significant differences in richness between urban and transition categories (t = 4.2, *P* = 0.0005), and between urban and mosaic units (t = 3.28, *P* = 0.0041). Regarding abundance, coincidently, the most abundant categories are the transition ones (*n* = 76) and the mosaic (*n* = 43), and the lowest was the urban (*n* = 2). There are significant differences between abundance of urban and transition categories (t = 2.12, *P* = 0.0478), and between urban and mosaic (t = 2.99, *P* = 0.0077; Supplementary material 5).

The relationships between rodent species, landscape units, and the landscape metrics were also examined with an exploratory ordering analysis, via a PCA. The components 1 and 2 explained 35.1 % and 17.2 % of the variation, respectively (Figure 4). The presence of rodent species in SCLC is a function of two components: 1) habitat heterogeneity, defined by the landscape metrics: landscape division index (DIVISION), Shannon's Diversity index (SHDI), contagion index (CONTAG), and aggregation index (AI; Table 2; Figure 4); and 2) urbanization, defined by the landscape metrics: forests cover (FOR), DIVISION, urban cover (URB), and aggregation index (AI; Table 2; Figure 4). There is a species' grouping pattern that corresponds to the grouping pattern of landscape units (Figure 4). The units of mosaic category are associated to the presence of *S. toltecus, R. fulvescens,* and *Oligoryzomys fulvescens*. Urban units are characterized by the presence of *Rattus rattus*. Transition units group together due to sharing *R. microdon, R. sumichrasti, R. norvegicus,* and *M. musculus.* Finally, conserved units group due to *P. beatae* and *P. mexicanus,* although not exclusively (Figure 4).

Regarding beta diversity, the Sorensen index (β_{SOR}) was 0.62; 52 % of this dissimilarity was due to species turnover (β_{SIM}) and the remaining 48 %, due to the component resulting from nesting (β_{SNE}). The landscape categories sharing the most species between them were the mosaic and transition units, with five species (*P. beatae, R. fulvescens, S. toltecus, R. rattus,* and *Mus musculus*). Conversely (according to the Sorensen dissimilarity index), the conserved LUs were the most dissimilar. Finally, six models explained the relation between metrics and rodent diversity. The landscape-composition variable that best explained species richness was the percentage of wetland coverage (AIC 55.9; Table 3). Abundance was better explained by the urban and forest cover variables (AIC 120.3) and Shannon's Diversity Index (SHDI, AIC 105.7; Table 3). In the case of landscape configuration metrics, DIVISION and CONTAG explained better the abundance of the species (AIC 98.094), and richness was better explained only by DIVISION (AIC 60.28).

Discussion

In the middle-sized city of SCLC, the greatest diversity in terms of rodent species richness and abundance, was found in the categories of landscape mosaic and transition. In contrast, the lowest richness was in the urban and conserved categories. According to our results, there are more rodent species in the most heterogeneous landscapes, *i. e.*, transition and mosaic landscape categories, where there is more than one type of land coverage and fragmentation occurs. The combination of urban, agricultural and wetland coverages contributed to the highest abundance and richness of rodent species in SCLC. This showed the importance of conserving those areas with wetland coverages and other natural vegetation coverages. Also, the majority of rodent species were distributed throughout the entire study area, regardless of the type of landscape, still some of them were more abundant in some landscape units.

Table 3. Estimated parameters for the best models of Generalized Linear Models for the effects of composition and configuration landscape on abundance and richness rodent in an urbanization gradient in San Cristóbal de Las Casas, Chiapas, México. SE = Standard error. AIC = Akaike criterion. *P* values < 0.05, are significant.

Landscape composition	**Parameters**	**Estimate**	**SE**	**AIC**	**Z-value**	***P*-value**
Abundance~URB+FOR	Intercept	4.81	0.36	120.3	13.36	< 0.00
	URB	-0.03	0.00		-5.96	0.00
	FOR	-0.03	0.00		-6.91	0.00
Abundance~SHDI	Intercept	0.64	0.27	105.7	2.37	0.01
	SHDI	1.94	0.27		7.08	0.00
q0 ~ WET	Intercept	0.90	0.17	55.2	5.09	0.00
	WET	0.01	0.00		1.85	0.06
q0 ~ SHDI	Intercept	0.09	0.44	51.9	0.20	0.83
	SHDI	1.16	0.47		2.43	0.01
Landscape configuration						
Abundance ~ DIVISION + CONTAG	Intercept	-4.82	1.65	98.0	-2.91	0.00
	DIVISION	7.37	1.33		5.54	0.00
	CONTAG	0.06	0.01		3.39	0.00
q0~DIVISION	Intercept	0.06	0.45	60.2	0.13	0.89
	DIVISION	1.94	0.80		2.41	0.01

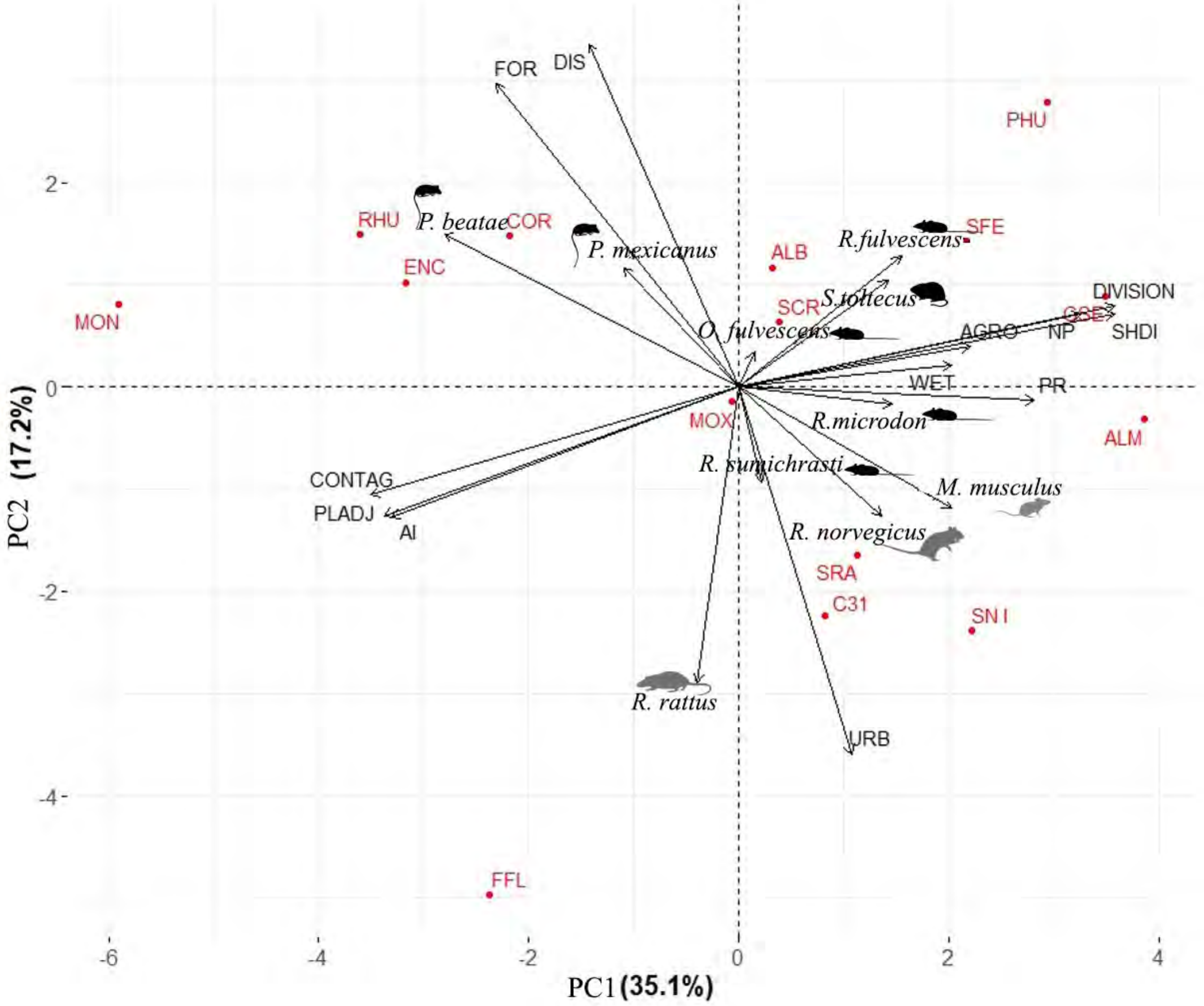

Figure 4. Plot loadings of PCA model. Arrows = loadings per landscape metrics and abundance of rodents. Points = landscape units. Black figures = native rodents. Gray figures = non native rodents.

The urbanization process in SCLC generates more than one landscape category, non-sequentially distributed, rather as spots in the study area and where the rodents are disseminated. The non-native rodents, *M. musculus* and *R. rattus*, were abundant in landscape categories with the highest proportions of urban coverage and were considered here as "urban dwellers". This data matches reports in other cities, although of different sizes, in Argentina, United States and the Netherlands (Castillo *et al.* 2003; Gomez *et al.* 2009; Johnson *et al.* 2016; Maas *et al.* 2020). In Argentina, the community of non-native rodents was accompanied by native species, in smaller proportions (Castillo *et al.* 2003; Gomez *et al.* 2009). This structure of the rodent community was attributed to the urbanization processes of Latin American cities; which grow disorderly and settle in areas with natural vegetation. This drives native and non-native species to cohabit (Castillo *et al.* 2003), just like happens in San Cristóbal de Las Casas.

Some scholars attributed this community structure to the configuration of cities. For example, old cities (more than 100 years) that conserve riparian areas, small parks, or even large trees possess greater structural diversity, favoring a greater diversity of mammals (Parsons *et al.* 2018). Contrary to areas with less structural diversity or more homogeneous (*e. g.*, monospecific forest areas) where there is less diversity of mammals. This reflects the patterns found in the city of SCLC, an old city preserving fragments with natural vegetation, agricultural plots and urban areas, generating a structurally complex landscapes, where native and non-native rodents cohabit.

Contrary to what was expected, the greatest richness and abundance of rodent species were concentrated in the mosaic and transition categories, and not in the conserved. This result coincides with that reported by García-Méndez *et al.* (2014) in SCLC. However, other studies carried out previously in the SCLC conserved units reported greater species richness. For example, Cruz Lara (2000), found five and three species of rodents in the Huitepec and Moxviquil reserves, respectively (versus one and two species reported in this study for the same reserves, respectively). Naranjo and Espinoza Medinilla (2001) reported a total of 11 species of the Cricetidae family and two of the Muridae, for the Huitepec Reserve. Finally, in 2018, Sántiz-Vázquez (2018) reported six species of cricetids in the Huitepec Reserve, two of which were also reported in this work. It is worth mentioning that these previous reports correspond to collections carried out systematically, monthly, during periods of six months or one year, which makes it likely that their numbers of species were greater than those reported in this study. Additionally, we believe that dogs, cats, chickens and other poultry (observed chasing mice) are contributing to the decrease in the number of rodents present in the reserve and park of SCLC.

Despite not being the richest sites, the conserved units harbored the greatest abundance of *P. mexicanus* and *P. beatae*, and no individuals of the non-native species *M. musculus* or *Rattus* species were found; although they had already been reported in the Huitepec Reserve (Naranjo and Espinoza Medinilla 2001). Even though the Mexican rodent, *P. mexicanus,* was present in two landscape categories in SCLC, these were the categories with the greatest forest coverage and with agricultural plots. This rodent species is widely distributed in the Mexican southeast and is considered a habitat generalist (Trujano-Ávarez and Álvarez-Castañeda 2010).

Our study indicates that habitat heterogeneity generates a landscape with intermediate disturbance, favoring an increase in species richness, as previously reported in other countries (Pardini *et al.* 2010). It is suggested that a structurally complex matrix in composition (*i. e.*, heterogeneous landscapes) can increase rodent species richness within such landscapes. This argument was also suggested by San José *et al.* (2014); who reported that species richness may benefit from the matrix that generates fragmentation of the tropical forest as a source of food and shelter. Similarly, Medellín and Equihua (1998) showed that rodents "residing in the forest" may be able to use the corridors that form the matrix and benefit from the resources of places such as pastures or crops.

Given this study shows that rodent species (native and non-native) are abundant in human settlements, it is important to consider some sanitary measures; since *R. norvegicus*. *R. rattus,* and *M. musculus* are reservoirs of pathogens with zoonotic potential (Panti-May *et al.* 2012, 2018). However, native species such as *P. mexicanus, S. toltecus, R. sumichrasti,* and *R. fulvescens* are also reservoirs of zoonotic viruses potentially transmissible to humans and possible causes of hemorrhagic fever outbreaks (Inizan *et al.* 2010; Milholland *et al.* 2018). This is particularly important if we consider that *S. toltecus* and *R. fulvescens* are considered here as "urban visitors" and were found in houses, *i. e.,* the contact with people is frequent.

This study highlights the necessity for more long-term studies focused on understanding landscape changes of the city of SCLC, as well as structural changes of the rodent community and their interactions with human populations. We suggest carrying out future monitoring activities that include virological analyses of the collected rodent specimens, to know the prevalence of zoonotic pathogens and establish the possible health risks of disorderly urban growth in SCLC.

Acknowledgements

The authors would like to thank people from San Cristobal de Las Casas, and PRONATURA sureste for giving us the permissions to access to the reserves. This study was part of a major project sponsored by Fondo Sectorial de Investigación para la Educación SEP-CONACyT (251053). G. Tapia-Ramírez received grants by The Rufford Foundation and Idea Wild. A. Hernández-Núñez, M. Notario Kumul, R. Cruz Sierra, N. Paniagua Gutiérrez, and D. Ortíz Pérez provided assistance in the field. Thanks to J. Bolaños for logistical support. We appreciate the annonymous reviewers' comments that improved the final version of this manuscript.

Literature cited

Álvarez-Castañeda, S. T., T. Álvarez, and N. González-Ruiz. 2017. Keys for identifying Mexican mammals. Johns Hopkins University Press. Baltimore, U.S.A.

Arce-Peña, N. P., *et al.* 2019. Landscape predictors of rodent dynamics in fragmented rainforests. Biodiversity and Conservation 28:655-669.

Aronson, M. F. J., *et al.* 2014. A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. Proceedings of the Royal Society B: Biological Sciences 281:1-8.

Aubry, A. 2008. San Cristóbal de Las Casas: su historia urbana, demográfica y monumental 1528-1990. Apoyo al Desarrollo de Archivos y Bibliotecas de México, A. C. México City, México.

Baker, P. J., *et al.* 2003. Factors affecting the distribution of small mammals in an urban area. Mammal Review 33:95-100.

Baselga, A., and C. D. L. Orme. 2012. Betapart: An R package for the study of beta diversity. Methods in Ecology and Evolution 3:808-812.

Calcagno, V., and C. de Mazancourt. 2010. Glmulti: An R package for easy automated model selection with (Generalized) Linear Models. Journal of Statistical Software 34:1-29.

Camacho-Valdez, V., *et al.* 2019. Land use change and urban ecosystem services: a case study of urban wetlands in a rapidly sprawling city in the Highlands of Chiapas, Mexico. Journal of Management and Sustainability 9:67-81.

Cantú Luna, J. B., and G. M. Fenner Sánchez. 2020. San Cristóbal de Las Casas: las consecuencias ambientales de un crecimiento ambicioso y descontrolado. Parte 1. Diversidad 18:75-87.

Castillo, E., *et al.* 2003. Commensal and wild rodents in an urban area of Argentina. International Biodeterioration and Biodegradation 52:135-141.

Congedo, L. 2020 Semi-automatic classification plugin documentation. Release 4:1-29.

Coronel-Arellano, H., et al. 2021. Raining feral cats and dogs? Implications for the conservation of medium-sized wild mammals in an urban protected area. Urban Ecosystems 24:83-94.

Cruz-Lara, L. E. 2000. Estudio de la variación estacional de distintas comunidades de pequeños roedores asociadas a los agroecosistemas en el municipio de San Cristóbal de Las Casas, Chiapas. UNAM. México City, México.

Doygun, H. 2009. Effects of urban sprawl on agricultural land: a case study of Kahramanmaras, Turkey. Environmental Monitoring and Assessment 158:471-478.

Dybas, C. L. 2017. The carnivorous come to town. BioScience 67:1018-1025.

Fahrig, L. 2013. Rethinking patch size and isolation effects: the habitat amount hypothesis. Journal of Biogeography 40:1649-1663.

Fernández, I. C., and J. A. Simonetti. 2013. Small mammal assemblages in fragmented shrublands of urban areas of central Chile. Urban Ecosystems 16:377-387.

García-Méndez, A., et al. 2014. Roedores y murciélagos en espacios verdes en San Cristóbal de Las Casas, Chiapas, México. Therya 5:615-632.

Gomez, D., C. Provensal, and J. Polop. 2009. Microhabitat use by the house mouse *Mus musculus* in an urban area. Acta Theriologica 54:183-192.

Instituto Nacional de Estadística y Geografía (INEGI). 2021. Número de habitantes por municipio. Información por entidad. http://www.cuentame.inegi.org.mx/monografias/informacion/chis/poblacion/. Accessed August 18, 2021.

Inizan, C. C., et al. 2010. Genetic evidence for a Tacaribe serocomplex virus, Mexico. Emerging Infectious Diseases 16:1007-1010.

Johnson, A. M., and T. J. Karels. 2016. Partitioning the effects of habitat fragmentation on rodent species richness in an urban landscape. Urban Ecosystems 19:547-560.

Johnson, S., et al. 2016. Characteristics of the built environment and the presence of the Norway rat in New York City: results from a neighborhood rat surveillance program, 2008-2010. Journal of Environmental Health 78:22-29.

Lättman, H., et al. 2014. Decline in lichen biodiversity on Oak Trunks due to urbanization. Nordic Journal of Botany 32:518-528.

Le, S., J. Josse, and F. Husson. 2020. FactoMineR: an R package for multivariate analysis. Journal of Statistical Software 25:1-18.

Liu, Z., C. He, and J. Wu. 2016. The relationship between habitat loss and fragmentation during urbanization: an empirical evaluation from 16 world cities. Plos One 11:1-17.

Lopucki, R., and I. Kitowski. 2017. How small cities affect the biodiversity of ground-dwelling mammals and the relevance of this knowledge in planning urban land expansion in terms of urban wildlife. Urban Ecosystems 20:933-943.

Maas, M., et al. 2020. Assessing trends in rat populations in urban and non-urban environments in the Netherlands. Journal of Urban Ecology 6:1-7.

Magle, S. B., et al. 2019. Advancing urban wildlife research through a multi-city collaboration. Frontiers in Ecology and the Environment 17:232-239.

Magurran, A. 1988. Ecological diversity and its measurement. Princeton University. New Jersey, U.S.A.

Marzluff, J. M., and K. Ewing. 2001. Restoration of fragmented landscapes for the conservation of birds: a general framework and specific recommendations for urbanizing landscapes. Restoration Ecology 9:280-292.

McGarigal, K., and E. Ene. 2015. Fragstats. University of Massachusetts. Amherst, U.S.A. Available at tapiaramglo@gmail.com.

McFarlane, R., A. Sleigh, and T. McMichael. 2012. Synanthropy of wild mammals as a determinant of emerging infectious diseases in the Asian-Australasian region. EcoHealth 9:24-35.

McKinney, M. L. 2006. Urbanization as a major cause of biotic homogenization. Biological Conservation 127:247-260.

Medellín, R. A., and M. Equihua. 1998. Mammal species richness and habitat use in rainforest and abandoned agricultural fields in Chiapas, Mexico. Journal of Applied Ecology 35:13-23.

Meerburg, B. G., G. R. Singleton, and A. Kijlstra. 2009. Rodent-borne diseases and their risk for public health. Critical Reviews in Microbiology 35:221-270.

Mella-Méndez, I. , et al. 2022. Predation of wildlife by domestic cats in a Neotropical city: a multi-factor issue. Biological Invasions. https://link.springer.com/article/10.1007/s10530-022-02734-5. Accessed February 20, 2021.

Milholland, M. T., et al. 2018. Global diversity and distribution of Hantaviruses and their hosts. EcoHealth 15:163-208.

Moreno, C., et al. 2011. Reanálisis de la diversidad alfa: alternativas para interpretar y comparar información sobre comunidades ecológicas. Revista Mexicana de Biodiversidad 82:1249-1261.

Naranjo, E., and E. Espinoza Medinilla. 2001. Los mamíferos de la Reserva Ecológica Huitepec, Chiapas, México. Revista Mexicana de Mastozoología 5:58-67.

Oksanen, J., et al. 2007. The vegan package. Communitty ecology package 10:1-719.

Ordiz, A., R. Bischof, and J. E. Swenson. 2013. Saving large carnivores, but losing the apex predator? Biological Conservation 168:128-133.

Panti-May, J. A., et al. 2012. Abundance and population parameters of commensal rodents present in rural households in Yucatan, Mexico. International Biodeterioration and Biodegradation 66:77-81.

Panti-May, J. A., et al. 2018. Características poblaciones de *Rattus rattus* y *Mus musculus* presentes en comunidades rurales de Yucatán, México. Tropical and Subtropical Agroecosystems 21:345-356.

Pardini, R., et al. 2010. Beyond the fragmentation threshold hypothesis: regime shifts in biodiversity across fragmented landscapes. Plos One 5:1-10.

Parsons, A. W., et al. 2018. Mammal communities are larger and more diverse in moderately developed areas. Elife 7:1-13.

QGIS Development Team. 2021. Geographic Information System. Open Source Geospatial Foundation. Beaverton, U.S.A.

The Ramsar convention secretariat. 2013. Ramsar Sites Information Service. Annotated list of wetlands of international importance: Mexico. Gland, Switzerland.

R Core Team. 2018. R: A Language and Environment for Statistical Computing. http://www.r-project.org. Viena, Austria. Accessed November 15, 2021.

Rizkalla, C. E., and R. K. Swihart. 2009. Forecasting the effects of land-use change on forest rodents in Indiana. Environmental Management 44:899-908.

Rodríguez Macedo, M. 2016. Efectos del tamaño y conectividad de los fragmentos sobre la composición de pequeños roedores en Laguna de Términos. ECOSUR. Campeche, México.

San-José, M., V. Arroyo-Rodríguez, and V. Sánchez-Cordero. 2014. Association between small rodents and forest patch and landscape structure in the fragmented Lacandona rainforest, Mexico. Tropical Conservation Science 7:403-422.

Santini, L., *et al*. 2019. One strategy does not fit all: determinants of urban adaptation in mammals. Ecology Letters 22:365-376.

Sántiz-Vázquez,E. C. 2018. Estudio poblacional de roedores en la Reserva Ecológica Huitepec, San Cristóbal de Las Casas, Chiapas. Universidad de Ciencias y Artes de Chiapas. Tuxtla Gutiérrez, México.

Serra-Medeiros, S., *et al*. 2021. Space use and activity of capybaras in an urban area. Journal of Mammalogy 102:814-825.

Sikes, R. S., and Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663-688.

Terradas, J., *et al*. 2011. Ecología Urbana. Investigación y Ciencia 52-60.

Trucíos-Caciano, R., *et al*. 2013. Análisis del cambio de uso de suelo en San Cristóbal de Las Casas. Revista Chapingo Serie Zonas Áridas XII: 45-50.

Trujano-Ávarez, A. L., and S. T. Álvarez-Castañeda. 2010. *Peromyscus mexicanus* (Rodentia: Cricetidae). Mammalian Species 42:111-118.

United Nations Human Settlements Progaramme (UN-HABITAT). 2020. World cities report 2020: the value of sustainable urbanization. UN-HABITAT. Nairobi, Kenya. https://doi.org/10.18356/c41ab67e-en. Accessed May 24, 2021.

Zar, J. H. 2010. Biostatistical Analysis. Pearson. Essex, England.

Associated editor: Sergio Ticul Álvarez Castañeda
Submitted: January 10, 2022; Reviewed: January 26, 2022
Accepted: March 8, 2022; Published on line: March 30, 2022

Supplementary material

https://www.revistas-conacyt.unam.mx/therya/index.php/THERYA/article/view/2100/2100_Supplementary%20material

THERYA, 2022, Vol. 13(2):195-203 DOI:10.12933/therya-22-2104 ISSN 2007-3364

Spatial and temporal distribution of bat mortality on a highway in southeast Brazil

Marcione Brito de Oliveira[1], and Cecília Bueno [2]*

[1] Postgraduate Program in Zoology, National Museum, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista s/n, São Cristóvão, C. P. 20940-040. Rio de Janeiro, RJ, Brazil. Email: oliveira01marcione@gmail.com (MBO).
[2] Universidade Veiga de Almeida, Núcleo de Estudos de Vertebrados Silvestres, Rua Ibituruna, 108, Maracanã, C. P. 20271-020. Rio de Janeiro, RJ, Brazil. Email: cecilia.bueno2020@gmail.com (CB).
*Corresponding author

Roads and highways can work as barriers to the movement of many species, thereby preventing the individuals from accessing feeding and reproduction sites and the immediate risk of colliding wild species with vehicles. Identifying the impacts of roads on wildlife can contribute to the establishment of actions that promote conservation. In Brazil, data on collisions between bats and vehicles are scarce and underestimated in the literature. We described bat roadkill from 2008 to 2019 on a stretch of the BR-040 highway, which crosses an area of Atlantic Forest. Roadkill species were identified and the sites with high collision frequencies were characterized. A total of 923 individuals of 57 species and five families of chiropterans were identified. Frugivore bats showed the largest number of affected individuals, with *Artibeus lituratus*, a common species in the study region, with the highest number of roadkills. The diet and foraging behaviour were the most likely factors explaining most of the bats killed on the highway. The highest roadkill rate was documented in the fall, and the critical points located nearby the APA Petrópolis and REBIO of Tinguá, environmental protection areas. We reinforce the need to mitigate these roadkills, ensuring that road systems, which constitute municipal, state and federal highways, are built to prevent major disturbance of habitat and displacement routes of these species. We believe in the need for mitigations, and considering the various species involved, we suggest speed bumps construction reducing the speed limit, installing bridges, and signaling the presence of wildlife, before the stretches identified as hotspots.

Los caminos y carreteras pueden funcionar como barreras para el movimiento de muchas especies, impidiendo así el acceso de los individuos a los sitios de alimentación y reproducción y el riesgo inmediato de colisión de especies silvestres con vehículos. Identificar los impactos de los caminos sobre la vida silvestre puede contribuir al establecimiento de acciones que promuevan la conservación. En Brasil, los datos sobre colisiones entre murciélagos y vehículos son escasos y subestimados en la literatura. Describimos murciélagos atropellados entre 2008 y 2019 en un tramo de la carretera BR-040, que atraviesa un área de Mata Atlántica. Se identificaron las especies de animales atropellados y se caracterizaron los sitios con alta frecuencia de colisión. Se identificaron un total de 923 individuos de 57 especies y cinco familias de quirópteros. Los murciélagos frugívoros mostraron el mayor número de individuos afectados, con *Artibeus lituratus*, una especie común en la región de estudio, con el mayor número de atropellamientos. La dieta y el comportamiento de búsqueda de alimento fueron los factores más probables que explicaron la mayoría de los murciélagos muertos en la carretera. La mayor tasa de atropellamientos se registró en otoño, y los puntos críticos se ubicaron en las cercanías de APA Petrópolis y REBIO de Tinguá, áreas de protección ambiental. Reforzamos la necesidad de mitigar estos atropellamientos, asegurando que los sistemas viales, que constituyen las carreteras municipales, estatales y federales, se construyan para evitar que se alteren el hábitat y las rutas de desplazamiento de estas especies. Creemos en la necesidad de mitigaciones y considerando las diversas especies involucradas. Sugerimos la construcción de topes para reducir el límite de velocidad, instalar puentes y señalizando la presencia de fauna, antes de los tramos identificados como hotspots.

Keywords: Chiroptera; collisions; mitigation; road ecology; southeast Brazil.

Introduction

The human population in recent decades has caused several changes in the environment, including road and highway construction that is currently a significant concern for biodiversity conservation (do Prado *et al.* 2006; Gumier-Costa and Sperber 2009; Bueno *et al.* 2015). Road impacts on wildlife include deaths from vehicle collisions, habitat destruction and fragmentation, barrier effect, edge effects and disturbances caused by light, noise and chemical pollution (Forman and Alexander 1998; Gibbs and Shriver 2002; Row *et al.* 2007; Kerth and Melber 2009; Stone *et al.* 2009; Zurcher *et al.* 2010; Berthinussen and Altringham 2012; Bueno *et al.* 2013; Esperandio *et al.* 2019). For several vertebrate species, some of the ecological consequences include a reduction in the gene flow between populations and a decrease in both migration rates and genetic diversity (Corlatti *et al.* 2009; Esperandio *et al.* 2019).

The order Chiroptera is the second largest among mammals, with more than 1,400 living species (Simmons and Cirranello 2020). In Brazil, this order is represented by 68 genera and 181 species subdivided into nine families (Garbino *et al.* 2020). The chiropterans play a fundamental role in maintaining the ecological processes of different vegetation domains, being excellent objects of study for characterizing habitats and their quality since they are also sensitive to changes (Fenton *et al.* 1992; Medellín *et al.* 2000;

Gorresen *et al.* 2005). However, most data on bat deaths on the roads comes from Europe, where several studies have been developed (Bafaluy 2000; Lesiński 2007; Gaisler *et al.* 2009; Kerth and Melber 2009; Berthinussen and Altringham 2012; Medinas *et al.* 2013; Ramalho and Aguiar 2020), while in Brazil, data on bat collisions are scarce (Pracucci *et al.* 2012; Pinheiro and Turci 2013; Almeida and Cardoso Junior 2014; Ceron *et al.* 2017; Valadão *et al.* 2018; Damásio *et al.* 2021; de Figueiredo Ramalho *et al.* 2021). The bat's low body mass, flight speed and nocturnal behavior make it difficult to find bat carcasses, resulting in the subsampling of the group in studies of road ecology (Bafaluy 2000; Lesiński *et al.* 2011; Berthinussen and Altringham 2012).

The bats forage using echolocation (Kalko *et al.* 1996; Siemers and Schnitzler 2000; Schaub *et al.* 2008) and hearing the sound of prey such as amphibians and insects (Marimuthu and Neuweiler 1987; Faure and Barclay 1992; Altringham and Kerth 2016). According to Schaub *et al.* (2008), the 'passive listening' strategy is adopted by bat species specialized in hunting arthropods in vegetation or on the ground, where other sounds of the environment mask the noises emitted by prey; thus, the sounds produced by cars can compromise the echolocation of bats nearby, reducing their feeding effectiveness. Therefore, roads act as barriers, reducing the bats' ability to access suitable places to forage or shelter, thus being a concerning factor, especially regarding migratory species (Zurcher *et al.* 2010; Lesiński *et al.* 2011; Ceron *et al.* 2017; Claireau *et al.* 2019).

Although some studies show that bats recognize vehicles as a threat (Baxter *et al.* 2006; Zurcher *et al.* 2010; Altringham and Kerth 2016), many others show that bats are killed on the roads by vehicle collisions (Bafaluy 2000; Gaisler *et al.* 2009; Ceron *et al.* 2017). Moreover, some studies recent provide only a list of affected species (Ceron *et al.* 2017; Novaes *et al.* 2018). Important variables that affect the collision rates between bats and vehicles, such as the diversity and abundance of specific plants in the landscape, the presence of flight corridors, habitat fragmentation and foraging routes (Gaisler *et al.* 2009; Medinas *et al.* 2013), are poorly documented in Brazil. Despite these factors, bat species moving on the road may vary from region to region, indicating other differences in the structure of the local landscape (Lesiński 2007; Kerth and Melber 2009).

Due to the lack of studies on collisions between vehicles and bats on the roads, the need to obtain data on mortality from being run over in different regions and landscapes is evident since the impact on natural populations can be severe and may cause a decline due to the cumulative effect of roadkills (Lesiński *et al.* 2011; Berthinussen and Altringham 2012; de Figueiredo Ramalho *et al.* 2021). In that regard, monitoring of the occurrence of collisions between vehicles and bats was carried out on a long stretch on the BR-040 highway in the period from 2008 to 2019, identifying the affected species and the points with the highest collision frequencies.

Materials and Methods

Study area. Data was collected on the BR-040 highway, which covers nine municipalities, from Rio de Janeiro to Juiz de Fora (Figure 1), accounting for 180.4 km. In the State of Rio de Janeiro, it goes from 0 km to 125.2 km, and in the State of Minas Gerais from 773.5 km to 828.7 km. Six municipalities are located in the state of Rio de Janeiro: Rio de Janeiro, Duque de Caxias, Petrópolis, Areal, Três Rios, Comendador Levy Gasparian, and three in the state of Minas Gerais: Simão Pereira, Matias Barbosa and Juiz de Fora. Along the studied stretch, the BR-040 highway crosses three Conservation Units of Atlantic Forest: Petrópolis Environmental Protection Area (APA Petrópolis), the Tinguá Biological Reserve (REBIO for Tinguá), and the Serra da Estrela Wildlife Refuge. The climate along the monitored highway is humid subtropical with a hot temperate summer and a dry winter, according to the Köppen classification, with precipitation concentrated in the summer, in an annual total that varies in the region between 1,300 and 2,200 mm (Alvares *et al.* 2013). We plot a final map with the critical areas of fatalities identified from analysis in Siriema software in the ArcGIS Desktop (Ormsby *et al.* 2010).

Monitoring and data collection. The data were compiled in the period from 2008 to 2019, and the stretches were travelled weekly at 40 km/h by two observers, in

Figure 1. BR-040, stretch from the municipality of Rio de Janeiro to Juiz de Fora.

addition to highway traffic inspectors monitoring 24 hours a day, seven days a week. The bats found dead were collected; when possible, they were identified at the lowest taxonomic level, stored in individual plastic bags, and frozen at the four User Service Centers. Subsequently, the specimens were sent to the Wild Animal Studies Center located at the Veiga de Almeida University, where they were prepared for deposition in the mammalogy section of the National Museum of Rio de Janeiro, a reference collection. Roadkill locations were georeferenced, and bats were photographed, sexed, measured, and identified more accurately in the laboratory. In this study, we included all individuals that could be identified within the order Chiroptera. Bats were identified with the help of identification keys provided by Gardner (2007), and Reis *et al.* (2017), and whenever necessary, identification was checked in the laboratory with available literature (Gregorin and Taddei 2002; Dias and Peracchi 2008; Velazco *et al.* 2010; Moratelli *et al.* 2013; Cirranello *et al.* 2016).

The collections of roadkill specimens were made under SISBIO License, Number: 30727-12. The use of these animals in this study complies with and is under the Operating License, Number: 1187/2013 and Authorization for Capture, Collection, and Transport of Biological Material - Abio (Renewal) No. 514/2014.

Data analysis. Siriema 2.0 software was used (Coelho *et al.* 2014) to identify the main stretches of significant groupings within a highway, the hotspots, and the analyses were 2D HotSpots and Ripley 2D K statistics. Ripley's K statistic describes the dispersion of data across a range of spatial scales (Ripley 1981; Levine 2004). To define the different scales evaluated, we used an initial radius of 100 m and an increment of 400 m for each step. To assess the meaning of possible aggregations, we subtracted the observed K values from the average obtained in 1,000 simulations of random roadkill distributions for each stop. The values above the 95% confidence limits obtained in the simulations indicate scales with significant aggregations (Levine 2004; Teixeira *et al.* 2013; Coelho *et al.* 2014). To find the critical hit-and-run points, we performed a two-dimensional analysis of the identification of the hotspots. Using an average of 1,000 simulations with a 95% confidence interval, we divided the highway into segments of 200 meters each (Teixeira *et al.* 2013; Coelho *et al.* 2014).

Data on the number of individuals run over in each of the four years and comparing the seasons were tested for normality of residues, using the Shapiro-Wilk test and homoscedasticity of residues, using the Bartlett test. Having the data sets in which the assumptions of normality and homoscedasticity of the residuals were accepted, ANOVA (one way) was performed, and a BoxPlot was plotted to verify differences between the results obtained in the present study. The significance level used in the tests was 0.05%, and the R software was used to perform all the statistical tests (R Core Team 2021).

Results

A total of 923 roadkilled individuals of 57 species of bats were recorded. Five families (Table 1) were observed; amongst them, the Phyllostomidae (*n* = 652; 71%) and Vespertilionidae (*n* = 204; 22 %) were the most common roadkilled. The species with the highest collision frequency were *Artibeus lituratus* (*n* = 216; 23.4 %), *Glossophaga soricina* (*n* = 61; 6.61 %), *Carollia perspicillata* (*n* = 55; 5.96 %), *Platyrrhinus recifinus* (*n* = 46; 4.98 %), *Sturnira lilium* (*n* = 41; 4.44 %), *Platyrrhinus lineatus* (*n* = 40; 4.33 %), *Phyllostomus hastatus* (*n* = 38; 4.12 %), *Nyctinomops laticaudatus* (*n* = 33; 3.58 %), *Artibeus fimbriatus* and *Anoura caudifer* (*n* = 31; 3.36 %); other species had less than 20 records each, and 151 individuals (16.36 %) could only be identified to the order level.

Frugivores represented the most roadkills, represented by 19 species and 52.87 % of the total number of individuals; however, the greatest recorded richness was that of insectivores, represented by 24 species and 14 % of individuals roadkills. The nectarivores were represented by five species and 10.83 % of the total roadkill. The other trophic guilds were hematophagous, with two species, and carnivore, omnivore and piscivore, represented by one species each. These represent a total of 5.9 % of roadkill recorded in the study. In most of the specimens, it was not possible to identify sex and age; however, in those where it was possible, the male/female rate was similar, and most of them were adults (Table 1). Differences were observed in the frequency of bat collisions between the seasons. The highest mortality was documented in the fall with 330 (35.75 %) individuals killed in collisions, followed by 251 (27.19 %) in the summer, 217 (23.51 %) in the winter and 125 (13.54 %) in the spring. Considering the number of individuals run over in relation to the seasons and each monitoring year (2014, 2015, 2016, 2017, and 2018). According to the Shapiro-Wilk test, the data are normally distributed (W = 0.98471, α = 2, *P* = 0.8891) and are homoscedastic according to the Bartlett test (2.1022, α = 3, *P* = 0.5515). Therefore, the data were submitted to the parametric ANOVA (One Way) test, where significant differences were observed between the data set of individuals hit by cars in relation to the seasons of the year in each monitored year (F = 1.337, α = 3, *P* = 0.297). The BoxPlot showed a difference, in terms of the number of individuals run over, between the spring season and the other seasons of the year (Figure 2).

In the analyses to determine the scales in which road deaths were significantly aggregated in space, we found different aggregation points for bats. The stretch in the city of Rio de Janeiro, that cover less than 1 km, was the only one that had no record of bat's roadkills. The spatial distribution of roadkill along the BR-040 highway was not random, indicating that the records concentrated on some specific points along the highway. Many stretches had significant aggregations, with the most probable and most significant extension being located between the Duque de Caxias and Petrópolis kilometers. This most critical stretch begins at the ascent of the sierra of Petrópolis. Other peaks of signifi-

Table 1. Species of bats roadkill during the study and the number of collisions according to their age and sexual classification. Undefined sex = SexInd; Undefined age category = AgeInd. Conservation status for species at global level follows IUCN (2020). Data Deficiente (DD); Least Concern (LC); Near Threatened (NT); No identification (S/ID).

Family / Species	Female	Male	SexInd	Adult	Young	AgeInd	IUCN
Emballonuridae							
Saccopteryx leptura	0	0	1	1	0	0	LC
Phyllostomidae							
Anoura caudifer	6	6	19	16	0	15	LC
Anoura geoffroyi	1	1	1	1	0	2	LC
Anoura sp.	0	0	1	0	0	1	
Artibeus cinereus	1	0	2	1	0	2	LC
Artibeus fimbriatus	5	9	17	16	0	15	LC
Artibeus lituratus	43	50	123	105	4	107	LC
Artibeus obscurus	0	0	1	0	0	1	LC
Artibeus planirostris	0	1	0	1	0	0	LC
Artibeus sp.	0	0	17	8	0	9	
Carollia perspicillata	17	11	27	24	2	29	LC
Chiroderma doriae	3	1	2	3	0	3	LC
Chiroderma sp.	0	0	1	1	0	0	
Chiroderma villosum	1	2	1	2	0	2	LC
Choeroniscus minor	1	0	0	1	0	0	LC
Chrotopterus auritus	0	0	4	0	0	4	LC
Desmodus rotundus	2	2	5	6	0	3	LC
Diphylla ecaudata	0	1	1	1	0	1	LC
Glossophaga soricina	18	13	30	35	1	25	LC
Lonchophylla peracchii	0	1	2	1	0	2	LC
Macrophyllum macrophyllum	0	0	1	0	0	1	LC
Micronycteris hirsuta	0	0	1	1	0	0	LC
Micronycteris megalotis	1	1	0	2	0	0	LC
Micronycteris microtis	0	0	1	0	0	1	LC
Micronycteris minuta	1	0	0	1	0	0	LC
Mimon bennettii	2	2	1	4	0	1	LC
Phyllostomus hastatus	5	7	26	24	1	13	LC
Platyrrhinus sp.	0	0	1	0	0	1	
Platyrrhinus lineatus	12	4	24	17	0	23	LC
Platyrrhinus recifinus	10	8	28	20	1	25	LC
Pygoderma bilabiatum	1	2	10	3	0	10	LC
Sturnira lilium	9	8	24	16	3	22	LC
Sturnira tildae	0	0	3	0	0	3	LC
Uroderma sp.	0	0	1	0	0	1	
Vampyressa pusilla	5	1	1	5	0	2	DD
Vampyrodes caraccioli	1	0	0	1	0	0	LC
Noctilionidae							
Noctilio leporinus	1	0	0	1	0	0	LC
Molossidae							
Eumops sp.	0	0	1	0	0	1	
Molossops neglectus	1	0	0	0	0	1	DD
Molossus molossus	1	3	11	5	0	10	LC
Molossus rufus	1	0	5	1	0	5	LC
Nyctinomops aurispinosus	0	0	1	0	0	1	LC
Nyctinomops laticaudatus	1	4	28	18	0	15	LC
Nyctinomops sp.	0	0	5	2	0	3	
Tadarida brasiliensis	0	0	1	0	0	1	LC
Molossidae (unidentified)	0	0	2	2	0	0	

Table 1. Continuation...

Family / Species	Female	Male	SexInd	Adult	Young	AgeInd	IUCN
Vespertilionidae							
Eptesicus brasiliensis	1	1	3	3	0	2	LC
Eptesicus diminutus	1	0	0	1	0	0	LC
Eptesicus sp.	1	0	2	2	0	1	
Histiotus velatus	1	0	0	1	0	0	DD
Dasypterus ega	1	1	4	2	0	4	LC
Myotis albescens	0	0	1	1	0	0	LC
Myotis izecksohni	1	1	1	2	0	1	DD
Myotis nigricans	1	3	5	3	0	6	LC
Myotis riparius	2	0	2	1	0	3	LC
Myotis ruber	0	1	0	1	0	0	NT
Myotis sp.	0	0	16	4	0	12	
Vespertilionidae (unidentified)	0	0	3	0	0	3	
S/ID	0	1	150	27	1	123	
Total	159	146	618	394	13	516	

cant aggregations were observed between the kilometers of Três Rios and Comendador Levy Gasparian (Figure 3). The most critical points on the road, according to the analyses, were in the Atlantic Forest Biodiversity Corridor, which includes the Tinguá Biological Reserve, the Serra da Estrela State Wildlife Refuge and the APA Petrópolis (Figure 4).

Discussion

Bats are valuable indicators of biodiversity and ecosystem health, as a result of his sensitivity to fragmentation, that can decrease the richness and abundance of the order in altered areas (Reis *et al.* 2003; Alurralde and Díaz 2021). Thus, habitat fragmentation could be an aggravating factor in the seed dispersal process carried out by Chiroptera species. Despite the relative richness and abundance observed during the monitoring of the current study, these values can be related to the time and size of the sample. However, still can be underestimated for the region due to the limitation of the study that only recorded species found dead by collisions. Species richness during this monitoring was similar to other studies in southeastern Brazil (Dias and Peracchi 2008; Esbérard and Bergallo 2008; Tavares *et al.* 2010; Perini *et al.* 2014), yet, we observed a high variation between the richness and diversity of these studies, which is probably due to differences in capture techniques, such as the use of mist nets or active search for shelters in areas with landscape mosaic varying from conserved to degraded environments (Dias and Peracchi 2008; Esbérard and Bergallo 2008; Tavares *et al.* 2010; Perini *et al.* 2014; Alurralde and Díaz 2021), and differences in collision probabilities by bat species in the present study.

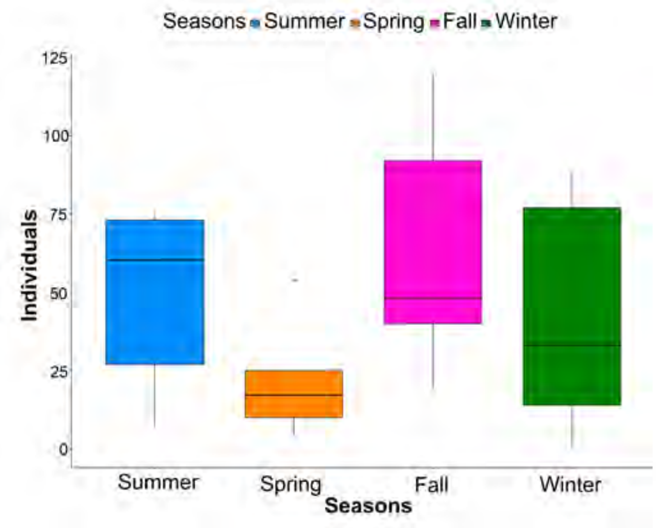

Figure 2. Boxplot (median with upper and lower quartiles) of the number of individuals of bats killed in collisions by season of the year on highway BR-040.

Frugivore bats are usually the most frequent group in collisions with vehicles on the roads, according to our data and other studies on South America (Pinheiro and Turci 2013; Ceron *et al.* 2017; Valadão *et al.* 2018; de Figueiredo Ramalho *et al.* 2021), where frugivores are more representative (Fenton *et al.* 1992; Martins *et al.* 2014). Species as *Artibeus lituratus*, *Carollia perspicillata,* and *Sturnira lilium* are not inhibited by fragmentation and are more abundant in disturbed areas than other bat species (Medellín *et al.* 2000; Reis *et al.* 2003; Alurralde and Díaz 2021). However, these species have been most affected by collisions with vehicles on the roads (Pinheiro and Turci 2013; Ceron *et al.* 2017; Valadão *et al.* 2018; de Figueiredo Ramalho *et al.* 2021). These species have been considered abundant in the study region (Dias and Peracchi 2008; Tavares *et al.* 2010; Luz *et al.* 2011), and have characteristics that make them good indicators of damaged areas, providing advantages for plants whose fruits are consumed by them, is a key factor for dispersion and genetic flow of plants in regions affected by fragmentation (Muller and dos Reis 1992; Reis *et al.* 2003; Bianconi *et al.* 2006).

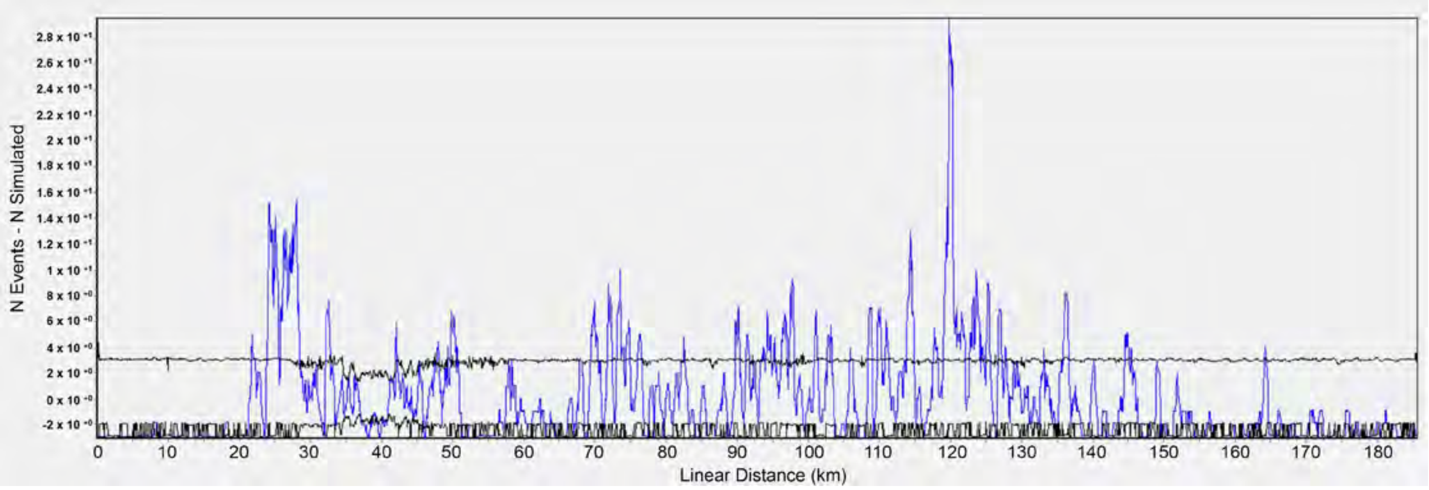

Figure 3. Bat roadkill aggregation intensity (blue line) and 95% confidence limits (black line) along 180.4 km of BR-040.

This higher rate of collisions with frugivores bats is probably due to some associated factors, such as flight height, displacement patterns in foraging, the temporal and spatial distribution of the fruits and the plants, as well as the landscape structure (Bernard and Fenton 2003; Clevenger *et al.* 2003; Bianconi *et al.* 2006; Gumier-Costa and Sperber 2009; Pracucci *et al.* 2012; Gomes *et al.* 2020). An essential factor is that many of these frugivorous species are consumers of pioneer plants, which are usually found in large densities in degraded areas, such as the vicinity and edges of roads (Muller and dos Reis 1992; Reis *et al.* 2003). The species most impacted by the collisions on BR-040, *Artibeus lituratus*, was also the most abundant in studies in the southeastern region (Esbérard and Bergallo 2008; Tavares *et al.* 2010; Luz *et al.* 2011; Perini *et al.* 2014). Although roadkills did not show marked seasonal trends, fewer bat collisions were documented during the spring. Moreover, there is evidence of the difference in the number of collisions by bats in the seasons (Lesiński *et al.* 2011), but little is known about the factors that can influence the number of roadkills by these species, needing further investigations.

Different species of bats can be impacted differently by the fragmentation of habitats resulting from a road network. Some factors may be contributing to this difference in the amount of roadkill by bat species, such as foraging strategies, diet, or resilience of species in degraded areas (Kalko *et al.* 1996; Stone *et al.* 2009; Bhardwaj *et al.* 2017; Claireau *et al.* 2019), and need be investigated. Rare species can potentially be affected even by small mortality rates (Fensome and Mathews 2016; Damásio *et al.* 2021). In the present study, many insectivores considered rare were found roadkilled. The study by Bhardwaj *et al.* (2021) pointed out that the activity of seven of ten insectivorous bats species decreased significantly within the proximity of a highway due to changes created in the surrounding environment by traffic, such as pollution, light, noise, and vibrations. The authors called the surrounding environment a "road effect zone," and in this area, the habitat is degraded or entirely unsuitable for wildlife, which leads to an indirect loss of habitat (Bhardwaj *et al.* 2021).

One of the stretches identified in this study as a highly significant hotspot is in an area of Atlantic Forest in the municipality of Petrópolis, a region inserted in the APA Petrópolis, the Serra da Estrela State Wildlife Refuge, and close to the Biological Reserve (REBIO) of Tinguá. Twenty-eight species of bats have been registered in REBIO so far, the most abundant being *C. perspicillata*, *S. lilium,* and *A. lituratus* (Dias and Peracchi 2008), also the most frequently recorded in the present study. We highlight the species *Myotis ruber*, which had a run-over record close to the REBIO area, and which is listed as almost threatened with extinction in the IUCN Red List (IUCN 2020). The most critical points were on the stretch that crosses Tinguá Biological Reserve, highlighting the need for more urgent conservation measures in this region. However, the simple counting of carcasses found on the roads is an underestimation of the actual rate of bats killed by being run over. Even in regular monitoring, some factors may influence this count. Bat carcasses can be completely destroyed by continuous vehicle traffic, some can be thrown off the road and lost in the vegetation, and some individuals could be removed by other animals for consumption (Pracucci *et al.* 2012; Cunha *et al.* 2015; Ramalho and Aguiar 2020).

The roads that cross parks and forest reserves can have an extremely negative impact, a barrier to the natural movement of the species, and establishing a population decline, as well as decreasing the genetic flow for the wildlife of these regions (Samson *et al.* 2016). Critically, bat collisions with vehicles have been observed mainly in these areas where endangered and endemic bat species occur (Lesiński 2007). Moreover, these regions also harbor other wild species, thus making roadkills more critical for the conservation of species (IUCN 2020). Efforts to mitigate these accidents in the region are carried out with wildlife warning signs, yet these had not shown been proved effective. Roads are already proven to be harmful to many wild animals (Trombulak and Frissell 2000; Fahrig and Rytwinski 2009; Gumier-Costa and Sperber 2009), and there are several approaches that can be used to reduce the effects of

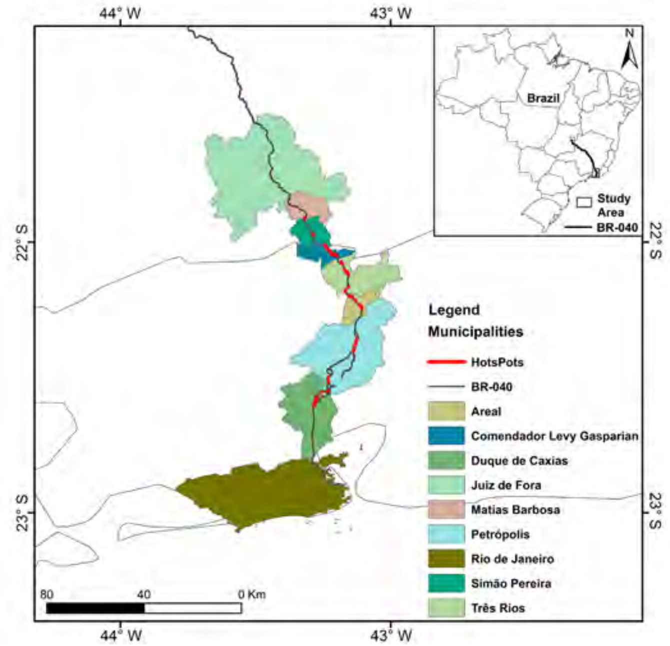

Figure 4. The sections marked in red indicate the critical areas of fatalities (HotSpots) identified from monitoring of run over bat fauna, analysis in Siriema software.

roads and mortality in wild animal populations (Jackson 2000; Glista *et al.* 2009). The use of underpasses and ecological bridges for wild fauna probably varies from species to species, depending on their behavior in terms of movement (Berthinussen and Altringham 2012; Bhardwaj *et al.* 2017). However, a combination of monitoring and ecological bridges has shown great effectiveness in decreasing roadkill rates (Bhardwaj *et al.* 2017).

Our results suggest that bats are particularly vulnerable to the impact of roads. Consequently, they may take a long time to recover from disturbances due to their low fertility, longevity, and foraging activity in large areas of the landscape (Findley 1993). Roads can affect the amount of area available to wildlife habitat, particularly through the fragmentation, and also affect the quality of this area by traffic of vehicles that cause collisions and deaths of wild animals (Bafaluy 2000; Bueno *et al.* 2015; de Figueiredo Ramalho *et al.* 2021). Other factors that influence this mortality are the lack of proper signs and awareness of drivers who travel on these highways. Hybrid mitigation is applied to reduce the adverse effects of roads on wildlife and comply with environmental legislation. These mitigations should account for several species, considering the broad diversification of habits showed by bats (Bernard and Fenton 2003; Bianconi *et al.* 2006; Gomes *et al.* 2020), and their sensitivity to increased lighting, which can change their activity pattern (Stone *et al.* 2009; Appel *et al.* 2017; Gomes *et al.* 2020). We suggest speed bumps construction to reduce the speed limit, install bridges, and signaling the presence of wildlife on the stretches identified as hotspots in our analyses, that is, with a higher risk of collision for these species of bats.

Acknowledgments

Marcione B. Oliveira received CNPq fellowship (Proc. 162273/2018-5) and Cecília Bueno thanks the Concer, BR-040 Concessionaire, for support to this research. We thank Igor Pfeifer Coelho, Camila L. Nacif, and Maria Clara S. Ribeiro for their suggestions on improving the manuscript.

Literature Cited

Almeida, V. M., and J. C. S. Cardoso Junior. 2014. Silvestres na Rodovia Vicinal Antônio Joaquim de Moura Andrade entre os Municípios de Mogi Guaçu-SP e Itapira-SP. Foco 5:99-116.

Altringham, J., and G. Kerth. 2016. Bats and roads. Pp. 35-62, *in* Bats in the Anthropocene: conservation of bats in a changing world (Voigt, C. C., and T. Kingston, eds.). Springer International Publishing. London, U.K.

Alurralde, S. G., and M. M. Díaz. 2021. Assemblage-level responses of Neotropical bats to forest loss and fragmentation. Basic and Applied Ecology 50:57-66.

Alvares, C. A., *et al.* 2013. Köppen›s climate classification map for Brazil. Meteorologische Zeitschrift 22:711-728.

Appel, G., A. López-Baucells, W. Ernest-Magnusson, and P. E. D. Bobrowiec. 2017. Aerial insectivorous bat activity in relation to moonlight intensity. Mammalian Biology 85:37-46.

Bafaluy, J. 2000. Mortandad de murciélagos por atropello en carreteras del sur de la provincia de Huesca. Galemys 12:15-23.

Baxter, D. J. M., J. M. Psyllakis, M. P. Gillingham, and E. L. O'Brien. 2006. Behavioural response of bats to perceived predation risk while foraging. Ethology 112:977-983.

Bernard, E., and M. B. Fenton. 2003. Bat mobility and roosts in a fragmented landscape in central Amazonia, Brazil. Biotropica 35:262-277.

Berthinussen, A., and J. Altringham. 2012. Do bat gantries and underpasses help bats cross roads safely? Plos One 7:e38775.

Bhardwaj, M. *et al.* 2021. Insectivorous bats are less active near freeways. Plos One 16:e0247400.

Bhardwaj, M. *et al.* 2017. Differential use of highway underpasses by bats. Biological Conservation 212:22-28.

Bianconi, G. V., S. B. Mikich, and W. A. Pedro. 2006. Movements of bats (Mammalia, Chiroptera) in Atlantic Forest remnants in southern Brazil. Revista Brasileira de Zoologia 23:1199-1206.

Bueno, C., C. O. M. Sousa, and S. R. Freitas. 2015. Habitat or matrix: which is more relevant to predict roadkill of vertebrates? Brazilian Journal of Biology 75:228-238.

Bueno, C., M. T. Faustino, and S. R. Freitas. 2013. Influence of landscape characteristics on capybara roadkill on highway BR-040, southeastern Brazil. Oecologia Australis 17:130-137.

Ceron, K. *et al.* 2017. Roadkilled bats (Mammalia: Chiroptera) in two highways of Santa Catarina state, Southern Brazil. Oecologia Australis 21:207-212.

Cirranello, A., N. B. Simmons, S. Solari, and R. J. Baker. 2016. Morphological diagnoses of higher-level phyllostomid taxa (Chiroptera: Phyllostomidae). Acta Chiropterologica 18:39-71.

Claireau, F. *et al.* 2019. Major roads have important negative effects on insectivorous bat activity. Biological Conservation 235:53-62.

Clevenger, A. P., B. Chruszcz, and K. E. Gunson. 2003. Spatial patterns and factors influencing small vertebrate fauna roadkill aggregations. Biological Conservation 109:15-26.

Coelho, A. V. P., I. P. Coelho, F. T. Teixeira, and A. Kindel. 2014. Siriema: road mortality software. Manual do Usuário V. 2.0. NERF, UFRGS, Porto Alegre, Brasil. https//:www.ufrgs.br/siriema. Accessed on 07 July 2020

Corlatti, L., K. HacklÄnder, and F. Frey-Roos. 2009. Ability of wildlife overpasses to provide connectivity and prevent genetic isolation. Conservation Biology 23:548-556.

Cunha, G. G., M. T. Hartmann, and P. A. Hartmann. 2015. Roadkills of vertebrate species in the Pampa Region, South Brazil. Ambiência 11:307-320.

Damásio, L. *et al*. 2021. Diversity and Abundance of Roadkilled Bats in the Brazilian Atlantic Forest. Diversity 13:335.

de Figueiredo Ramalho, D. *et al*. 2021. Factors influencing bat road casualties in a Neotropical savanna. Perspectives in Ecology and Conservation 19:189-194.

Dias, D., and A. L. Peracchi. 2008. Quirópteros da Reserva Biológica do Tinguá, estado do Rio de Janeiro, sudeste do Brasil (Mammalia: Chiroptera). Revista Brasileira de Zoologia 25:333-369.

do Prado, T. R., A. A. Ferreira, and Z. F. S. Guimarães. 2006. Efeito da implantação de rodovias no cerrado brasileiro sobre a fauna de vertebrados. Acta Scientiarum. Biological Sciences 28:237-241.

Esbérard, C. E. and H. G. Bergallo. 2008. Influência do esforço amostral na riqueza de espécies de morcegos no sudeste do Brasil. Revista Brasileira de Zoologia 25:67-73.

Esperandio, I. B. *et al*. 2019. Do roads act as a barrier to gene flow of subterranean small mammals? A case study with *Ctenomys minutus*. Conservation Genetics 20:385-393.

Fahrig, L., and T. Rytwinski. 2009. Effects of roads on animal abundance: An empirical review and synthesis. Ecology and Society 14:21.

Faure, P. A., and R. M. Barclay. 1992. The sensory basis of prey detection by the long-eared bat, *Myotis evotis*, and the consequences for prey selection. Animal Behaviour 44:31-39.

Fensome, A. G., and F. Mathews. 2016. Roads and bats: a meta-analysis and review of the evidence on vehicle collisions and barrier effects. Mammal Review 46:311-323.

Fenton, M. B. *et al*. 1992. Phyllostomid bats (Chiroptera: Phyllostomidae) as indicators of habitat disruption in the neotropics. Biotropica 24:440-446.

Findley, J. S. (Eds.). 1993. Bats: a community perspective. Cambridge University Press. Cambridge, U.K.

Forman, R. T. T., and L. E. Alexander. 1998. Roads and their major ecological effects. Annual Review of Ecology and Systematics 29:207-231.

Gaisler, J., Z. Řehák, and T. Bartonička. 2009. Bat casualties by road traffic (Brno-Vienna). Acta Theriologica 54:147-155.

Garbino, G. S. T. *et al*. 2020. Updated checklist of Brazilian bats: versão 2020. Comitê da Lista de Morcegos do Brasil—CLMB. Sociedade Brasileira para o Estudo de Quirópteros (Sbeq). https://www.sbeq.net/lista-de-especies. Accessed on 30 September 2020

Gardner, A. L. (Eds.). 2007. Mammals of South America, Volume I. Marsupials, xenarthrans, shrews, and bats. The University of Chicago Press. Chicago, U.S.A.

Gibbs, J. P., and W. G. Shriver. 2002. Estimating the effects of road mortality on turtle populations. Conservation Biology 16:1647-1652.

Glista, D. J., T. L. DeVault, and J. A. DeWoody. 2009. A review of mitigation measures for reducing wildlife mortality on roadways. Landscape and Urban Planning 91:1-7.

Gomes, D. G., G. Appel, and J. R. Barber. 2020. Time of night and moonlight structure vertical space use by insectivorous bats in a Neotropical rainforest: an acoustic monitoring study. PeerJ 8:e10591.

Gorresen, P. M., M. R. Willig, and R. E. Strauss. 2005. Multivariate analysis of scale-dependent associations between bats and landscape structure. Ecological Applications 15:2126-2136.

Gregorin, R., and V. A. Taddei. 2002. Chave artificial para identificação de molossídeos brasileiros (Mammalia, Chiroptera). Mastozoologia Neotropical 9:13-32.

Gumier-Costa, F., and C. F. Sperber. 2009. Atropelamentos de vertebrados na Floresta Nacional de Carajás, Pará, Brasil. Acta Amazonica 39:459-466.

IUCN. 2020. The IUCN Red List of Threatened Species. Version 2020-1. https://www.iucnredlist.org. Accessed on 30 June 2020

Jackson, S. D. 2000. Overview of transportation impacts on wildlife movement and populations. Wildlife and highways: seeking solutions to an ecological and socio-economic dilemma. The Wildlife Society 7-20.

Kalko, E. K. V., C. O. Handley, and D. Handley. 1996. Structure, diversity, and long-term dynamics of a Neotropical bat community. Pp. 503-553, *in* Long-Term studies of vertebrate communities (Cody, M. L., and J. A. Smallwood, eds.). Academic Press. New York, U.S.A.

Kerth, G., and M. Melber. 2009. Species-specific barrier effects of a motorway on the habitat use of two threatened forest-living bat species. Biological Conservation 142:270-279.

Lesiński, G. 2007. Bat road casualties and factors determining their number. Mammalia 71:138-142.

Lesiński, G., A. Sikora, and A. Olszewski. 2011. Bat casualties on a road crossing a mosaic landscape. European Journal of Wildlife Research 57:217-223.

Levine, N. 2004. CrimeStat III: A Spatial Statistics Program for the Analysis of Crime Incident Locations. Ned Levine and Associates, Houston, TX, and the National Institute of Justice. Washington, DC, U.S.A.

Luz, J. L., L. D. M. Costa, E. C. Lourenço, and C. E. L. Esbérard. 2011. Morcegos (Mammalia, Chiroptera) da Reserva Rio das Pedras, Rio de Janeiro, Sudeste do Brasil. Biota Neotropica 11:95-101.

Marimuthu, G., and G. Neuweiler. 1987. The use of acoustical cues for prey detection by the Indian False Vampire Bat, Megaderma lyra. Journal of Comparative Physiology A 160:509-515.

Martins, M. P. V., J. M. Torres, and E. A. C. Dos Anjos. 2014. Dieta de morcegos filostomídeos (Mammalia, Chiroptera, Phyllostomidae) em fragmento urbano do Instituto São Vicente, Campo Grande, Mato Grosso do Sul. Papeis Avulsos de Zoologia 54:665-670.

Medellín, R. A., M. Equihua, and M. A. Amin. 2000. Bat diversity and abundance as indicators of disturbance in neotropical rainforest. Conservation Biology 14:1666-1675.

Medinas, D., J. T. Marques, and A. Mira. 2013. Assessing road effects on bats: The role of landscape, road features, and bat activity on roadkills. Ecological Research 28:227-237.

Moratelli, R., A. L. Gardner, J. A. Oliveira, and D. E. Wilson. 2013. Review of *Myotis* (Chiroptera, Vespertilionidae) from northern South America, including description of a new species. American Museum of Natural History. American Museum Novitates 2013:1-36.

Muller, M. F., and N. R. dos Reis. 1992. Partição de recursos alimentares entre quatro espécies de morcegos frugívoros (Chiroptera, Phyllostomidae). Revista Brasileira de Zoologia 9:345-355.

Novaes, R. L. M. *et al*. 2018. On a collision course: the vulnerability of bats to roadkills in Brazil. Mastozoología Neotropical 25:115-128.

Ormsby, T. *et al*. 2010. Getting to Know ArcGIS Desktop. Redlands, ESRI Press. California, U.S.A.

Perini, F. A., V. C. Tavaores, and C. Nascimento. 2014. Bats from the city of Belo Horizonte, Minas Gerais, southeastern Brazil. Chiroptera Neotropical 9:169-173.

Pinheiro, B. F., and L. C. B. Turci. 2013. Vertebrados atropelados na estrada da Variante (BR-307), Cruzeiro do Sul, Acre, Brasil. Natureza on line 11:68-78.

Pracucci, A., C. Alves da Rosa, and A. Bager. 2012. Variação sazonal da fauna selvagem atropelada na rodovia MG 354, Sul de Minas Gerais – Brasil. Biotemas 25:73-79.

Ramalho, D. F., and L. Aguiar. 2020. Bats on the Road—A Review of the Impacts of Roads and Highways on Bats. Acta Chiropterologica 22:417-433.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reis, N. R. *et al*. 2017. História Natural dos Morcegos Brasileiros Chave de identificação de Espécies. Technical Books Editora. Rio de Janeiro, Brasil.

Reis, N. R., M. L. S. Barbieri, I. P. de Lima, and A. L. Peracchi. 2003. O que é melhor para manter a riqueza de espécies de morcegos (Mammalia, Chiroptera): um fragmento florestal grande ou vários fragmentos de pequeno tamanho? Revista Brasileira de Zoologia 20:225-230.

Ripley, B. D. 1981. Spatial Statistics. John Wiley and Sons. New York, U.S.A.

Row, J. R., G. Blouin-Demers, and P. J. Weatherhead. 2007. Demographic effects of road mortality in black ratsnakes (*Elaphe obsoleta*). Biological Conservation 137:117-124.

Samson, A. *et al*. 2016. Effect of vehicular traffic on wild animals in Sigur Plateau, Tamil Nadu, India. Journal of Threatened Taxa 8:9182-9189.

Schaub, A., J. Ostwald, and B. M. Siemers. 2008. Foraging bats avoid noise. Journal of Experimental Biology 211:3174-3180.

Siemers, B., and H. Schnitzler. 2000. Natterer's bat (*Myotis nattereri* Kuhl, 1818) hawks for prey close to vegetation using echolocation signals of very broad bandwidth. Behavioral Ecology and Sociobiology 47:400-412.

Simmons, N. B., and A. L. Cirranello. 2020. Bat species of the world: a taxonomic and geographic database [online]. https://batnames.org/. Accessed on 10 December 2020

Stone, E. L., G. Jones, and S. Harris. 2009. Street Lighting Disturbs Commuting Bats. Current Biology 19:1123-1127.

Tavares, V. C. *et al*. 2010. Bats of the state of Minas Gerais, southeastern Brasil. Chiroptera Neotropical 16:675-705.

Teixeira, F. Z. *et al*. 2013. Are road-kill hotspots coincident among different vertebrate groups? Oecologia Australis 17:36-47.

Trombulak, S. C., and C. A. Frissell. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. Conservation Biology 14:18-30.

Valadão, R. M., L. F. Bastos, and C. P. de Castro. 2018. Atropelamentos De Vertebrados Silvestres Em Quatro Rodovias No Cerrado, Mato Grosso, Brasil. Multi-Science Journal 1:62-74.

Velazco, P. M., A. L. Gardner, and B. D. Patterson. 2010. Systematics of the *Platyrrhinus helleri* species complex with descriptions of two new species. Zoological Journal of the Linnean Society 159:785–812.

Zurcher, A. A., D. W. Sparks, and V. J. Bennett. 2010. Why the bat did not cross the road? Acta Chiropterologica 12:337-340.

Associated editor: Sergio Solari
Submitted: January 16, 2022; Reviewed: February 18, 2022
Accepted: March 5, 2022; Published on line: March 31, 2022

THERYA, 2022, Vol. 13(2):205-224 DOI:10.12933/therya-22-1173 ISSN 2007-3364

Evidence of the genetic and spatial structure of *Nasua narica* in Central America and northern South America from mitogenomic analysis

María Fernanda Jaramillo[1], and Manuel Ruiz-García[1]*

[1] Laboratorio de Genética de Poblaciones Molecular-Biología Evolutiva. Unidad de Genética. Departamento de Biología. Facultad de Ciencias. Pontificia Universidad Javeriana. Carrera 7A No 43-82. Bogotá D. C., Colombia. Email: ocmafe@hotmail.com (MFJ), mruizgar@yahoo.es (MRG).

*Corresponding author

Carnivores are extremely important in ecosystem dynamics. Coatis (Procyonidae) are a group of Neotropical species with highly developed social behavior. One coati species is the Central American or white-nosed coati (*Nasua narica*). This work describes the analysis of two sets of mitochondrial data for a sample of *N. narica* covering most of the geographic distribution range of the species. The first data set analyzed 74 specimens for three mitochondrial loci; the second, 59 specimens for complete mitochondrial genomes. Our phylogenetic analyses revealed six distinct genetic groups of *N. narica* in southern México, Central America, and South America, which, together with three additional groups found in northern México and southern USA in a previous study, resulted in a total of nine genetically distinct groups of *N. narica*. The first genetic group (G1), which began to differentiate 4.1 to 3.2 million years ago, was located on the Pacific coast of Ecuador and northern Colombia. A second genetic group (G6) was detected in northern Colombia, Panama, and southern Costa Rica, being introgressed by mitochondrial DNA from the mountain coati (*Nasuella olivacea*). The third genetic group (G3) was located in Costa Rica, Nicaragua, El Salvador, Honduras, and southern Guatemala. The fourth genetic group (G4) was located in north-central Guatemala and Belize. The fifth genetic group (G5) was distributed in southern México (Chiapas, Tabasco, Campeche, Quintana Roo, and Yucatán) and northern Guatemala. Finally, the sixth genetic group (G2) was found only in Mérida (Yucatán, México). Groups G2 to G5 became mitochondrially diversified over 1.9 to 1.1 million years. All groups showed high mitochondrial genetic diversity, although the South American genetic group (G1) had the highest diversity. The northern genetic groups (G4, G5) had lower genetic diversity, except for the Merida group, which is likely composed of other undetected subgroups. The existence of six (nine, considering another study) well-developed groups in *N. narica* is related to female phylopatry and climatic changes during the Pleistocene. A spatial autocorrelation analysis showed a very high structure, well in line with the south-to-north colonization of the American continent by *N. narica*.

Los carnívoros son extremadamente importantes en la dinámica de los ecosistemas. Los coatíes (Procyonidae) son un grupo de especies neotropicales con un comportamiento social muy desarrollado. Una especie de coatí es el coatí centroamericano o de nariz blanca (*Nasua narica*). Este trabajo describe el análisis de dos conjuntos de datos mitocondriales para una muestra de *N. narica* que cubre la mayor parte del rango de distribución geográfica de la especie. El primer conjunto de datos analizó 74 especímenes para tres loci mitocondriales; el segundo, 59 especímenes para genomas mitocondriales completos. Nuestros análisis filogenéticos revelaron seis grupos genéticos distintos de *N. narica* en el sur de México, América Central y América del Sur, que, junto con tres grupos adicionales encontrados en el norte de México y el sur de EE. UU. en un estudio anterior, dieron como resultado un total de nueve genéticamente distintas grupos de *N. narica*. El primer grupo genético (G1), que comenzó a diferenciarse hace 4,1 a 3,2 millones de años, se ubicó en la costa del Pacífico de Ecuador y el norte de Colombia. Un segundo grupo genético (G6) fue detectado en el norte de Colombia, Panamá y el sur de Costa Rica, siendo introgresado por ADN mitocondrial del coatí de montaña (Nasuella olivacea). El tercer grupo genético (G3) se ubicó en Costa Rica, Nicaragua, El Salvador, Honduras y el sur de Guatemala. El cuarto grupo genético (G4) se ubicó en el centro-norte de Guatemala y Belice. El quinto grupo genético (G5) se distribuyó en el sur de México (Chiapas, Tabasco, Campeche, Quintana Roo y Yucatán) y norte de Guatemala. Finalmente, el sexto grupo genético (G2) se encontró únicamente en Mérida (Yucatán, México). Los grupos G2 a G5 se diversificaron mitocondrialmente durante 1,9 a 1,1 millones de años. Todos los grupos mostraron una alta diversidad genética mitocondrial, aunque el grupo genético sudamericano (G1) presentó la mayor diversidad. Los grupos genéticos del norte (G4, G5) tuvieron menor diversidad genética, excepto el grupo Mérida, que probablemente esté compuesto por otros subgrupos no detectados. La existencia de seis (nueve, considerando otro estudio) grupos bien desarrollados en *N. narica* está relacionada con la filopatría femenina y los cambios climáticos durante el Pleistoceno. Un análisis de autocorrelación espacial mostró una estructura muy alta, en línea con la colonización de sur a norte del continente americano por *N. narica*.

Keywords: Coati; mitochondrial genes; *Nasua* sp.; phylogeography; Pleistocene; population genetics; spatial patterns.

Introduction

Regardless of the time when North and South America became connected in geological times and the physical substrate that was established in Central America (islands, isthmus of Panama, etc.) for the Great American Biotic Exchange (GABI), the orographic, biotic, biogeographical, and climatological complexity of the zone (southern North America, Central America, and northern South America) is of such diversity and magnitude that evolutionary processes in this area, including diversification and speciation, have occurred at a high speed (Marshall 2007; Hardy *et al.* 2013). Therefore, the evolutionary study of the species with a broad distribution range in this geographic area can reveal essential information for understanding the colonization, adaptation, diversification, and speciation processes of migrant organisms between previously separate continents (Webb 2006; Morgan 2008).

A species that meets these characteristics is the white-nosed coati (*Nasua narica* Linnaeus 1766, Order Carnivora, Family Procyonidae) since it has a wide distribution from North America to northern South America (González-Maya *et al.* 2011). The evolutionary and biogeographical history of procionids is controversial from the molecular and paleontological perspectives (Soibelzon and Prevosti 2013; Nigenda-Morales *et al.* 2019; Ruiz-García *et al.* 2019b, 2020a). In fact, procionids are one of the taxonomic groups that can provide surprising insights into how and when GIBA occurred (Koepfli *et al.* 2007; Forasiepi *et al.* 2014).

Nasua narica is distributed from Arizona and New Mexico (USA) through Central America, including the Pacific coast of Colombia, Ecuador, and northern Peru (Emmons 1990; Emmons and Feer 1997; Tirira 2007, 2011). However, Gompper (1995) excluded the Ecuadorian and Peruvian Pacific areas because this author considered that *Nasua nasua* lives there. Although Gompper (1995) excluded *N. narica* from the South American mammal fauna, other authors, such as those previously mentioned, and the results of the present study postulate the existence of *N. narica* in northwestern South America, which is why we used South American specimens of this species.

Nasua narica is a strongly gregarious species that forms social groups including up to 30 females and juveniles per group, while males tend to be solitary and are the only ones to disperse after reaching sexual maturity (Gompper 1997; Gompper *et al.* 1997, 1998). On the other hand, females are strongly philopatric and frequently do not disperse outside the area where they were born (Valenzuela and Ceballos 2000). This species potentially participates in controlling small-sized pests such as rodents and arthropods, besides being a seed disperser and a potential prey for large cats such as jaguar (*Panthera onca*) and puma (*Puma concolor* (Gompper 1995, 1997).

Four subspecies have been recognized according to body size, differences in coat color, and cranial characteristics (Hershkovitz 1951; Hall 1981; Decker 1991; Gompper 1995). These differences may be related to geographic barriers and ecological differences (Hershkovitz 1951). The four subspecies are: 1) *N. n. molaris* (Merriam 1902; Type locality: Manzanillo, Colima, México), distributed from the north of the Trans-Mexican Volcanic Belt to Arizona and New Mexico USA; 2) *N. n. narica* (Linneus 1766; Type locality: "America", restricted to Achotal, Isthmus of Tehuantepec, Veracruz, México), distributed south of the Trans-Mexican Volcanic Belt to south Panama; 3) *N. n. nelsoni* (Merriam 1901; Type locality: Cozumel Island, Quintana Roo, México), distributed exclusively in the type locality; and 4) *N. n. yucatanica* (Allen 1904; Type locality: Chichen Itzá, Yucatán, México), confined to the Yucatán Peninsula. McFadden *et al.* (2008) designated the insular subspecies as a different species (*N. nelsoni*) based on a morphometric analysis.

To date, only two works have analyzed the genetic structure of *N. narica*. The first, by Silva-Caballero *et al.* (2017), used a fragment of 800 base pairs (bp) of the mitochondrial gene (mt) *Cyt-b* and 12 nuclear microsatellites in 60 specimens from five different populations in México (Nayarit, Jalisco, Morelos, Tabasco, and Quintana Roo). Moderate and high levels of genetic diversity were found for both types of molecular markers (mitochondrial: haplotypic diversity, H_d = 0.968; microsatellites: expected heterozygosity, H_E = 0.774). A total of 22 haplotypes were detected in the five areas analyzed, and each of the populations with unique haplotypes, except for three haplotypes shared between the two geographically closest populations (Puerto Morelos, Quintana Roo, and La Venta, Tabasco). Both genetic markers clearly separated the five populations studied and identified a strong genetic structure between them, suggesting isolation by distance.

The second study was conducted by Nigenda-Morales *et al.* (2019) with sequences of three mt genes (*Cyt-b*, *NAD5*, and *16S rRNA*) and 11 nuclear microsatellites in 85 specimens from Arizona and New Mexico (USA), México (Jalisco, Morelos, Yucatán, and Cozumel Island), Belize, Guatemala, Costa Rica, and Panama. They found a strong genetic structure with five distinct Evolutionary Units (EU). The southernmost (Panama) was the most differentiated EU, with genetic distances between 9.9 % and 10.8 % from the other four EU, and which appeared 3.8 million years ago (Ma). A second EU consisted of specimens from Costa Rica, Guatemala, Belize, Cozumel Island, and Yucatán (México). A third EU included specimens from Morelos (central México), while the fourth EU included specimens from Jalisco (western México); finally, the fifth EU comprised specimens from southwest USA. The diversification of this group was calculated to have occurred 1.2 Ma. The genetic flow took place from south to north and from east to west.

The present study used two sets of mitochondrial genes (three loci: *ND5*, *Cyt-b*, and *D-loop*; and complete mitogenomes) analyzed in 74 and 59 specimens, respectively, of *N. narica* sampled in México, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panama, Colombia, and Ecuador. We focused on mtDNA to expand the scope of previous results with a larger number of data (mitogenomes) and samples of specimens from South America that clearly belong to this species because of their external morphology (phenotype). It is also possible to compare our results with those of Nigenda-Morales *et al.* (2019) since the present study included Colombia and Ecuador, whereas the study referred to was limited to northern Mesoamerica.

Mitochondrial DNA genes allow reconstructing phylogenetic relationships to determine recent intra- or interspecific genetic divergence processes, while nuclear genes reveal deeper relationships (Collins and Dubach 2000, 2001; Cortés-Ortiz *et al.* 2003; Ruiz-García *et al.* 2016). In fact, mtDNA has been extremely effective for detecting new groups or taxa that had been left out with other techniques (Krause *et al.* 2010; Derenko *et al.* 2012; Sawyer *et al.* 2015). Mitochondrial genes have high mutation accumulation rates, short coalescence times, absence of introns and recombination, and haploid inheritance (Avise *et al.* 1987). In addition, although they represent a single linked locus, the selection pressure and evolutionary rates are heterogeneous, depending on the genes considered (Nabholz *et al.* 2012). An additional advantage is that the number of mtDNA copies per cell is high, thus making it easier to obtain sequences and mitogenomes from low-quality samples (teeth, hair, skin, etc.; Guschanski *et al.* 2013). However, caution should be exercised with mtDNA because gene trees do not always match species trees (Freeman and Herron 1998). Also, mtDNA only shows the evolution of female lineages, so hybridization events produced by males as vectors of gene flow go unnoticed (Burrell *et al.* 2009).

Therefore, considering that this work covers the broadest distribution range analyzed to date for *N. narica* and that, for the first time, complete mitogenomes are analyzed for this species, the objectives are to 1) determine the number of lineages in *N. narica* throughout its geographic range, and whether the putative morphological subspecies described for *N. narica* match the molecular groups detected; 2) estimate genetic heterogeneity among these groups and the ancestor-descendant relationship between them; 3) determine the relationship of historical geographic, geological, and climatological events to the divergence times found in the genetic groups of *N. narica*; 4) estimate the levels of genetic diversity within them; and 5) determine significant patterns of spatial structure throughout the area analyzed.

Materials and Methods

Sample collection. We analyzed 74 specimens of *N. narica* from México (*n* = 25), Guatemala (*n* = 21), Belize (*n* = 3), Honduras (*n* = 7), El Salvador (*n* = 3), Nicaragua (*n* = 1), Costa Rica (*n* = 3), Panama (*n* = 1), Colombia (*n* = 4), Ecuador (*n* = 5), and Robinson Crusoe Island (Chile; *n* = 1, Figure 1 and Supplementary Table 1). Eight specimens of *Nasuella olivacea* from Colombia were used as an external group, as well as 29 specimens of *Nasua nasua* from Colombia (*n* = 4), Ecuador (*n* = 5), Perú (*n* = 11), Bolivia (*n* = 3), and Brazil (*n* = 6). For mitogenomes, there were 59 specimens from México (*n* = 21), Guatemala (*n* = 14), Belize (*n* = 3), Honduras (*n* = 7), El Salvador (*n* = 3), Nicaragua (*n* = 1), Costa Rica (*n* = 3), Panama (*n* = 1), Colombia (*n* = 2), Ecuador (*n* = 3), and Robinson Crusoe Island (Chile) (*n* = 1). Samples of six specimens of *N. olivacea* from Colombia were used as an external group, as well as 23 specimens of *N. nasua* from Colombia (*n* = 4), Ecuador (*n* = 2), Peru (*n* = 10), Bolivia (*n* = 3), and Brazil (*n* = 4).

The samples come from specimens hunted by indigenous communities and road-killed animals in southern México, and Central and South America (fragments of skin, teeth, and hairs with bulbs). Permission to collect biological materials was requested from the authorities of the communities (in addition to the corresponding ministries). During the sampling process, hunters from the local communities were interviewed to determine the exact source of the samples; these were usually collected within 5 to 15 kilometers of the interview site. Samples were gathered over 22 years (1996–2018).

DNA extraction, amplification, and sequencing. DNA extraction from skin and muscle samples was performed using the phenol-chloroform procedure (Sambroock *et al.* 1989). DNA from follicle hairs and teeth was extracted with Chelex 100 resin (Bio-Rad, Hercules, California, USA) using the protocol of Walsh *et al.* (1991), while the DNA from bones was extracted with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). These procedures were used for all three mitochondrial loci. Three mt loci were amplified: 1) 407 bp of the *Cyt-b* gene (Irwin *et al.* 1991), 2) 1,800 bp of gene *ND5* (Trigo *et al.* 2008), and 3) 306 bp from the *D-loop* region (Hoelzel *et al.* 1994). The total sequenced length was 2,513 bp.

We used the PCR (Polymerase Chain Reaction) in a final volume of 25 µL: 2µL of 1 mM $MgCl_2$ (CorpoGen), 1 µL of 0.2 mM dNTPs (BioLabs), 1 µL of 0.1 mM of each primer, one Taq Polymerase Unit (CorpoGen), 100–200 ng of DNA template (in 2–4 µL of DNA), 2 µL of 10X Buffer, and 14–16 µL of double-distilled H_2O. The PCR temperatures for gene amplification were 95 °C for 5 minutes, followed by 40 cycles at 94 °C for one minute: 52 °C (*Cyt-b*), 55 °C (*ND5*), and 56 °C (*D-loop*) for one minute, 72 °C for one minute, and a final extension at 72 °C for ten minutes. The amplification products, including positive and negative controls, were run on a 2 % agarose gel stained with ethidium bromide and visualized with ultraviolet light on a transilluminator. Both directions were sequenced using BigDye Terminator v3.1 (Applied Biosystems, Inc., Foster City, California, USA), whose products were analyzed on an ABI 3730 sequencer (Applied Biosystems, Inc., Foster City, California, USA). The sequences were assembled and edited with Sequencher 4.7 (Gene Codes, Corp., Ann Arbor, Michigan, USA).

Nucleotide sequences of the mt*ND5* and *Cyt-b* genes were translated into amino acid sequences to exclude the possibility of nuclear mitochondrial DNA segments (Numts; Lopez *et al.* 1994). All amino acid translations performed showed correct start and stop codons, and a total absence

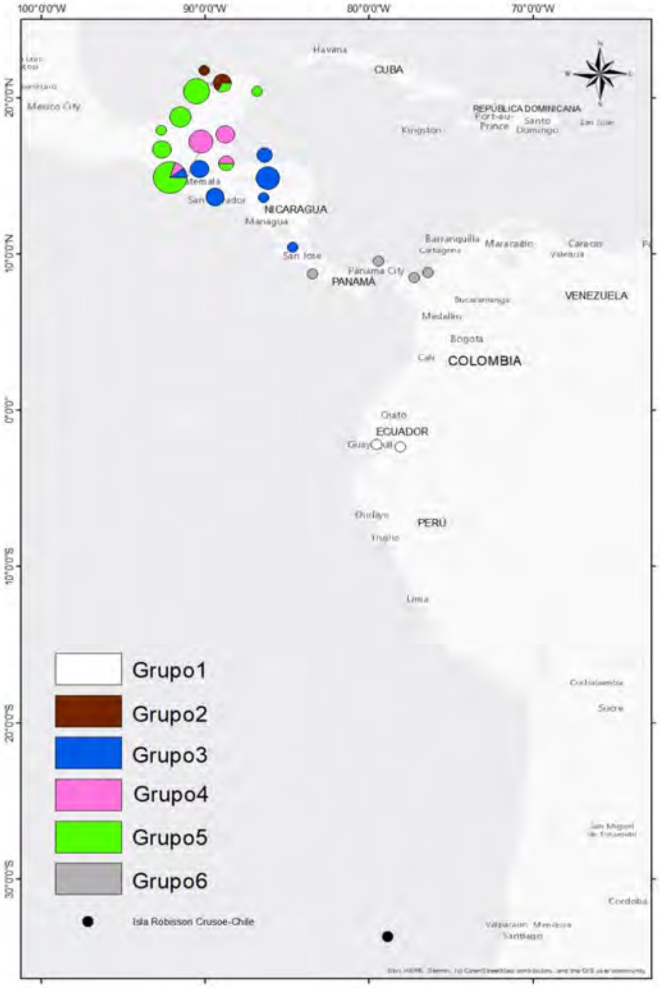

Figure 1. Map of Central America and northern South America where 74 specimens of white-nosed coati (*Nasua narica*) were sampled for three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*), 59 of which also sampled for the complete mitogenomes. Circles are proportional to the sample size. The colors of circles mark the different molecular groups detected and are the same colors shown in phylogenetic trees and the haplotype network.

of termination codons. All the mutations observed were synonymous, which is well-related to the absence of Numts in the two mt genes mentioned.

For the mitogenomic analysis, DNA was extracted and isolated from the tissues mentioned above using the QIAamp DNA Micro Kit (Qiagen, Inc. Hilden, Germany) according to the manufacturer's protocol. Mitogenomes were sequenced by "long-template PCR", which minimizes Numts amplification (Thalmann *et al.* 2004; Raaum *et al.* 2005). The PCRs to obtain mitogenomes were performed with the LongRange PCR Kit (Qiagen, Inc. Hilden, Germany), with a reaction volume of 25 µL. The components of each reaction were 2.5 µL of 10x LongRange PCR Buffer, 500 mM of each dNTP, 0.6 mM of each primer, 1 unit of LongRange PCR Enzyme, and 100–250 ng of DNA template. The temperature conditions of the PCRs were 94 °C for 5 min, followed by 45 cycles of 94 °C for 30 s, with an annealing temperature of 50–57 °C (depending on the primer set) for 30 s and an extension of 72 °C for 8 min. Afterward, there were 30 denaturation cycles at 93 °C for 30 s, with annealing temperatures of 45–52 °C (depending on the primer set) for 30 s, and an extension of 72 °C for 5 min, with a final extension of 72 °C for 8 min. Four sets of primers were used to generate overlapping amplicons: PROCYONID1F (ATGAGTAATCAGCCCTTGAT) and PROCYONID1R (ATGCATCCCACGTCAATCAT), approximately 5,000 bp; PROCYONID2F (AAGTAATATGTCTGACATAA) and PROCYONID2R (TCATCTGCATCTATTCTGA), approximately 4,000 bp, PROCYONID3F (CATTTAGAAGCTAATTAAGC) and PROCYONID3R (GTGCAACTCGAAATAAATGT), approximately 4,000 bp; PROCYONID4F (TAATTGTAATAAAGCTATTT) and PROCYONID4R (TGGCACATCTCGATGGAGTA), approximately 3,200 bp. These allowed observing the circularity of the mitochondrial genome (Bensasson *et al.* 2001; Thalmann *et al.* 2004). Both DNA strands were sequenced directly using BigDye Terminator v3.1 (Applied Biosystems, Inc., Foster City, California, USA). The sequencing products were analyzed in an ABI 3730 DNA Analyzer system (Applied Biosystems, Inc., Foster City, California, USA). The sequences were assembled and edited with the Sequencher 4.7 program (Gene Codes, Corp., Ann Arbor, Michigan, USA). The overlapping regions were examined for possible irregularities, such as termination codons. The absence of such irregularities is a valid indication of the absence of Numts. Genes were concatenated (about 16,200 bp) after removing any problematic region with the Gblocks 0.91 program (Talavera and Castresana 2007) under a relaxed approach. Individual alignments were concatenated with SequenceMatrix v1.7.6 (Vaidya *et al.* 2011) to create the final alignment.

The sequences were deposited in the NCBI GenBank under access numbers MT587713-MT587788, MW410859-MW410914, and MW419814-MW419853.

Phylogenetic analyses. The sequences were concatenated for phylogenetic analyses of the three mt loci studied because they showed the same phylogenetic signal. To this end, we used the ILD test ("Incongruence-length difference test"; Farris *et al.* 1995) in the mILD program (Planet and Sarkar 2005). The programs jModeltest v2.0 (Darriba *et al.* 2012) and MEGA 6.05 (Tamura *et al.* 2013) were applied to determine the best nucleotide substitution model, using the Akaike information criterion (AIC; Akaike 1974) and the Bayesian information criterion (BIC; Schwarz 1978).

A Maximum Likelihood (ML) analysis was performed as phylogenetic inference using the RAxML v.7.2.6 program (Stamatakis 2006) with the partition scheme selected by the PartitionFinder 2.0 program (Lanfear *et al.* 2012). This program was used to simultaneously determine the optimal model for nucleotide substitution (which coincided with that of jMoldeltest v2.0) and sequence partition scheme. For partitions, codons 1 + 2, and codon 3 were combined for each gene (for both *Cyt-b* and *ND5* in the case of the data set with three loci and for each of the coding genes in the mitogenomic case. Additionally, RNA genes were included in the mitogenomic analysis and the control region for both data sets). The best-fit models were selected using BIC under a 'greedy' search scheme with a subset of models specific to RAxML. The GTR + G + I model

(Tavaré 1986) was used for the ML tree search, as discussed in the *Results* section. The support for nodes was estimated using the "rapid-bootstrapping" algorithm with 1,000 non-parametric bootstrap replicates (Stamatakis *et al.* 2008). Clades were considered to have good nodal support when bootstrap values were greater than 70 % (loose limit; Hillis and Bull 1993).

The relationships between the haplotypes found were determined using the Median Joining Network algorithm (MJN; Bandelt *et al.* 1999) of the Network 4.6 program (Fluxus Technology Ltd). One advantage of MJN over phylogenetic trees is that it explicitly allows the coexistence of ancestral and descendant haplotypes, while phylogenetic trees treat all sequences as terminal taxa (Posada and Crandall 2001). This allows identifying which haplotypes were the first to originate and which are the most recently derived haplotypes (Freeland *et al.* 2011). Divergence times were calculated with the *p* statistic (Morral *et al.* 1994) and its standard deviation (Saillard *et al.* 2000) since this statistic can be transformed into years. This statistic is unbiased and highly independent of past demographic events. This approach is called a "borrowed molecular clock" and directly uses the nucleotide substitution rates estimated in other taxa (Pennington and Dick 2010). For the set of three mt genes, we used an evolutionary rate of 1.75 % per million years, representing one mutation every 22,742 years. For all mitogenomic data, the evolutionary rate used was 2.34 % per million years, equivalent to one mutation every 2,638 years. These evolutionary rates were reported for the family Canidae (Wayne *et al.* 1997). In the present work, this methodology was used to estimate divergence times as previous studies (Ruiz-García *et al.* 2020a, 2021a, b) have used Bayesian inference methods to investigate the divergence between the species of *Nasua*, *Nasuella*, and *Bassaricyon*. However, the use of the MJN is preferable because the present study preferably analyzed the divergence times within *N. narica*, and there is a scarce fossil record for coatis. In fact, there are no fossil remains attributable to either *N. narica* or *Nasuella,* and those attributable to *N. nasua* do not exceed 0.125 MYA (Woodburne 2010), which significantly underestimates all studies of divergence times within procionids (Koepfli *et al.* 2007; Nigenda-Morales *et al.* 2019; Ruiz-García *et al.* 2019b, 2020a, 2021a, b).

Genetic heterogeneity and diversity. The statistics H_{ST}, K_{ST}, K_{ST}*, γ_{ST}, N_{ST}, and F_{ST} (Hudson *et al.* 1992) were calculated to determine the global genetic heterogeneity among the six genetic groups of *N. narica* detected with the phylogenetic methods used for both the set of three mt loci and the mitogenomic set. Indirect estimates of the genetic flow between the six genetic groups were obtained assuming an infinite island model (Wright 1965). Statistical significance was estimated using a permutational test with 10,000 replicates. Additionally, genetic heterogeneity and genetic flow statistics were estimated in pairs for the six groups detected. To this end, the F_{ST} statistic was used with Markov chains with 10,000 dememorization parameters, 20 batches, and 5,000 iterations per batch. All analyses were carried out using the DNAsp 5.1 (Librado and Rozas 2009) and Arlequin 3.5.1.2 (Excoffier and Lischer 2010) programs.

We estimated the number of haplotypes (NH), haplotypic diversity (*H*d), nucleotide diversity (*π*), and the statistic Ø per sequence in the DNAsp 5.1 program (Librado and Rozas 2009) for the total sample and for each of the six genetic groups detected by the phylogenetic analyses.

Spatial autocorrelation analysis. A total of four spatial analyses were applied to the set of three mt loci and three to the set of mitogenomes. The first analysis was a Spatial Analysis of Molecular Variance (SAMOVA) with the SAMOVA 1.0 program (Dupanloup *et al.* 2002) to assess the spatial structure in a geographic context. This program seeks to define geographically homogeneous groups of populations and maximize differentiation with other geographic groups. This would potentially facilitate the identification of genetic barriers between population groups. The method is based on a simulated annealing procedure that helps maximize the proportion of total genetic variance due to differences between population groups. We analyzed the number of different populations (*k*; from two to six), and the statistical significance was estimated with 1,000 permutations.

The second analysis was isolation by distance with the Mantel test (Mantel 1967) performed in the Alleles In Space 1.0 (AIS) program (Miller 2005). The matrix of genetic distances of the Kimura two-parameter model (Kimura 1980) between the different individuals of *N. narica* sequenced was compared with the matrix of Euclidean geographic distances between them. Statistical significance was estimated with 10,000 permutations.

The third procedure was a spatial autocorrelation analysis using the *Ay* statistic with AIS 1.0 (Miller 2005). This can be interpreted as the average genetic distance between pairs of individuals falling within a particular distance class (DC). *Ay* takes a value of 0 when all individuals within a DC are genetically identical and a value of 1 when all individuals within a DC are completely different. The probability for each DC was obtained using 10,000 permutations. To carry out this analysis, we defined 10 DCs constructed using DCs of identical size (in km) with unequal sample size per DC for the set of three mt loci (DC 1: 0–183 km; DC 2: 183–366 km; DC 3: 366–549 km; DC 4: 549–732 km; DC 5: 732–915 km; DC 6: 915–1,098 km; DC 7: 1,098–1,281 km; DC 8: 1,281–1,464 km; DC 9: 1,464–1,647 km; DC 10: 1,647-1,831 km), and 10 DCs constructed with unequal CDs but with approximately the same sample sizes per DC for mitogenomic data (DC 1: 0–26 km; DC 2: 26–83 km; DC 3: 83–183 km; DC 4: 183–270 km; DC 5: 270–337 km; DC 6: 337–381 km; DC 7: 381–547 km; DC 8: 547–730 km; DC 9: 730–1,089 km; DC 10: 1,089–2,298 km). The specimens were connected to the Gabriel and Sokal network (Gabriel and Sokal 1969) and other types of networks (Ruiz-García 1993, 1994, 1997, 1999; Ruiz-García and Álvarez 2000).

The fourth analysis was the Monmonier algorithm (Monmonier 1973; MMDA) with the AIS 1.0 program (Miller 2005). This geographic regionalization method was used to detect putative geographic barriers to gene flow by iterative identification of contiguous sets of high genetic distances across connectivity networks (Doupanloup *et al.* 2002; Manel et al. 2003; Manni *et al.* 2004). The Delaunay triangulation was used (Watson 1992; Brouns *et al.* 2003) to generate the connectivity network between sampling points. A graphic representation of the putative geographic barriers inferred by the algorithm was overlaid on the connectivity network to facilitate the detection of likely geographic obstacles reflected in the sequence data. In this case, the procedure was used to identify the five most important possible putative geographic barriers for the set of three mt loci.

Results

Phylogenetic analysis for three mitochondrial loci and for complete mitogenomes. The optimal nucleotide substitution models for the set of three mt loci were TN93 + G for CIA (-Ln = 11,861.36) and GTR + G + I for CIB (-Ln = 17,332.27); for the mitogenomic set, the optimal nucleotide substitution model was GTR + G + I for CIA (-Ln = 55,535.88) and TN93 + G for CIB (-Ln = 63,912.52). For the phylogenetic analyses, we used the GTR + G + I model since it was one of the two optimal models in all cases and is a model implemented in the programs used.

In the ML tree (Figure 2) with three mt loci, the *N. nasua* clade had good nodal support and was the sister group of the other clades (Bootstrap Support, BS = 100). An interesting finding is the inclusion in this clade of three specimens that *a priori* would correspond to *N. narica* based on their phenotype and geographic origin. The first is a specimen from Robinson Crusoe Island (Chile). The other two specimens were collected in northern Colombia. In both cases, the analysis with three mt genes showed that they were highly related (BS = 95). The remaining clades found in *N. nasua* were already analyzed in detail by Ruiz-García *et al.* (2021a).

The clade of *N. olivacea* also had high nodal support (BS = 95) and was the sister group of *N. narica*; this may be considered evidence that the genus *Nasuella* could be included in the genus *Nasua* (Ruiz-García *et al.* 2021b). On the other hand, a group of five specimens of *N. narica* distributed in southern Costa Rica, Panama, and northern Colombia (Antioquia and Choco) — the typical distribution of *N. narica* — with an undoubted morphotype of this species, nested within the *N. olivacea* clade. This group (BS = 72), which we named G6, has a mitochondrial DNA clearly introgressed by *N. olivacea*.

The *N. narica* group was monophyletic (BS = 74) and was composed of five distinct genetic groups. The first (G1; BS = 79) comprised two specimens from the trans-Andean (Pacific) part of Ecuador (Guayas province). The second group (G2; BS = 97) is composed of two specimens sampled in Yucatán (México). The third genetic group (G3; BS = 81) is formed by specimens sampled in northern Costa Rica, Nicaragua, El Salvador, Honduras, and southern Guatemala. It should be noted that an Ecuadorian Amazonian specimen with the phenotype of *N. narica* (Macas, province of Morona-Santiago) was included in this clade. The fourth group (G4; BS = 91) consisted of specimens distributed in the northwestern part of Guatemala and Belize; however, two specimens from the Pacific coast of Ecuador (Guayas province) were also included in this group. Finally, the fifth genetic group (G5; BS = 90) comprised specimens from northwestern Guatemala and southern México, with specimens from Quintana Roo (including Cozumel Island), Campeche, Chiapas, Tabasco, and Yucatán.

The ML tree (Figure 3) with mitogenomic data was similar to the previous one, with minor differences. *Nasua nasua* was the sister clade of all others (BS = 87). The specimen from Robinson Crusoe Island (Chile) was included in this taxon. Contrary to the ML tree calculated with three mt loci, one specimen of *N. narica* from central-northern

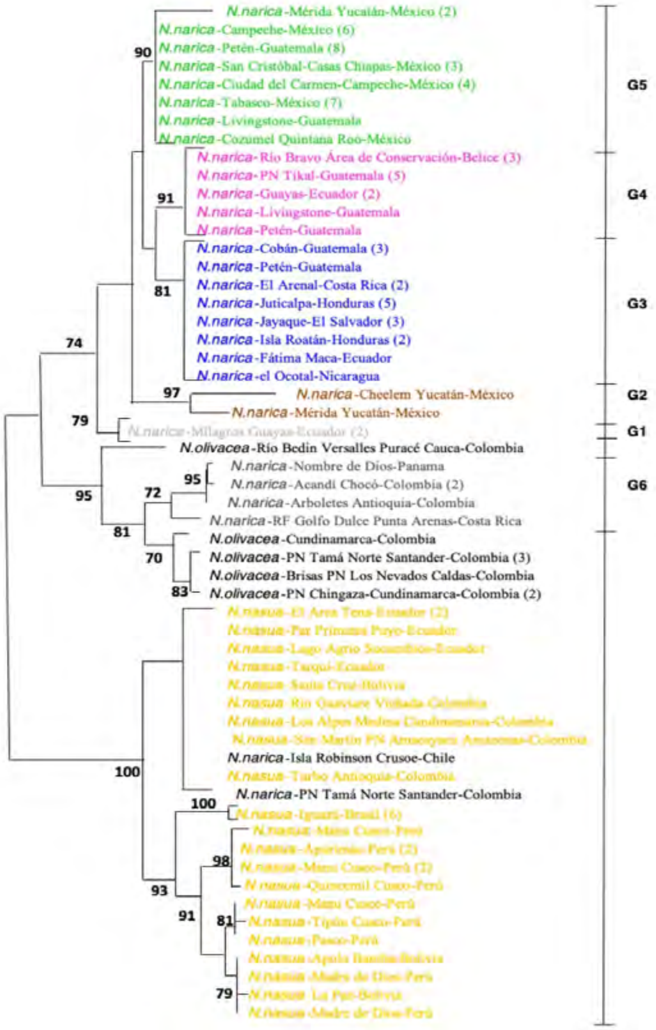

Figure 2. Maximum Likelihood Tree showing the phylogenetic relationships of 74 specimens of white-nosed coati (*Nasua narica*) for three mitochondrial genes (*ND5, Cyt-b*, and *D-loop*). The mountain or Andean coati, *Nasuella olivacea*, and the South American coati, *Nasua nasua,* were used as external groups. Numbers on nodes are bootstrap percentages greater than 70 %. The number of specimens sampled in a given locality is shown in parenthesis.

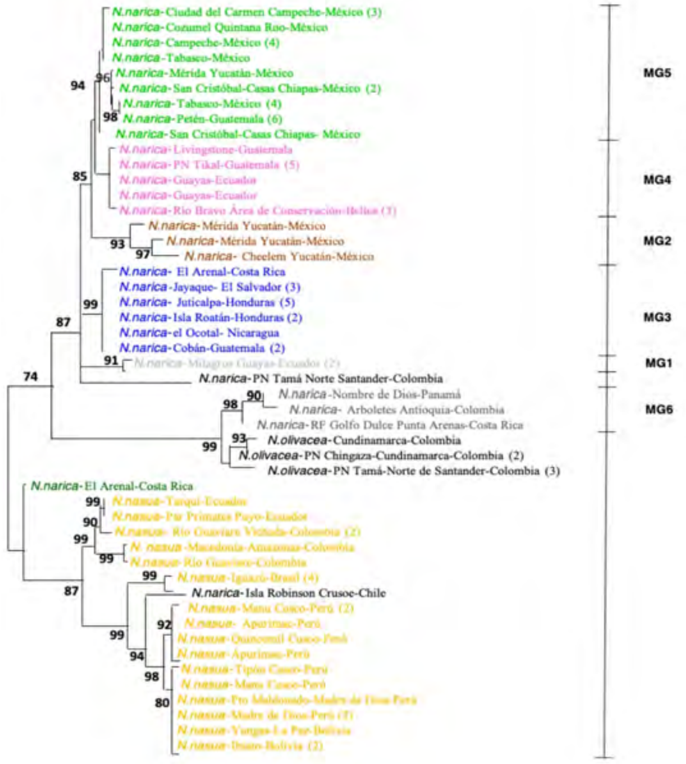

Figure 3. Maximum Likelihood Tree constructed with the complete mitogenomes of 59 specimens of white-nosed coati (*Nasua narica*). The mountain or Andean coati (*Nasuella olivacea*) and the South American coati (*Nasua nasua)* were used as external groups. Numbers on nodes are bootstrap percentages greater than 70 %. The number of specimens sampled in a given locality is shown in parenthesis.

Costa Rica was shown to be the most divergent within the *N. nasua* clade. The *N. olivacea* clade (BS = 99) was the sister group of *N. narica* (BS = 87); it included the genetic group (MG6) of *N. narica* (BS = 98), with which the complete mitogenomes confirmed the genetic introgression of one group of *N. olivacea* in the distribution range of *N. narica* in southern Central America and northern Colombia. In the present tree, the specimen from PN Tamá (North of Santander, Colombia) forms a polytomy with the genetic groups MG1 (BS = 91) and MG3 (BS = 99). This was followed by the subsequent divergence of groups MG2 (BS = 93; in this case, a third specimen from the Yucatán Peninsula was added, which had been included in G5 in the previous tree), MG4 (BS = 85), and MG5 (BS = 94).

The haplotype network (Figure 4) for the set of three mt loci showed that the earliest ancestral *N. narica* haplotypes would have derived from *N. nasua* haplotypes; these specimens already displayed the distinctive phenotype of *N. narica* and with haplotypes very close to one of the external groups. These were found in the trans-Andean and Pacific zones of Ecuador (H2 and H4), along with the haplotype of the specimen from the Ecuadorian cis-Andean (Amazon) zone (H3) that had been included in G3 in the phylogenetic trees for the three mt loci. In other words, the first *N. narica* (with explicit phenotypes of this species) with haplotypes already differentiated from those of *N. nasua* and closer to the Central American *N. narica* are found in Ecuador, in the

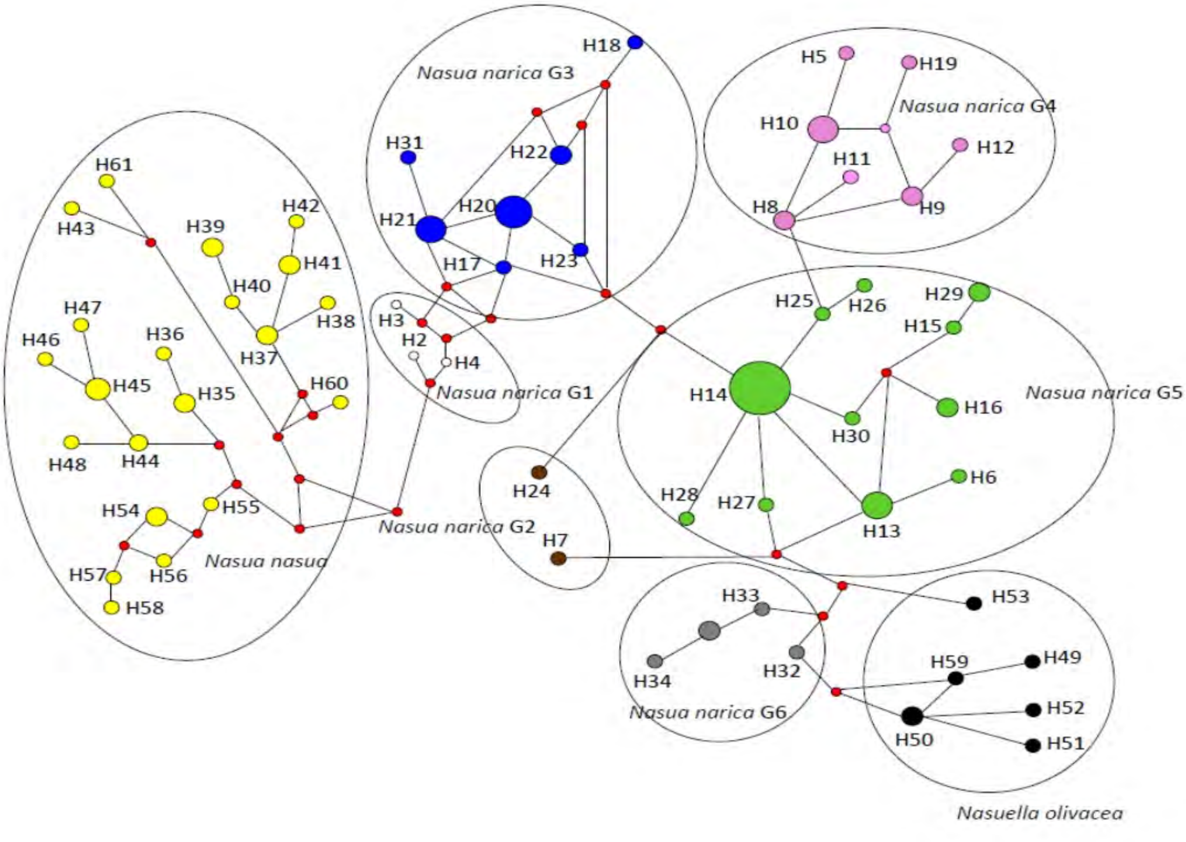

Figure 4. Median Joining Network procedure applied to the haplotypes found in 74 specimens of white-nosed coati (*Nasua narica*) for three mitochondrial genes (*ND5, Cyt-b*, and *D-loop*). The haplotypes of eight mountain or Andean coatis (*Nasuella olivacea*) and 29 South American coatis (*Nasua nasua*), were used as external groups. White = haplotypes of G1 specimens; brown = haplotypes of G2 specimens; blue = haplotypes of G3 specimens; pink = haplotypes of G4 specimens; light green = haplotypes of G5 specimens; gray = haplotypes of G6 specimens; black = haplotypes of *N. olivacea* specimens; yellow = haplotypes of *N. nasua* specimens; small red circles indicate intermediate haplotypes that were not found.

trans-Andean and cis-Andean areas (G1). From this group (haplotypes that were gradually differentiated from the external group), G3 would have emerged, distributed in the south-central zone of Central America, with H20 occupying the central position in the analysis (El Salvador, Honduras, and southern Guatemala). G5 would have later derived from G3, with H14 at the central position in the analysis (northern Guatemala and México, including Chiapas, Tabasco, Campeche, and part of Yucatán). G4 derived from G5 (northwestern Guatemala and Belize, although these groups also included haplotypes from the trans-Andean zone of Ecuador). Finally, G6 clearly showed that it was introgressed by *N. olivacea*, as reflected by phylogenetic trees. In this network of haplotypes, G2, clearly visible in the trees mentioned above, does not form a well-consolidated group. One haplotype (H24) appears as derived from a non-sampled haplotype between G3 and G5, and another haplotype (H7) appears as derived from G5.

Divergence times within and between N. narica *groups.* For the matrix of three mt loci, using the MJN, the time elapsed for the G1 haplotype to give rise to all the Central American haplotypes derived from it was 4.06 ± 0.93 million years ago (Mya). On the other hand, for the mitogenomic data set, the divergence between the intermediate haplotype of the *N. narica* specimen from central-northern Costa Rica and all other Central American haplotypes detected occurred 3.16 ± 0.34 Mya. The time elapsed since G1 gave rise to the first G3 haplotype was estimated at 2.20 ± 0.27 Ma for the set of three mt loci and 1.21 ± 0.02 Mya between the intermediate Costa Rican haplotype and the first MG3 haplotype, respectively. The time of divergence since the G3 generated all the other northernmost haplogroups in Central America was 1.93 ± 0.54 Mya and the time between the G3 haplotype and the emergence of G5 from it was 1.07 ± 0.30 Mya. The same divergence times with mitogenomic data were 0.82 ± 0.15 Mya and 0.36 ± 0.01 Mya, respectively. The time elapsed since G5 gave rise to the first G4 haplotype was estimated at 0.12 ± 0.27 Mya for the set of three mt loci and 0.09 ± 0.02 Mya for the mitogenomic set, respectively. Additionally, the time of divergence between the nearest *N. narica* haplotypes and the *N. narica* haplotypes introgressed by *N. olivacea* was 4.4 ± 0.15 Mya for the set of three mt loci and 2.34 ± 0.08 Mya for the mitogenomic set, respectively. Finally, the temporal separation between the present *N. olivacea* haplotypes and those of G6 (MG6) (*N. narica* introgressed by the latter species) was 2.32 ± 0.34 Mya for the set of three mt loci and 0.73 ± 0.08 Mya for the mitogenomic data. In general, for most estimates, the divergence times estimated with mitogenomic data are shorter than those estimated with the set of three mt loci. Additional divergence times are shown in Table 1.

Genetic heterogeneity and diversity within and among Nasua narica *groups.* The analysis of genetic heterogeneity for the six genetic groups of *N. narica* detected from phylogenetic analyses for the matrix of three mt loci showed a significant global genetic differentiation ($\gamma_{ST} = 0.734$, $p < 0.0001$; $F_{ST} = 0.658$, $p < 0.0001$; Table 2). The global genetic flow estimates showed low values among these groups, taken globally ($Nm_{\gamma ST} = 0.18$; $Nm_{FST} = 0.26$). The same analysis including only the four Central American groups (excluding G1 and G6) also showed high and significant values ($\gamma_{ST} = 0.599$, $p < 0.0001$; $F_{ST} = 0.496$, $p < 0.0001$; Table 2), and genetic flow estimates were also low ($Nm_{\gamma ST} = 0.33$; $Nm_{FST} = 0.51$). These results are consistent with the mitogenomic data ($\gamma_{ST} = 0.755$, $p < 0.00001$; $F_{ST} = 0.775$, $p < 0.00001$; Table 2), with low genetic flow values ($Nm_{\gamma ST} = 0.16$; $Nm_{FST} = 0.14$).

The analysis of paired genetic heterogeneity of genetic groups with data from three mt loci showed that all comparison pairs were significant (Table 3), except for the pair G1 vs. G2 ($p = 0.331$). The highest genetic flow was estimated between G1and G2 ($Nm = 0.504$) and the lowest between G3 and G6 ($Nm = 0.038$; Table 4). The mitogenomic data showed similar results. The only pair of groups with a nonsignificant difference was between MG1 and MG2 ($p = 0.103$; Table 3). The highest estimate of genetic flow was between the pair of groups MG1 and MG2 ($Nm = 0.578$) and the lowest between G5 and G6 ($Nm = 0.021$; Table 4). Therefore, there is very high genetic heterogeneity among the six groups detected with phylogenetic methods.

The levels of global genetic diversity for both three-loci-based data and mitogenomic data (Supplementary Table 2) are high. By group, G1 (MG1), G2 (MG2), and G6 (MG6) showed the highest levels of genetic diversity for both the three-loci mt matrix and the mitogenomic matrix. The groups that showed the lowest levels of genetic diversity were G3 and G5 for both the data with three mt loci and mitogenomic data.

Spatial structure in N. narica. With the three mt loci, the maximum differentiation was observed between four groups ($F_{CT} = 0.702$, $p < 0.0001$), *i. e.*, G6, G1, G2, and the set of G3, G4, and G5. With mitogenomes, the maximum differentiation occurred between two groups ($F_{CT} = 0.817$, $p < 0.0001$), namely, between MG6 and all the other groups of *N. narica*, regardless of their geographic location (Supplementary Table 3).

Table 1. Divergence times within the different groups of white-nosed coati (*Nasua narica*) detected through its geographic distribution range in Central America and northern South America. A) Based on three mitochondrial genes (*ND5, Cyt-b*, and *D-loop*); B) based on complete mitogenomes. SD = Standard Deviation. Time in millions of years.

Groups of *Nasua narica*	Divergence Times ± SD
A	
Within G1	2.99 ± 0.69
Within G3	0.25 ± 0.11
Within G4	0.49 ± 0.18
Within G5	0.60 ± 0.13
Within G6	2.47 ± 0.77
B	
Within MG1	1.34 ± 0.16
Within MG3	0.15 ± 0.04
Within MG4	0.41 ± 0.09
Within MG5	0.12 ± 0.03
Within MG6	0.37 ± 0.09

Table 2. Statistics of genetic heterogeneity and gene flow comparing simultaneously the different groups of white-nosed coati (*Nasua narica*) detected in Central America and northern South America. A) Based on three mitochondrial genes (*ND5, Cyt-b*, and *D-loop*) for six different groups (four in Central America and two in northern South America); B) based on three mitochondrial genes (*ND5, Cyt-b*, and *D-loop*) for four groups in Central America; C) based on mitogenomes for all groups detected. ** $p < 0.01$, df = degrees of freedom. *Nm* = Gene Flow statistic. Nm^1 = Gene flow obtained with the γ_{ST} statistic; Nm^2 = gene flow obtained with the N_{ST} statistic; Nm^3 = gene flow obtained with the F_{ST} statistic.

Genetic Heterogeneity and gene flow statistics	Values	Probabilities
A		
X^2	360.000 $df = 165$	0.00001**
H_{ST}	0.1732	0.00001**
K_{ST}	0.6718	0.00001**
$K_{ST}*$	0.5162	0.00001**
Z_S	492.11	0.00001**
Z_S*	5.8609	0.00001**
S_{nn}	0.9563	0.00001**
γ_{ST}	0.7344	0.00001**
N_{ST}	0.6689	0.00001**
F_{ST}	0.6578	0.00001**
Nm^1	0.18	
Nm^2	0.25	
Nm^3	0.26	
B		
X^2	195.000 $df = 81$	0.00001**
H_{ST}	0.1651	0.00001**
K_{ST}	0.5269	0.00001**
$K_{ST}*$	0.4456	0.00001**
Z_S	465.6	0.00001**
Z_S*	5.8262	0.00001**
S_{nn}	0.967	0.00001**
γ_{ST}	0.5995	0.00001**
N_{ST}	0.4963	0.00001**
F_{ST}	0.4956	0.00001**
Nm^1	0.33	
Nm^2	0.51	
Nm^3	0.51	
C		
X^2	996.000 $df = 240$	0.00001**
H_{ST}	0.0112	0.00069**
K_{ST}	0.7068	0.00001**
$K_{ST}*$	0.3718	0.00001**
Z_S	2262.6	0.00001**
Z_S*	5.211	0.00001**
S_{nn}	0.9464	0.00001**
γ_{ST}	0.755	0.00001**
N_{ST}	0.7862	0.00001**
F_{ST}	0.7755	0.00001**
Nm^1	0.16	
Nm^2	0.14	
Nm^3	0.14	

The results of the Mantel test were significant for the matrices of three mt loci and mitogenomes. In the first case, geographic distance significantly explained 30.59 % of the genetic distances between the specimens analyzed (r = 0.553, $p = 0.00009$); in the second, geographic distance significantly explained 23.81 % of genetic distances ($r = 0.488$, $p = 0.0014$). In general, evidence of isolation by distance was detected in the geographic distribution of *N. narica*.

The spatial autocorrelation analysis showed evidence of spatial structure. For the matrix with three mt loci, the global correlogram was statistically significant ($V = 0.0361$, $p < 0.000001$). The first two distant classes (DCs) showed a significant positive spatial autocorrelation (DC 1: p = 0.000001; DC 2: $p = 0.000001$). This indicates that in areas around 360 km in in diameter there are specimens more genetically similar to each other than expected at random. From DC 6, all spatial autocorrelation values were significantly negative (DC 6: $p = 0.0021$; DC 7: $p = 0.0026$; DC 8: p = 0.0001; DC 9: $p = 0.0003$; DC 10: $p = 0.0002$). That is, from 1,098 km to 1,831 km, genetic differentiation progressively increased. Similar results were obtained with mitogenomic data. The overall correlogram was statistically significant (V = 0.0146, $p < 0.0008$), with the first six DCs showing a significant positive spatial autocorrelation (DC 1: $p = 0.00001$; DC 2: $p = 0.026$; DC 3: $p = 0.0175$; DC 4: $p = 0.0338$; DC 5: p = 0.000001; DC 6: $p = 0.0365$). This indicated a strong genetic similarity between specimens geographically separated by a distance of about 380 km between them. This value is very similar to the one reported above for the first spatial autocorrelation analysis. DCs 9 and 10 yielded a significant negative spatial autocorrelation (DC 9: $p = 0.0071$; DC 10: $p = 0.0023$). That is, genetic differentiation increased from 729 km to 2,297 km. Therefore, both analyses show a clear monotonic cline in northern South America and Central America.

The analysis with the Monmonier algorithm (Figure 5) was performed for only three mt loci. The first barrier detected differentiated the geographic area corresponding to G6 specimens introgressed by *N. olivacea*. The second barrier demarcated a geographic area correlated with the area where part of the G4 specimens are interspersed (Belize) with part of the G5 specimens (Cozumel Island and part of Yucatán). The third barrier bordered the geographic area corresponding to G2 specimens (Yucatán). The fourth barrier delimited the geographic area that included G1 specimens (Ecuadorian Pacific). The fifth barrier marked the geographic area that was aligned with the trans- and cis-Andean zones of Ecuador but with specimens more genetically related to the Central American group G3. The geographic structure of *N. narica* in Central America and northern South America is very pronounced.

Discussion

Systematics of N. narica. This article reports the population phylogeographic and genetic analysis encompassing the broadest geographic extension of the distribution range of *N. narica* to date and uses complete mitogenomes for this species for the first time. From a strictly systematic standpoint, this analysis provides new evidence for the re-interpretation of the systematics of this species.

The genetic distances between the different *N. narica* groups found are smaller than those observed in *N. nasua* (Ruiz-García *et al.* 2020a, 2021a). The values ranged from 1.6 % to 4.5 %, typical of well-differentiated populations or subspecies (Kartavtsev 2011). This is consistent with the fact that the ancestor of the present *N. narica* is considerably more recent than the ancestor of the present *N. nasua*, which, in turn, shows that the mitochondrial evolution of coatis occurred in South America in the first place and then in Central America. This conclusion contrasts with the traditional paleontological view that the current coatis migrated from North to South America in the early Pleistocene. The arrival of the first procionids in South America produced endemic forms (Argentina) such as *Cyonasua* and *Chapalmalania* (Soibelzon and Prevosti 2013) around 7.3 Mya. However, paleontologists consider that these procionids became extinct and did not give rise to the present *Nasua*. They are considered to have arrived in South America in a second migratory wave of procionids during GABI 4 (Woodburne 2010), which occurred during the Lujanense (126,000–8,500 before present, BP) and Platense (8,500–1,500 BP) ages. However, *N. nasua* haplotypes started to differentiate *in situ* in northwestern South America some 13–10 Mya (Ruiz-García *et al.* 2020a, 2021a, b), preceding the diversification of *N. narica* haplotypes (also in northwest South America) 4.1–3.2 Mya. These findings indicate a south-to-north migration of the current coatis, opposite to the direction suggested by paleontologists.

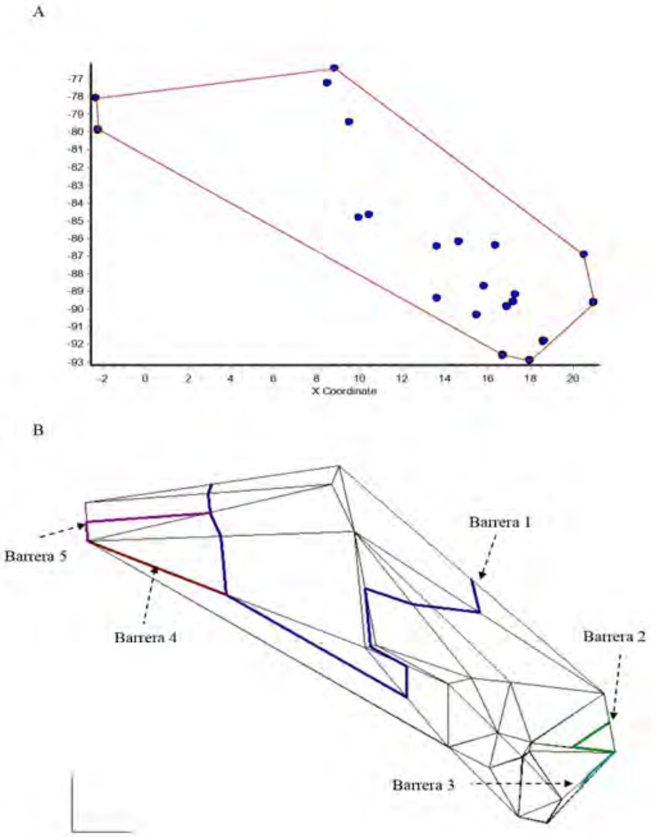

Figure 5. Analysis of the Monmonier algorithm to detect the five most important geographic barriers in the geographic distribution range of the samples of white-nosed coati (*Nasua narica*) analyzed from Central America and northern South America. A) Geographic area analyzed; B) Geographic barriers for 74 specimens of *N. narica* sequenced for three mitochondrial genes (*ND5, Cyt-b*, and *D-loop*).

Four morphological subspecies of *N. narica* have been considered in recent decades (Gompper 1995): *N. n. molaris*, *N. n. nasua*, *N. n. nelsoni*, and *N. n. yucatanica*. In particular, *N. n. nelsoni* has been considered a full species (*N. nelsoni*). However, the present study showed at least six genetically distinct groups.

The first group (G1, MG1) is distributed in northwestern South America. We have detected haplotypes of this group in the Ecuadorian Pacific and, in the mitogenomic study, also in one specimen from northern Colombia (PN Tamá, North of Santander). Traditionally, the coatis of the trans-Andean and Pacific Colombian and Ecuadorian areas have been classified as *N. narica* (Emmons 1990; Emmons and Feer 1997; Tirira 2007, 2011); however, some authors (Gompper 1995; Nowak 1999) suggest that the coati living in the Pacific area of Colombia and Ecuador is *N. nasua* based on its morphological traits.

Our research showed that some of those specimens might be intermediate forms between *N. nasua* and *N. narica*. The specimens in this group from the Ecuadorian Pacific had external phenotypes (coloration and fur pattern) typical of *N. narica*. However, Ruiz-García *et al.* (2021a) observed that most specimens with the *N. narica* phenotype in the trans-Andean and Pacific areas of Ecuador had the mitochondrial haplotypes typical of *N. nasua*. The study also detected specimens in the Ecuadorian Pacific with intermediate morphotypes between *N. nasua* and *N. narica*. That study also showed that the *N. narica* haplotypes of the Ecuadorian Pacific and North Santander (Colombia) had originated from an *N. nasua* haplotype distributed in the Colombian and Ecuadorian Amazon. One specimen from the Colombian Pacific (not included in this study; Buenaventura, Valle del Cauca) with an intermediate phenotype between *N. narica* and *N. nasua* showed inconclusive results according to different molecular analyses performed (Ruiz-García *et al.* 2021a). In fact, in the present study, we detected one specimen of PN Tamá (located in an area where *N. nasua* and *N. narica* allegedly converge), which, despite having the distinct *N. narica* morphotype, resembled *N. nasua* in the analysis with three mt genes (not so in the mitogenomic analysis). The analysis with three mt genes also included one specimen (Turbo, Antioquía, Colombia; border area between Colombia and Panama) with a morphotype closer to *N. nasua* but within a distribution area typical of *N. narica*. The analysis based on three mt loci associated this specimen with the previous specimen within the *N. nasua* clade. Unfortunately, that specimen could not be sequenced for its complete mitogenome because of the poor quality of its DNA. However, there is evidence on the Pacific coast of Ecuador and Colombia, and in northern Colombia, of specimens with a full *N. narica* morphotype and with mitochondrial haplotypes intermediate between *N. nasua* and Central American *N. narica*, albeit closer to the latter. Ruiz-García *et al.* (2020a, 2021ab) showed that the haplotypes of *N. nasua* and *N. olivacea* diverged in South America before those of *N. narica*. The

Table 3. F_{ST} statistic for paired groups of white-nosed coati (*Nasua narica*) analyzed in Central America and northern South America. Below the diagonal, based on three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*) (G); above the diagonal, based on mitogenomes for all groups detected (MG). * $p < 0.05$; ** $p < 0.01$.

Groups of *Nasua narica*	G1 (MG1)	G2 (MG2)	G3 (MG3)	G4 (MG4)	G5 (MG5)	G6 (MG6)
G1 (MG1)		0.463	0.786**	0.564*	0.803**	0.910
G2 (MG2)	0.498		0.734**	0.629**	0.723**	0.823
G3 (MG3)	0.822**	0.833**		0.697**	0.723**	0.952**
G4 (MG4)	0.771**	0.788*	0.764**		0.578**	0.928**
G5 (MG5)	0.817**	0.834**	0.652**	0.615**		0.960**
G6 (MG6)	0.849*	0.810*	0.928**	0.912*	0.735*	

existence of intermediate haplotypes between *N. nasua* and *N. narica* are particularly well-detected in the haplotype networks of the Ecuadorian Pacific and northern Colombia.

The case of the assumed *N. narica* from Robinson Crusoe Island in the Chilean Pacific might represent a hybridization event. In the 1950s, a couple of Germans introduced coatis to fight rodent pests on the island. The morphotype of these island animals matches *N. narica*. However, this study, together with that of Ruiz-García *et al.* (2021a), showed that the mtDNA of that specimen behaved like *N. nasua*. The coatis introduced to those islands likely came from the Ecuadorian Pacific with the typical phenotype of *N. narica* but with the mtDNA of *N. nasua* that characterizes part of the coatis inhabiting the Pacific slope of Ecuador.

The problem with *N. narica* from Ecuador and Colombia is that they have not been assigned a sub-specific denomination because it has recently been considered that this species is not present in the Pacific zone of these two countries. It would be very important to perform morphological and molecular analyses of the *N. nasua* holotypes or paratypes described for the trans-Andean and Pacific areas of Ecuador. The holotype described for this Ecuadorian area was first named *N. nasua manium* (Thomas 1912), but a more recent paratype was also described for the same area, *N. nasua gualeae* (Lönnberg 1921; Type locality: Gualea, province of Pichincha, Ecuador). If either of these two taxa could be analyzed at molecular level and had *N. narica* mtDNA, then the G1 (MG1) specimens could be assigned to *N. narica manium* or *N. narica gualeae*, with preference for the first name.

Therefore, our results drastically change the traditional view of the existence of only *N. narica* on the Colombian and Ecuadorian Pacific coast and challenge the idea of the absence of this species in that geographic area, as claimed by other authors (Gompper 1995; Nowak 1999). Indeed, most coatis living in the Pacific trans-Andean zone of Colombia and Ecuador belong to *N. nasua*. However, a small proportion of individuals in this geographic area show the oldest *N. narica* haplotypes found to date with a morphotype very similar to the Central American populations (present study and Ruiz-García *et al.* 2021a). Additionally, in the Ecuadorian Pacific, we detected two specimens (for the set of three mt loci, only one for mitogenomes) with the *N. narica* phenotype and the typical Central American G4 (MG4) haplotypes. Furthermore, an Ecuadorian cis-Andean animal (Macas, Morona-Santiago province, Ecuadorian Amazon) showed the typical Central American G3 haplotype. This would indicate that an *N. narica* haplotype would have crossed the Andes mountain range; however, we currently ignore whether this crossing was natural or resulted from humans transporting animals through the Ecuadorian Andean Cordillera. The fact that the Macas specimen showed a haplotype with some nucleotide differentiation relative to the G1 haplotypes leads us to think that the crossing of the Cordillera was not recent, likely a natural process.

Therefore, nuclear genes should be analyzed to determine the degree of hybridization or introgression (if any) between the small proportion of coatis with *N.* aff. *narica* phenotype and mtDNA (defined in this study as *N. narica*) and the largest proportion of specimens, many of them also with phenotypes with *N. narica* traces but with mtDNA belonging to different *N.* aff. *nasua* haplogroups (defined herein as *N. nasua*), especially in the Ecuadorian area considered. Nuclear DNA sequencing could contribute to defining the coati systematics. Additionally, our results indicate that in the Pacific area and northern Colombia there is another group of *N. narica* (G6, MG6) introgressed with mtDNA of *N olivacea*. These new findings are relevant to the conservation policies of coatis in Colombia and Ecuador.

In Central America, the situation would be as follows: 1) *N. n. molaris*, from the north-central and Pacific areas of México to southern USA, was not represented in our samples. However, Silva-Caballero *et al.* (2017) showed with the mt *Cyt-b* gene, on the one hand, a clade including the haplotypes of the Mexican Pacific coast (Punta Raza and Chamela-Cuixmala, Nayarit and Jalisco, respectively) and, on the other hand, a clade comprising the haplotypes of central México (El Tepozteco; Morelos) and southeast México (La Venta and Puerto Morelos, Tabasco and Quintana-Roo, respectively). In fact, the highest F_{ST} statistic was between one of the Pacific populations (Chamela-Cuixmala) and the central population (El Tepozteco; $F_{ST} = 0.125$, $p < 0.05$). The same trend was observed with nuclear microsatellites. With the R_{ST} statistic (0.995; $p < 0.05$), the most differentiated populations were those of Chamela-Cuixmala (Mexican Pacific) and Puerto Morelos in the Yucatán Peninsula; with the F_{ST} statistic 0.258; $p < 0.05$), the most differentiated populations were Punta Raza (Mexican Pacific) and El Tepozteco (Central México). Likewise, Nigenda-Morales *et al.* (2019) detected five distinct groups in the sample of *N. narica* studied with both mitochondrial sequences and nuclear microsatellites. Three of these groups were detected in the geographic area attributed *a priori* to *N. n. molaris*. These three groups were distributed in the Mexican Pacific, Central México, and southwestern United States. The *N. n. molaris* holotype comes from Colima in the Mexican Pacific. Therefore, the Mexican Pacific group can be related to *N. n. molaris*. In contrast, the other two groups found by Nigenda-Morales *et al.* (2019) in central-northern México and southwestern USA may correspond to *pallida* (Allen 1904), *tamaulipensis* (Goldman 1942), *vulpecula* (Erxleben 1777), or *solitaria* var. *mexicana* (Weinland 1860); the respective holotypes should be analyzed to confirm those denominations. 2) *N. n. narica*, whose holotype comes from the State of Veracruz corresponds well with our fifth genetic group (G5, MG5). It also corresponds to one of the groups detected by Nigenda-Morales *et al.* (2019) in the Yucatán Peninsula and Guatemala. 3) *N. n. yucatanica*, whose holotype comes from "Merida in the Yucatán Peninsula", may be represented by our second genetic group (G2, MG2). However, this group, which comprises a few specimens, shows a high internal genetic heterogeneity and a variable relationship with other groups according to the analyses used. An extensive molecular study is needed in the Yucatán and north Quintana Roo since this area appears to have a highly marked genetic structure at the micro-geographic level. We have detected specimens in the area of Mérida separated by a few kilometers, belonging to groups G2 and G5, suggesting the possibility of areas with hybrid specimens of these two groups in that Mexican state. 4) *N. n. nelsoni* or *N. nelsoni* is typical of Cozumel Island. We only studied one specimen from this area, which was included in G5. Likewise, Nigenda-Morales *et al.* (2019) analyzed nine specimens from this island and found no obvious differentiation between these specimens and those from the Yucatán and north Guatemala discussed above. Therefore, molecular evidence seems to reject the validity of *N. n. nelsoni* (*N. nelsoni*). The only possibility for this taxon to be valid is that the specimen studied by us, and the nine specimens analyzed by Nigenda-Morales *et al.* (2019) had been recently introduced by human activities from southern México to Cozumel, and that an original population persists in the island, which had not been sampled in either of the two works. Otherwise, this taxon would have no systematic validity since it could have been introduced to the island in relatively recent historical times. The smaller body size of the coati of Cozumel Island may have been generated over a few generations through natural selection as an adaptation to a small island with few food resources. We agree with Glatston (1994) that this coati was introduced to Cozumel by the Mayans, although we do not agree that *N. nelsoni* is a full species. Similarly, the coati sampled on Roatán Island (Honduras) had the same haplotype as the others found in G3 (MG3), suggesting that it was recently introduced in that locality by human action.

However, three of the genetic groups detected here (and one of those found by Nigenda-Morales *et al.* 2019, which corresponds to our G6-MG6) do not match any putative morphological subspecies of *N. narica*. Two of these groups, G3 and G4, were not observed by Nigenda-Morales *et al.* (2019) because these authors did not collect samples in Honduras, southern Guatemala, El Salvador, Nicaragua, and Costa Rica (only a single specimen was analyzed). Thus, the subspecific name for these two groups remains to be established. A more complex matter is the likely subspecies designation of G6 (MG6). This group, distributed across southern Costa Rica, Panama, and the border area between northern Colombia and Panama, is introgressed with mt DNA of *N. olivacea*. We propose an ancient introgression with mtDNA from *N. olivacea* to the genetic stock of *N. narica* in the border zone between South America and the south of Central America. In the first place, this hypothesis is supported, by the MJN analysis showing a temporal separation between G6 (MG6) haplotypes and *N. olivacea* haplotypes, both located in the same clade at 2.3 Mya for the set of three mt loci and 0.8 Mya for the mitogenomic set. Secondly, the introgressed specimens lack an intermediate morphotype between *N. narica* and *N. olivacea*, but showed the typical morphotype of *N. narica*. Third, the five specimens studied in southern Costa Rica, Panama, and the area bordering Colombia, and the 13 specimens analyzed by Nigenda-Morales *et al.* (2019), showed *N. olivacea* mtDNA in all cases. If a recent hybridization event had caused this phenomenon, we would expect the *N. olivacea* mtDNA in one specimen, but not in all specimens sampled in that area. A possible subspecific denomination could be *N. narica panamensis* (Allen 1904). However, only molecular analysis of the respective holotype may solve this issue because an alternative possibility is that this subspecific denomination may correspond to G3.

In summary, considering Nigenda-Morales *et al.* (2019) and the present study, at least nine genetically differentiated *N. narica* groups are detectable across its geographic range. Three of the morphological subspecies described

Table 4. Estimated gene flow between six groups of white-nosed coati (*Nasua narica*) analyzed in Central America and northern South America. Below the diagonal, based on three mitochondrial genes (*ND5*, *Cyt-b*, and *D-loop*) (G); above the diagonal, based on mitogenomes for all groups detected (MG).

Groups of *Nasua narica*	G1 (MG1)	G2 (MG2)	G3 (MG3)	G4 (MG4)	G5 (MG5)	G6 (MG6)
G1 (MG1)		0.578	0.136	0.387	0.122	0.049
G2 (MG2)	0.504		0.182	0.295	0.182	0.107
G3 (MG3)	0.108	0.100		0.218	0.191	0.025
G4 (MG4)	0.148	0.134	0.155		0.365	0.039
G5 (MG5)	0.118	0.099	0.267	0.312		0.021
G6 (MG6)	0.088	0.117	0.038	0.048	0.041	

would match three of the genetic groups mentioned, one morphological subspecies described should be invalid, and six new subspecific names should be proposed. The large number of groups found in the present study may respond to different geographic, geological, and ecological causes. In the case of coatis, their behavior is also relevant since they have high levels of phylopatry and a limited dispersal capacity (Frantz *et al.* 2010). They form groups with a small territorial range of 0.5 to 4 km^2 (Valenzuela and Ceballos 2000; Hirsch and Gompper 2017). Being barely dispersed from the birth site, the groups, mainly formed by females, are strongly bonded. Occasionally, males that moved up to 20 km away from their birth sites have been detected (Lanning 1976). However, estimates of gene flow between populations in México (Silva-Caballero *et al.* 2017) or Central and South America (Nigenda-Morales *et al.* 2019 and the present study) show that the coati groups are virtually genetically isolated.

Colonization dynamics and evolutionary times of the different N. narica *groups found.* The results obtained are useful for reconstructing the evolutionary history of *N. narica*; to this end, different hypotheses are proposed regarding the origin of each of the *N. narica* groups detected. The analysis of three mt loci suggests the following colonization schemes: 1) The first group to emerge was the Ecuadorian (G1) (→ = gave rise), which gave rise to two separate colonization events in Central America. The oldest was of G1 → Yucatán Peninsula (G2) and, on the other hand, G1 → south-central Central America (G3) (northern Costa Rica, Nicaragua, El Salvador, Honduras, and part of Guatemala). G3 → G4 (southern Guatemala and Belize) → G5 (northern Guatemala and southern México). In this hypothesis, some differential nuances can be considered to explain the existence of two Ecuadorian trans-Andean specimens in G4 and one Ecuadorian cis-Andean specimen in G3. There are two alternatives. The first is that the oldest haplotypes of G3 and G4 were generated *in situ* in South America, followed by subsequent colonization events to Central America. Some of the original haplotypes that expanded through Central America would still "survive" in the Ecuadorian trans-Andean area. The second is that these haplotypes of G3 and G4 emerged *in situ* in Central America from G1, but there was a subsequent migration of individuals with haplotypes originating in Central America, again, to South America. Besides, in northern South America, an *N. narica* group was introgressed with mt DNA of *N. olivacea* and another colonization event took place from northern South America to southern Central America. This hypothesis is consistent with three mitochondrial colonization events from northern South America to Central America, with the G5 haplotypes (southern México and northern Guatemala) being the most recent ones and the G1 haplotypes (Pacific coast of Ecuador) as the original ones. 2) Based on the MJN analysis, only two colonizing events from northern South America to Central America would be considered. In this case, G1 → G3 → G5 → G4, and G2, and the additional colonization with the introgression of *N. olivacea*. All other details would be identical to the previous hypothesis. Other species of Neotropical mammals have also shown at least two colonization events from South America to Central America. An example is the case of the howler monkey (*Alouatta*; Ruiz-García *et al.* 2017), armadillo (*Dasypus novemcinctus*; Arteaga *et al.* 2012), or anteater (*Tamandua* sp.; Ruiz-García *et al.* 2021c). Using mitogenomic data, an alternative hypothesis can be proposed: 3) An *N. nasua* haplogroup in northern South America (Ruiz-Garcia *et al.* 2021a) gave rise to intermediate haplotypes between the current *N. nasua* haplotypes and the current *N. narica* haplotypes, some of which could still be detectable in southern Central America (southern Costa Rica). However, the haplotypes from the Central American *N. narica* would have originated in the north of present-day Colombia, as evidenced by the specimen of the PN Tama (North of Santander), which → MG1 (trans-Andean Ecuador) → MG3 → MG2, MG4, and MG5. In addition, the colonization of specimens introgressed with the mtDNA of *N. olivacea* (MG6) would have occurred. Therefore, this hypothesis proposes two colonizing events from South America to Central America, adding the possibility of a small colonizing event of intermediate haplotypes and even of haplotypes closer to *N. nasua* that occurred before the two major colonization events.

Ruiz-García *et al.* (2021a) proposed the beginning of mitochondrial evolution of the genus *Nasua* (including *Nasuella*) in the Andean zone of northwest South America 13–10 Ma (Miocene). This is consistent with the start of an intense uplifting in the central and northern Andes estimated to have occurred between 13 and 11 Mya (Antonelli *et al.* 2009; Hoorn *et al.* 2010) and with the completion of the Pebas system, which was characterized by the complete, or almost complete, flooding of the western Amazon during the Middle Miocene (Hoorn *et al.* 2010). From 11 to 7 Mya, another rapid uplifting of the Andean zone took place (Quechua II and III phases; Noble *et al.* 1990; Antonelli *et al.* 2009), and the Pebas System was replaced by the Acre fluvial system (Hoorn *et al.* 2010). These geomorphological and hydrological changes may have contributed to the emergence of different genetic clusters within *N. nasua* and the genesis of *N. olivacea*. However, this means that the ancestors of the current coatis had already reached South America during the Middle Miocene, supporting the hypothesis that the closure of the Central American Seaway occurred 15–13 Mya (Farris *et al.* 2011; Montes *et al.* 2012a b, 2015; Carrillo *et al.* 2015; Ruiz-García *et al.* 2018a).

According to the results reported here, the oldest *N. narica* haplotypes in South America seem to have emerged 4.5–3.2 Mya (Pliocene), with the Andes having played a major role. During the Pliocene, the eastern Andes mountain range in Colombia did not reach 40 % of their current elevation (Hoorn *et al.* 2010). The uplifting of the Andes continued until 2.7 Mya (Andriessen *et al.* 1994; Gregory-Wodzicki 2000). Therefore, the Andes mountain range itself may have been a physical and climatic barrier for multiple species that previously would have been widely distributed in northwestern South America (Miller *et al.* 2008; Rull 2011). This period was characterized by glaciations in Argentina (3.5 Mya; Mercer 1984), Bolivia (3.27 Mya; Clapperton 1981), Chile (3–2 Mya; Caviedes and Paskoff 1975). According to the standard GABI model, the Isthmus of Panama emerged completely around 3.5–3.0 Mya (Middle Pliocene) with the definitive closure of the Central American Seaway (Coates and Stallard 2013; O'Dea *et al.* 2016), which could enable some colonizing events by *N. narica* from South America to Central America. Therefore, the case of coatis is contrary to the hypothesis supported by the fossil record, which suggests the predominance of a north-to-south colonization process that occurred 2.8–2.4 Mya (Simpson 1980; Webb 2006; Woodburne 2010). However, the Central American Seaway closure model during the Middle Miocene (15–13 Mya) seems more likely as it predicts active colonization from South America to North America over the past 6 Mya (Bacon *et al.* 2015; Marko *et al.* 2015), as observed for the coati in the present study.

The formation of the truly Central American haplotypes (excluding those of G6-MG6) took place between 1.9-1.1 Mya (Pleistocene), but the time of the introgression of *N. olivacea* into an *N. narica* group also occurred in this period (2.3–0.8 Mya). Nigenda-Morales *et al.* (2019) placed the divergence of the Central American groups at around 1.3 Mya, within the time range established in the present study. During this period, particularly in the range of 1.3–0.8 Mya, called Pre-Pastonian (maximum glacial peak of the great Gunz glaciation), the Central American groups of *N. narica* started to emerge. This period was extremely dry, which significantly reduced the extent of forests and boosted much of the genetic structure in different species of Neotropical mammals (*Lagothrix* sp., Ruiz-García *et al.* 2014, 2019a, 2020b; *Puma yagouroundi*, Ruiz-García *et al.* 2018b; *Lycalopex* sp., Ruiz-García *et al.* 2013).

The genetic structuring of *N. narica* in Central America during the Pleistocene is consistent with the refuge hypothesis (RH; Haffer 1969, 1997, 2008; Vanzolini 1970; Vanzolini and Williams 1970) of alternating humid-dry periods as a result of the Milankovitch cycles. This led to periods of forest contraction where much of the fauna was relegated. During dry periods, forests became shelter patches isolated by shrub zones, savannas, or arid steppes. This fostered the divergence between populations of the same species that became isolated in different refuges and the emergence of new lineages within them. Conversely, forests expanded in the humid periods, and the different lineages coexisted again in sympatric territories. At that time (1.30–1.10 Mya), sixth and seventh North American glaciations occurred (1.1–0.9 Mya) that affected southern México and northern Guatemala (Barendregt and Duk-Rodkin 2011; Rutter *et al.* 2012) and could have led to the differentiation of the G3 haplotypes from G4 and G5. Subsequently, the Reid glaciation (0.3–0.13 Mya; Barendregt and Duk-Rodkin 2011; Rutter *et al.* 2012), with severe dry peaks in North and Central America (Dansgaard *et al.* 1993; Hodell *et al.* 2008), also significantly reduced the extension of forests in Central and North America where *N. narica* lived, leading to the diversification within groups G3 and G5.

In Central America, more recent divergence processes have already occurred. For instance, the MJN showed that G5 (MG5) may have produced G4 (MG4) about 0.12–0.09 Mya. Although Hooghiemstra (1984) defined up to 27 potential climate changes with a periodicity of 100,000 years in the Colombian Andes (which also may had been reflected in Central America), there is only definitive evidence of the start of a great glaciation 116,000 years ago (Van der Hammen and González 1963; Van der Hammen *et al.* 1981; Helmens 1988; Florez 1992).

Genetic diversity and spatial structure. The levels of mitochondrial genetic diversity of *N. narica* were high but lower than those estimated for *N. nasua*, *N. olivacea*, or *P. flavus* (Ruiz-García *et al.* 2019b, 2020a, 2021a). This is correlated with the more recent origin of the ancestor of *N. narica*. In general, the populations that emerged more recently and peripheral to their geographic range have lower genetic diversity levels. The populations of other mammal species distributed in South America and which later colonized Central America and the south of the USA have lower genetic diversity levels than South American populations (long-nosed armadillo, *Dasypus novemcinctus*, Huchon *et al.* 1999; Arteaga *et al.* 2012; puma, *Puma concolor*, Culver *et al.* 2000; jaguarundi, *Puma yagouroundi*, Holbrook *et al.* 2013; Ruiz-García *et al.* 2018b).

In addition, a geographic pattern is observed in the distribution of genetic diversity. The groups living in northwestern South America (G1), and the southernmost part of Central America, introgressed by *N. olivacea* (G6), have the highest genetic diversities, whereas the northernmost groups of Central America have lower genetic diversity values. The group of Yucatán (México; G2) is an exception.

The spatial structure of *N. narica* was conspicuous and widespread throughout its range (Silva-Caballero *et al.* 2017, Nigenda-Morales *et al.* 2019), which appears to be common in the procionids studied from a population genetics stsandpoint (*e. g.*, *N. nasua*, *N. olivacea*, or *P. flavus*; Ruiz-García *et al.* 2019b, 2020a, 2021a). In addition to the philopatric behavior of coati females and the climatic and ecological changes already described, geographic barriers may also have been drivers of the high spatial structure found in *N. narica*. Three physical barriers would help understand the spatial structure found in this study. The area between the Sierra Madre Oriental and the Isthmus of Tehuantepec south to the Nicaraguan depression is home to the groups G5, G2, G4, and part of the specimens of G3. In the area between the Nicaraguan depression and the Talamanca Mountain range (southern Costa Rica and northern Panama, whose emergence is dated at 5–3 Mya; MacMillan *et al.* 2006; Mann *et al.* 2007), we found another part of the specimens of G3 and one specimen of G6. In the area between the Talamanca Mountain range and the Darien region, we found the introgressed specimens of G6. Some of these barriers emerged at times consistent with the estimated divergence times between the *N. narica* groups and have been effective geographic barriers for different taxa. The Talamanca Mountain range has been a geographic barrier for reptiles (Daza *et al.* 2010) and mammals (Bradley *et al.* 2008; Harding and Dragoo 2012; Hardy *et al.* 2013). The same is true for the Nicaraguan depression, characterized by marine transgressions during the Pliocene and Pleistocene (Bagley and Johnson 2014), which have been effective in isolating rodent species (Gutiérrez-García and Vázquez-Domínguez 2012). The territory delimited between the Motagua-Polochic-Jocotan fault (southern Guatemala) and the Isthmus of Tehuantepec (México) has been considered an area of endemic fauna delimited by these geographic barriers (Daza *et al.* 2010; Gutiérrez-García and Vázquez-Domínguez 2013), although this area originated 6–3 Mya, that is, before the arrival of *N. narica* to that area of Central America.

However, these physical barriers only partially explain the groups found in this study. Within the first area delimited by the physical barriers just mentioned (Sierra Madre Oriental and the Isthmus of Tehuantepec south to the Nicaraguan depression), we defined three groups that, in principle, are not separated by physical barriers, and we even found specimens of G3, *i. e.*, the only group found in the second geographic area delimited by the geographic barriers mentioned above (between the Nicaraguan depression and the Talamanca mountain range). The G6 specimens were found basically in the third geographic area mentioned (between the Talamanca mountain range and the Darien region); however, one specimen was found on the other slope of the Talamanca mountain range, whereas the Colombian specimens of the same group were found on the other side of the Darien region. Therefore, we consider that the behavior of the species and climatic changes were more relevant for defining the spatial structuring of *N. narica* than orographic barriers.

Our findings based on mtDNA can be interpreted as a first step toward solving the issue regarding the systematics of *N. narica*. This study provides the most comprehensive mitochondrial analysis and includes the widest geographic range covered to date for the species. However, the study of nuclear markers is essential to determine the hybridization and gene flow levels between the different mitochondrial groups of *N. narica* and achieve a complete evolutionary understanding of this species. Similarly, sampling efforts should be expanded to better understand the origin of this species.

Acknowledgments

The authors wish to thank D. Alvarez, A. Castellanos, J. Brito, and P. Escobar-Armel for their assistance in obtaining samples of *N. narica* over the past 22 years. This work was funded by Project 6839 (Pontificia Universidad Javeriana). Thanks to the Ministerio del Ambiente Ecuatoriano (MAE) in Santo Domingo de Tsáchilas (Ecuador), the Instituto Nacional de Biodiversidad (INABIO) (Ecuador), the Instituto von Humboldt (Colombia), and the Dirección General de Zoológicos y Vida Silvestre of México City (México) for their collection permits in those countries. Thanks also to the many peasants and Mayan communities of Panama, Costa Rica, Nicaragua, Honduras, El Salvador, Belize, Guatemala, and southern México for their assistance in collecting *N. narica* samples. María Elena Sánchez-Salazar translated the manuscript into English.

Literature Cited

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716–723.

Allen, J. A. 1904. New mammals from Venezuela and Colombia. Bulletin of the American Museum of Natural History 20:327-335.

Andriessen, P. A. *et al.* 1994. Absolute chronology of the Pliocene-Quaternary sediment sequence of the Bogotá area, Colombia. Quaternary Science Reviews 12:483-501.

Antonelli, A., *et al.* 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. Proceedings National Academy of Sciences USA 106:9749-9754.

Arteaga, M. C., *et al.* 2012. Genetic structure and diversity of the nine-banded armadillo in Mexico. Journal of Mammalogy 93:547–559.

Avise, J.C., *et al.* 1987. Intraspecific phylogeographic: the mitochondrial DNA bridge between population genetics and systematics. Annual Review Ecology and Systematics 18:489-522.

Bacon, C. D., *et al.* 2015. Biological evidence supports an early and complex emergence of the Isthmus of Panama. Proceedings of the National Academy of Sciences of the United States of America 112:6110-6115.

Bacon, C. D., *et al.* 2016. Quaternary glaciation and the Great American Biotic Interchange. Geology 44:375-378.

Bagley, J. C., and J. B. Johnson. 2014. Phylogeography and biogeography of the lower Central American Neotropics: diversification between two continents and between two seas. Biological Reviews 89:767-790.

Bandelt, H. J., P. Forster, and A. Rohl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37-48.

Barendregt, R. W., and A. Duk-Rodkin. 2011. Chronology and extent of Late Cenozoic ice sheets in North America: a magnetostratigraphical assessment. Developments in Quaternary Science 15:419-426.

Bensasson, D., *et al.* 2001. Mitochondrial pseudogenes: evolution's misplaced witnesses. Trends in Ecology and Evolution 16:314–321

Bradley, R. D., D. D. Henson, and N. D. Durish. 2008. Re-evaluation of the geographic distribution and phylogeography of the *Sigmodon hispidus* complex based on mitochondrial DNA sequences. Southwestern Naturalist 53:301–310.

Brouns, G., A. De Wulf, and D. Constales. 2003. Delaunay triangulation algorithms useful for multibeam echosounding. Journal of Surveying Engineering129:79–84.

Burrell, A. S., *et al.* 2009. Mitochondrial evidence for the hybrid origin of the kipunji, *Rungwecebus kipunji* (Primates: Papionini). Molecular Phylogenetics and Evolution 51:340–348.

Carrillo, J. D., *et al.* 2015. Neotropical mammal diversity and the Great American Biotic Interchange: spatial and temporal variation in South America's fossil record. Frontiers in Genetics 5:451.

Caviedes, C. N., and R. Paskoff. 1975. Quaternary glaciations in the Andes of north-central Chile. Journal of Glaciology 14:155-170.

Clapperton, C. H. M. 1981. Quaternary glaciation in the Cordillera Blanca, Perú and the Cordillera Real, Bolivia. Revista CIAF 6:93-111.

Coates, A. G, and R. F. Stallard. 2013. How old is the Isthmus of Panama? Bulletin of Marine Science 89:801-813.

Collins, A. C., and J. M. Dubach. 2000. Phylogenetic relationships of spider monkeys (*Ateles*) based on mitochondrial DNA variation. International Journal of Primatology 21:381–420.

Collins, A. C., and J. M. Dubach. 2001. Nuclear DNA variation in spider monkeys (*Ateles*). Molecular Phylogenetics and Evolution 19:67–75.

Cortés-Ortiz, L., *et al.* 2003. Molecular systematics and biogeography of the Neotropical monkey genus, *Alouatta*. Molecular Phylogenetics and Evolution 26:64-81.

Culver, M., *et al.* 2000. Genomic ancestry of the American puma (*Puma concolor*). Journal of Heredity 91:186-197.

Dansgaard, W., *et al.* 1993. Evidence for general instability of past climate from a 250-kyr ice-core record. Nature 364:218–220.

Darriba, D., *et al.* 2012. jModelTest2: more models, new heuristics and parallel computing. Nature Methods 9:772.

Daza, J. M., T. A. Castoe, and C. L. Parkinson. 2010. Using regional comparative phylogeographic data from snake lineages to infer historical processes in Middle America. Ecography 33:343-354.

Decker, D. M. 1991. Systematics of the coatis, Genus *Nasua* (Mammalia: Procyonidae). Proceedings of the Biological Society of Washington 104:370-386.

Derenko, M., *et al.* 2012. Complete Mitochondrial DNA Analysis of Eastern Eurasian Haplogroups Rarely Found in Populations of Northern Asia and Eastern Europe. Plos One 7:e32179.

Dupanloup, I., S. Schneider, and L. Excoffier. 2002. A simulated annealing approach to define the genetic structure of populations. Molecular Ecology 11:2571-2581.

Emmons, L. H. 1990. Carnivores (Procyonidae). Pp. 136-138, *in* Neotropical rainforest mammals. University of Chicago Press. Chicago, U.S.A.

Emmons, L. H., and F. Feer. 1997. Neotropical rainforest mammals: a field guide. University of Chicago Press. Chicago, U.S.A.

Erxleben, J. C. P. 1777. Systema regni animalis per classes, ordines, genera, species, varietates cum synonymia et histoire animalium. Classis I. Mammalia. Impensis Weygandianis, Lipsiae.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567.

Farris, D. W., *et al.* 2011. Fracturing of the Panamanian Isthmus during initial collision with South America. Geology 39:1007–1010.

Farris, J. S., *et al.* 1995. Testing significance of incongruence. Cladistics 10:315-319.

Flórez, A. 1992. Los nevados de Colombia: glaciales y glaciaciones. Análisis Geográficos IGAC 22:1–95.

Forasiepi, A. M., *et al.* 2014. Carnivorans at the great american biotic interchange:new discoveries from the northern neotropics. Naturwissenschaften 101:965-974.

Frantz, A.C., *et al.* 2010. Using genetic methods to investigate dispersal in two badger (*Meles meles*) populations with different ecological characteristics. Heredity 104:493-501.

Freeland, J. R., H. Kirk, and S. D. Petersen. 2011. Molecular ecology. Wiley-Blackwell. Oxford, U.S.A.

Freeman, S. H. J., and J. C. Herron. 1998. Evolutionary analysis. Prentice Hall. Upper Saddle River, U.S.A.

Gabriel, K. R., and R. R. Sokal. 1969. A new statistical approach to geographic variation analysis. Systematic Zoology 18:259–278.

Glatston, A. R. 1994. The red panda, olingos, coatis, raccoons, and their relatives. Status survey and conservation action plan for procyonids and ailurids. IUCN/SSC Mustelid, viverrid and procyonid specialist group. International Union for Conservation of Nature and Natural Resources. Bougy, Switzerland.

Goldman, E. A. 1942. Notes on the coatis of the Mexican mainland. Proceedings of the Biological Society of Washington 55:7982.

Gompper, M. E. 1995. *Nasua narica*. Mammalian Species 487:1-10.

Gompper, M. E. 1997. Population ecology of the white-nosed coati (*Nasua narica*) on Barro Colorado Island, Panama. Journal of Zoology 241:441-455.

Gompper, M. E., J. L. Gittleman, and R. K. Wayne. 1997. Genetic relatedness coalitions and social behaviour of white-nosed coatis, *Nasua narica*. Animal Behaviour 53:781-797.

Gompper, M. E., J. L. Gittleman, and R. K. Wayne. 1998. Dispersal, philopatry, and genetic relatedness in a social carnivore: comparing males and females. Molecular Ecology 7:157-163.

González-Maya, J. F., *et al.* 2011. Recent confirmed records and distribution of the White-nosed Coati *Nasua narica* in Colombia. Small Carnivore Conservation 45:26–30.

Gregory-Wodzicki, K. M. 2000. Uplift history of the central and northern Andes: a review. Geological Society of American Bulletin 112:1091–1105.

Guschanski, K., *et al.* 2013. Next-generation museomics disentangles one of the largest primate radiations. Systematic Biology 62:539–554.

Gutiérrez-García, T. A., and E. Vázquez-Domínguez. 2012. Biogeographically dynamic genetic structure bridging two continents in the monotypic Central American rodent *Ototylomys phyllotis*. Biological Journal of the Linnean Society 107:593-610.

Haffer, J. 1969. Speciation in Amazonianforest birds. Science 165:131-137.

Haffer, J. 1997. Alternative models of vertebrate speciation in Amazonia: an overview. Biology Conservation 6:451-476.

Haffer, J. 2008. Hypotheses to explain the origin of species in Amazonia. Brazilian Journal of Biology 68:917-947.

Hall, E. R. 1981. The mammals of North America. Second Ed. John Wiley and Sons. New York, U.S.A.

Harding, L. E., and J. W. Dragoo. 2012. Out of the tropics: a phylogeographic history of the long-tailed weasel, *Mustela frenata*. Journal of Mammalogy 93:1178-1194.

Hardy, D. K., *et al.* 2013. Molecular phylogenetics and phylogeographic structure of Sumichrast's harvest mouse (*Reithro-*

dontomys sumichrasti: Cricetidae) based on mitochondrial and nuclear DNA sequences. Molecular Phylogenetics and Evolution 68:282-292.

Helmens, K. F. 1988. Late Pleistocene glacial sequence in the area of the high plain of Bogota (Eastern cordillera, Colombia). Palaeogeography, Palaeoclimatology, Palaeoecology 67:263–283.

Hershkovitz, P. 1951. Mammals from British Honduras, Mexico, Jamaica and Haiti. Fieldiana Zoology 31:547-569.

Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42:182–192.

Hirsch, B. T., and M. E. Gompper. 2017. Causes and consequences of coati sociality. Pp. 11-33, *in* Biology and Conservation of Musteloids (Macdonald, D. W., C. Newman, and L. A. Harrington, eds.). Oxford University Press, Oxford, UK.

Hodell, D. A., *et al*. 2008. An 85-ka record of climate change in lowland Central America. Quaternary Science Reviews. 27:1152-1165.

Hoelzel, A., R., *et al*. 1994. Rapid evolution of a heteroplasmic repetitive sequence in the Mitochondrial DNA control region of carnivores. Journal of Molecular Evolution 39:191-199.

Holbrook, J. D., *et al*. 2013. Population Genetics of Jaguarundis in Mexico: Implications for Future Research and Conservation. Wildlife Society Bulletin 37:1-6.

Hooghiemstra, H. 1984. Vegetational and climatic history of the high plain of Bogota, Colombia: a continuous record of the last 3.5 millions years. Pp. 62–96, *in* The quaternary of Colombia. vol. 5 (Van Der Hammen, T., ed.). Vaduz, Lichtenstein.

Hoorn, C., *et al*. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science 330:927-931.

Huchon, D., F. *et al*. 1999. Armadillos exhibit less genetic polymorphism in North America than in South America: nuclear and mitochondrial data confirm a founder effect in *Dasypus novemcinctus* (Xenarthra). Molecular Ecology 8:1743–1748.

Hudson, R. R., D. D. Boss, and N. L. Kaplan. 1992. A statistical test for detecting population subdivision. Molecular Biology and Evolution 9:138-151.

International Commission on Stratigraphy. 2007. International stratigraphic chart. http://www.sratigraphy.org/chus.pdf. Consulted 7 January 2020.

Irwin, D. M., T. D. Kocher, and A. C. Wilson. 1991. Evolution of the cytochrome *b* gene of mammals. Journal of Molecular Evolution 32:128–144.

Kartavtsev, Y. 2011. Divergence at *Cyt-b* and *Co-1* mtDNA genes on different taxonomic levels and genetics of speciation in animals. Mitochondrial DNA 22:55-65.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-121.

Koepfli, K. P., *et al*. 2007. Phylogeny of the Procyonidae (Mammalia: Carnivora): molecules, morphology and the Great American Interchange. Molecular Phylogenetics and Evolution 43:1076-1095.

Krause, J., *et al*. 2010. The complete mitochondrial DNA genome of an unknown hominin from southern Siberia. Nature 464:894–897.

Lanfear, R., *et al*. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29:1695–1701.

Lanning, D. V. 1976. Density in Movements of the Coati in Arizona. Journal of Mammalogy 57:609-611.

Librado, P., and J. Rozas. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Linnaeus, C. 1766. Systema naturae per regna tria nature, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Regnum animale. Twelfth ed. Laurentii Salvii, Holmiae 1:1-824.

Lönnberg, E. 1921. A second contribution to the mammalogy of Ecuador with some remarks on *Caenolestes*. Arkiv för Zoologi 14:1-104.

López, J. V., et al. 1994. Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. Journal of Molecular Evolution 39:174-190.

MacMillan, I., P. B. Gans, and G. Alvarado. 2006. Middle Miocene to present plate tectonic history of the southern Central American volcanic Arc. Tectonophysics 392:325-348.

Mann, P., R. D. Rogers, and L. Gahagan. 2007. Overview of plate tectonic history and its unresolved tectonic problems, Pp. 205–241, *in* Central America: Geology, Resources and Hazards (Bundschuh, J., and G. E. Alvarado, eds.). Taylor and Francis. Philadelphia, U.S.A.

Manel, S., *et al*. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology and Evolution 18:189–197.

Manni, F., E. Guerard, and E. Heyer. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Human Biology 76:173–190.

Mantel, N. A. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209-220.

Marko, P. B., R. I. Eytan, and N. Knowlton. 2015. Do large molecular sequence divergences imply an early closure of the Isthmus of Panama? Proceedings of the National Academy of Sciences of the United States of America 112: E5766.

Marshall, J. S. 2007. The geomorphology and physiographic provinces of Central America. Pp. 1-51, *in* Central America: Geology, Resources and Hazards (Bundschuh, J., and G. E. Alvarado, eds.). Taylor and Francis, Philadelphia, U.S.A.

McFadden, K. W., *et al*. 2008. Evolutionary history of the critically endangered Cozumel dwarf carnivores inferred from mitochondrial DNA analyses. Journal of Zoology 276:176-186.

Mercer, J. H. 1984. Changes in the ice cover of temporate and tropical South-America during the last 25,000 years. Zentralblatt für Geologie und Paläontologie Teil I 12:1661-1665.

Merriam, C. H. 1901. Six new mammals from Cozumel Island, Yucatan. Proceedings of the Biological Society of Washington 14:99-104.

Merriam, C. H. 1902. Five new mammals from Mexico. Proceedings of the Biological Society of Washington 15:67-69.

Miller, M. J., *et al*. 2008. Out of Amazonia again and again: episodic crossing of the Andes promotes diversification in a lowland forest flycatcher. Proceedings of the Royal Society of London, B. Biological Sciences 275:1133–1142.

Miller, M. P. 2005. Alleles In Space: Computer software for the joint analysis of interindividual spatial and genetic information. Journal of Heredity 96:722-724.

Monmonier, M. S. 1973. Maximum-difference barriers: an alternative numerical regionalization method. Geographical Analysis 5:245–261.

Montes, C., *et al*. 2012a. Arc-continent collision and orocline formation: closing of the Central American seaway. Journal of Geophysical Research 117:B04105.

Montes, C., *et al*. 2012b. Evidence for Middle Eocene and younger land emergence in central Panama: Implications for Isthmus closure. Geological Society of America Bulletin 124:780-799.

Montes, C., *et al*. 2015. Middle Miocene closure of the Central American sea way. Science 348:226–229.

Morgan, G. S. 2008. Vertebrate fauna and geochronology of the Great American Biotic Interchange in North America. Pp. 93-140, *in* Neogene Mammals (Lucas, S. G., J. A. Morgan, S. Spielmann, and D. R. Prothero, eds.). Museum of Natural History and Science Bulletin 44. New Mexico, U.S.A

Morral, N., J. Bertrantpetit, and X. Estivill. 1994. The origin of the major cystic fibrosis mutation (delta F508) in European populations. Nature Genetics 7:169-175.

Nabholz, B., H. Ellegren, and J. B. Wolf. 2012. High levels of gene expression explain the strong evolutionary constraint of mitochondrial protein-coding genes. Molecular Biology and Evolution 30:272–284.

Nigenda-Morales, S. F., *et al*. 2019. Phylogeographic and diversification patterns of the white-nosed coati (*Nasua narica*): Evidence for south-to-north colonization of North America. Molecular Phylogenetics and Evolution 13:149–163.

Noble, D. C., *et al*. 1990. Cenozoic stratigraphy, magmatic activity, compressive deformation, and uplift in northern Peru. Geological Society American Bulletin 102:1105-1113.

Nowak, R. M. 1999. Walker's Mammals of the World, 6 edn. Johns Hopkins University Press. Baltimore, U.S.A.

O'Brien, S. J. 1994. A role for molecular genetics in biological conservation. Proceedings of the National Academy of Science USA 91:5748-5755.

O'Dea, A., *et al*. 2016. Formation of the Isthmus of Panama. Science Advances 2:e1600883.

Pennington, R. T., and C. W. Dick. 2010. Diversification of the Amazonian flora and its relation to key geological and environmental events: a molecular perspective. Pp. 373-385, *in* Amazonia, Landscape and Species Evolution: A Look into the Past (Hoorn, C., and F. Wesselingh, eds.). Wiley- Blackwell. Oxford, U.S.A.

Planet, P. J., and I. N. Sarkar. 2005. A tool for constructing and analyzing matrices of pairwise phylogenetic character incongruence tests. Bioinformatics 21:4423-4424.

Posada, D., and K. A. Crandall. 2001. I ntraspecific gene genealogies: trees grafting into networks. Trends in Ecology and Evolution 16:37–45.

Raaum, R. L., *et al*. 2005. Catarrhine primate divergence dates estimated from complete mitochondrial genomes: concordance with fossil and nuclear DNA evidence. Journal of Human Evolution 48:237–257.

Ruiz-García, M. 1993. Analysis of the evolution and genetic diversity within and between Balearic and Iberian cat populations. Journal of Heredity 84:173-180.

Ruiz-García, M. 1994. Genetic profiles from coat genes of natural Balearic cat populations: An eastern Mediterranean and North- African origin. Genetics Selection Evolution 26:39-64.

Ruiz-García, M. 1997. Genetic relationships among some new cat populations sampled in Europe: a spatial autocorrelation analysis. Journal of Genetics 76:1-24.

Ruiz-García, M. 1999. Genetic structure of different cat populations in Europe and South America at a microgeographic level: importance of the choice of an adequate sampling level in the accuracy population genetics interpretations. Genetics and Molecular Biology 22:493-505.

Ruiz-Garcia, M., and D. Alvarez. 2000. Genetic microstructure in two Spanish cat populations. I: Genic diversity, gene flow and selection. Genes & Genetic Systems 75:269-280.

Ruiz-García, M., *et al*. 2019a. First molecular phylogenetic analysis of the *Lagothrix* taxon living in Southern Peru and Northern Bolivia: *Lagothrix lagothricha tschudii* (Atelidae, Primates), a new subspecies. Folia Primatologica 90:215-239.

Ruiz-García, M., *et al*. 2018a. Mitogenomics phylogenetic relationships of the current sloth's genera and species (Bradypodidae and Megalonychidae). Mitochondrial DNA Part A 29:281-299.

Ruiz-García, M., M. F. Jaramillo, and J. M. Shostell. 2019b. Mitochondrial phylogeography of kinkajous (Procyonidae, Carnivora): maybe not a single ESU. Journal of Mammalogy 100:1631-1652.

Ruiz-García, M., *et al*. 2020a. The Phylogeographic Structure of the mountain coati (*Nasuella olivacea*; Procyonidae, Carnivora) in Colombia and Ecuador, and phylogenetic relationships with the other coati species (*Nasua nasua* and *Nasua narica*) by means of mitochondrial DNA. Mammalian Biology 100: 501-523.

Ruiz-García, M., M. F. Jaramillo, and J. M. Shostell. 2021a. How many taxa are within the genus *Nasua* (including *Nasuella*; Procyonidae, Carnivora)? The mitochondrial reconstruction of the complex evolutionary history of the coatis throughout the Neotropics. Journal of Phylogenetics and Evolutionary Biology (in press).

Ruiz-García, M., *et al*. 2021b. The genus *Nasuella* should be included in the genus *Nasua* (Procyonidae, Carnivora): mitochondrial DNA and karyotypic evidence. Journal of Vertebrate Biology 71:21040.

Ruiz-García, M., M. Pinedo-Castro, and J. M. Shostell. 2014. How many genera and species of woolly monkeys (Atelidae, Platyrrhine, Primates) are?: First molecular analysis of *Lagothrix flavicauda*, an endemic Peruvian primate species. Molecular Phylogenetics and Evolution 79:179-198.

Ruiz-García, M., M. Pinedo-Castro, and J. M. Shostell. 2018b. Mitogenomics of the jaguarundi (*Puma yagouaroundi*, Felidae, Carnivora): Disagreement between morphological subspecies and molecular data. Mammalian Biology 93:153-168.

Ruiz-García, M., *et al* . 2020c. Invalidation of taxa within the silvery wooly monkey (*Lagothrix lagothricha poeppigii*, Atelidae, Primates). Mitochondrial DNA Part A 31:147-162.

Ruiz-García, M., *et al*. 2021b. Comparative mitogenomics phylogeography of two Anteaters genera (*Tamandua* and *Myrmecophaga*; Myrmecophagidae, Xenarthra): Some discrepant evolutionary traits. Zoological Research 42:525-547.

Ruiz-García, M., D. Rivas-Sánchez, and N. Lichilín. 2013. Phylogenetics relationships among four putative taxa of foxes of the *Pseudoalopex* genus (Canidae, Carnivora) and molecular population genetics of *Ps. culpaeus* and *Ps. sechurae*. Pp. 97-128, *in* Molecular population genetics, evolutionary Biology and Biological conservation of Neotropical Carnivores (Ruiz-García, M., and J. M. Shostell, eds.). Nova Science Publishers Inc. New York, U.S.A.

Ruiz-García, M., *et al*. 2016. Phylogenetic relationships of Pithecidae and temporal splits in reference to Cebidae and Atelidae by means of mitogenomics. Pp. 345-368, *in* Phylogeny, molecular population genetics, evolutionary biology and conservation of the Neotropical Primates (Ruiz-García, M., and J. M. Shostell, eds.). Nova Science Publishers Inc. New York, U.S.A.

Ruiz-García, M., *et al*. 2017. Phylogeography of the mantled howler monkey (*Alouatta palliata*; Atelidae, Primates) across its geographical range by means of mitochondrial genetic analyses and new insights about the phylogeny of *Alouatta*. Folia Primatologica 88:421-454.

Rull, V. 2011. Neotropical biodiversity: timing and potential drivers. Trends in Ecology and Evolution 26:508–513.

Rutter, N., *et al*. 2012. Glaciations in North and South America from the Miocene to the last Glacial Maximum, comparisons, linkages and uncertainties. Springer. New York, U.S.A.

Saillard, J., *et al*. 2000. mtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. American Journal of Human Genetics 67:718-726.

Sambrock, J., E. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory manual. 2nd edition. V1. Cold Spring Harbor Laboratory Press. New York, U.S.A.

Sawyer, S., *et al*. 2015. Nuclear and mitochondrial DNA sequences from two Denisovan individuals. Proceedings of the National Academy of Science USA 112:15696–15700.

Schwarz, G. E. 1978. Estimating the dimension of a model. Annals of Statistics 6:461-464.

Simpson, G. G. 1980. Splendid Isolation: The curious history of South American mammals. Yale University Press. New Haven, U.S.A.

Silva Caballero, A., *et al*. 2017. Patterns of genetic diversity of the white-nosed coati reveals phylogeographically structured subpopulations in Mexico. Natural Resources 8:31-53.

Soibelzon, L. H., and F. Prevosti. 2013. Fossils of South American Land arnivores (Carnivora, Mammalia). Pp 509–527 *in* Molecular population genetics, evolutionary biology and biological conservation of Neotropical carnivores (Ruiz-García, M., and J. M. Shostell, eds.). Nova Science Publisher. New York, U.S.A.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–1243.

Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML Web servers. Systematic Biology 57:758–771.

Talavera, G., and J. Castresana. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56:564–577.

Tamura, K., G. *et al*. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30:2725-2729.

Tavaré, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences 17:57–86.

Thalmann, O., *et al*. 2004. Unreliable mtDNA data due to nuclear insertions: a cautionary tale from analysis of humans and other apes. Molecular Ecology 13:321–335.

Thomas, O. 1912. Two new species of *Nasua*. Annals and Magazine of Natural History, Series 8, 10:228-230.

Tirira, D. 2007. Guía de campo de los mamíferos del Ecuador. Editorial Murciélago Blanco. Quito, Ecuador.

Tirira, D. 2011. Libro rojo de los mamíferos de Ecuador. Publicación especial 8. Fundación Mamíferos y Conservación. Quito, Ecuador.

Trigo, T. C., *et al*. 2008. Inter-species hybridization among Neotropical cats of the genus *Leopardus*, and evidence for an introgressive hybrid zone between *L. geoffroyi* and *L. tigrinus* in southern Brazil. Molecular Ecology 17:4317–4333.

Vaidya, G., D. J. Lohman, and R. Meier. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27:171–180.

Valenzuela, D., and G. Ceballos. 2000. Habitat selection, home range, and activity of the White-nosed coati (*Nasua narica*) in a Mexican tropical dry forest. Journal of Mammalogy 81:810-819.

Van Der Hammen, T., *et al*. 1981. Glacial sequence and environmental history in the Sierra Nevada del Cocuy (Colombia). Palaeogeography, Palaeoclimatology, Palaeoecology 32:247–340.

Van Der Hammen, T., and E. González. 1963. Historia del clima y vegetación del Pleistoceno superior y del Holoceno de la sabana de Bogotá. Boletín de Geología 11:189–266.

Vanzolini, P. E. 1970. Zoología sistemática, geografía e a origem das espécies. Instituto Geográfico de São Paulo. São Paulo, Brazil.

Vanzolini, P. E., and E. E. Williams. 1970. South American anoles: geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). Arquivos de Zoologia 19:1–298.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506-513.

Watson, D. F. 1992. Contouring: A Guide to the Analysis and Display of Spatial Data. Pergamon Press. New York, U.S.A.

Wayne, R., *et al*. 1997. Molecular systematics of the Canidae. Systematic Biology 46:622-653.

Webb, S. D. 2006. The Great American Biotic Interchange: patterns and processes. Annals of the Missouri Botanical Garden 93:245–257.

Weinland, D. F. 1860. Ueber den Mexikanischen nasenharen. Zoologische Garten 11:189-193.

Woodburne, M. O. 2010. The great American biotic interchange: dispersals, tectonics, climate, sea level and holding pens. Journal of Mammalian Evolution 17:245-264.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395-420.

Associated editor: Jesús Fernández
Submitted: April 26, 2021; Reviewed: May 14, 2021
Accepted: November 14, 2021; Published on line: May 23, 2022

THERYA, 2022, Vol. 13(2):225-234 DOI:10.12933/therya-22-2145 ISSN 2007-3364

Potential Distribution of the Neotropical Otter (*Lontra longicaudis annectens*) in the State of Yucatán, México

Alicia Andrea Ortega-Padilla[1], Juan Pablo Gallo-Reynoso[2]*, Verónica Farías-González[3] Javier Enrique Sosa- Escalante[4], Silvia Hernandez-Betancourt[1], Gloria Ponce-García[2], and Tania Elizabeth Quintana-Salvador[2]

[1] Campus de Ciencias Biológicas y Agropecuarias, Departamento de Zoología, Cuerpo Académico Bioecología Animal. Universidad Autónoma de Yucatán. Carretera Mérida Xmatkuil Km 15.5, C. P. 97100. Mérida, Yucatán, México. Email: aliceort75@gmail.com (AAO-P) and hbetanc@correo.uady.mx (SH-B).

[2] Centro de Investigación en Alimentación y Desarrollo, A.C. Unidad Guaymas. Carretera al Varadero Nacional km 6.6, C. P. 8548. Guaymas, Sonora, México. E-mail: jpgallo@ciad.mx (JPG-R), gloria.ponce.garcia@gmail.com (GP-G) and elisaalvador@hotmail.com (TEQ-S).

[3] Laboratorio de Recursos Naturales, Unidad de Biología, Tecnología y Prototipos. Facultad de Estudios Superiores Iztacala, UNAM, C. P. 54090. Estado de México, México. Email: v.farias@unam.mx (VF-G)

[4] Centro para la Gestión de la Sustentabilidad. Calle 78 5780, C. P. 97217. Mérida, Yucatán, México. Email: javiersosae@hotmail.com (JES-E).

*Corresponding author

En el Estado de Yucatán los registros de nutria neotropical (*Lontra longicaudis annectens*) son insuficientes para determinar si existen diferentes tipos de hábitat para la especie, su estatus de conservación y los riesgos potenciales que enfrenta. Nuestros objetivos son el generar un modelo de distribución potencial de la nutria neotropical para el Estado de Yucatán, y el verificar la presencia de la nutria neotropical en sitios predichos por el modelo. Se generó un modelo de distribución potencial de la nutria neotropical con MaxEnt con registros de presencia de la especie y variables ambientales de EarthEnv a 1 km^2 de resolución. Se verificó la presencia de la nutria en siete sitios predichos por el modelo mediante visitas en el mes de octubre de los años 2017 al 2019 y 2021. Se caracterizó el hábitat y se colectaron muestras biológicas, así como evidencia video-fotográfica de las señales de presencia de las nutrias y de su hábitat. La distribución potencial de la nutria cubrió 3,487 km^2, el 8 % de la superficie del Estado de Yucatán, en ríos, lagunas y ecosistemas generalmente costeros, sin embargo, el modelo permitió identificar la idoneidad ambiental del hábitat para la nutria en áreas al interior de la península, y la validación en campo de los sitios predichos produjo los primeros registros de la especie en cenotes y lagunas epicontinentales. Los sitios presentaron disponibilidad de presas, vegetación riparia para refugio y madrigueras, y conectividad acuático-terrestre con manantiales de agua dulce. Se obtuvieron observaciones directas de nutrias en dos de los sitios visitados, así como señales indirectas de su presencia en los siete sitios, las cuales consistieron en heces, letrinas, geles, huellas, rastros, residuos de alimento, madrigueras de descanso y veredas hacia el cuerpo de agua. Los resultados señalan la necesidad de continuar con el monitoreo minucioso en las áreas potenciales predichas por el modelo para poder sugerir medidas de conservación para la nutria neotropical y su hábitat en el estado de Yucatán.

Records of Neotropical otters (*Lontra longicaudis annectens*) are insufficient in the state of Yucatán, México, to determine the different habitat types where otters live, the conservation status of the species and the potential threats. Therefore, our objectives were to generate a map of the potential distribution of the Neotropical otter in Yucatán and verify the otter's presence in sites predicted by the model. A potential distribution species model of the Neotropical otter was generated with MaxEnt using species' records and EarthEnv environmental variables with 1 km^2 resolution. Otter's presence was verified in seven sites predicted by the model by field surveys conducted during October months of 2017 to 2019 and 2021. The habitat was characterized, and biological samples and video-photographic evidence of otter's presence and its habitat were collected. Potential distribution of the Neotropical otter covered 3,487 km^2, 8 % of Yucatán State's surface area, in rivers, lagoons, and coastal ecosystems with mangrove forest and wetlands; nevertheless, sites with suitable habitat and potential areas for the presence of otters were observed in epicontinental lagoons in the interior of the State. The potential distribution model allowed to identify the environmental suitability for otter's habitat in the interior of the peninsula, whereas field surveys made to validate the sites predicted by the model led us to report the first records of the neotropical otter in cenotes (sinkholes) and epicontinental lagoons. The sites presented availability of prey species, high density of riparian vegetation for shelter and dens, and terrestrial-aquatic interconnectivity between freshwater springs. Direct observations of otters were obtained in two of the visited sites, as well as indirect signals in seven of them, which consisted of feces latrines, annal jellies, footprints, tracks, food residues, active resting dens, and shore trails. Our results point out the need to continue thorough monitoring in potential areas predicted by the model to be able to suggest conservation measures for the Neotropical otter and its habitat in Yucatán State.

Keywords: Algorithm; potential distribution; monitoring; epicontinental; coastal lagoons.

Introduction

The Neotropical otter (*Lontra longicaudis annectens*) has a wide distribution range in México; it can be found in the Pacific slope and coast including the mainland coasts of the central and southern Gulf of California, in the Gulf of México slope and coast, and in the Caribbean slope; the species inhabits in many and diverse habitat types along creeks, rivers, lakes, lagoons, wetlands, and reservoirs (Gallo-Reynoso 1997; Briones-Salas *et al.* 2008; Hernández-Romero 2016; Gallo-Reynoso and Meiners 2018). The species' altitudinal range spans from sea level to 2,500 masl and inhabits river basins across zones covered by different vegetation types including coastal marshes, mangrove forest, thorny scrubland, tropical deciduous forest, tropical evergreen forest, mountain cloud forest, and oak-pine forest (Gallo-Reynoso and Meiners 2018; Hernández-Romero *et al.* 2018).

The Neotropical otter is listed as endangered species in México (NOM-059-SEMARNAT-2010; SEMARNAT 2019) and is included in the Agreement for Priority Species and Population Conservation in México (SEMARNAT 2014). It is listed under subsection I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2017) and in the red list of the International Union for Conservation of Nature (IUCN 2019).

In Yucatán, due to the karstic nature of the soil, surface water bodies are relatively shallow and streams flow underground with water outcrops and wells locally called *cenotes* (sinkholes). Cenotes may be of various types: the most typical are cavern-type cenotes with stalactites and stalagmites, but some are open cenotes, similar to small lagoons (Cervantes-Martínez 2007). This particular freshwater system in Yucatán favors suitable habitats for the Neotropical otter and may foster the habitat connectivity required for the species to establish viable populations, since otters have been recorded in Campeche, Quintana Roo, and Yucatán (Gallo-Reynoso 1997; Sánchez and Gallo-Reynoso 2007; Calmé and Sanvicente 2009; Santiago-Plata *et al.* 2013; Sosa-Escalante *et al.* 2013; Gallo-Reynoso and Meiners 2018; Vazquez-Maldonado *et al.* 2021).

It is worth to mention that time gaps between records in the same localities in the State of Yucatán may indicate preferential habitat types (Sosa-Escalante and Martínez-Meyer 2014). For example, the first recorded occurrence of Neotropical otter in Yucatán was reported by Leopold (1965) at Ría Celestún, 64 km W Mérida. The presence of specimens at Ría Celestún Biosphere Reserve was confirmed in 2000 (Sánchez and Gallo-Reynoso 2007). Thus, the species was recorded on two different occasions separated by circa 35 years (1965, 2000) in the same natural protected area. Although the Neotropical otter is mentioned as present in the Management Program of Ría Celestún Biosphere Reserve, there are no reports available of the species conservation status (CONANP 2000). From the above, it follows the need to investigate if the previous records of Neotropical otter at Ría Celestún, and the record of 2000 at San Crisanto in Yucatán (Leopold 1965; Sánchez and Gallo-Reynoso 2007) may correspond to marginal (*i. e.,* isolated) populations, and/or if the species might be facing serious conservation issues in Yucatán. Thereby, it is important to identify the potential distributional range and potential suitable habitat for the Neotropical otter in Yucatán, to confirm its current distribution. The objectives of this study were to generate a model of the potential distribution of the Neotropical otter in the State of Yucatán and verify the otter's presence in sites predicted by the model.

Materials and Methods

Study Area. The Yucatán peninsula is located in southeastern México and separates the Gulf of México to the north and west from the Caribbean Sea and the northern part of Central America to the east; Yucatán is on the northern part of the peninsula, between Campeche and Quintana Roo. Climate is subhumid with summer rains, mean annual precipitation of 1,600 mm and mean annual temperature of 26°C; the dry season spans from March to May and the rainy season from June to October (García 2004; Estrada and Cobos 2014). Dominant winds from the north prevail from November to February, with a tropical cyclone season (tropical storms and hurricanes) from August to November (Herrera-Silveira 2006; INEGI 2008). Extensive areas in the Yucatán peninsula are covered by deciduous and evergreen tropical forests; nevertheless, Yucatán also have various vegetation communities that include coastal dune vegetation, mangrove forest, floodplain forest, savannah, wetland vegetation known locally as *petén*, *popal*, *tular*, and secondary vegetation (Flores-Guido *et al.* 2010; Pérez-Sarabia 2017; Zavala-Cruz *et al.* 2016).

A calcareous platform made up of a series of plains and plateaus of karstic origin with underground drainage constitute the State. The northern part has low elevations, less than 250 m, except for the Sierra de Ticul with an elevation of 350 masl (Herrera-Silveira 2006; Pérez-Sarabia 2017). In the southern part of the Yucatán and the area bordering with Campeche and Quintana Roo, the relief consists of plateaus and karstic valleys of recent formation (Herrera-Silveira 2006; Pérez-Sarabia 2017). The underground system connects areas of water recharge within deciduous and medium tropical forests with coastal areas with mangrove forests where cenotes are also located (Estrada-Medina *et al.* 2014). The areas where karstic rock has dissolved by water filtration through geologic time gave place to large cavern-type cenotes (Estrada-Medina *et al.* 2014). Some cenotes are deep, closed caverns; other cavern-type cenotes are partially open where portions of the cavern roof have collapsed; and cenotes with roofs that collapsed completely in the past are now fully open and exposing the underground water (Estrada-Medina *et al.* 2014).

Rainwater permeates underground due to the carbonate rocks of the peninsula platform, as well as through abundant fractures in the karstic rock which hamper the formation of surface currents like creeks or rivers. As such, a few rivers are formed in the borders between Yucatán, Campeche, and Quintana Roo, increasing in quantity in southern Campeche towards Tabasco, the same is presented in southern Quintana Roo towards Belize, and connecting with many coastal lagoons and deltaic systems (Zavala-Cruz *et al.* 2016; Pérez-Sarabia 2017).

Some freshwater springs arise inside mangrove forests and petenes, as well as surface water reservoirs called "*aguadas*", which are topographic depressions that allow rain to be accumulated due to a fine layer of compacted clay sediment that prevents the filtration of rain to underground waters. Another type of spring water is called *"sartenejas",* which are reservoirs of underground water, some of which have been used by the local Maya inhabitants to excavate and build cisterns with wide fringes to delimit water sources and channels to conduct rainwater (Herrera-Silveira 2006).

Potential distribution map. We gathered georeferenced observations from international data sources GBIF (Global Biodiversity Information Facility 2016) and VertNet (Constable *et al.* 2010), from national collections on biodiversity (*e. g.*, the online catalogue of the Coleccion Nacional de Mamíferos (CNMA) of the Instituto de Biología, Universidad Nacional Autónoma de México and from scientific literature such as articles and reports (Martínez-Meyer and Sánchez-Cordero 2006; Sánchez and Gallo-Reynoso 2007; Guzmán-Soriano *et al.* 2013).

The potential distribution of the Neotropical otter was modeled considering the georeferenced otter records and environmental variables that favor its presence in Yucatán. Spatial autocorrelation was eliminated from the record database with the geographic information system ArcMap 10.2 (produced by ESRI 2014) and the tool "Spatially Rarefy Occurrence Data for SDMToolbox" (Brown 2014) to prevent over-adjustment in areas subjected to more intensive sampling (Veloz 2009; Boria *et al.* 2014; ESRI 2014b). The refined database was split into two subsets: 1) correspond to species records within its geographic range, excluding Yucatán (hereafter 'calibration base') and 2) that considered the records only for the Yucatán peninsula Biotic Province or YPBP (hereafter 'evaluation base'). The two subsets were defined to have independent records of the species to evaluate the estimated environmental suitability and the potential distribution area. As predictors of the potential distribution modeling, several environmental variables from EarthEnv (Domisch *et al.* 2015) were used, which contain suitable information on freshwater organisms at ~1 km^2 resolution (Table in Appendix 1). All variables were delimited accordingly with the area of the two scenarios mentioned above. A MaxEnt algorithm (Maximum Entropy; Phillips *et al.* 2006) were used to generate the model because it requires presence data only and allows the use of categorical variables and flexible parameterization (Elith *et al.* 2011).

The calibration area of the model was delimited from the shapefile of Terrestrial Ecoregions of the World (Olson *et al.* 2001); from it, a polygon was drafted representing the hypothesis of historic accessibility for Neotropical otter populations, constituted by the ecoregions that contained records of *L. longicaudis annectens* within the Neotropical region ('M' area in the BAM diagram; Soberón and Peterson 2005). M was partitioned in two polygons according to the data subsets previously split into two stages: 'stage one', which included the complete distribution range of the species, excluding Yucatán, which corresponded to the 'calibration base'; and 'stage two', which included the Yucatán peninsula, corresponding to the 'evaluation base'.

The model was calibrated with the information from stage one using the observations from the calibration base as inputs. Then, the model was transferred to stage two (Yucatán) to represent the environmental suitability for Neotropical otter in Yucatán based on environmental characteristics of its distribution range for the whole country. The effectiveness of the model was corroborated by using the projection of the evaluation base in the resulting model for stage two; and to validate the models, an analysis of the partial ROC curve was performed, which was configured to perform 1000 "bootstrap" tests using the iPartialROC function (Barve 2008) from the ENMGadets package in R (R Development Core 2017). To estimate the potential distribution of the species, we reclassified the data from the MaxEnt model and generated binary probability models (absence/presence), using ArcMap 10.2.

Otter's presence verification in the field. In October of years 2017, 2018, 2019 and 2021, we visited a total of seven sites which included coastal ecosystems of marshes and mangrove forests, cenotes, and epicontinental lagoons (Table 1). The sites were selected accordingly to the presence of suitable habitat predicted by the potential distribution map previously generated (Figure 1).

Otter vocalizations sessions were held in locations where the presence of otters was predicted by the model. Previously, we recorded otter vocalizations consisting of barks and high-pitched barks of an individual otter from the Miguel Álvarez del Toro Zoo, in Tuxtla Gutierrez, México. These vocalizations were played every 10 minutes, for one minute, during the surveys, until there was a response or after 30 minutes with no response.

In every visit, we recorded geographic data with a GPS (Garmin GPSmap 78s) to obtain locations, distances, routes, altitude, and survey effort. The perimeter of cenotes, coastal lagoons, continental lagoons, marshes, and wetlands was surveyed on foot and/or navigating on a canoe or a kayak, searching for direct sightings and indirect signs of Neotropical otter's presence. At the seven sites surveyed, habitat characteristics were described, and the habitat requirements of Neotropical otters were verified including freshwater presence, a stable water level, water transparency, subaquatic vegetation, presence of suitable prey types inside and outside of the water body, tree coverage, and suitable areas to establish breeding and resting dens like rocky areas and holes excavated in banks of cenotes or lagoons, or among dense vegetation.

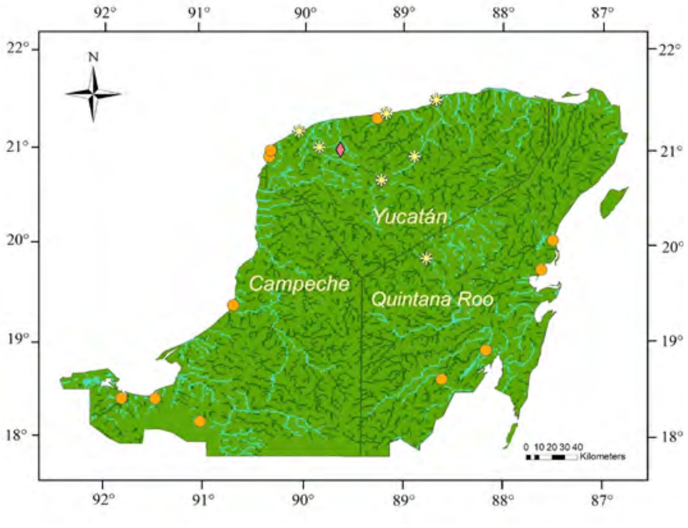

Figure 1. Potential distribution of the Neotropical otter habitat in Yucatán (Dark green), with historical records of this species (orange circles); from Sánchez and Gallo-Reynoso (2007) and Gallo-Reynoso and Meiners (2018). Favorable environmental sites in blue. Surveyed sites are indicated (yellow stars) for seven localities: San Crisanto, Cenote Tunkás, Yalahau Lagoons State Park (Parque Estatal Lagunas de Yalahau), Chichankanab Lagoon, Sisal, San Antonio Chel, and El Islote (Dzilam de Bravo). Binary map. Coordinate System WGS84. Environmental layers from Earth Env. Domisch *et al.* 2015.

The information on tracks, footprints, latrines, and dens was recorded in a database. We collected biological samples and specimens (spraints, anal jellies, food residues, and plant specimens), and recorded photographic and video material that showed the characteristics of the studied areas. With a GoPro Hero2 video camera, we recorded underwater video to understand the underwater habitat characteristics such as presence of underwater vegetation, species of fish present and features of each site such as the depth of water, as well as otter vocalizations. Collected materials were deposited at the Laboratorio de Biología de la Conservación at the Parque Científico y Tecnológico de Yucatán. Also, in the surveyed areas, we searched for connection between water bodies (*i. e.*, several lagoons that were separated by 500 to 1,000 m) that would facilitate the movement of Neotropical otters.

Results

Otter's potential distribution. The potential distribution of the Neotropical otter in Yucatán covered an estimated area of approximately 3,487 km^2, equivalent to 8 % of the area of Yucatán and 2.6 % of the Yucatán peninsula. The potential distribution modeling allowed us to identify suitable habitat for the Neotropical otter in inland areas of the Yucatán peninsula; although most known records were located near the coast in mangrove forest and wetland vegetation known as *petén*, *popal*, and *tular*. Seven sites predicted with potential habitat for otter in Yucatán were visited to confirm the presence of otters.

Otter's presence verification in the field. San Crisanto (site 1). With a total survey effort of 5 km, the paths between the artisanal salt evaporation plots were checked on foot, the mangrove area was not visited because it was flooded by the high tide. The vegetation consisted of mangroves (*Rizophora mangle*, *Laguncularia racemosa*), and coastal dune vegetation (shrubs and grasses) in the salt evaporation plots, particularly a pioneer vegetation zone (*Soriana maritima*, *Sesuvium portulacastrum*, *Sporobolus virginicus*, *Distichlis spicata*).

Feeding sites were found with presence of bones, feathers, and wings mostly of Blue-winged Teal (*Spatula discors*); one site presented remains of an American flamingo juvenile (*Phoenicopterus ruber*). We found a series of footprints and tracks of Neotropical otter in one of the clay shores of the marsh and a spraint on the vegetation (Figure 2).

Cenote Tunkás (site 2). With a total effort of 2.3 km, we surveyed the perimeter of an open cenote with a slope of approximately 7 m from the upper edge to the water body. The vegetation in Cenote Tunkás was tropical deciduous forest with *petén* along the shore and aquatic vegetation with dense riparian vegetation in a gallery forest composed by *Metopium brownei, Bursera simaruba, Phitecelobium albicans, Thrinax radiata,* and *Nymphaea ampla*, to mention some of the species that provided shade to the cenote.

In the bank, paths leading from the cenote to an otter's burrow were found devoid of vegetation and the path edge vegetation flattened at ground level, which indicates constant use by otters. Two burrows were found near the water's edge, outside one of them tracks were found; an annal gland jelly mark was found on top of a rock in the middle of the slope near one of the burrows (Figure 2). During the survey the high-pitched bark of an otter was heard, and the recording was reproduced to entice the otter to answer the calls, which the otter did.

Yalahau Lagoons State Park (site 3). This site was visited during the month of October for four years (2017 to 2019 and 2021). The survey conducted in 2017 included a lagoon's bank section, with a total effort of 2.1 km. The following years the area surveyed was increased to include the total perimeter of the lagoon of 8 km. The vegetation at Yalahau Lagoon was composed by *popal* and tropical deciduous forest subjected to annual floods, with species such as *Haematoxylum campechianum*, *Dalbergia glabra*, and *Mimosa bahamensis*. As evidence, an otter spraint was collected from a rocky outcrop among grasses in the lagoon's bank, even though the water level was overflowed due to the extra seasonal rains.

During the 2018 survey, an otter latrine was found on a rock, in the northern portion of the lagoon (Figure 2). The islands of hydrophilic vegetation that are near the northern portion of the body of water were also visited, during this activity we heard an otter's bark, thus we reproduced the zoo's vocalization recording to incite the otter to answer the call to confirm the presence of the species; then, the individual came close enough to obtain a visual record. For the 2019 survey, the latrine was abandoned probably due to the overflow of the lagoon's water level, as many areas of riparian vegetation.

Table 1. Characteristics of the seven sites visited to verify the Neotropical otter's (*Lontra longicaudis annectens*) presence in areas with predicted suitable environmental conditions in the State of Yucatán, southeastern México.

Site name, geographic coordinates, and location	Site description
1. San Crisanto. 21° 20.914 N, –89° 10.281 W. 51 km E of the city of Progreso, north Yucatán.	Coastal locality with marshes, wetlands, mangrove forests, cenotes, estuaries, and coastal areas, as well as evaporation parcels for obtaining artisanal salt within a mangrove preservation zone. San Crisanto Mangroves Management Unit for Wildlife Preservation (Unidad de Manejo para la Conservación de Vida Silvestre [UMA] Manglares de San Crisanto [SEMARNAT-UMA-EX0196-YUC-11; 21.34735 N, -89.18113 W]).
2. Cenote Tunkás. 20° 54.558 N, –88° 52.013 W. 30 km W of Cenotillo.	Open cenote with an approximate diameter of 75 m. This water body is located within a complex of 20 cenotes, lagoons, and aguadas located in the northeastern portion of the Yucatán State where large-scale tourism is not yet developed.
3. Yalahau Lagoon. 20° 39.435 N, –89° 13.034 W. Yalahau Lagoons State Park, 60 km SE of the city of Mérida.	Yalahau Lagoon in the main lagoon of Yalahau Lagoons State Park, a Yucatán State Natural Protected Area encompassing three epicontinental lagoons, cenotes and aguadas located in the central part of the State of Yucatán (DOF 2004).
4. Chichankanab Lagoon. 19° 52.113 N, -88° 45.886 W. 150 km SW of the city of Mérida.	Chichankanab Lagoon is located at the central-west portion of the State of Quintana Roo, limiting with the State of Yucatán, and is approximately 30 km long stretching from La Presumida to Kantemó. Chichankanab is the largest epicontinental lagoon system of the Yucatán peninsula.
5. Sisal. 21° 09.554 N, –90° 02.930 W. 53 km NW of the city of Mérida.	Sisal is near Punta Piedra in a tide-flooded area with gravel bottom, several kilometers away from the "Paraíso Sisal" residential complex. A portion of the wetland is part of the "El Palmar" State Ecological Reserve.
6. San Antonio Chel. 20° 59.903 N, –89° 50.659 W. 7.6 km east of Hunucmá.	Abandoned quarry flooded with water from a cenote and surrounded by native vegetation.
7. El Islote. 21° 28.656 N, –88° 39.975 W. 25 km NE of Dzilam. Dzilam de Bravo State Reserve.	El Islote is located between the coastal lagoon and the eastern coast of the Dzilam de Bravo State Reserve, close to a place called "Las Bocas", characterized by extensive sandy areas devoid of vegetation and with mangrove trees bordering the lagoon.

In 2020, due to the COVID 19 pandemic, no surveys were carried out because the lagoon's state park was closed during the contingency. Activities resumed in October 2021, when indirect records of the species were found in the northern portion of the water body, a site close to where we observed an otter in 2018. In 2021 we also surveyed other two smaller lagoons close to the main one and in all of them we found indirect evidence of otter's presence consisting in tracks and feeding sites.

Chichankanab Lagoon (site 4). We covered 4.8 km on canoe and 1.7 km on foot. The vegetation at the western bank was tropical deciduous forest with *popal*. The tropical forest species at the eastern bank were *Lonchocarpus castilloi*, *Alseis yucatanensis*, *Brosimum alicastrum*, *M. zapota*, *Ceiba pentandra*, and *H. campechianum*, and banks were surrounded by red mangrove (*R. mangle*; Merediz-Alonso 2004). Three places with abundant otter signs consisting of feces on the roots and branches of mangrove trees were found on the banks of the lagoon. However, many of these were washed away after the rain that occurred that same day.

Sisal (site 5). We surveyed 10 km of coastal lagoons and mangrove forest at coastal areas in the El Palmar State Ecological Reserve. The vegetation was dominated by coastal dune bushes and mangrove, with *Suaeda linearis, Lycium carolinianum, Tribulus cistoides, Canavalia rosea, Euphorbia mesembrianthemifolia, Ernodea littoralis*, *Ipomea pes caprae*, *Coccoloba uvifera*, *R. mangle*, and *T. radiata*. Neotropical otter footprints were found over a sand-gravel substrate near Cocodrilo Lagoon at low tide.

San Antonio Chel (site 6). The survey consisted of 1.5 km on an abandoned limestone quarry with an excavated pit filled with water. The surrounding area included a flat section of fine gravel of about 3,000 m^2 with vegetation corresponding to floodplain forest with *H. campechianum, Cameraria latifolia, M. brownei, M. zapota, B. simaruba, C. aesculifolia, Cochlospermun vitifolium,* button mangrove (*Conocarpus erecta*), *D. glabra,* Neotropical otter footprints and tracks were found over a sand and gravel substrate near the pit.

El Islote (site 7). In October 2019, 10 km were surveyed in mangrove and beach sandbars areas near the coast of Dzilam de Bravo State Reserve. The area was mostly devoid of vegetation; however, it covered part of the mangrove forest in the swamp. The vegetation included red mangrove, button mangrove, and black mangrove (*Avicennia germinans*), some dune vegetation with sea grape or uvero de playa (*C. uvifera*) and grasses. We found indirect signs of otters consisting of footprints on a humid sandy substrate between the coastal lagoon's shore and coastal dunes. In October 2021, a combined boat and foot survey was done in the same area, on this occasion the tide was high, and the cenotes inside the mangrove forest were also overflood due to extra-seasonal rains, therefore we found no evidence of otters on the mangrove branches and roots.

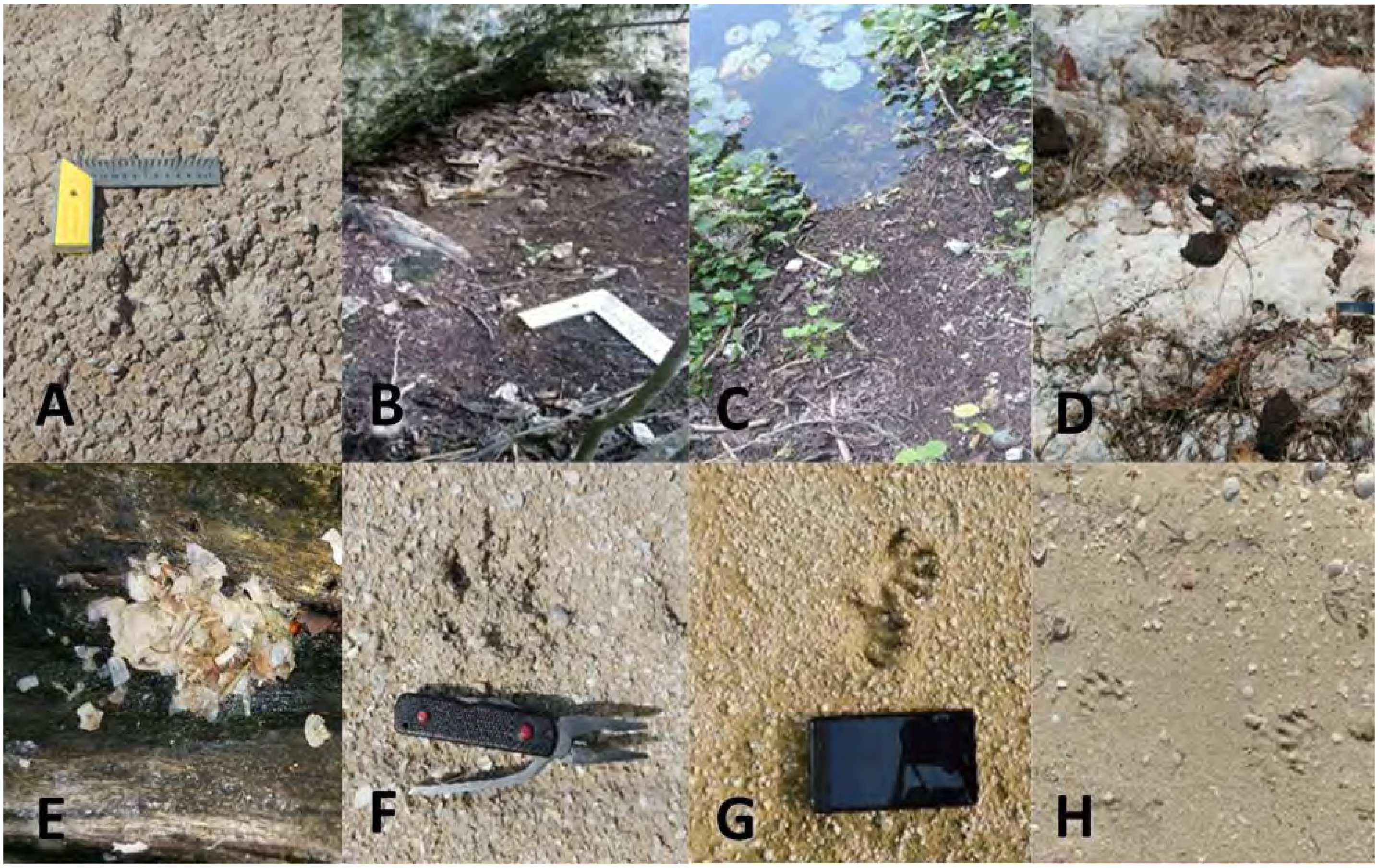

Figure 2. Records of *Lontra longicaudis* in Yucatán. A) Tracks at San Crisanto. B) and C) Den and otter trail from the water to the den at Cenote Tunkás. D) Latrine at Yalahau Lagoons State Park (Parque Estatal Lagunas de Yalahau). E) A spraint dispersed by recent rain on a trunk at Chichankanab Lagoon, Quintana Roo (border with Yucatán). Note the presence of fish scales and fresh remains of water crab. F) Footprint at Cocodrilo Lagoon near Sisal. G) Footprint at San Antonio Chel. H) Footprint at El Islote near Dzilam de Bravo.

Discussion

According to the potential distribution model, the available habitat for the otter were generally located in the coastal lagoons in the western and northern coasts of Yucatán, and in a geological structure named "Ring of Cenotes" which are the remnants of the meteorite that impacted the area of northern Yucatán at Chicxulub *ca* 66 Myr and produced thousands of cenotes and other geological features (Connors *et al.* 1996). The "Ring of Cenotes" probably connects suitable habitat with many cenotes of several types including open cenotes which are nearby, and land depressions subject to flood during rainy season which form temporal lagoons (Aguilar *et al.* 2016).

Direct and indirect evidence of otter's presence was found in seven verified sites predicted with habitat suitability by the potential distribution model. These sites showed availability of prey species, including crustaceans, fish, reptiles, and birds, which are the main food sources for otters, as well as adequate vegetation cover, density and interconnectivity between land, water, and freshwater sources; these two aspects are essential for the survival of otters.

Since otter's presence was confirmed in mangrove forests, epicontinental lagoons and cenotes, our results were consistent with previous studies on Neotropical otter ecology performed in other regions of Mexico (Macías-Sánchez 2003; Mayagoitia-González *et al.* 2012; Hernández-Romero 2016).

We report the first records of Neotropical otter in different inland areas of the Yucatán peninsula and noteworthy direct observations and the second record of Neotropical otter in the coastal locality of San Crisanto after a 20-year span (Sánchez and Gallo-Reynoso 2007). Otter's presence was corroborated in Ría Celestún after an 18-year span, and new coastal records were found at Sisal, Dzilam de Bravo (El Islote), as well as new records at Chichankanab epicontinental lagoon.

The Chichankanab Lagoon is probably connected to the hydrological basin of the State of Quintana Roo; this basin includes several lagoon systems connected to Laguna de Bacalar, where a Neotropical otter was filmed in April 2020 (Hernandez 2020), and Laguna de los Milagros where otters have been previously recorded (Gallo-Reynoso 1997; Gallo-Reynoso and Meiners 2018).

Outstandingly, at San Crisanto marshes, the cenote is located among salt-extraction parcels, which neutralize the high concentration of dissolved salts in the area and made it suitable for otters since they can tolerate salinity levels of 2 to 3 ppm; the same condition was recorded at Celestún, Sisal, and Dzilam de Bravo (El Islote).

The marking activity of the Neotropical otter is known to decrease during the rainy season, due to the flooding of latrine areas (Gallo-Reynoso *et al.* 2016). We visited Yalahau Lagoons State Park when supposedly the rainy season would be over by October, but we found high-water level resulting from heavy rains of an extended rainy season during 2017 to 2019 and 2021 surveys; nevertheless, we found an active latrine in 2018. These rains overflood the rocks, branches, roots and fallen trunks, thus washing away any spraint residues on latrine-suitable areas.

Our results pointed out that the Neotropical otter is associated with large freshwater bodies such as open cenotes, aguadas, epicontinental lagoons, coastal lagoons, and marshes in Yucatán which are major geological features of the state (Connors *et al.* 1996; Aguilar *et al.* 2016). It is important to continue searching for otters in the central areas of the Yucatan peninsula to unravel possible mobility between sites occupied by otters. The potential distribution model suggested habitat availability at many nearby locations, even though the lack of runoff waters and rivers hinders otter's mobility. Therefore, it remains unclear if otter populations might be marginal (*i. e.*, isolated) because the connectivity between these available habitats for otters is unknown, although we found otters distributed across the Ring of Cenotes and in other areas of Yucatán which points out to the presence of a certain connectivity due to geological features (Aguilar *et al.* 2016), that should be thoroughly investigated.

The distribution of the Neotropical otter in Yucatán is poorly known in the System of Natural Protected Areas of Yucatán (SANPY) and currently lacks a management plan or a specific conservation strategy for the species (Gallo-Reynoso 2013; Sosa-Escalante *et al.* 2013). The results of our research can be used to support the prioritization of sites for the conservation of the Neotropical otter, such as the Yalahau Lagoons State Park and the Ring of Cenotes and Chichankanab lagoon.

Conservation implications. The current growth and expansion of human populations and related urbanization generates a high demand for subsurface freshwater, along with groundwater pollution due to organic and solid wastes, agricultural and urban wastewater; all of which drain to surface and subsurface waters and pose a major threat for the Neotropical otter and its preys in the studied area (Arcega-Cabrera *et al.* 2014; Polanco-Rodríguez *et al.* 2015).

In areas of environmental suitability for Neotropical otter's presence, we suggest organizing workshops to raise awareness on the biological importance and environmental services provided by this species in the different ecosystems, which might be translated into community strategies for habitat and water quality conservation (Hernández-Romero 2016). Otter populations have successfully recovered in several parts of the world thanks to legal protection programs and strict legislation addressing pollution of water bodies (Carone *et al.* 2014; Rheingantz *et al.* 2021), according to international instruments acknowledged by Mexico (Agenda 21, Río 1992).

It is recommended that the natural protected areas of Yucatán (Sisal, Yalahau Epicontinental Lagoons and Dzilam among others), and Federal protected areas such as Ria Celestún Biosphere reserve, where we found Neotropical otter presence, be acknowledged as refuge areas for their protection based on the Mexican General Law for Wildlife (Ley General de Vida Silvestre), and the Biosphere Reserve Ria Lagartos should be examined for Neotropical otter's presence.

Acknowledgments

We are thankful to all the participants of the course "Ecología de las nutrias 2017" that took place at VI Jornadas Mastozoológicas of Asociación Mexicana de Mastozoología, for their help on the field. To C. Selem-Salas for her help on logistics of the 2017 expedition. We are grateful to D. M. Antochiw-Alonzo, M. Guerrero, J. Pettis, and S. Bautista for their help on the field on San Crisanto in 2000. To L. Vázquez, for her help in 2017, M. Pérez Medrano for his help in 2018 and S. Gallo for his help in 2021 field surveys.

Literature cited

Aguilar, Y., *et al.* 2016. Density of karst depressions in Yucatán State, Mexico. Journal of Cave and Karst Studies 78:51-60.

Arcega-Cabrera, F., *et al.* 2014. Fecal sterols, seasonal variability, and probable sources along the ring of cenotes, Yucatan, Mexico. Journal of Contaminant Hydrology 168:41-49.

Barve. N. 2008. Tool for Partial-ROC ver (1.0). Biodiversity Institute, Lawrence ,U.S.A.

Boria, R. A., L. E. Olson, S. M. Goodman, and R. A. Anderson. 2014. Spatial filtering to reduce sampling bias can improve the performance of ecological niche models. Ecological Modeling 275:73-77.

Briones-Salas, M., J. Cruz-Alfaro, J. P. Gallo-Reynoso, and V. Sánchez-Cordero. 2008. Abundancia de la nutria neotropical (*Lontra longicaudis annectens* Major, 1897) en el Río Zimatán en la Costa de Oaxaca, México. Pp. 354-376, *in* Avances en el Estudio de los Mamíferos de México Vol. II (Lorenzo, C., E. Medinilla, and J. Ortega, eds.). Publicaciones Especiales. Asociación Mexicana de Mastozoología. México, Distrito Federal.

Brown, J. L. 2014. SDMtoolbox: a python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. Methods in Ecology and Evolution 5:694-700.

Calmé, S., and M. Sanvicente. 2009. Distribución, uso de hábitat y amenazas para la nutria neotropical (*Lontra longicaudis annectens*): un enfoque etnozoológico. Pp. 124-130, *in* El sistema ecológico de la Bahía de Chetumal/ Corozal: Costa occidental del mar Caribe. (Espinoza-Avalos, J., G. A, Islebe, and H. A. Hernández-Arana, eds.). El Colegio de la Frontera Sur. Chetumal, México.

Carone, M., *et al.* 2014. A multi-temporal approach to model endangered species distribution in Europe. The case of the Eurasian otter in Italy. Ecological Modeling 274:21-28.

Cervantes Martínez, A. 2007. El balance hídrico en cuerpos de agua cársticos de la Península de Yucatán. Teoría y Praxis 3:143-152.

CITES (Convención sobre el Comercio Internacional de Especies Amenazadas de Fauna y Flora Silvestres). 2017. Apéndices I, II y III. Convención sobre el Comercio Internacional de Especies Amenazadas de Fauna y Flora Silvestres, Programa de las Naciones Unidas para el Ambiente. Available at: https://cites.org/sites/default/files/eng/app/2017/E-Appendices-2017-10-04.pdf

CONAFOR (Comisión Nacional Forestal). 2014. Inventario Estatal Forestal y de Suelos de Yucatán (IEFYS) 2013. Secretaría de Medio Ambiente y Recursos Naturales. Distrito Federal, México.

CONANP (Comisión Nacional de Áreas Naturales Protegidas). 2000. Programa de Manejo Reserva de la Biósfera Ría Celestún, México. Secretaría de Medio Ambiente y Recursos Naturales. Distrito Federal, México.

Connors, M., *et al.* 1996. Yucatán Karst features and the size of Chicxulub crater. Geophysical Journal International 127:F11-F14.

Constable, H., *et al.* 2010. VertNet: A New Model for Biodiversity Data Sharing. PLoS Biol 8:e1000309

Diario Oficial del Gobierno del Estado de Yucatán. 2004. Reglas administrativas a las cuales se sujetan las actividades que se desarrollen en el Área Natural Protegida denominada Parque Estatal Lagunas de Yalahau. Acuerdo Número 44. 26 de Junio. Mérida, México.

Diario Oficial del Gobierno del Estado de Yucatán. 2013. Decreto que establece el Área Natural Protegida denominada Reserva Estatal Geohidrológica del Anillo de Cenotes. Decreto Número 117. 28 de Octubre. Mérida, México.

Domisch, S., G. Amatulli, and W. Jetz. 2015. Near-global freshwater-specific environmental variables for biodiversity analyses in 1 km resolution. Scientific Data 2:150073.

Eckert, C. G., K. E. Samis, and S. C. Lougheed. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Molecular Ecology 17:1170-1188.

Elith, J., *et al.* 2011. A statistical explanation of MaxEnt for ecologists. Diversity and Distributions 17:43-57.

Estrada Medina, H., and V. Cobos Gasca. 2014. Programa de medidas preventivas y de mitigación de la sequía de la Cuenca de la Península de Yucatán. Universidad Autónoma de Yucatán. Versión 2:5. Available at: aliceort75@gmail.com

Flores-Guido S., R. Durán-García, and J. J. Ortiz-Díaz. 2010. Comunidades vegetales terrestres. Pp. 125-129, *in* Biodiversidad y Desarrollo Humano en Yucatán (Durán, R. and M. Méndez, eds). Centro de Investigaciones Científicas de Yucatán, Programa de Pequeñas Donaciones del Fondo para el Medio Ambiente Mundial, CONABIO, Secretaría de Desarrollo Urbano y Medio Ambiente del Estado de Yucatán. Mérida, México.

Gallo-Reynoso, J. P. 1989. Distribución y estado actual de la nutria o perro de agua (*Lutra longicaudis annectens* Major, 1897), en la Sierra Madre del Sur, México. M. Sc. Thesis, Universidad Nacional Autónoma de México. Ciudad de México, México. Available at: jpgallo@ciad.mx

Gallo-Reynoso, J. P. 1997. Situación y distribución de las nutrias en México, con énfasis en *Lontra longicaudis annectens* Major, 1897. Revista Mexicana de Mastozoología 2:10-32.

Gallo-Reynoso, J. P. 2013. Presentación de la sección especial de nutrias. Therya 4:187-190.

Gallo-Reynoso, J. P., *et al.* 2016. Camera traps and otter latrines, what do they tell us? Congreso Nacional de Mastozoología. Tuxtla Gutiérrez, Chiapas. Octubre de 2016.

Gallo-Reynoso, J. P., and M. Meiners. 2018. Las nutrias de río en México. Biodiversitas, CONABIO 140:1-7. Septiembre-Octubre.

García, E. 2004. Modificaciones al sistema de clasificación climática de Köppen. Instituto de Geografía, Quinta edición. Serie Libros Universidad Nacional. Autónoma de México. Ciudad de M{exico, México.

Global Biodiversity Information Facility (GBIF). 2016. Occurrence Download. http://doi.org/10.15468/dl.hpta6x

Guzmán-Soriano, D., *et al.* 2013. Registros notables de mamíferos para Campeche, México. Acta Zoológica Mexicana (n. s.) 29:269-286

Hall, E. R. 1981. The mammals of North America. Wiley Interscience Publication. New York, U.S.A.

Hernández, I. 2020. Un precioso venado se pasea por la Riviera Maya. Aparece un venado en la playa, pero también una nutria en Bacalar. Quinta Fuerza. 15 de abril de 2020. https://quintafuerza.mx/quintana-roo/video-un-precioso-venado-se-pasea-por-riviera-maya/

Hernández-Romero, P. C. 2016. Morfometría, filogenia y nicho ecológico de la nutria neotropical, *Lontra longicaudis* a lo largo de su distribución. Doctoral Dissertation. Instituto de Ecología. Xalapa, México.

Hernández-Romero, P. C., F. J. Botello-López, N. Hernández-García, and J. Espinoza-Rodríguez. 2018. New altitudinal record of Neotropical otter (*Lontra longicaudis* Olfers, 1818) and conflict with fish farmers in Mexico. IUCN Otter Specialist Group Bulletin 35:193-197.

Herrera-Silveira, J. 2006. Lagunas costeras de Yucatán (SE, México): investigación, diagnóstico y manejo. Ecotropicos 19:94-108.

Instituto Nacional de Estadística, Geografía e Informática (INEGI). 2008. Características edafológicas, fisiográficas, climáticas e hidrológicas de México. Available at: http://docplayer.es/12617571-Caracteristicas-edafologicas-fisiograficas-climaticas-e-hidrograficas-de-mexico.html Consultado el 12/05/17

IUCN (International Union for Conservation of Nature). 2019. The IUCN Red List of Threatened Species. Available at: https://www.iucnredlist.org/

Leopold. A. S. 1965. Fauna silvestre de México. Instituto Mexicano de Recursos Naturales Renovables. Distrito Federal, México.

Macías-Sánchez, S. 2003. Evaluación del hábitat de la nutria neotropical (*Lontra longicaudis* Olfers, 1818) en dos ríos de la zona centro del Estado de Veracruz, México. M. Sc. Thesis. Instituto de Ecología. A.C. Xalapa, México

Martínez-Meyer, E., and V. Sánchez-Cordero. 2006. Uso de datos de colecciones mastozoológicas. Pp. 177-186, *in* Colecciones Mastozoológicas de México. (Lorenzo, C., E. Espinoza, M. Briones, and F. A. Cervantes, eds.). Instituto de Biología, UNAM and Asociación Mexicana de Mastozoología, A. C. Distrito Federal, México

Merediz-Alonso, G. 2004. Ficha informativa de los humedales de Ramsar (FIR) Laguna de Chichankanab. Available at: https://en.wikipedia.org/wiki/List_of_Ramsar_sites_in_Mexico

Mayagoita-González, P., *et al.* 2012. Uso de hábitat y perspectivas de *Lontra longicaudis* en un área protegida de Tamaulipas, México. Therya 4:243-256.

Olson, D. *et al.* 2001. Terrestrial ecoregions of the world: a new map of life on Earth. Bioscience 51:933-938.

Peterson A. T., M. Papes, and J. Soberón. 2008. Rethinking receiver operating characteristic analysis applications in ecological niche modeling. Ecological Modeling 213:63-72.

Pérez-Sarabia, J. E., *et al*. 2017. El Conocimiento Florístico De La Península De Yucatán, México. The Floristic knowledge of the Yucatan Peninsula, Mexico. Polibotánica 44:39-49.

Phillips, S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modeling 190:231-259.

Polanco-Rodríguez, A. G. *et al*. 2015. Contamination by organic pesticides in the aquifer of the Ring of Cenotes in Yucatán, México. Water and Environment Journal 29:140-150.

R Core Team. 2001. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rheingantz, M. L., *et al*. 2021. *Lontra longicaudis*. The IUCN Red List of Threatened Species 2021: e.T12304A164577708.

Sánchez, O., and J. P. Gallo-Reynoso. 2007. Evaluación del riesgo de extinción de *Lontra longicaudis* de acuerdo al numeral 5.7 de la NOM-059-SEMARNAT-2001. Pp. 61-89, *in* Método de evaluación del riesgo de extinción de las especies silvestres en México (Sánchez, O., R. Medellín, A. Aldama, B., Goettsch, J. Soberón, and M. Tambutti, eds.). INE-SEMARNAT. Distrito Federal, México

Santiago-Plata, V. M. *et al*. 2013. Aspectos ecológicos de la nutria neotropical (*Lontra longicaudis annectens*) en el camino La Veleta en la Laguna de Términos, Campeche, México. Therya 4:265-280.

SEDUMA (Secretaría de Desarrollo Urbano y Medio Ambiente). 2004. Programa de Manejo Área Natural Protegida Parque Estatal Lagunas De Yalahau. Yucatán, México.

SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). 2010. Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en peligro. Diario Oficial de la Federación. Secretaría de Medio Ambiente y Recursos Naturales. 30 de Diciembre.

SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). 2014. Acuerdo por el que se da a conocer la lista de especies y poblaciones prioritarias para la conservación. Diario Oficial de la Federación del 5 de marzo de 2014. Distrito Federal, México.

SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). 2019. Modificación del Anexo Normativo III, Lista de especies en riesgo de la Norma Oficial Mexicana NOM-059 SEMARNAT-2010, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo, publicada el 30 de diciembre de 2010. Diario Oficial de la Federación del 14 de noviembre de 2019. Ciudad de México, México.

Soberón J., and A. T. Peterson. 2005. Interpretation of models of fundamental ecological niches and species' distributional areas. Biodiversity Informatics 2:1-10.

Sosa-Escalante, J. E., J. M. Pech-Canché, M. C. MacSwiney, and S. Hernández-Betancourt. 2013. Mamíferos terrestres de la península de Yucatán, México: riqueza, endemismo y riesgo. Revista Mexicana de Biodiversidad 84:949-969.

Sosa-Escalante, J., and E. Martínez-Meyer. 2014. Propuesta para priorizar los esfuerzos oficiales de conservación de los mamíferos terrestres y sus hábitats: El caso de la Península de Yucatán. En: Memorias del XII Congreso Nacional de Mastozoología. Asociación Mexicana de Mastozoología A. C., Benemérita Universidad Autónoma de Puebla, México.

Urquiza, T. 2013. Taller: Información sobre biodiversidad para la conservación medioambiental. Estación Biológica La Selva. CONABIO.

Vázquez-Maldonado, L. A., A. Delgado-Estrella, and J. P. Gallo-Reynoso. 2021. Knowledge and Perception of the Neotropical otter (*Lontra longicaudis annectens*) by local inhabitants of a protected area in the state of Campeche, México. IUCN/SCC Otter Specialist Group Bulletin 38:155-172.

Veloz, S. D. 2009. Spatially autocorrelated sampling falsely inflates measures of accuracy for presence only niche models. Journal of Biogeography 36:2290–2299.

Zavala-Cruz, J., *et al*. 2016. Paisajes geomorfológicos: Base para el levantamiento de suelos en Tabasco, México. Geomorphological landscapes: framework for soil surveys in Tabasco, México. Ecosistemas y Recursos Agropecuarios 3:161-171. Available at: www.ujat.mx/era

Associated editor: Sergio Ticul Álvarez Castañeda
Submitted: April 14, 2021; Reviewed: May 20, 2021
Accepted: March 20, 2022; Published on line: May 26, 2022

Appendix 1

Variables used to make the potential distribution model for the Neotropical otter (*Lontra longicaudis annectens*) in the State of Yucatán, southeastern México.

No.	Variable	Name
1	Climate	Monthly minimum temperature (average)
2	Climate	Monthly maximum temperature (average)
3	Climate	Monthly upstream precipitation (sum)
4	Climate	Monthly minimum temperature (distance-weighted average)
5	Climate	Monthly maximum temperature (distance-weighted average)
6	Climate	Monthly upstream precipitation (distance-weighted sum)
7	Climate	Hydroclimatic variables (average and sum)
8	Climate	Hydroclimatic variables (distance-weighted average and sum)
9	Topography	Upstream elevation (min, max, range, avg)
10	Topography	Upstream slope (min, max, range, avg)
11	Topography	Stream length and flow accumulation
12	Land cover	Upstream landcover coverage (minimum)
13	Land cover	Upstream landcover coverage (maximum)
14	Land cover	Upstream landcover coverage (range)
15	Land cover	Upstream landcover coverage (average)
16	Soil	Upstream landcover coverage (distance-weighted average)
17	Soil	Upstream soil (minimum)
18	Soil	Upstream soil (maximum)
19	Soil	Upstream soil (range)
20	Soil	Upstream soil (average)
21	Soil	Upstream soil (distance-weighted average)

THERYA, 2022, Vol. 13(2):235-243 DOI:10.12933/therya-22-1168 ISSN 2007-3364

Effect of anthropogenic noise on the echolocation pulses of the bats *Molossus sinaloae* and *Mormoops megalophylla*

Ana Cristel Lara-Nuñez[1], José Antonio Guerrero[1], and Areli Rizo-Aguilar[2]*

[1] Facultad de Ciencias Biológicas, Universidad Autónoma del Estado de Morelos. Av. Universidad 1001, Col. Chamilpa, C. P 62209. Cuernavaca, Morelos, México.

[2] Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma del Estado de Morelos. Av. Universidad 1001, Col. Chamilpa, C. P 62209. Cuernavaca, Morelos, México. Email: laranuz@gmail.com (ACL-N) areli.rizo@uaem.mx (AR-A) aguerrero@uaem.mx (JAG).

*Corresponding author

Anthropogenic noise interferes with the acoustic signals of various wildlife species. For bats that use echolocation, noise can mask the information received in the echo. The effect of anthropogenic noise on the time and frequency components of echolocation pulses emitted by the aerial insectivorous bats *Molossus sinaloae* and *Mormoops megalophylla* in urban and natural habitats were evaluated. We hypothesized that the frequency components of pulses would increase in response to masking, while time components would not change significantly. To this end, acoustic recordings of both species were made in the two types of habitats using ultrasonic detectors; simultaneously, the intensity of the ambient noise was measured. Frequency (kHz) and time (ms) were analyzed for each echolocation pulse using the BatSound 4.2 software. Consistent with our hypothesis, the results showed that under background noise of 75 dB in an urban environment, *M. sinaloae* increased the low and high frequencies of its echolocation pulses by 5.8 kHz on average. For *M. megalophylla*, no increase in pulse frequencies was observed. Contrary to our expectation, the time components of pulses for *M. sinaloae* were modified, being of shorter duration in urban sites. Increasing the maximum amplitude-frequency by *M. sinaloae* may be a response to the Lombard effect, *i. e.*, the increase in vocal amplitude in response to increased background noise. It is important to carry out studies focused on understanding the modification of echolocation pulses, mainly for species living in urban environments.

El ruido antropogénico interfiere con las señales acústicas de diversas especies de fauna silvestre. Para los murciélagos que usan la ecolocalización, el ruido puede enmascarar la información recibida en el eco. Se evaluó el efecto del ruido antropogénico en los componentes de tiempo y frecuencia de los pulsos de ecolocalización de los murciélagos insectívoros aéreos *Molossus sinaloae* y *Mormoops megalophylla* dentro de un hábitat urbano y uno natural. Hipotetizamos que los componentes de frecuencia de los pulsos incrementarían como respuesta al enmascaramiento mientras que los componentes de tiempo no variarían significativamente. Para ello se realizaron grabaciones acústicas de ambas especies en los dos tipos de hábitat usando detectores de ultrasonidos, simultáneamente se midió la intensidad del ruido ambiental. Se analizaron para cada pulso de ecolocación parámetros de frecuencia (kHz) y tiempo (ms) en el software BatSound 4.2. De acuerdo con nuestra hipótesis los resultados mostraron que, bajo el ruido de fondo a una intensidad de 75 dB en el ambiente urbano, *M. sinaloa*e incrementó las frecuencias de sus pulsos de ecolocalización, tanto bajos como altos, en 5.8 kHz en promedio. Para *M. megalophylla*, no se observaron incrementos en las frecuencias de sus pulsos. Contrario a lo esperado, los componentes de tiempo para los pulsos de *M. sinaloae* sí se vieron modificados, siendo de menor duración para los sitios urbanos. El incremento de la frecuencia de máxima amplitud para *M. sinaloae*, podría ser una respuesta al efecto Lombard, que es el incremento en la amplitud vocal en respuesta al aumento del ruido de fondo. Es de importancia la realización de estudios enfocados a entender la modificación de los pulsos de ecolocalización, principalmente para las especies que viven en ambientes urbanos.

Keywords: Acoustic masking; Lombard effect; noise pollution; pulses variation.

Introduction

Several studies have shown that chronic exposure to noise can have adverse effects on wildlife (Kight and Swaddle 2011), which occur at the physiological level or affect communication, behavior patterns, and the structure of communities (Barber *et al.* 2010; Brumm 2010; Kight and Swaddle 2011; Siemers and Schaub 2011; Francis and Barber 2013; Shannon *et al.* 2015). In communication, noise interferes with the tone, intensity, and structure of signal emissions, so that its reception lacks information, thus producing an acoustic masking effect (Barber *et al.* 2010; Naguib 2013). To compensate for this effect, different species of animals have made vocal adjustments in the acoustic signals emitted (Nemeth *et al.* 2013; Rios-Chelén *et al.* 2015; Shen and Xu 2016; Brumm and Zollinger 2017). For instance, birds can increase the frequency and amplitude of the song and reduce the number of syllables (Slabbekoorn and Peet 2003; Brumm 2004; Nemeth *et al.* 2013; Rios-Chelén *et al.* 2015). It has also been observed that some species of lizards may increase the duration of syllables or use a higher number of syllables containing more intense tones (Brumm and Zollinger 2017).

Similar to other vertebrates, bats have adapted to life in urban areas, so it is possible to find bat shelters and hunting sites within or in the periphery of cities (Kunz and Reynolds 2003; Russo and Ancillotto 2015). The emission of high-frequency sounds allows bats to perceive shapes and textures of the surrounding environment, thus creating an acoustic image that provides them with information for spatial orientation and the detection, identification, and location of food (Neuweiler 2000; Schnitzler and Kalko 2001). For some bat species, noise represents an aversive stimulus. It has been observed that some species avoid foraging in areas exposed to high noise levels, and that foraging becomes less efficient due to increased search effort (Schaub *et al.* 2011; Bunkley and Barber 2015; Luo *et al.* 2015a). Echolocation pulses are susceptible to different acoustic stimuli that may potentially interfere with echo reception (Gillam and Montero 2016), so that noise can mask this information in low-frequency echolocation pulses. In response, bats can adjust the components of their echolocation pulses by increasing the duration, bandwidth (Bunkley *et al.* 2015), and amplitude (Hage and Metzner 2013a; Hage *et al.* 2013b; Luo *et al.* 2015b). However, other factors such as flight height, social interactions, reproductive age, geographic isolation, foraging habitat, postnatal development, and environmental temperature and humidity influence the variation of echolocation pulses (Chaverri and Quirós 2017; Grillot *et al.* 2014; Guillam *et al.* 2009; Jiang *et al.* 2015; Kraker-Castañeda *et al.* 2019; Varela-Boydo *et al.* 2019; Voigt-Heukce *et al.* 2010).

The bats *Molossus sinaloae* (family Molossidae) and *Mormoops megalophylla* (family Mormoopidae) have been reported as urban exploiters *sensu* McKinney (2002), as they take advantage of resources available in cities for shelter and food (Rodríguez-Aguilar *et al.* 2017). Individuals inhabiting urban areas are constantly subjected to high anthropogenic noise levels, mainly from vehicular traffic (> 50 dB, Luo *et al.* 2015a). *M. sinaloae* is a fast-flight forager in open spaces that emits low-frequency, narrow-bandwidth and long-duration echolocation pulses characterized by the alternation of frequencies, visualized as steps in the spectrogram, and displays high plasticity in pulse emission (Jung *et al.* 2014). *M. megalophylla* is a forager in vegetation clearings and edges (Schnitzler and Kalko 2001). Its pulses are easily recognizable, consisting of a main component of constant frequency that ends with a downward curve of modulated frequency showing harmonics in which the first (fundamental signal) is of low intensity while the second and third are usually of high intensity (Rydell *et al.* 2002).

Lara-Núñez (2015, unpublished data), in a work carried out in Cuernavaca, Morelos, México, found that the frequencies of the echolocation pulses of *M. sinaloae* and *M. megalophylla* were higher than those described for individuals of these species recorded in a natural habitat (Orozco-Lugo *et al.* 2013). Considering the above, this study evaluated whether anthropogenic noise produces an acoustic masking effect on the time and frequency components of the echolocation pulses of *M. sinaloae* and *M. megalophylla*, by comparing the pulses of each species recorded in two sites with contrasting conditions of anthropogenic noise levels, an urban area and a natural area, located in the state of Morelos, México. Our hypothesis is that the frequency and time components of the pulses of both species would increase in response to masking.

Materials and methods

Study areas and sites. To compare the levels of ambient noise and their effect on the components of echolocation pulses, two study areas were selected: an urban area (with anthropogenic noise) and a natural area (without anthropogenic noise; Supplementary material 1). The urban area selected was the city of Cuernavaca, which extends across an area of 151 km^2 and is home to 366,320 inhabitants (INEGI 2015). It covers an altitudinal range from 1,100 masl in the southern zone to 1,800 masl in the northern zone (INEGI 2009); the dominant climate is warm subhumid with a mean annual temperature of 21.1° C (IMTA 2014). The city is characterized by the transition between its main types of vegetation, from low deciduous tropical forest or deciduous tropical forest in the southern zone to pine-oak forest in the north (Dorado *et al.* 2012). The natural area selected was Sierra de Huautla (SH), located in the south of the state of Morelos. Its altitudinal range extends from 700 to 2,000 masl, characterized by a warm subhumid climate with summer rains. The main type of vegetation is low deciduous tropical forest; however, there are also areas with medium subdeciduous forest, oak forest, and a small area of pine forest (Dorado *et al.* 2005). The distance separating the two study areas is 53 km.

In Cuernavaca, four sampling sites were set for the recordings: Acapantzingo, Centro, Palmas, and Galerías Cuernavaca shopping mall. In each, a sound meter was used to record preliminary measurements of environmental noise under different conditions of vehicular traffic and environmental noise. The selected sites include housing and commercial infrastructure and government offices with an open layout; the avenues and roads that cross them are open spaces between buildings, which are mostly less than 10 m in height. In SH, three sites were selected for the recordings, one located on the road to the village of El Limón, one on the stream that runs through the town of Quilamula, and the third located in El Limón Biological Station. These are open sites surrounded by low deciduous tropical forest where trees reach heights of 4 to 10 m.

Acoustic sampling of bats. Ultrasound recordings of bats were made in May, June, October, November, and December 2016. Fixed points were set at each sampling site, where recordings were made continuously over one and a half hours from the sunset hour indicated by a GPS device (Garmin eTrex). The recording period was established by prioritizing personal safety when staying during such hours of the day in urban sites. Recordings were made in real time with the Echo Meter 3+ (Wildlife Acoustics) ultrasonic detector, at a sampling rate of 256 kHz and a 16-bit resolution, using an SDHC memory card with 4 GB storage capacity, and positioning the detector at 45 degrees at the elbow level. Recordings were made once a month at each sample site, following a random order.

Analysis of recordings. Search sequences were selected based on the emission of more than five consecutive pulses because pulses were more uniform in structure during the search phase, unlike the approach-and-capture phase. The pulses in the spectrogram were considered high-quality when the display of each pulse was complete from start to end, making sure that no more than one conspecific or heterospecific individual interfered. The recordings were analyzed with the software BatSound 4.2 (Pettersson Elektronik) following the protocol outlined below.

Spectrograms were displayed at a 16-bit resolution, with a sampling rate of 44,100 kHz/s, at a time expansion of 10x, Fast Fourier Transforms (FFT) with 512 samples, in a 95% overlapping Hanning window. Five frequency (kHz) and two time (ms) parameters were measured. The initial frequency (IF; frequency at the start of the pulse) was measured by placing the frequency cursor in the center of the start of the pulse. The final frequency (FF; frequency at the end of the pulse) was measured by placing the frequency cursor in the center at the end of the pulse. To measure the mid-pulse frequency (MPF; frequency at the middle of the pulse length), the pulse duration was divided by two, placing the frequency cursor at that point and recording the value at the intersection with time. The maximum amplitude frequency (MAF; frequency emitted at the point of highest pulse energy) was measured as the value of the highest peak recorded over the entire pulse length, as shown in the cumulative power spectrum. Also recorded were the bandwidth (BW; difference between the initial and final frequencies) and the pulse interval (PI; time elapsed from the start of one pulse to the start of the next pulse), the latter measured by placing the cursors at the start of each pulse. The pulse duration (PD; the time from pulse start to end) was measured by placing the cursors at the start and end of each pulse. For *M. megalophylla*, the second harmonic was measured according to Rydell *et al.* (2002) because this harmonic attained the highest intensity while the fundamental one was very weak and, in most echolocation sequences, it was imperceptible in the spectrogram; in the case of *M. sinaloae*, no harmonics were observed, but only the fundamental frequency.

Noise measurement. Ambient noise was measured in parallel with the ultrasound recordings at 10-minute intervals, from the time when each recording started, using a sonometer (BENETECH, GM 1351), an instrument that measures sound intensity in decibels (dB, unit of measure). These measurements were recorded at all sites in both natural and urban areas, to obtain a measure of the level of environmental noise experimented by the bat species in the study sites.

Statistical analysis. For statistical analyses, the data for each parameter obtained from the echolocation sequences of the search fase were averaged. Given that *M. sinaloae* shows alternation of pulses during commuting and search for prey, low pulses and high pulses were compared separately in the statistical analyses and the difference in initial frequency between both pulses was evaluated as a measure of pulse alternation.

We analyzed the differences in noise levels between urban and natural sites, as well as between sampling periods, to determine whether the effect of noise is homogeneous over the spatial and temporal scales studied. To this end, we first evaluated the data normality and homoscedasticity assumptions using the MASS package (Venables and Ripley 2002) in R 4.01 (R Core Team 2021). These analyses showed that the variables considered have a normal distribution, but their variance is heterogeneous. Hence, we used the robust Welch's Analysis of Variance, which is designed to treat heteroscedastic data (Wilcox 2012), followed by a multiple comparison test of trimmed means. These analyses were implemented in the package WRS2 (Mair and Wilcox 2020).

Since the noise analyses showed no significant differences either between sites or between sampling periods (see *Results*), the analysis of each component of the echolocation pulses was carried out by sorting sites into two levels: urban (the four sites sampled in the city of Cuernavaca) and natural (SH sites). We evaluated the data normality and homoscedasticity following the same procedure described for noise levels, finding that the pulse variables have normal distribution and heterogeneous variances. Thus, univariate comparisons between urban and natural levels were made using the robust ANOVA model. A value of $\alpha = 0.05$ was used for all the analyses. In addition, a multivariate analysis of variance was used to assess the differences between the urban and natural categories, after the data were transformed into natural logarithms to homogenize the variances, followed by a discriminant analysis to illustrate the differences and test the *a priori* classification of the groups by type of habitat. This analysis allows classifying independent variables with mutually exclusive characteristics in a group established *a priori* from a dependent variable. These multivariate analyses were run using the program PAST 4.02 (Hammar *et al.* 2001).

Results

Seventy-five noise measurements were recorded over eight nights in urban sites, and 58 over six nights in natural sites. The average environmental noise measured was 75 (± 4.2) dB in the urban area and 45 (± 1.4) dB in the natural area. Robust analyses of variance and multiple comparison tests showed significant differences between urban and natural sites (F = 180.08, P < 0,001), but comparisons of noise levels within urban sites and within natural sites, as well as between sampling periods, showed that these differences were not significant (urban $F = 4.91$, $P > 0.05$; natural $F = 3.2$, $P > 0.05$; Figure 1).

A total of 795 sequences for both species were identified in 1530 minutes of recording during the sampling months, of which we analyzed 33 for *M. sinaloae* and 27 for *M. megalophylla*. From these sequences, a total of 545 echolocation pulses were measured for the two species, 332 for the

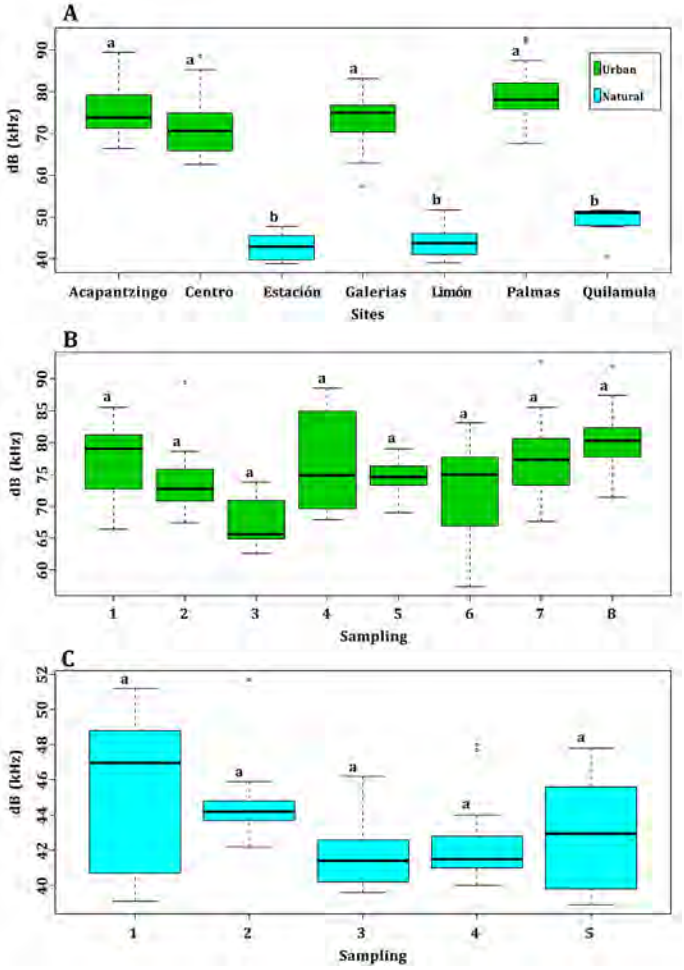

Figure 1. Box plots showing noise levels according to sampling sites in the natural and urban environments (A), and to sampling periods in the urban (B) and the natural (C) environments. The black line represents the median; boxes represent the ranges between quartiles, and whiskers represent minimum and maximum values.

urban area and 213 for the natural area. The parameters of each echolocation pulse were stored in a database, sorted by species and type of area where recordings were made. An average per recording was obtained for each parameter per sampling site in Cuernavaca and SH.

For *M. sinaloae*, 266 pulses, 93 for the natural area and 173 for the urban area, were analyzed in 33 sequences. In the urban area, the frequency and time parameters showed higher values than those obtained in the natural area (Table 1). The variables measured in the high echolocation pulses of *M. sinaloae* were contrasted between the two different types of habitats and noise intensity (urban 75 dB *vs.* natural 45 dB). The analyses of variance showed significant differences between the five frequency components: initial rate ($F = 114.38$, $P < 0.05$), final frequency ($F = 115.30$, $P < 0.05$), bandwidth ($F = 23.25$, $P < 0.05$), mid-pulse frequency ($F = 106.70$, $P < 0.05$), and maximum amplitude frequency ($F = 85.63$, $P < 0.05$). In all cases, higher frequencies were observed in the urban area (Figure 2 A-E). For the time parameters, pulse duration was significantly shorter in the urban area ($F = 17.15$, $P < 0,001$; Figure 2 F), while no significant differences were observed in the time elapsed between the emission of consecutive pulses ($F = 4.02$, $P > 0.05$). Similar results were found for low pulses, where robust analyses of variance showed that the five frequency components were significantly higher in the urban area: initial frequency ($F = 77.93$; $P < 0.05$), final frequency ($F = 67.91$; $P < 0.05$), bandwidth ($F = 43.55$, $P < 0.05$), mid-pulse frequency ($F = 73.86$; $P < 0.05$), and maximum amplitude frequency ($F = 64.59$; $P < 0.05$) (Figure 3 A-E). On the other hand, pulse duration and interval of low pulses were significantly shorter in the urban area ($F = 67.24$, $P < 0.05$; $F = 17.15$, $P < 0.05$; Figure 3 F and G).

The difference in the initial frequency between the low and high echolocation pulses of *M. sinaloae* also showed significant differences ($F = 13.81$, $P < 0.05$). In the urban area, the difference between the low and high pulses was 7.2 kHz on average, while for the natural area, the difference between the two pulses was 5.7 kHz on average (Figure 3 H).

The discriminant analysis of the low pulses of *M. sinaloae* showed a significant difference between groups (Wilks $\lambda = 0.45$; $P = 0.0028$). Of the low pulses corresponding to the urban area, 84 % were correctly assigned, while of the pulses emitted in the natural area, 100 % were correctly assigned (Supplementary material 2). Differentiation was also significant for the high pulses (Wilks $\lambda = 0.32$; $P < 0.05$); 92 % of the high pulses emitted in the urban area and 86 % in the natural area were correctly assigned (Supplementary material 3).

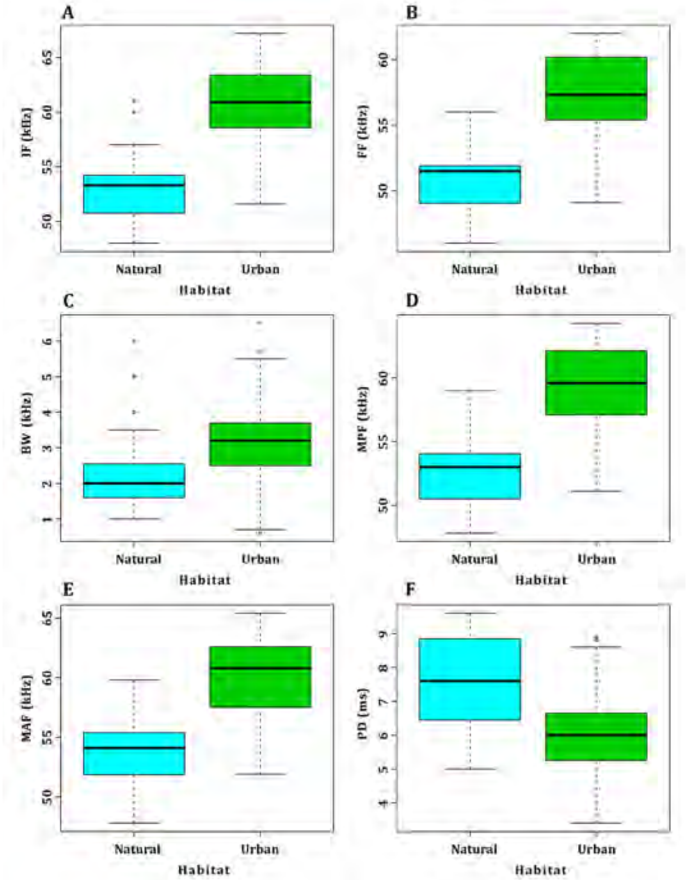

Figure 2. Box plots of the frequency and time components of the echolocation high pulses of *M. sinaloae* contrasted in the two environments studied: natural and urban. (A) Initial frequency, (B) final frequency, (C) bandwidth, (D) mid-pulse frequency, (E) maximum amplitude frequency, and (F) pulse duration. Four frequency components had higher values in the urban area. The black line represents the median; boxes represent the ranges between quartiles, and whiskers represent minimum and maximum values.

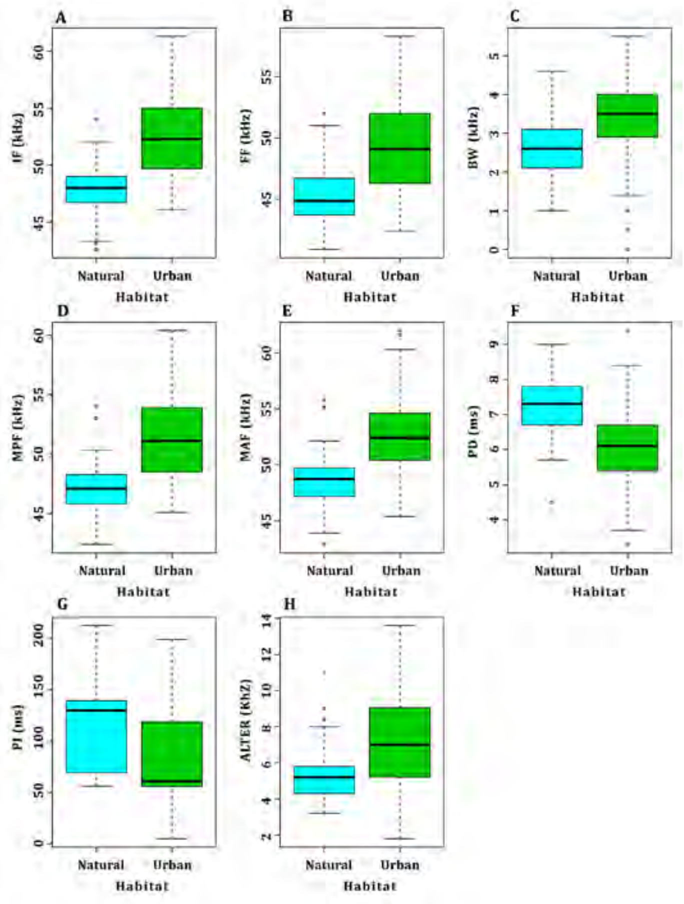

Figure 3. Box plots of the frequency and time components of the echolocation low pulses of *M. sinaloae* contrasted in the two environments studied: natural and urban. (A) Initial frequency, (B) final frequency, (C) bandwidth, (D) mid-pulse frequency, (E) maximum amplitude frequency, (F) pulse duration, (G) interval between pulses, and (H) alternation between high and low pulses. The black line represents the median; boxes represent the ranges between quartiles, and whiskers represent minimum and maximum values.

For *M. megalophylla*, 279 pulses, 120 for the natural area and 159 for the urban area, were analyzed in 27 sequences. The time and frequency components of the pulses showed similar values between the urban and natural areas (Table 2). The robust ANOVA between pulse components and urban and natural areas showed significant differences only in initial frequency ($F = 148.35$; $P < 0.05$), final frequency ($F = 95.46$; $P < 0.05$), and mid-pulse frequency ($F = 101.24$; $P < 0.05$); these were higher in the Cuernavaca urban area (Figure 4 A-D). Bandwidth ($F = 2.78$; $P = 0.74$), and the time components pulse duration ($F = 0.48$; $P = 0.57$) and interval ($F = 0.86$; $P = 0.35$) showed non-significant differences between the two areas. The discriminant analysis of the pulses of *M. megalophylla* showed a non-significant difference between groups (Wilks $\lambda = 0.53$; $P = 0.104$; Supplementary material Figure 7). For this species, 81 % of the total data corresponding to the urban area and 87 % of those corresponding to the natural area were correctly assigned.

Discussion

The results showed that, under background noise at an average intensity of 75 dB in the urban environment, *M. sinaloae* increased the frequencies of its echolocation pulses, both low and high, by 5.8 kHz on average. This suggests that, according to the hypothesis of the present study, when facing higher background noise levels, *M. sinaloae* emits echolocation pulses of higher frequencies since anthropogenic noise masks echolocation signals, significantly affecting the components of the pulses emitted by *M. sinaloe*, while the interval duration and pulse duration decreased.

Although it has been documented that bats exhibit variations in the design of the time and frequency components of their echolocation pulses due to factors associated with geographic isolation, flight height, and foraging habitat (Gillam *et al.* 2009; Jiang *et al.* 2015; Kraker-Castañeda *et al.* 2019; Russo *et al.* 2018), particularly in species of the family Molossidae, there are changes in the frequency and alternation of navigation pulses associated with the variations in geographic location and flight altitude (Gillam *et al.* 2009; Jung *et al.* 2014). However, in none of the cases documented to date, the initial, final, and maximum amplitude frequency exceed 5 kHz between locations separated by more than 100 km or at altitudes from 30 m to 800 m. In this study, recordings were made in open spaces in both natural and urban habitats, so that the changes in frequency and time parameters are not determined by habitat structure (Kraker-Castañeda *et al.* 2019). Although environmental conditions such as temperature and humidity can influence the variation of echolocation pulses, the difference in kHz and ms is unclear (Chaverri and Quirós 2017). In view of these results, we consider that the increase in the frequency components of the pulses of *M. sinaloae* — mainly the maximum amplitude frequency, which is where the maximum energy of the pulse is concentrated — may be a response to the

Table 1. Mean and standard deviation of each component measured in the echolocation pulses of *M. sinaloae* in the sampling areas.

Environment	Type of Pulses	IF (KHz)	FF (kHz)	BW (kHz)	MPF (kHz)	MAF (kHz)	PD (ms)	PI (ms)
Urban	Low	52.8	49.4	3.3	51.7	52.6	5.8	84.2
		± 3.7	± 3.5	± 0.7	± 3.5	± 4.2	± 0.9	± 38.1
	High	60.1	57	3	59	59.8	5.9	87.9
		± 3.6	± 3.2	± 1	± 3.4	± 3.4	± 0.9	± 23.2
Natural	Low	47.7	44.9	2.7	47.1	48.4	7.2	111.4
		± 2	± 2	± 0.6	± 2.1	± 2.2	± 0.4	± 30.1
	High	53	50.5	2.5	52.2	53.2	7.4	117.4
		± 3	± 2.3	± 0.9	± 2.6	± 2.8	± 1.1	± 29.5

Table 2. Mean and standard deviation of each component measured in the echolocation pulses of *M. megalophylla* in the two sampling areas.

Environment	IF (kHz)	FF (kHz)	BW (kHz)	MPF (kHz)	MAF (kHz)	PD (ms)	PI (ms)
Urban	69.5 ± 1.9	65 ± 2.8	4.4 ± 2.7	68.8 ± 1.8	69 ± 2.5	6.1 ± 0.7	81 ± 20
Natural	67.4 ± 0.8	62.7 ± 0.9	4.7 ± 0.3	67 ± 0.8	67.7 ± 0.8	6.4 ± 0.5	70.4 ± 12.3

Lombard effect, defined as the increase in vocal amplitude when facing background noise (Zollinger and Brumm 2011; Luo *et al.* 2017). This type of response has been observed in male concave-eared torrent frogs (*Onorrana tormota*) as increased fundamental frequencies and amplitude of vocalizations when noise intensity increased by 10 dB (Shen and Xu 2016). In birds, the great tit (*Parus major*) increased the minimum frequency of its sound associated with higher background noise levels (Slabbekoorn and Peet 2003).

In the case of the bat *M. megalophylla*, statistically significant changes were observed only in the initial, final, and mid-pulse frequencies. A potential factor also influencing the difference observed in the response to noise between both bat species is the type of foraging, since *M. megalophylla* forages near or between the vegetation, *i. e.*, it flies in areas with dense vegetation structure. As open-space foragers, molossids emit pulses of low frequency, long duration, narrow band, and with long time lapses between pulses (Schnitzler and Kalko 2001). For *M. sinaloae*, background noise is equivalent to flying in an enclosed space, so it should be able to distinguish the echo of its prey from the echo of traffic noise. Thus, by emitting pulses of high frequency, broadband, and short duration, it retrieves more accurate information about the location and characterization of the environment in which it flies, facilitating the detection of background objects, preys, and avoiding collisions (Schnitzler and Kalko 2001). This increase in pulse frequencies was also observed for *Tadarida brasiliensis*, a species for which the amplitude, duration, and bandwidth of echolocation pulses increased when individuals were exposed to digitally generated noise at 85 dB (Tressler and Smotherman 2009).

However, noise limits signal detection, thus reducing the echolocation range (Tyack and Janik 2013); also, the distance for prey detection becomes shorter in areas with background noise, so the increased frequencies and shorter pulses respond to prey detection at a shorter range.

The results also showed that, in the urban environment, there is a greater difference in the frequency between high and low pulses. Different hypotheses have been put forward about the use of pulse alternation in different bat species (Kingston *et al.* 2003), the most plausible being that emitting pulses at different frequencies maximizes the detection distance, allowing a precise discrimination of the echoes of calls (Jung *et al.* 2006). For this study, the sequences with the highest number of high pulses were those corresponding to the urban environment, so that alternating pulse frequencies would improve the classification of traffic noise echoes; however, the difference between pulses is greater as pulse frequencies increase, representing a higher energy expenditure for the bat, likely related to the additional work by the muscles of the abdominal wall involved in the production of echolocation pulses (Currie *et al.* 2020).

Our results do not associate the increased frequencies in the pulses of *M. megalophylla* with noise. The values recorded in Cuernavaca are similar to those recorded in Sierra de Huautla in this study, where anthropogenic noise is lower. The duration of the pulses (6.4 ± 0.5 ms) recorded in the Reserve is similar to the duration (6.9 ms) reported in a previous study in the same area (Orozco-Lugo *et al.* 2013) and to the 5.55 ± 2.19 ms reported for a location in the state of Oaxaca (Briones-Salas *et al.* 2013). The variation in frequency is likely associated with harmonics, integer

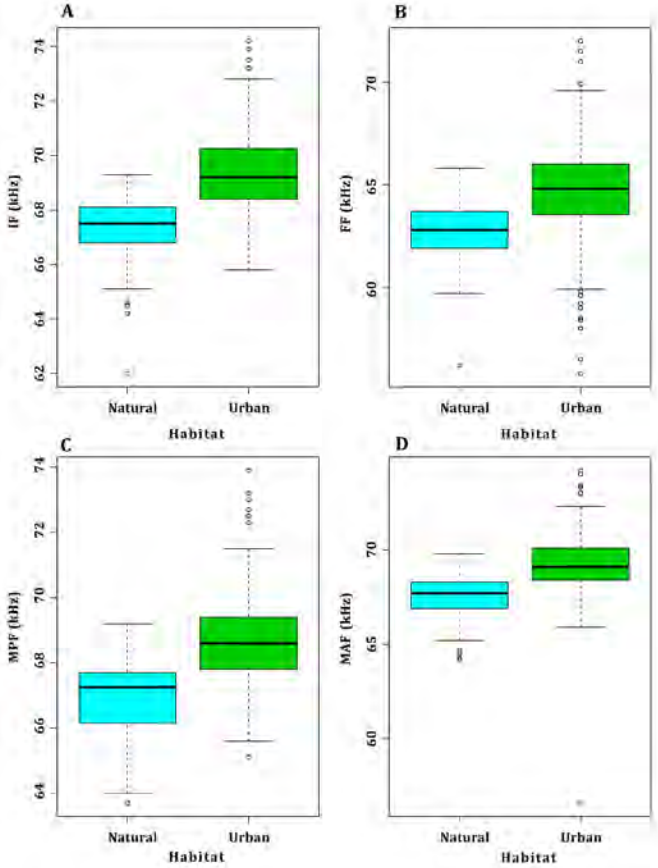

Figure 4. Box plots of the frequency and time components of the echolocation pulses of *M. megalophylla* contrasted in the natural and urban areas. (A) Initial frequency, (B) final frequency, (C) mid-pulse frequency, and (D) maximum amplitude frequency. The black line represents the median; boxes represent the ranges between quartiles, and whiskers represent the minimum and maximum values.

multiples of the lowest harmonic, or the fundamental signal (Jones and Teeling 2006). Commonly, in the echolocation pulses of the family Mormoopidae, a fundamental harmonic is emitted with frequencies around 30 kHz followed by three harmonics ranging from 60 to 120 kHz (Griffiths 1978). Specialized structures in the larynx of Mormoopids amplify the second harmonic and can suppress the fundamental signal (Griffiths 1978). The incidence of harmonic emission depends on the foraging environment and the positioning of the ultrasonic microphone toward the bat (Fenton *et al.* 2011). In open spaces, some species can emit narrow-band multi-harmonic signals dominated by non-fundamental harmonics (Jones and Teeling 2006). *Eptesicus fuscus* emits three harmonics above the fundamental one when flying in enclosed spaces, and one harmonic above the fundamental signal in open spaces (Fenton *et al.* 2011). When *M. megalophylla* flies in an open space, only the second harmonic is recorded. Research on bats addressing the effect of anthropogenic noise is still scarce. For this reason, it is important to carry out further studies to determine and understand the changes in echolocation frequencies and duration of their pulses because, depending on these modifications, bats will be able to thrive in an environment increasingly transformed by anthropogenic activities.

Acknowledgments

To CONACYT, for the scholarship number 740769 granted to undertake the Master's studies of the main author, from which this work derives. The Center for Biodiversity Research and Conservation of the Universidad Autónoma del Estado de Morelos, for the facilities granted for the conduct of the project. To the people who collaborated in the field work and E. Farfán, for their valuable assistance in data collection. To the three anonymous reviewers, whose contributions helped improve the manuscript. M. E. Sánchez-Salazar translated the manuscript into English.

Literature Cited

Barber, J. R., K. R. Crooks, and K. M. Fristrup. 2010. The costs of chronic noise exposure for terrestrial organisms. Trends in Ecology & Evolution 25:180-189.

Briones-Salas, M., M. Peralta-Pérez, and M. García-Luis. 2013. Acoustic characterization of new species of bats for the State of Oaxaca, Mexico. Therya 4:15-32.

Brumm, H. 2004. The impact of environmental noise on song amplitude in a territorial bird. Journal of Animal Ecology 73:434-440.

Brumm, H. 2010. Anthropogenic noise: implications for conservation. Pp. 89-93, *in* Encyclopedia of animal behavior (Breed, M. D., and J. More, eds.). Academic Press. Oxford, U.K.

Brumm, H., and S. A. Zollinger. 2017. Vocal plasticity in a reptile. Proceedings of The Royal. Society 284:1-6.

Bunkley, J. P., and J. R. Barber. 2015. Noise reduces foraging efficiency in pallid bats (*Antrozous pallidus*). Ethology 121:1-6.

Bunkley, J. P., *et al.* 2015. Anthropogenic noise alters bat activity levels and echolocation calls. Global Ecology and Conservation 3:62-71.

Chaverri, G., and O. E. Quirós. 2017. Variation in echolocation call frequencies in two species of free-tailed bats according to a temperature and humidity. The Journal of the Acoustical Society of America 142:146-150.

Currie. S. E., *et al.* 2020. Echolocation at high intensity imposes metabolic costs of flying bats. Nature Ecology and Evolution 4:1174-1177.

Dorado, O., *et al.* 2005. Programa de conservación y manejo de la Reserva de la Biosfera Sierra de Huautla. Comisión nacional de Áreas Naturales Protegidas. Ciudad de México, México.

Dorado, O., *et al.* 2012. Árboles de Cuernavaca nativos y exóticos. Cuernavaca, Morelos, México. Trópico Seco Ediciones, Universidad Autónoma del Estado de Morelos. Cuernavaca, México.

Fenton, M. B., M. D. Skowronski, L. P. McGuire, and P. A. Faure. 2011. Variation in the use of harmonics in the calls of laryngeally echolocating bats. Acta Chiropterologica 13:169-178.

Francis, C. D., and J. R. Barber. 2013. A framework for understanding noise impacts on wildlife: an urgent conservation priority. Frontiers in Ecology and the Environment 11:305-313.

Gillam, E. H., *et al.* 2009. Bats aloft: variability in echolocation call structure at high altitudes. Behavior Ecology Sociobiology 64:69-79.

Gillam, E. H., and B. K. Montero. 2016. Influence of call structure on the jamming avoidance response of echolocating bats. Journal of Mammalogy 97:14-22.

Griffiths, T. A. 1978. Modification of *M. cricothyroideus* and the larynx in the Mormoopidae, with reference to amplification of high-frequency pulses. Journal of Mammalogy 59:724-730.

Grilliot, M. E., S. C. Burnett, and M. T. Mendoca. 2014. Sex and season differences in the echolocation pulses of big brown bats (*Eptesicus fuscus*) and their relation to mating activity. Acta Chiropterologica 16:379-386.

Hage, S. R., *et al.* 2013a. Ambient noise induces independent shifts in call frequency and amplitude within the Lombard effect in echolocating bats. Proceedings of the National Academy of Sciences 110:4063-4068.

Hage, S. R., and W. Metzner. 2013b. Potential effects of anthropogenic noise on echolocation behavior in horseshoe bats. Communicative & Integrative Biology 6:1-3.

Hammar, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: paleontological statistics software package for education and data analysis. Paleontologia Electronics 4:9.

Instituto Mexicano de Tecnología del Agua (IMTA). 2014. Programa de medidas preventivas de mitigación de la sequía. SEMARNAT. Cuernavaca, México.

Instituto Nacional de Estadística y Geografía (INEGI). 2009. Prontuario de información geográfica municipal de los Estados Unidos Mexicanos. Cuernavaca, Morelos, México: INEGI.

Instituto Nacional de Estadística y Geografía (INEGI). 2015. Panorama sociodemográfico de Morelos. http://cuentame.inegi.org.mx. Consulted 3 june 2017.

Jiang, T., H. Wu, and J. Feng. 2015. Patterns and causes of geographic variation in bat echolocation pulses. Integrative Zoology 10:241-256.

Jones, G., and E. C. Teeling. 2006. The evolution of echolocation bats. Trends in Ecology & Evolution 21:149-156.

Jung, K., E. K. V. Kalko, and O. von Helversen. 2006. Echolocation calls in Central American emballonurid bats: signal design and call frequency alternation. Journal of Zoology 272:125-137.

Jung, K., J. Molinari., and E. K. V. Kalko. 2014. Driving factors for the evolution of species-specific echolocation call design in new world free-tailed bats (Molossidae). Plos One 9:1-9.

Kight, C. R., and J. P. Swaddle. 2011. How and why environmental noise impacts animals: an integrative, mechanistic review. Ecology Letters 14:1052-1061.

Kingston, T., G. Jones, Z. Akbar., and T. H. Kunz. 2003. Alternation of echolocation calls in 5 species of aerial-feeding insectivorous bats from Malaysia. Journal of Mammalogy 84:205-215.

Kraker-Castañeda, C. A., Santos-Moreno, C. Lorenzo, and G. M. C. MacSwiney. 2019. Effect of intrinsic and extrinsic factors on the variability of echolocation pulses of *Myotis nigricans* (Schinz, 1821) (Chiroptera: Vespertilionidae). Bioacoustics 28:366-380.

Kunz, T.H., and D. S. Reynolds. 2003. Bats colonies in buildings. Pp. 91-102, *in* Monitoring Trends in bat Populations of the United States and Territories: Problems and Prospects (O´Shea, T. J., and M. A. Bogan, eds.). Springfield, U.S.A.

Luo, J., H. R. Goerlitz, and L. Wiegrebe. 2015a. Linking the sender to the receiver: vocal adjustments by bats to maintain signal detection in noise. Scientific Reports 5:1-11.

Luo, J., B. M. Siemers, and K. Koselj. 2015b. How anthropogenic noise affects foraging. Global Change Biology 21:3278-3289.

Luo, J., A. Lingner, U. Firzlaff, and L. Wiegrebe. 2017. The Lombard effect emerges early in young bats: implications for the development of audio-vocal integration. Journal of Experimental Biology 220:1032-1037.

Mair, P., and R. R. Wilcox. 2020. Robust statistical methods in R using the WRS2 Package. Behavior Research Methods 52:464-488.

McKinney, M. L. 2002. Urbanization, biodiversity and conservation. BioScience 52:883-890.

Naguib, M. 2013. Living in a noisy world: indirect effects of noise on animal communication. Behaviour 150:1069-1084.

Nemeth, E., *et al*. 2013. Bird song and anthropogenic noise: vocal constraints may explain why birds sing higher-frequency songs in cities. Proceeding of the Royal Society B 280:1-7.

Neuweiler, G. 2000. Echolocation. Pp. 140-206, *in* The biology of bats (Neuweiler, G., ed). Oxford University Press, New York, U.S.A.

Orozco-Lugo, L., A. Guillén-Servet, D. Valenzuela-Galván, and H. T. Arita. 2013. Descripción de los pulsos de ecolocalización de once especies de murciélagos insectívoros aéreos de una selva baja caducifolia en Morelos, México. Therya 4:33-46.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ríos-Chelén, A. A., G. Lee, and G. L. Patricelli. 2015. Anthropogenic noise is associated with changes in acoustic but not visual signals in red-winged blackbirds. Behavioral Ecology and Sociobiology 69:1139-1151.

Rodríguez-Aguilar, G., C. L. Orozco-Lugo, I. Vleut, and L. B. Vázquez. 2017. Influence of urbanization on the occurrence and activity of aerial insectivorous bats. Urban Ecosystems 29:477-488.

Russo, D., and L. Ancillotto. 2015. Sensitivity of bats to urbanization: A review. Mammalian Biology 80:205-212.

Russo, D., L. Ancillotto, and G. Jones. 2018. Bats are still not birds in the digital era: echolocation call variation and why it matters for bat species identification. Canadian Journal of Zoology 96:63-78.

Rydell, J., H. T. Arita, M. Santos, and J. Granados. 2002. Acoustic identification of insectivorous bats (order Chiroptera) of Yucatan, Mexico. Journal of Zoology 257:27-36.

Schaub, A., J. Ostwald, and B. Siemers. 2011. Foraging bats avoid noise. The Journal of Experimental Biology 211:3174-3180.

Schnitzler, H. U, and E. K. V. Kalko. 2001. Echolocation by insect-eating bats. BioScience 51:557-569.

Shannon, G., *et al*. 2015. A synthesis of two decades of research documenting the effects of noise on wildlife. Biological Reviews 91:982-1005.

Shen, J. X., and Z. M. Xu. 2016. The Lombard effect in male ultrasonic frogs: regulating antiphonal signal frequency and amplitude in noise. Scientific Reports 6:1-8.

Siemers, B. M., and A. Schaub. 2011. Hunting at the highway: traffic noise reduces foraging efficiency in acoustic predators. Proceedings of the Royal Society B 278:1646-1652.

Slabbekoorn, H., and M. Peet. 2003. Birds sing at a higher pitch in urban noise. Nature 424: 267.

Tressler, J., and M. S. Smotherman. 2009. Context-dependent effects of noise on echolocation pulse characteristics in free-tailed bats. Journal of Comparative Physiology A 195:923-934.

Tyack, P. L., and V. M Janik. 2013. Effects of noise on acoustic signal production in marine mammals. Pp. 251-271, *in* Animal communication and noise. Animal signals and communication (Brumm, H., eds.). Springer. Berlin, Alemania.

Varela-Boydo, F., L. Ávila-Torresagatón, A. Rizo-Aguilar, and J. A. Guerrero. 2019. Variation in echolocation calls produced by *Myotis velifer* (Chiroptera: Vespertiliondae) during postnatal development. Therya 10:55-58.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. Fourth Edition. Springer. New York, U.S.A.

Voigt-Heucke, S. L., M. Taborsky, and D. K. N. Dechmann. 2010. A dual function of echolocation calls to identify familiar and unfamiliar individuals. Animal Behaviour 80:59-67.

Wilcox, R. R. 2012. Introduction to robust estimation and hypothesis testing. Academic. San Diego, U.S.A.

Zollinger, S. A., and H. Brumm. 2011. The Lombard effect. Current Biology 21:614-615.

Associated editor: Sergio Solari

Submitted: April 14, 2021; Reviewed: May 20, 2021

Accepted: March 5, 2022; Published on line: May 29, 2022

Supplementary material

https://www.revistas-conacyt.unam.mx/therya/index.php/THERYA/article/view/1168/1168_Supplementary_material

THERYA, 2022, Vol. 13(2):245-252 DOI:10.12933/therya-22-2103 ISSN 2007-3364

Evaluation of the distribution pattern on a Neotropical microcarvivora

Rodolfo Rodríguez-Ruiz[1], Alejandro Juárez-Agis[1]*, Silberio García Sánchez[1], Branly Oliver Salome[1], and Víctor Hugo Reza Galicia[2]

[1] Universidad Autónoma de Guerrero, Escuela Superior de Ciencias Ambientales, Área de Recursos Naturales. Campus Llano Largo. Carretera Cayaco- Puerto Marques s/n, C. P. 39906. Acapulco de Juárez, Guerrero, México. Email: rr9155364@gmail.com (RR-R); 13457@uagro.mx (AJ-A); silberio_garcia134@hotmail.com (SGS); branlyos@gmail.com (BOS).

[2] Benemérita Universidad Autónoma de Puebla, edificio Valsequillo 1, Km 1.7, carretera a San Baltazar Tétela, C. P. 72960. San Pedro Zacachimalpa, Puebla, México. Email: vhreza.galicia@gmail.com (VHRG).

* Corresponding author

The Pacific region, one of the most disturbed areas in México, is home to the pygmy spotted skunk (*Spilogale pygmaea*), a local endemic species and one of the smallest carnivores in the world. This small carnivore is currently listed as a species subjected to special protection in México and the International Union for Conservation of Nature. The objective of this work was to model environmental suitability and estimate the potential distribution of *S. pygmaea* in México. Predictive models were created using climatic, anthropic, and topographic variables with the Maxent tool. Models were assessed through partial Receiver Operating Characteristic (ROC) performance by omission rate and AUC. Finally, land use within the predicted potential area (potential distribution) was analyzed using the 2015 land cover layer of México issued by CONABIO. According to the model, *S. pygmaea* has a potential distribution from southern Sinaloa to Chiapas, comprising Michoacán and Guerrero towards the Balsas River basin in relation to dry forests. The predicted area was 95,600 ± 0.02 km^2, representing a restricted distribution in México. Many localities have low environmental suitability (<0.4) and ecosystem modification and fragmentation, mainly influenced by livestock density. *Spilogale pygmaea* may be considered rare due to the lack of sampling, which jeopardizes the conservation of this group given its fragmented habitat. Additionally, *S. pygmaea* is attracted to areas with human settlements, potentially leading to human-animal conflicts. Natural areas, along with information sharing on the presence and importance of the species in nearby communities, may be an effective strategy to benefit this small carnivore.

La región del Pacífico es una de las zonas más perturbadas de México y área de distribución del zorrillo pigmeo (*Spilogale pygmaea*) una especie endémica de esta región y uno de los carnívoros de menor tamaño del mundo. Actualmente, este pequeño carnívoro se encuentra en un estatus de protección especial en México y por la International Union for Conservation of Nature. El objetivo de este trabajo fue modelar la idoneidad ambiental y estimar la distribución potencial de *S. pygmaea* en México. Se generaron modelos predictivos utilizando variables climáticas, antrópicas y topográficas desde la herramienta Maxent. Los modelos se evaluaron según la ROC parcial, el rendimiento midiendo la tasa de omisión y el AUC. Por último, se realizó un análisis de uso de suelo dentro del área potencial predicha (distribución potencial), para esto, se utilizó la capa de cobertura de suelo de México para el 2015 de CONABIO. De acuerdo al modelo de distribución potencial, *S. pygmaea* se puede distribuir desde el sur de Sinaloa hasta Chiapas y entrar por Michoacán y Guerrero hacia la Cuenca del Balsas en torno a las selvas secas en el país, con un área predicha de 95,600 ± 0.02 km^2, presentando una distribución restringida en el país. Muchas localidades presentan una baja idoneidad ambiental (< 0.4) y algunas zonas presentan alteración y fragmentación de los ecosistemas, lo cual es principalmente influenciado por la densidad de ganado. *Spilogale pygmaea* se ha considerado como poco común en su distribución, sin embargo, esto pudiera estar influenciado por la falta de muestreos, lo cual puede representar un problema para la conservación de esta especie, ya que su hábitat se encuentra fragmentado. Por otro lado, *S. pygmaea* se ve influenciado positivamente hacia las zonas pobladas, lo cual puede generar conflicto humano-animal. Las áreas naturales en conjunto con la divulgación sobre la presencia e importancia de la especie en las comunidades cercanas pueden ser una estrategia que beneficie a este pequeño carnívoro.

Keywords: Conservation; carnivores, endemic; potential distribution; Mephitidae; *Spilogale pygmaea.*

Introduction

The pygmy spotted skunk, *Spilogale pygmaea,* is one of the smallest carnivores in the family Mephitidae and the genus *Spilogale* (Medellín *et al.* 1998; Wilson and Reeder 2005). This species is endemic to México, and its distribution has been reported from southern Sinaloa to the Pacific coast of the state of Oaxaca (Medellín *et al.* 1998), from sea level to 1,630 masl (Ceballos and Miranda 2000), mainly in deciduous forests (Cantú-Salazar *et al.* 2009; Domínguez-Castellanos and González 2011). It is considered a rare species throughout its distribution range (Medellín *et al.* 1998).

This small skunk belongs to the carnivore guild (Dragoo *et al.* 1993; Wilson and Reeder 2005), but also consumes a great diversity of insects and fruits, depending on food availability (Cantú-Salazar *et al.* 2005). Thus, it plays a key role within its environment, acting as a pest controller and an efficient seed disperser (Medellín *et al.* 1998).

In México, habitat fragmentation and loss (Sarukhan 2008) threaten this small carnivore. The main disturbances affecting the distribution of this species include urbanization, agriculture, livestock, and forest fires, all of which are common in the Pacific Coast (Trejo 2005; Ceballos *et* al. 2010; Botello *et al.* 2015; Mas *et al.* 2017). These issues can fragment natural populations and reduce connectivity between them (Tlapaya and Gallina 2010; Capdevila-Argüelles *et al.* 2013), which may lead to the loss of *S. pygmaea* populations in some localities.

S. pygmaea is currently listed as a species subject to special protection in NOM-059-SEMARNAT 2010 and as vulnerable by the International Union for Conservation of Nature (Helgen *et al.* 2016). Given the anthropic modifications in its environment, it is important to know the potential distribution and the impact of anthropic pressures on an endemic species such as *S. pygmaea*. However, for rare species, understanding the distribution and drivers of these patterns is challenging and resource-intensive (Perkins-Taylor and Frey 2020).

Environmental suitability and potential distribution models support inferences about the distribution of species (Austin 2002; Pérez-Irineo *et al.* 2019) from biological and environmental information (Guisan and Zimmermann 2000; Franklin 2010). These techniques are based on modeling the niche of a species in an *n*-dimensional space (Elith *et al.* 2006; Soberón *et al.* 2017), identifying the environmental requirements of the species (Soberón and Nakamura 2009) and visualizing them on maps to identify potential regions where these species may be distributed (Lindenmayer *et al.* 1991). The hypotheses derived from these models usually estimate the potential distribution in a predicted area or environmental suitability ranges (Soberón and Peterson 2005). These results can be an important support for species conservation strategies (Mercado and Wallace 2010; Cuervo-Robayo and Monroy-Vilchis 2012; Carrillo-Reyna *et al.* 2015).

Assessing the potential distribution of species in the Anthropocene is essential considering the current loss of biodiversity and habitats. This information may support the development of conservation strategies at local and national levels. The objective of this work was to model environmental suitability and estimate the potential distribution of *S. pygmaea* in México based on presence records and scenopoetic and interactive variables.

Materials and methods

To build environmental suitability models, a region of interest was delineated based on the area accessible to the species (Soberón and Peterson 2005; Peterson *et al.* 2011), using the classification of Biogeographic Regions of México issued by CONABIO (1997). The Pacific Coast, Balsas Depression, and Sierra Madre del Sur were considered as these are the layers that match the known distribution of the species (González and Arroyo-Cabrales 2012).

The model was built using data collected based on georeferenced records from publications, theses, and museum data (Cortés-Marcial and Briones-Salas 2014; Buenrostro-Silva *et al.* 2015; Urrea-Galeano *et al.* 2016; Juárez-Agis *et al.* 2020; Briseño-Hernández and Naranjo 2021). We also consulted presence records collected by Ballesteros-Barrera *et al.* (2016) and Lavariega and Briones-Salas (2019; available at: www.conabio.gob.mx/informacion/gis/). Private presence data were gathered from users of the NaturaLista platform. The data not provided were considered for model assessment only because they are shown as a random point within an area of ±20 km^2 for species at risk.

The records were refined by removing spatially correlated and duplicate records. The SDMToolbox space tool was used in ArcMap® 10.3 (Brown 2014). Records were discarded at a minimum distance of 1.2 km (approximately equivalent to the size of a pixel), resulting in 76 presence records.

Variables were selected according to their importance and influence on the species (Buenrostro-Silva *et al.* 2015). The climatic layers by Cuervo-Robayo *et al.* (2013) were considered environmental variables. Only the distance to water bodies was included as a topographic variable. Vegetation attributes included distance to deciduous forest or grassland (secondary and natural) and percentage of tree cover; the anthropogenic attributes considered were distance to urban and agricultural areas and livestock density (Appendix 1).

The distance variables were estimated using the National Forest Inventory, Series VI (INEGI 2016). Polygons were divided according to the attribute, and the Euclidean distance was calculated for each polygon. Once all the geodata were obtained, the resolution of variables was adjusted based on climatic variables, resulting in an approximate size of 800 m^2.

The importance of the variables to the model was assessed by a jackknife analysis in Maxent and by Pearson's correlation between variables through the Ntbox package (Osorio-Olvera *et al.* 2020) in Rstudio; those with a ratio >0.7 were discarded, identifying the variable with the highest percent contribution to the model and then removing the variable correlated with it (Warren *et al.* 2014; Perkins-Taylor and Frey 2020). The result was a set of seven climatic variables, three vegetation and anthropic variables, and one topographic variable (Appendix 1).

Models were built using the MaxEnt algorithm (Phillips *et al.* 2017), which has shown a good performance in predicting the potential distribution of species from presence records and environmental variables (Elith *et al.* 2006; Phillips *et al.* 2006). In addition, MaxEnt is an effective tool when limited data are available (Byeon *et al.* 2018), as in the case of *S. pygmaea*.

Potential models were built using 70 % (n = 53) and assessed using 30 % (n = 23) of records. We tested 29 possible combinations of entity classes including linear, quadratic, product, threshold, and hinge, which allow adjusting simple interactions (Elith *et al.* 2011). Models were evaluated according to their statistical significance. The area under the curve (AUC) was obtained directly from the model assessment through the "Receiver Operating Characteristic" (ROC) curve, which measures sensitivity and specificity; values closest to 1 indicate perfect discrimination (Byeon *et al.* 2018). The Partial ROC (Peterson *et al.* 2008) was determined by resampling 1,000 boostrap interactions using 50 % of the independent data from presence records, and setting a 5 % omission error with the Ntbox platform (Osorio-Olvera *et al.* 2020).

The simplest model was selected, considering as reliable AUC values above 0.75 (Elith *et al.* 2011) and Partial ROC >1 (Peterson *et al.* 2008). The model selected was exported with a logistic output, which offers an interpretation associated with environmental suitability (Mateo *et al.* 2011).

A cutoff threshold was applied to estimate the potential distribution, evaluate the predicted area, and exclude false positives, using 70 % of the records used to build the model. The minimum value was selected as cutoff level (Pearson *et al.* 2007; Espinoza-García *et al.* 2014).

A land-use analysis was performed within the predicted potential area using the 2015 land-cover layer of México (CONABIO 2020). First, the land-use layer was trimmed based on the potential distribution. Subsequently, land-use attributes were sorted into three categories: conserved vegetation (deciduous forest, mixed forest, temperate forest), agricultural areas, and urban zones. Finally, the area occupied by each category was calculated.

The results were interpreted and discussed by overlapping the final maps on the layer of México (INEGI 2019), to facilitate the state conservation policy.

Results

The states with the largest number of records were Jalisco ($n = 17$) and Guerrero ($n = 28$), both with 57.8 % of the data (Figure 1). The records were obtained mainly from scientific collections in museums ($n = 54$) and the rest from the NaturaLista platform ($n = 16$) and paper reports ($n = 6$). New localities were reported in Michoacán (Urrea-Galeano *et al.* 2016) and the most southern record in Juchitán, Oaxaca (Cortés-Marcial and Briones-Salas 2014).

Figure 1. Potential distribution of *S. pygmaea* and presence records used in the model.

The optimal model selected was created from three class types (hinge, linear, and quadratic). Its evaluation showed optimal AUC (0.89) and partial ROC (1.59 ± 0.14) values; random AUC and partial AUC values were statistically different ($P < 0.0001$).

The variables with the greatest contribution to the model were distance to water bodies (31.3 %), maximum temperature of the warmest month (14.0 %), and precipitation of the driest month (12.8 %), which together explain 58.1 % of the model. The influence observed showed a positive relationship with water bodies and a negative relationship with temperatures of the warmest month >35 °C and with areas with precipitation of the driest month >5 mm (Table 1).

In the case of anthropic variables, a positive influence was observed with agricultural areas and the periphery of urban zones (Table 1). However, livestock density responded negatively, affecting the presence of the species.

The data analysis revealed that *S. pygmaea* prefers areas adjacent to rivers and lagoons, limiting its distribution to livestock areas. It is associated with subhumid

Table 1. Percent contribution of the variables in the environmental suitability model for *S. pygmaea*.

Variable	Percent contribution (%)	Importance of permutation (%)
Distance to water bodies	31.3	26.3
Maximum temperature of the warmest month	14.0	20.9
Precipitation of the driest month	12.8	15.0
Precipitation of the wettest month	9.0	17.7
Livestock density	8.4	2.7
Average day range	6.9	0
Distance to deciduous forests	6.3	0.6
Percentage of tree cover	3.1	4.5
Minimum temperature of the coldest month	2.0	0.9
Distance to grasslands	1.9	1.0
Distance to urban areas	1.5	0.5
Annual mean precipitation	1.3	6.8
Isothermality	0.7	2.2
Distance to agricultural land	0.7	0.8

warm climates with the temperatore of the warmest month between 19 °C and 32 °C, and avoids areas with high precipitation. The environmental suitability map indicated that the areas of greatest suitability (>0.4) are located around the *Huatulco* and *Lagunas de Chacahua* National Parks, Oaxaca; in areas surrounding Acapulco de Juarez, Guerrero; adjacent to the Balsas basin between Michoacán and Guerrero, where it penetrates inland to the mid Balsas, Guerrero, restrained by extreme temperatures (>35 °C). In Colima, its potential distribution ranges from the Malaque tropical forest along the coast of Jalisco to the deciduous tropical forest surrounding Puerto Vallarta, the tropical forests in Nayarit, and the *Meseta de Cacaxtla* Natural Protected Area in Sinaloa (Figure 2).

The optimal threshold for this model was 0.2, with a potential distribution of approximately 95,600 ± 0.02 km^2, comprising 4.9 % of the mainland area of México (INEGI 2018). The states with the largest potential distribution area are Michoacán (45,300 ± 0.05 km^2), Guerrero (30,000 ± 0.1 km^2), and Jalisco (26,500 ± 0.1 km^2).

However, regarding the conserved habitat alone (*i. e.*, forests and tropical forests within the potential distribution range) comprises 60,900 ± 0.03 km^2, representing a loss of 34,700 ± 0.02 km^2 (agricultural area = 34,500 ± 0.2 km^2; urban area = 260 ± 2.3 km^2) as a result of anthropic fragmentation.

Discussion

Historically, *Spilogale pygmaea* has been considered an uncommon species across its distribution range (Medellín *et al.* 1998). This perception might be due to the lack of sampling since reports are scarce. It is worth highlighting that the reports found are new localities for Michoacán (Charre-Medellín 2012; Urrea-Galeano *et al.* 2016), Oaxaca (Cortés-Marcial and Briones-Salas 2014), and Guerrero (Briseño-Hernández and Naranjo 2021); however, the interest and focus of these studies was on larger mammals. Based on our results, *S. pygmaea* may be distributed up to Chiapas in the south and through the Balsas River basin up to to Morelos and Puebla.

The records obtained on the citizen science platforms provide a useful tool when there is insufficient information on the species to conduct an analysis like the one reported herein. However, these data should be used with caution (in model assessment) as there may be uncertainty in localities or species identification. Therefore, data should be refined based on specialized literature. Historical bases of museums or in the literature are considered reliable sources, keeping in mind that environmental and land-use changes may be unsuitable for some species, thus affecting the development of conservation strategies (Pliscoff and Fuentes-Castillo 2011; Espinoza-García *et al.* 2014).

According to our results, much of the area predicted as potentially suitable provides an unfavorable scenario for *S. pygmaea* (Balvanera *et al.* 2000; Crooks 2002; Botello *et al.* 2015) since it involves fragmented zones, which could hamper the conservation of the species (Cantú-Salazar *et al.* 2005). Land-use change and livestock raising have a negative impact on colonization between patches (Lira-Torres and Briones-Salas 2011). Although the distribution of this small carnivore is influenced by its affinity to disturbed areas, this does not indicate that the species thrives or remains in these areas since habitat modifications or reduction have adverse effects on its permanence and distribution patterns. In the case of species with a restricted range, distribution patterns may be influenced by anthropogenic effects (McDonald *et al.* 2018; Perkins-Taylor and Frey 2020). An aspect not addressed in this study but that is also a consequence of anthropic activities is the pollution of water bodies. Today, this issue is on the rise (Rodríguez *et al.* 2013), and although the water requirement of the pygmy spotted skunk may be lower relative to other mammals, this resource is vital (Charre-Medellín 2012) and may affect its populations.

Spilogale pygmaea has a varied diet (Cantú-Salazar *et al.* 2005), nocturnal habits (Sánchez-Cordero and Martínez-Meyer 2000), and the ability to move across patches (Nupp and Swihart 2000; Gehring and Swihart 2004). These habits could reduce the risk of conflicts with humans in semi-urban and agricultural areas, increasing its chances of survival. However, although *S. pygmaea* may potentially be found in areas of low environmental suitability, ignorance about this species might lead to interactions and problems with humans, as observed with other species (Alvarado-Barboza and Gutiérrez-Espeleta 2013).

The assessment of climatic variables shows that water bodies, marked seasonality, precipitation of the driest month of 5 mm, and warm climates favor the presence of this species; these characteristics are typical of dry forests where *S. pygmaea* is abundant (Buenrostro-Silva *et al.* 2015; Lira-Torres *et al.* 2012; Cantú-Salazar *et al.* 2009; Domínguez-Castellanos and González 2011; Cortés-Marcial *et al.* 2014; Charre-Medellín 2012; Bradie and Leung 2017).

Within the potential distribution range of this species, the *Lagunas de Chacahua* National Park, Chamela-Cuix-

Figure 2. Environmental suitability of *S. pygmaea* in México.

mala Biosphere Reserve, Zicuirán-Infiernillo Biosphere Reserve, *Meseta de Cacaxtla* Protected Natural Area, *Marismas Nacionales Nayarit* Biosphere Reserve, Huatulco and *El Veladero* National Parks and the Sierra de Manantlán Biosphere Reserve are important areas for conservation that provide shelter from habitat loss (Gallina *et al.* 2007; Jiménez *et al.* 2015). The presence of *S. pygmaea* in the first five areas is worth highlighting (Cantú-Salazar *et al.* 2009; Buenrostro-Silva *et al.* 2015; Urrea-Galeano *et al.* 2016; CONANP 2016; Guzmán-Pacheco 2019). Therefore, based on our results, we recommend the monitoring of non-confirmed areas to implement actions aiming to conserve this and other species.

Currently, most conservation efforts target larger mammals (>15 kg) because many are considered umbrella species due to their wide distribution (Thornton *et al.* 2016). Smaller mammals that are not necessarily covered by the protection of these large umbrellas, either because of their reduced range or, as in the case of *S. pygmaea*, because of their fragmented distribution, are left outside of government conservation initiatives (Thornton *et al.* 2016).

There is little information available about the distribution range of *S. pygmaea*. The potential distribution is well represented on the Pacific coast; however, few areas have high environmental suitability. The distance to water bodies positively influences environmental suitability, while high livestock density has the opposite effect. Although *S. pygmaea* is associated with semi-urban areas, this does not mean that its permanence is guaranteed. Semi-urbanized areas should be monitored to understand better the distribution of this species and the influence of these areas on it. It is important to focus on small species, allocate areas for conservation and maintain the connectivity between their populations (Mills and Allendorf 1996). Also, outreach and education strategies should be implemented in nearby human communities to inform the local inhabitants about the importance of this species.

Acknowledgments

We thank Á. Balbuena Serrano, O. Erick Ramírez Bravo for their support with the manuscript, E. Teyssier Teuitli and L. Ortega Guzmán for providing presence records for the analysis.

Literature cited

Alvarado-Barboza, G., and G. Gutiérrez-Espeleta. 2013. Conviviendo con los mapaches: del conflicto a la coexistencia. Biocenosis 27:1-2.

Austin, M. P. 2002. Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. Ecological modelling 157:101-118.

Ballesteros-Barrera *et al*. 2016. Distribución potencial de las especies de mamíferos con distribución restringida presentes en México. CONABIO. Ciudad de México, México. Disponible en bbc7@xanum.uam.mx.

Balvanera, P., *et al*. 2000. Las selvas secas. Ciencias 057:19-24.

Botello, F., V. Sánchez-Corder, and M. A. Ortega-Huerta. 2015. Disponibilidad de hábitats adecuados para especies de mamíferos a escalas regional (Estado de Guerrero) y nacional (México). Revista Mexicana de Biodiversidad 86:226-237.

Bradie, J., and B. Leung. 2017. A quantitative synthesis of the importance of variables used in MaxEnt species distribution models. Journal of Biogeography 44:1344-1361.

Briseño-Hernández, I., and E. J. Naranjo. 2021. Outstanding records of mammals from two protected areas of central Guerrero, México. Therya Notes 2:99-104.

Brown, J. L. 2014. SDM toolbox: a python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. Methods in Ecology and Evolution 5:694-700.

Buenrostro-Silva, A., D. S. Pérez, and J. García-Grajales. 2015. Mamíferos carnívoros del Parque Nacional Lagunas de Chacahua, Oaxaca, México: Riqueza, abundancia y patrones de actividad. Revista Mexicana de Mastozoología (n. e.) 5:39-54.

Byeon, D. H., S. Jung, and W. H. Lee. 2018. Review of CLIMEX and MaxEnt for studying species distribution in South Korea. Journal of Asia-Pacific Biodiversity 11:325-333.

Cantú-Salazar, L., *et al*. 2005. Diet and food resource use by the pygmy skunk (*Spilogale pygmaea*) in the tropical dry forest of Chamela, Mexico. Journal of Zoology 267:283-289.

Cantú-Salazar, L., *et al*. 2009. Dry season den use by Pygmy Spotted Skunk (*Spilogale pygmaea*) in a tropical deciduous forest of Mexico. Biotropica 41:347-353.

Capdevila-Argüelles, L., B. Zilletti, and V. Á. Suárez-Álvarez. 2013. Causas de la pérdida de biodiversidad: Especies Exóticas Invasoras. Memorias Real Sociedad Española de Historia Natural 2:55-75.

Carrillo-Reyna, N. L., H. Weissenberger, and R. Reyna-Hurtado. 2015. Distribución potencial del Tapir Centroamericano en la Península de Yucatán. Therya 6:575-596.

Ceballos, G. and A. Miranda. 2000. Guía de los mamíferos de la Costa de Jalisco, México/ A field guide to the mammals to the Jalisco coast, México. Fundación Ecológica de Cuixmala, A. C. y Universidad Nacional Autónoma de México. Distrito Federal, México.

Ceballos, G., *et al*. 2010. Áreas prioritarias para la conservación de las selvas secas del Pacífico mexicano. Pp. 387-551, *in* Diversidad, amenazas y áreas prioritarias para la conservación de las selvas secas del Pacífico de México (Ceballos G., L. Martínez, A. García, E. Espinoza, J. Bezaury-Creel, and R. Dirzo, eds.). Fondo de Cultura Económica. Distrito Federal, México.

Charre-Medellín, J. F. 2012. Uso de manantiales por los mamíferos silvestres en bosques tropicales de Michoacán. Tesis de Maestría. Universidad Michoacana de San Nicolás de Hidalgo. Disponible en jfcharre@yahoo.mx.

Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. 2020. Cobertura del Suelo de México a 30 metros, 2015, edición: 1.0. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México. Disponible en http://www.conabio.gob.mx/informacion/metadata/gis/.

Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. 1997. Provincias biogeográficas de México. Escala 1:4000 000. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México. Disponible en: http://www.conabio.gob.mx/informacion/gis/.

Comisión Nacional de Áreas Naturales Protegidas. 2016. Programa de Manejo Área de Protección de Flora y Fauna Meseta de Cacaxtla. Comisión Nacional de Áreas Naturales Protegidas. Ciudad de México, México.

Comisión Nacional de Áreas Naturales Protegidas. 2017. Áreas Naturales Protegidas Federales de México. Secretaría de Medio Ambiente y Recursos Naturales, Comisión Nacional de Áreas Naturales Protegidas. Ciudad de México, México.

Cortés-Marcial, M., and M. Briones-Salas. 2014. Diversidad, abundancia relativa y patrones de actividad de mamíferos medianos y grandes en una selva seca del Istmo de Tehuantepec, Oaxaca, México. Revista de Biología Tropical 62:1433-1448.

Crooks, K. R. 2002. Relative sensitivities of mammalian carnivores to habitat fragmentation. Conservation Biology 16:488-502.

Cuervo-Robayo, A. P., *et al*. 2013. An update of high-resolution monthly climate surfaces for México. International Journal of Climatology 34:2427-2437.

Cuervo-Robayo, A. P., and O. Monroy-Vilchis. 2012. Distribución potencial del jaguar *Panthera onca* (Carnivora: Felidae) en Guerrero, México: persistencia de zonas para su conservación. Revista de Biología Tropical 60:1357-1367.

Defries, R. S., *et al*. 2000. A new global 1-km dataset of percentage tree cover derived from remote sensing. Global Change Biology 6:247-254.

Domínguez-Castellanos, Y., and G. J. C. González. 2011. Variación temporal y espacial en la estructura de la comunidad de pequeños mamíferos en un bosque tropical seco. Revista Mexicana de Mastozoología (Nueva Época) 1:19-38.

Dragoo, J. W., *et al*. 1993. Phylogenetic relationships among the skunks: a molecular perspective. Journal of Mammalian Evolution 1:255-267.

Elith, J., *et al*. 2006. Novel methods improve prediction of species distributions from occurrence data. Ecography 29:129-151.

Elith, J., *et al*. 2011. A statistical explanation of MaxEnt for ecologists. Diversity and Distributions 17: 43-57.

Espinoza-García, C. R., *et al*. 2014. Distribución potencial del coatí (*Nasua narica*) en el noreste de México: implicaciones para su conservación. Therya 5:331-345.

Franklin, J. 2010. Mapping species distributions: spatial inference and prediction. Cambridge University Press. Austin, Texas U.S.A.

Gallina, S., S. Mandujano, and C. Delfín-Alfonso. 2007. Importancia de las Áreas Naturales Protegidas para conservar y generar conocimiento biológico de las especies de venados en México. Hacia una Cultura de Conservación de la Biodiversidad Biológica 6, 187-196.

Gehring, T. M., and R. K. V. Swihart. 2004. Home range and movements of long-tailed weasels in a landscape fragmented by agriculture. Journal of mammalogy 85:79– 86.

González, G. J. C., and J. Arroyo-Cabrales. 2012. Lista Actualizada de los mamíferos de México 2012. Revista Mexicana de Mastozoología (n. e.) 2:27-80

Guisan, A., and N. E. Zimmermann. 2000. Predictive habitat distribution models in ecology. Ecological Modelling 135:147-186.

Guzmán-Pacheco, H. M. 2019. Determinación de la abundancia relativa, distribución de indicios, patrones de actividad y composición de la dieta de perros ferales (*Canis lupus familiaris*) en el parque nacional Huatulco. Tesis de Maestría. Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad-Oaxaca.

Helgen, K., *et al*. 2016. *Spilogale pygmaea*. In: IUCN 2017. The IUCN Red List of Threatened Species. Version 2017.3. http://www.iucnredlist.org. Consultado el 8 de septiembre 2021.

Instituto Nacional de Estadística y Geografía. 2016. Conjunto de Datos Vectoriales de Uso de Suelo y Vegetación. Escala 1:250 000. Serie VI (Capa Union), escala: 1:250 000. Edición: 1. Instituto Nacional de Estadística y Geografía. Aguascalientes, México.

Instituto Nacional de Estadística y Geografía. 2018. Anuario estadístico y geográfico por entidad federativa. Aguascalientes, México. Disponible en: http://internet.contenidos.inegi.org.mx/contenidos/Productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/AEGPEF_2018/702825107017.pdf.

Instituto Nacional de Estadística y Geografía. 2019. División política estatal 1:250000. 2019, escala: 1:250000. Edición: 1. Instituto Nacional de Estadística y Geografía. Aguascalientes, México.

Jiménez, D. T. O., *et al*. 2015. La importancia de las áreas naturales protegidas en nuestro país. Pp 41–60, *in* Las Áreas Naturales Protegidas y la Investigación Científica en México. (Ortega-Rubio, A., *et al*.). Centro de Investigaciones Biológicas del Noroeste, Universidad Autónoma de Yucatán, Universidad de Michoacán de San Nicolás de Hidalgo, La Paz, México.

Juárez-Agis, A., *et al*. 2020. Los Zorrillos en México un Grupo Vulnerable poco conocido. Biodiversitas 153: 12-16

Lavariega, M. C. and M. Briones-Salas. 2019. *Spilogale pygmaea* (zorrillo pigmeo). Registros de presencia, edición: 1. Proyecto: JM011, Modelado de la distribución geográfica de mamíferos endémicos y en categoria de riesgo de Oaxaca. CONABIO. Xoxocotlán, Oaxaca.

Lindenmayer, D. B., *et al*. 1991. The conservation of Leadbeater's possum, *Gymnobelideus leadbeateri* (McCoy): a case study of the use of bioclimatic modelling. Journal of Biogeography 371-383.

Lira-Torres, I., C. Galindo-Leal, and M. Briones-Salas. 2012. Mamíferos de la Selva Zoque, México: riqueza, uso y conservación. Revista de Biología Tropical 60:781-797.

Lira-Torres, I., and M. Briones-Salas. 2011. Impacto de la ganadería extensiva y cacería de subsistencia sobre la abundancia relativa de mamíferos en la Selva Zoque, Oaxaca, México. Therya 217-244.

Mas, J. F., *et al*. 2017. Evaluación de las tasas de deforestación en Michoacán a escala detallada mediante un método híbrido de clasificación de imágenes SPOT. Madera y Bosques 23:119-131.

Mateo, R. G., Á. M. Felicísimo, and J. Muñoz. 2011. Modelos de distribución de especies: Una revisión sintética. Revista Chilena de Historia Natural 84:217-240.

McDonald, P. J., A. Stewart, and C. R. Dickman. 2018. Applying the niche reduction hypothesis to modelling distributions: a case study of a critically endangered rodent. Biological Conservation 217:207-212.

Medellín, R. A., G. Ceballos, and H Zarza. 1998. *Spilogale pygmaea*. Mammalian species 600:1-3.

Mercado, N. I., and R. B. Wallace. 2010. Distribución de primates en Bolivia y áreas prioritarias para su conservación. Tropical Conservation Science 3:200-217.

Mills L. S., and F. W. Allendorf. 1996. He one-migrant-per-generation rule in conservation and management. Conservation Biology 10:1509-1518.

Nupp, T. E. and R. K. Swihart. 2000. Landscape-level correlates of small-mammal assemblages in forest fragments of farmland. Journal of Mammalogy 81:512-526.

Osorio-Olvera, L., *et al*. 2020. Ntbox: an R package with graphical user interface for modelling and evaluating multidimensional ecological niches. Methods in Ecology and Evolution 11:1199-1206.

Pearson, R. G., *et al*. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. Journal of Biogeography 34:102-117.

Pérez-Irineo, G., C. Ballesteros Barrera, and A. Santos-Moreno. 2019. Densidad, idoneidad ambiental y nicho ecológico de cuatro especies de felinos americanos (Carnivora: Felidae). Revista de Biología Tropical 67:667-678.

Perkins-Taylor, I. E., and J. K. Frey. 2020. Predicting the distribution of a rare chipmunk (*Neotamias quadrivittatus oscuraensis*): comparing MaxEnt and occupancy models. Journal of Mammalogy 101:1035-1048.

Peterson A.T., *et al*. 2011. Ecological niches and geographic distributions. Princeton: Princeton University Press. Princeton, U.S.A.

Peterson, A. T., M. Papes, and J. Soberón. 2008. Rethinking receiver operating characteristic analysis applications in ecological niche modeling. Ecological Modelling 213:63-72.

Phillips, S. J., R. P. Anderson and R. E. Schapire. 2006. Maximum entropy modeling in species geographic distributions. Ecological Modeling 190:231-259.

Phillips, S.J., M. Dudík, and R. E. Schapire. 2017. Maxent software for modeling species niches and distributions (Version 3.4.1). Available from url: http://biodiversityinformatics.amnh.org

Pliscoff, P. and T. Fuentes-Castillo. 2011. Modelación de la distribución de especies y ecosistemas en el tiempo y en el espacio: una revisión de las nuevas herramientas y enfoques disponibles. Revista de Geografía Norte Grande 48:61–79.

Robinson, T. P., *et al*. 2014. Mapping the Global Distribution of Livestock. PLoS ONE 9(5):e96084.

Rodríguez Herrera, A. L, *et al*. 2013. La contaminación y riesgo sanitario en zonas urbanas de la subcuenca del río de la Sabana, ciudad de Acapulco. Gestión y Ambiente 16:85-96.

Sánchez-Cordero, V., and E. Martínez-Meyer. 2000. Museum specimen data predict crop damage by tropical rodents. Proceedings of the National Academy of Sciences 97:7074-7077.

Sarukhan, J. 2008. Capital natural de México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO). Distrito Federal, México.

Secretaría de Medio Ambiente y Recursos Naturales. 2010. Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Que establece especificaciones para la realización de actividades de colecta científica de material biológico de especies de flora y fauna silvestres y otros recursos biológicos en el territorio nacional. Secretaría del Medio Ambiente y Recursos Naturales. México. 31 de enero de 2001. Diario Oficial de la Federación 30 diciembre, 2010.

Soberón J. and A.T. Peterson. 2005. Interpretation of models of fundamental ecological niches and species distributional areas. Biodiversity Informatics 2:1-10.

Soberón J. and M. Nakamura. 2009. Niches and distributional areas: concepts, methods, and assumptions. Proceedings of the National Academy of Sciences 106:19644-19650.

Soberón, J., L. Osorio-Olvera, and T. Peterson. 2017. Diferencias conceptuales entre modelación de nichos y modelación de áreas de distribución. Revista mexicana de Biodiversidad 88:437-441.

Thomas, O. 1898. *Spilogale pygmaea*. Proceeding of the Zoological Society of London. 898-899

Thornton, D., *et al*. 2016. Assessing the umbrella value of a range-wide conservation network for jaguars (*Panthera onca*). Ecological Applications 26:1112-1124.

Tlapaya, L. and S. Gallin. 2010. Cacería de mamíferos medianos en cafetales del centro de Veracruz, México. Acta Zoológica Mexicana (n. s.) 26:259-277.

Trejo, I. 2005. Análisis de la diversidad de la selva baja caducifolia en México. Pp. 111-122, *in* Sobre diversidad biológica: El significado de las diversidades alfa, beta y gamma. Monografias Tercer Milenio, Zaragoza.

Urrea-Galeano, L. A, *et al*. 2016. Registro de *Puma yagouaroundi* en la Reserva de la Biosfera Zicuirán-Infiernillo, Michoacán. Revista Mexicana de Biodiversidad 87:548-551.

Warren, D. L, *et al*. 2014. Incorporating model complexity and spatial sampling bias into ecological niche models of climate change risks faced by 90 California vertebrate species of concern. Diversity and Distributions 20:334-343.

Wilson, D. E., and D. M. Reeder. 2005. *Spilogale pygmaea*. Mammal species of the world: a taxonomic and geographic reference, primera edición. Johns Hopkins University Press. Baltimore, U.S.A.

Associated editor: Lazaro Guevara
Submitted: January 16, 2022; Reviewed: February 5, 2022
Accepted: May 11, 2022; Published on line: May 29, 2022

Appendix 1

Variables used in the environmental suitability model and potential distribution of the pygmy spotted skunk (*Spilogale pygmaea*).

Variable	Unit of measure	Source
Average day range	°C (degrees Celsius)	Cuervo-Robayo *et al.* (2013)
Isothermality		
Maximum temperature of the warmest month		
Minimum temperature of the coldest month		
Annual Mean precipitation	mm^3	
Precipitation of the wettest month		
Precipitation of the driest month		
Distance to deciduous forests	Distance (decimal degrees)	National Forest Inventory, Series VI (INEGI 2016)
Distance to grasslands		
Distance to water bodies		
Distance to urban areas		
Distance to livestock areas		
Percentage of tree cover	Percentage	(Defries *et al*. 2000)
Livestock density	Density (individuals/km^2)	Robinson *et al.* (2014)