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La Portada

El murciélago zapotero (*Artibeus jamaicensis*) es una de las especies de murciélagos más abundantes en el trópico de México, y de los trópicos de América. La especie se caracteriza por su tamaño y la presencia de líneas faciales y malares en el rostro (fotografía: Patricia Cortés Calva).

Nuestro logo "Ozomatli"

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

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DERECHOS DE AUTOR Y DERECHOS CONEXOS, año 12, No. 1, enero-abril del 2021, 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 29 enero 2021.

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

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Editorial

The early history of netting bats

The Japanese-style mist net that mammalogists and ornithologists use extensively came into regular use by scientists in the 1950s and early 1960s and its use in capturing bats and birds unharmed is now worldwide. The history of the innovative mist net, which was originally made of silk and brought to the U.S. by ornithologist Oliver L. Austin, Jr., shortly after WW II, was reviewed recently by Genoways *et al.* (2020). However, the mist net was not the first net to be used for the scientific capture of bats and birds—that was the Italian trammel net.

The Italian trammel net had a long history of being used to capture birds for culinary purposes (Macpherson 1897). The Italian Peninsula, like the prefectures along the coast of the Sea of Japan where the Japanese *kasumi-ami* net is believed to have been developed in the middle of the 17th century, is along one of the major migratory flyways between Europe and Africa.

Apparently, the first biologists to successfully use a net to capture bats were Josselyn Van Tyne and Adolph Murie, then at the University of Michigan, Ann Arbor. In 1931, Van Tyne and Murie worked together sampling birds and mammals at the Uaxactún Mayan archaeological site in north-central Guatemala. Prior to departure on this trip, ornithologist Oliver L. Austin, Jr., loaned Van Tyne a linen trammel bird net that Austin had imported from Italy.

Van Tyne's primary goal in Guatemala was to collect birds (Van Tyne 1935), but he left the trammel net in place overnight, catching bats serendipitously, with the first capture being on 13 April 1931. Over the next month, he and Murie captured 55 bats representing four genera and five species of phyllostomids (*Artibeus*, *Carollia*, *Desmodus*, and *Glossophaga*—Van Tyne 1933). Adolph Murie included the bats taken with Van Tyne using the trammel net in his report on the mammal survey in Guatemala and Belize (Murie 1935). Although this is almost certainly the first time a net was used to catch bats that were preserved as scientific specimens, it was not an innovation that changed mammalogy because the use of trammel nets seemingly was not embraced by others.

Francesco Chigi (1933) first described the use of the trammel net to catch birds for banding in Italy (Figure 1). Chigi (1933:59) stated, "For taking birds in nets special skill is necessary, besides a knowledge of the habits of the different species, hence only the most expert bird-catchers are able to use nets with the maximum of efficiency."

Trammel nets were of varying lengths, with the one used by Van Tyne and Murie being 40 feet long by 6 feet high. Those described in Chigi (1933) look longer and taller. Trammel nets distinguish themselves from other sorts of nets because instead of a single wall (or brail) of netting, they have two or more walls, sometimes constructed of different materials as well as varying sizes of mesh. The two outside nets are made of strong cotton or linen with a mesh size of 35 cm by 25 cm (Chigi 1933) or 7 inches by 7 inches (Van Tyne 1933), whereas the single internal net was made of the same material but lighter and finer textured, with mesh sizes of 3.5 cm by 2.5 cm (Chigi 1933) or 0.75 inch by 0.75 inch (Van Tyne 1933). All three nets were suspended by metal rings along a heavy rope or cord stretched between two trees or stout poles. The outer trammels were slightly flared and staked to the ground and kept taut, but the inner net was allowed to hang freely. Van Tyne (1933:145) warns that under the nets, "clear completely a 'right of way' . . . leaving the ground bare. If this is not done thoroughly, much time will be wasted later in disengaging twigs and leaves from the net." The concept was that any bird or bat approaching the net would fly through the first outer net, strike the middle net forming a pocket, and this pocket would loop through the second outer net holding the captured individual tightly.

Philip Hershkovitz, who was a graduate student in mammalogy at the University of Michigan in the early 1930s and again in the late 1930s, would have known of Van Tyne's and Murie's success in capturing bats with the trammel net in Guatemala. He wrote in 1986 that the trammel net was known as early as the early 1800s but had not been used in fieldwork (Hershkovitz 1987). The fact that four articles appeared in just a three-year period in the mid 1930s in both the ornithological and mammalogical literature, but did not result in the adoption of nets to collect bats and birds is a historical enigma. To add to this mystery, Van Tyne (1935:9) reported that the net they used in Guatemala was loaned to him by his friend, "Oliver L. Austin, Jr., of the United States Biological Survey." This is, indeed, the same Oliver Austin who, a little more than a decade later, set off the widespread use of Japanese mist nets (Genoways *et al.* 2020). In the 1930s, the U.S. Biological Survey had active collecting programs in both mammals and birds, but clearly, the trammel net was not widely adopted. A partial answer to our mystery may lie with the complicated three-layer design of the trammel nets.

Figure 1. A trammel net set about fifteen miles from Rome in a corridor at the Station of Castel Fusano, founded through the National Fascist Federation of Italian Sportsmen. Modified from Chigi (1933:63, figure 2). The long light net in the center of the photo is one of the outside cotton or linen nets; a thinner, lighter net can be seen to the right of the post. Note the well-cleaned net lane and stout post anchoring the end of the net.

Another reason for the non-adoption of the trammel net may lie with bad timing. In the U.S., the stock market had crashed in 1929, Franklin Roosevelt was elected President in 1933 to combat the Great Depression, and the Dust Bowl was in full swing, with World War II beginning to loom in Europe. Adolph Hitler became chancellor in Germany in 1933, Italy under Mussolini invaded Ethiopia in 1935; in 1932 Japan annexed a portion of Manchuria and the seeds of war with China were planted. When Austin (1947) described the use of Japanese mist nets, the war was over. He was familiar with the Italian trammel net and the mist net, and it was the mist net that he shared with his colleagues in 1950. It was the post-World War II publications introducing the *kasumi-ami* Japanese-style mist net that had an immense impact on mammalogy and ornithology (Austin 1947; Dalquest 1954; Clench and Hardy 1989; Genoways *et al.* 2020). There was a boom in higher education in the U.S. as returning veterans took advantage of the G.I. Bill. A cohort of these new students had been exposed to medical and other scientific aspects of war, resulting in pursuit of careers in mammalogy, ornithology, parasitology, and other scientific fields. The timing was right, Japanese mist nets were becoming available, and were easier to set and manage although, being imported from Japan, they were not always readily available and somewhat expensive. Tom Kunz (2005:159) informs us that in the mid-1960s, his first mist nets were purchased for "about $8.00 each" [about $66.00 in 2021 dollars].

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David Huckaby provided insights into the early history of the use of trammel nets and Cody Thompson and Brett Benz provided background on the Museum of Zoology, University of Michigan's records. Ed Heske critically reviewed an earlier draft of this manuscript. The photograph of the trammel net used as Figure 1 was edited by Matthew Girard to compose the figure.

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Literature cited

Austin, O. L., Jr. 1947. Mist netting for birds in Japan. General Headquarters, Supreme Commander for the Allied Powers, Natural Resources Section Report, Tokyo 88:1–24.

Chigi, F. 1933. Methods of capturing birds at the ornithological station Castel Fusano, Italy. Bird-Banding: A Journal of Ornithological Investigation 4:59–67 (translated into English from Italian by Margaret Morse Nice, a prominent ornithologist of the era).

Clench, M. H., and J. W. Hardy. 1989. In Memoriam: Oliver L. Austin, Jr. Auk 106:706–723.

Dalquest, W. W. 1954. Netting bats in tropical Mexico. Transactions of Kansas Academy of Science 57:1–10.

Genoways, H. H., S. B. McLaren, and R. M. Timm. 2020. Innovations that changed mammalogy: the Japanese mist net. Journal of Mammalogy 101:627–629.

Hershkovitz, P. 1987. A history of the recent mammalogy of the Neotropical region from 1492 to 1850. Pp. 11–98 in Studies in Neotropical mammalogy: essays in honor of Philip Hershkovitz (Patterson, B. D., and R. M. Timm, eds.). Fieldiana: Zoology (New Series) 39:viii + 1–506.

Kunz, T. H. 2005. Becoming a mammalogist: on the wings of heroes. Pp. 141–170 in Going afield: lifetime experiences in exploration, science, and the biology of mammals (Phillips, C. J., and C. Jones, eds.). Museum of Texas Tech University, Lubbock, Texas.

Macpherson, H. A. 1897. A history of fowling. David Douglas. Edinburgh, Scotland.

Murie, A. 1935. Mammals from Guatemala and British Honduras. Miscellaneous Publications of the Museum of Zoology, University of Michigan 26:1–30.

Van Tyne, J. 1933. The trammel net as a means of collecting bats. Journal of Mammalogy 14:145–146.

Van Tyne, J. 1935. The birds of northern Petén, Guatemala. Miscellaneous Publications of the Museum of Zoology, University of Michigan 27:1–46.

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Population characteristics of *Akodon montensis* (Sigmodontinae) in response to habitat degradation and food availability

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Small mammal populations can be affected by habitat degradation, causing changes in their abundance, density and movement. *Akodon montensis*, a persistent host for *Orthohantavirus*, is a common rodent species in primary and secondary forest habitats and is considered a generalist species. This paper analyzes how habitat degradation and resource availability affect the population characteristics of the species. Six plots were classified into three levels of degradation, with sampling conducted in June and November 2015. After the June sampling, three plots were selected for the increase of food resources for three months, to assess how this factor affects the population. Abundance was estimated with the capture-mark-recapture method and density was estimated by dividing abundance by the effective sampling area. Home range was calculated using the Minimum Convex Polygon method, and the Maximum Distance Traveled as the longest average movement between two sampling stations where an individual was encountered. More degraded habitats supported lower average density and abundance of *A. montensis* compared to less degraded habitats. Increasing food availability led to increases in abundance in the more degraded habitats and decreases in the least degraded. Changes in home range were most evident, decreasing in the least degraded plots after the increase in resources. The sex ratio did not differ from equity in any plot, nor with respect to any of the factors studied. Population characteristics of the species are determined by several factors, including habitat quality and food distribution and abundance. If changes occur in these factors (either artificially or naturally) then movement, abundance and population density are affected in response to such changes. Although some results were not statistically significant, an apparent interaction was observed between habitat quality and resource availability, thereby influencing the abundance and density of *A. montensis*.

Las poblaciones de pequeños mamíferos pueden verse afectadas por las degradaciones en el hábitat, ocasionando cambios en la abundancia, densidad y movimiento de los mismos. *Akodon montensis*, un persistente hospedero para el *Orthohantavirus*, es una especie de roedor bastante común en hábitats de bosque primario y secundario, y es considerado como una especie generalista. Este trabajo analiza cómo la degradación del hábitat y la disponibilidad de recursos alimenticios, afectan las características poblacionales de la especie. Seis parcelas se clasificaron en tres niveles de degradación, realizándose muestreos en junio y noviembre del 2015. Luego del muestreo de junio, tres parcelas fueron seleccionadas como experimentales con el aumento de recursos durante tres meses, para evaluar cómo afecta este factor a la población, y las otras tres parcelas se mantuvieron como control sin el aumento de recursos. La abundancia se estimó con el método de captura-marca-recaptura y la densidad se estimó dividiendo la abundancia por el área efectiva de muestreo. Se calculó el área de acción mediante el método de Polígono Mínimo Convexo. Hábitats más degradados registraron un menor promedio de densidad y abundancia comparando con las degradaciones más bajas. Con el aumento de recursos, la disponibilidad de alimento generó aumentos en la abundancia de los hábitats más degradados mientras que disminuyeron en el menos degradado. Se observaron principalmente cambios en el área de acción, que disminuyeron en las parcelas menos degradadas luego del aumento de recursos. La proporción de sexos no presentó diferencias a la equidad en ninguna parcela, ni con respecto a alguno de los factores estudiados. Las características poblacionales de las especies están determinadas por factores como la calidad del hábitat, la distribución y abundancia de alimento. Cambios (artificiales o naturales) en estos factores, afectan el movimiento, la abundancia y densidad de las poblaciones. Aunque algunos resultados no fueron estadísticamente significativos, se observa cierta interacción entre la calidad del hábitat y disponibilidad de recursos, que influyen principalmente en la abundancia y densidad de *A. montensis*.

Keywords: abundance; habitat quality; home range; maximum distance moved; resource augmentation; sex ratio.

Introducción

La fragmentación y transformación del hábitat han aumentado considerablemente debido al incremento de las actividades humanas, lo que afecta la diversidad, abundancia, densidad y distribución de las poblaciones silvestres. Los ensambles de pequeños mamíferos se ven ampliamente afectados por estas alteraciones, favoreciendo en gran medida a especies generalistas o de amplia distribución, y provocando a su vez un empobrecimiento de la diversidad (Cebollada-Pütz et al.2012; Santos-Filho *et al.* 2012; García-Estrada *et al.* 2015).

Esos cambios generados en el hábitat estarían afectando la ecología de hospederos naturales de patógenos zoonóticos, aumentando su incidencia y prevalencia en ciertos paisajes (Dearing y Dizney 2010), ocasionando un aumento en la abundancia y cambios en la distribución de dichas especies, incrementando así el posible contacto y transmisión de los patógenos (Suzán *et al.* 2008; Rubio *et al.* 2014).

Aunque los estudios al respecto son limitados y hasta controversiales, en los subtrópicos húmedos la precipitación parece tener un efecto menor sobre las poblaciones de hospederos de patógenos (Prist 2016), ya que se relacionan más con la estructura del hábitat (Prado 2015; Prist 2016). Cuando las alteraciones antrópicas, como por ejemplo la agricultura, enriquecen el medio ambiente para los hospederos, pareciera aumentar también su abundancia, y por lo tanto la prevalencia de los patógenos (Goodin *et al.* 2006; Dearing y Dizney 2010). También se generan cambios en el comportamiento y movimiento de las especies, los desplazamientos y área de acción de acuerdo con la distribución y disponibilidad de recursos alimenticios, siendo menores los cambios en aquellos hábitats con mayor disponibilidad de recursos y aumentan en hábitats más pobres (Desy *et al.* 1990; Batzli 1992).

La nutrición, el comportamiento espacial y la depredación se han propuesto como principales factores limitantes de la densidad poblacional de los roedores, pero no existe un acuerdo general sobre la importancia relativa de estos factores, debido a que sus interacciones no han sido estudiadas con mayor profundidad (Desy y Batzli 1989). Los cambios en las poblaciones a través del tiempo y del espacio, se producen en respuesta a las variaciones ambientales (condiciones físicas, hábitat, recursos, competidores y depredadores) y éstos a su vez se reflejan en el comportamiento y fisiología de los individuos de la población (crecimiento, reproducción, supervivencia y movimiento; Batzli 1992).

El roedor sigmodontino *Akodon montensis* se encuentra frecuentemente en hábitats perturbados en el Bosque Atlántico (Oliveira *et al.* 2014; D'Elía y Pardiñas 2015). A pesar de su carácter generalista, al ser un hospedero de *Orthohantavirus* es importante comprender si las alteraciones en el hábitat afectan a sus características poblacionales, para comprender su comportamiento ante los cambios.

El objetivo de esta investigación fue analizar cómo las características poblacionales (abundancia, densidad, clases de edad, proporción de sexo y área de acción) de la especie se ven afectadas por diferentes niveles de degradación del hábitat o ante un cambio en la disponibilidad de los recursos alimenticios.

Materiales y Métodos

Área de estudio. La Reserva Natural del Bosque Mbaracayú (RNBM) posee 64,405 hectáreas que se localizan al noreste de la región oriental del Paraguay, en el Departamento de Canindeyú, entre los -24° 00' y -24° 18' S, y -55° 17' y -55° 32' O (Figura 1). La misma se localiza en un área de transición entre las ecorregiones Bosque Atlántico Interior y Cerrado (FMB/BM 2005).

Diseño de muestreo. Seis parcelas fueron seleccionadas sobre la base de resultados de estudios previos, donde se encontraron roedores seropositivos para anticuerpo contra *Orthohantavirus* (Eastwood *et al.* 2018; Barreto Cáceres y Owen 2019). Se colocaron parcelas de 12 x 12 estaciones, cada estación separada por 10 m, con dos trampas Sherman™ cada una colocada a nivel del suelo. Como cebo se utilizó una mezcla avena con mantequilla de maní. El esfuerzo de trampeo fue 1,440 noches-trampa por parcela por muestreo, y 17,280 noches-trampa en total.

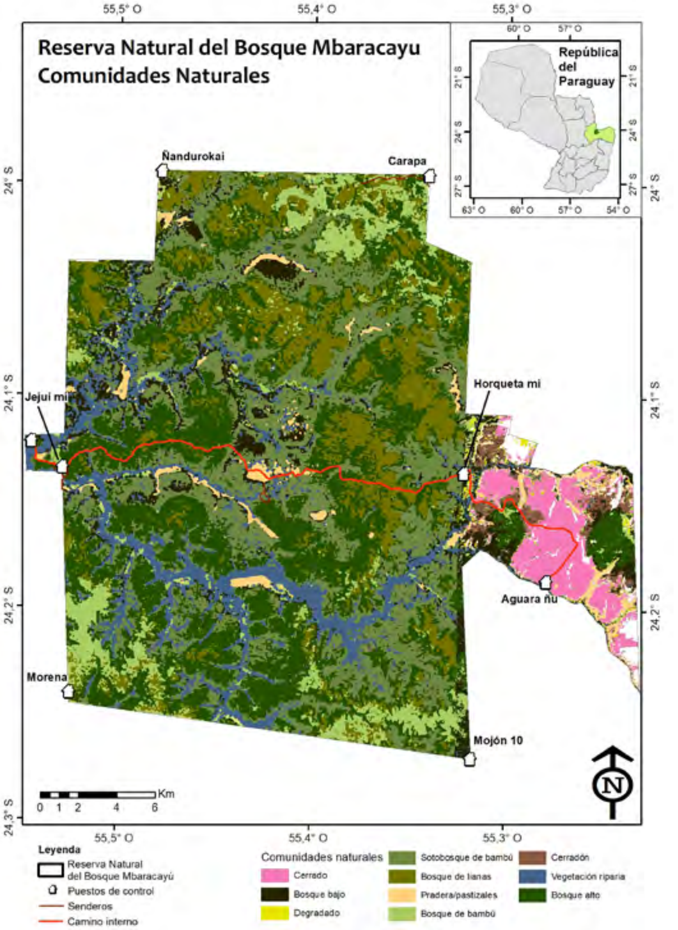

Figura 1. Ubicación de la Reserva de Biósfera del Bosque Mbaracayú (Canindeyú, Paraguay), hábitats encontrados dentro de la reserva y ubicación de las parcelas. Ubicación de la Reserva está entre -24.000°, -24.275° S, -55.322°, -55.543° O. Coordenadas de los centros de las seis parcelas: A) -24.1239° S, -55.5048° O. B) -24.1412° S, -55.3664° O. C) -24.1316° S, -55.5020° O; D) -24.1314° S, -55.4326° O. G) -24.1306° S, -55.5369° O. H) -24.1212° S, -55.4651° O. Figura modificada de Owen *et al.* (2018).

Las parcelas fueron evaluadas sobre la base de las características estructurales de la vegetación: porcentaje de cobertura de hierbas, porcentaje de madera en suelo, altura del dosel, árboles caídos cercanos, distancia al árbol más cercano y presencia de árboles de *Citrus aurantium* (una especie invasiva, indicador de degradación del bosque), medidas en cada estación. Los promedios de estos datos de cada estación se utilizaron como medidas generales para cada parcela, y fueron analizados mediante Análisis de Componentes Principales con Árbol de Recorrido Mínimo (Principal Component Analysis with Minimum Spanning Tree), hecho con NTSYSpc (Rohlf 2018). Se establecieron como indicadores de la degradación del hábitat características que se relacionan con mayor porcentaje de cobertura de hierbas, presencia de árboles de *Citrus aurantium* y árboles caídos cercanos, menos restos de madera en el suelo, menor altura del dosel y distancias menores entre los árboles cercanos a cada estación (Owen *et al.* 2019a).

Sobre la base de estos análisis citados, se designaron tres pares de parcelas de acuerdo con la calidad del hábitat, definidas en términos de degradación: menos degradado (B y H), medio degradado (A y D) y más degradado (C y G). Tres parcelas (H, D, y C) fueron seleccionadas para el aumento experimental de los recursos alimenticios y las otras tres (B, A y G) permanecieron como parcelas control (sin aumento de recursos). Para facilitar la comparación de varias publicaciones basadas en esta investigación de campo, las designaciones de parcela (A, B, C, D, G, H) se corresponden con las de las otras publicaciones realizadas en la misma área.

Las parcelas fueron muestreadas en 2015 en dos sesiones, cinco noches consecutivas en cada una, la primera entre junio y julio, antes del aumento de recursos alimenticios, y la segunda en noviembre luego de tres meses de aumento de recursos. Para la colecta de los datos se utilizó el método de captura-marca-recaptura, donde a cada individuo se le colocó entre los hombros un *Passive Integrated Transponder* (microchip) subdérmico para su posterior identificación. De cada individuo capturado se registraron todos los datos estándar: identificación específica, peso, sexo, estado reproductivo y edad (adultos o subadultos, teniendo en cuenta características externas para el estado reproductivo, pelaje subadulto o adulto y el peso del individuo). Estudios previos en la misma reserva han verificado que solamente una especie de *Akodon* ocurre en el área. Para su identificación, se tuvo en cuenta que *A. montensis* se distingue de *A. azarae* debido a su mayor tamaño, y de *A. cursor* debido a la ausencia de la vesícula biliar (característica verificada en los especímenes colectados en el área en estudios recurrentes).

Aumento de recursos. El alimento seleccionado para el aumento de los recursos fue la mezcla "Super Mix" disponible como alimento para mascotas, que consiste en maíz en grano, maní con cáscara, girasol, granos balanceados y arroz en cáscara. Se diseñaron "alimentadores" (ver Figura 2), para que sólo pequeños mamíferos pudieran acceder a las semillas, y se colocaron en las parcelas correspondientes. Aunque la comida fue elegida sobre la base de pruebas de "cafetería" con *Akodon*, cualquier pequeño roedor tenía acceso libre a los alimentadores. Resultados sobre las características de la comunidad de los mamíferos pequeños aparecen en Barreto Cáceres y Owen (2019) y Owen *et al.* (2019b).

El aumento de recursos se realizó a través de la colocación de 16 estaciones de comida en un patrón uniforme, en las tres parcelas experimentales. La cantidad de alimento, 150 gr de alimento en cada estación de comida, se calculó sobre la base de la biomasa estimada de *Akodon montensis* en cada parcela, basado en los resultados de los muestreos de junio-julio. Las mismas fueron vaciadas y rellenadas cada siete días durante tres meses, iniciando al finalizar el muestreo de junio-julio y terminando antes del muestreo de noviembre.

Estimación de la población. La estimación de la abundancia se calculó por el método de captura-marca-recaptura de individuos aplicando modelos que trabajan con poblaciones cerradas. El mismo se realizó con el *software* de estimación MARK (White 2014).

Figura 2. Alimentador. Se colocaron 16 estaciones de comida (un alimentador por estación) en un patrón uniforme, en cada una de las tres parcelas con aumento alimenticio.

Para la estimación de la densidad poblacional se dividió la abundancia por el área efectiva de trampeo. El área efectiva se calculó para cada parcela en cada sesión de trampeo, usando la mitad del promedio de las distancias máximas recorridas por los individuos de *A. montensis*. Esta distancia se añadió como una franja al perímetro de cada parcela (Schnell *et al.* 1980), y con este dato se calculó la densidad en función al área efectiva de cada parcela y en cada muestreo. Análisis de X^2 se realizaron para determinar las diferencias de sexo, edad, las densidades y abundancias: 1) entre los diferentes niveles de degradación y 2) entre los tratamientos de disponibilidad de recursos. Para ello se utilizó el *software* Infostat (Infostat 2008).

Estimación del área de acción y la distancia máxima recorrida. Los cálculos del área de acción se realizaron con el *software* R, usando el método de Polígono Mínimo Convexo (PCM) con el paquete "adehabitatHR" (Calenge 2011). Fueron utilizados sólo los datos de los individuos adultos que tuvieron a partir de tres capturas en puntos diferentes, con ambos sexos combinados. La Distancia Máxima Recorrida (DMR) se calculó como la distancia más larga entre dos estaciones de muestreo recorrido por un individuo (Hernández-Betancourt *et al.* 2003).

Se utilizó el análisis ANOVA de una vía para analizar la relación entre los tamaños de área de acción y la DMR de acuerdo con: 1) los niveles de degradación y 2) la disponibilidad de recursos. El ANOVA de dos vías se realizó para examinar la interacción entre el movimiento de los individuos, la calidad del hábitat y la disponibilidad de recursos. Para ambos análisis se utilizó el *software* R (Calenge 2011).

Los procedimientos con animales en este estudio se llevaron a cabo bajo los Permisos de Colecta Científica No. 011/2014 y 132/2015 (Secretaría del Ambiente - actualmente el Ministerio de Ambiente y Desarrollo Sostenible - Paraguay), y se siguieron las pautas del Comité de Cuidado y Uso de Animales de la American Society of Mammalogists para el uso de mamíferos silvestres en investigación y educación (Sikes *et al.* 2011). Todos los procedimientos con animales fueron aprobados (Aprobación

No. 14024 - 03) por el Comité Institucional de Cuidado y Uso Animal de la Universidad de Texas Tech (IACUC-TTU), que sigue la 8ª Edición de la Guía para el Cuidado y Uso de Animales de Laboratorio (NRC 2011).

Resultados

Características poblacionales de acuerdo con los niveles de degradación. Se observaron diferencias en los datos de abundancia y densidad poblacional en los tres niveles de degradación, ambos valores fueron mayores en las parcelas con el nivel de degradación bajo (Tabla 1). Según las pruebas de X^2, tanto la abundancia como la densidad, entre niveles de degradación presentan diferencias significativas ($P = 0.001$ y $P = 0.002$).

La distancia máxima recorrida fue menor en las parcelas de degradación baja, y aumenta a medida que aumenta la degradación. El área de acción fue similar entre los niveles de degradación bajo y alto, y menor en comparación con la parcela de degradación medio (Tabla 1). Sin embargo, las pruebas ANOVA de una vía realizadas para ambas medidas no presentan una diferencia significativa entre los niveles de degradación [F (2, 4) = 2.752, $P < 0.0748$].

La proporción de sexos no mostró diferencias en ninguna de las parcelas, ni niveles de degradación ($P > 0.05$). En las clases de edad puede observarse escasa diferencia entre los niveles de degradación, siendo similares entre los niveles de degradación bajo y alto, y levemente mayor respecto a los subadultos en las de degradación media (Tabla 1). Sin embargo, no existen diferencias significativas ($P > 0.05$).

Características poblacionales en relación con la disponibilidad de alimento. La parcela de degradación baja, posterior al aumento de recursos, registró una disminución en la abundancia, mientras que en aquellas de degradación media y alta se observó aumento de la abundancia (Tabla 1). La prueba de X^2 dio un valor de $P < 0.0001$ considerándose cambios significativos en el antes y después del aumento de recursos. La densidad en la parcela de degradación baja se mantuvo similar antes y después del aumento de recursos, mientras que en las parcelas de degradación media y alta se observó un aumento considerable (Tabla 1). Sin embargo, las pruebas de X^2 ($P = 0.057$) no mostraron diferencias significativas.

Con el aumento de recursos, la distancia máxima recorrida disminuyó en todas las parcelas, siendo más visibles en las parcelas de degradación media y alta, mientras que el área de acción disminuyó en las parcelas de degradación baja y media, y aumentó en la de degradación alta (Tabla 1). En ambos casos, los análisis no presentaron diferencia significativa (distancia máxima recorrida $P = 0.109$, área de acción $P = 0.933$).

La proporción de sexos se mantuvo igual en todas las parcelas antes y después del aumento de recursos. En cuanto a las clases de edad, se observó un aumento en el porcentaje de subadultos, luego del aumento de recursos, siendo mucha la diferencia sobre todo en las parcelas de degradación baja y alta (Tabla 1), pero sin valores de P significativos. Este mismo comportamiento se observa en las parcelas control, pero las diferencias no fueron tan marcadas.

Área de acción de acuerdo con la calidad del hábitat y la disponibilidad de recursos. De los dos factores estudiados, el área de acción fue el más influenciado por la degradación del hábitat ($P = 0.075$), mientras que el aumento de los recursos alimenticios no demostró generar cambios significativos ($P = 0.322$).

Después del aumento de recursos se observó una disminución en el área de acción, incluso estratificado por nivel de degradación del hábitat, siendo más marcado en el caso del hábitat de degradación media (Figura 3), donde el nivel medio incluso tiene un descenso más marcado cruzando el nivel de degradación alta. Sin embargo, aunque esta tendencia es evidente observando los gráficos, los valores no resultaron estadísticamente significativos a través del análisis de ANOVA de dos vías (valores de P: degradación = 0.314, aumento de recursos = 0.150, degradación*aumento = 0.546).

Discusión

Características poblacionales de acuerdo con los niveles de degradación. Abundancia y densidad. Akodon montensis es considerada generalista ya que los estudios muestran que se encuentra en distintos hábitats y asociada positivamente con diferentes factores de éstos (Goodin *et al.* 2009; Melo *et al.* 2013). Además, también está presente en bosques perturbados (Püttker *et al.* 2008), como se ha observado en

Tabla 1. Datos poblacionales de *Akodon montensis*, comparando las dos sesiones de muestreo. * Parcelas con aumento de recursos en el muestreo de noviembre. ** Datos con diferencias significativas.

		Abundancia		Densidad (indiv/ha)		Distancia máxima recorrida (m)		Área de acción (m^2)		Proporción de sexos (% hembras)		Proporción de edades (% subadultos)	
Degradación	**Parcela**	**Jun**	**Nov**	**Jun**	**Nov**	**Jun**	**Nov**	**Jun**	**Nov**	**Jun**	**Nov**	**Jun**	**Nov**
Baja	B	55	61	20.1	13.6	24.0	28.0	1,269	1,793	0.5	0.5	0.2	0.5
	H*	73**	33**	23.5	22.2	28.0	20.0	1,258**	281**	0.3	0.3	0.3	0.6
Media	A	87	77	29.8	27.2	26.0	25.0	2,431	1,425	0.4	0.5	0.5	0.6
	D*	10**	33**	1.9**	10.3**	46.6	29.0**	1,916	1,114	0.5	0.5	0.5	0.6
Alta	G	32	36	8.6	12.3	35.0	26.0	1,000	802	0.4	0.5	0.3	0.3
	C*	45	54	8.6**	16.6**	46.0**	29.5**	1,183**	2,757**	0.5	0.5	0.2	0.5

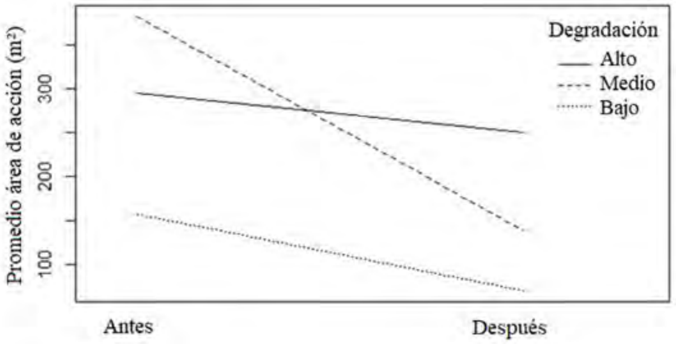

Figura 3. Gráfico de interacción entre el nivel de degradación y el aumento de recursos, mostrando los cambios en el área de acción promedio, debido al aumento de recursos.

este trabajo. Sin embargo, y si bien los análisis estadísticos no resultaron significativos, se observa una tendencia de la especie a ser más recurrente en hábitats menos degradados. La degradación estructural al nivel de microhábitat en los bosques involucra la pérdida de algunos recursos utilizados por los mamíferos (y otros animales) y que cubren requerimientos tales como sitios de anidamiento, refugio o descanso, y también sustratos para la alimentación, su pérdida puede reducir la disponibilidad de hábitats considerados de buena calidad, afectando a las poblaciones a diferentes escalas (Vergara *et al.* 2014).

Los resultados en este estudio demuestran que tanto la abundancia como la densidad se ven afectados por la calidad del hábitat. Esto significa que en los hábitats con menor degradación se encuentran disponibles elementos como cobertura del suelo, altura del dosel, cobertura de hierbas, entre otros, que brindan una "buena calidad" al hábitat para estos roedores. Mientras que en los hábitats con mayor degradación estaría ocurriendo una pérdida gradual y extensiva de estos elementos ocasionando una disminución de la abundancia y la densidad de las poblaciones. Los cambios en la población se reflejan en el comportamiento de los individuos, y éstos a su vez, se dan en respuesta a las diferentes variables que se encuentran en el ambiente (Batzli 1992).

Distancia máxima recorrida y área de acción. Tanto el área de acción como la DMR son medidas que reflejan la movilidad de los individuos de una población y muestran como esa población va respondiendo a los diferentes cambios del hábitat. El tamaño del área de acción (o ámbito hogareño) de las especies está determinado por varios factores, como ser la calidad del hábitat, la distribución y abundancia de alimento, la densidad poblacional, sexo y edad y la actividad reproductiva (Gentile *et al.* 1997; Ribble y Stanley 1998; Pires *et al.* 1999; Priotto y Steinmann 1999). La movilidad de las poblaciones puede estar sujeta a varios factores como la calidad del hábitat o la distribución y disponibilidad de alimento, así en hábitats con menos recursos el área de acción sería mayor comparado con hábitats más ricos en recursos (Corbalán y Ojeda 2005). En este estudio el área de acción no presentó diferencias significativas entre los diferentes niveles de degradación analizados; entonces para *A. montensis*, independiente de la degradación, el hábitat no llega a ser tan pobre para afectar de manera significativa el área de acción en estos diferentes hábitats. Esto concuerda con algunos estudios en los que algunas especies de roedores muestran adaptaciones a las diversas condiciones del hábitat (Klemann y Pelz 2006). Probablemente al ser *A. montensis* una especie generalista, le da cierta ventaja para mantener un área de acción en hábitats con diferentes niveles de degradación que le permitan cubrir la mayoría de los requerimientos necesarios, como alimentación, apareamiento y refugio.

Si bien no se registró una diferencia significativa para la DMR, entre los diferentes niveles de degradación, se observó una tendencia en los resultados, donde la DMR es mayor a medida que el hábitat está más degradado. Se podría considerar que en áreas degradadas los individuos necesitan moverse distancias más largas, ya sea en busca de alimento o inclusive pareja.

Proporción de sexos y clases de edad. La proporción de machos y hembras y la proporción de edades (subadultos/adultos) se mantuvo prácticamente sin variación en los distintos niveles de degradación. Si bien las diferencias entre las clases de edad no fueron muy marcadas, se observó una mayor proporción de subadultos en los hábitats menos degradados, y una menor proporción en un hábitat más degradado. Para algunas especies de *Akodon,* la actividad reproductiva está asociada con la cobertura vegetal provista por el hábitat. Esto se relacionaría con los requisitos alimentarios específicos y como recurso importante para la protección, relacionadas al rendimiento de las hembras reproductivas (Escudero *et al.* 2014). En hábitats menos degradados la cobertura vegetal se encuentra como un factor de calidad del hábitat, lo que supondría una condición favorable para la actividad reproductiva, explicando hasta cierto punto la proporción mayor de subadultos registrados en hábitats menos degradados.

Características poblacionales en relación con la disponibilidad de alimento. Abundancia y densidad. La disponibilidad de recursos en el hábitat afecta a las poblaciones (Batzli 1992), y de acuerdo con este trabajo, las características poblacionales relacionadas a ese recurso son principalmente la abundancia y la densidad. Ambas variables aumentaron luego del enriquecimiento de recursos principalmente en las parcelas de perturbación media y alta, coincidiendo con el hecho de que, si aumentan los recursos alimenticios, aumentan la prevalencia de los roedores en el hábitat (Desy *et al.* 1990; Dearing y Dizney 2010). En cuanto a la parcela de baja degradación, se observó una disminución de la abundancia mientras que la densidad no sufrió modificaciones.

En las cosechas de maíz y trigo, en campos cercanos o relacionados a agroecosistemas en general, se observan efectos sobre la abundancia, movimiento y comportamiento de los roedores, ocasionando un aumento de los roedores de estos durante las cosechas (Cavia *et al.* 2005; Fraschina 2011; Muratore *et al.* 2019). Esto sugiere que mediante la suplementación y complementación del paisaje, se pueden mitigar los efectos de pérdida o degradación de los hábitats, y los hábitats antropogénicos que van cambiando los hábitats nativos, van compensando en cierta medida la pérdida de recursos, teniendo en cuenta sobre todo que los roedores generalistas pueden complementar sus necesidades alimentarias debido a su amplia gama alimentaria (Vergara *et al.* 2014).

La interacción competitiva entre todos los miembros de una comunidad es uno de los mecanismos principales por el que la disponibilidad de recursos influye en la estructura poblacional (González-Romero *et al.* 2005), lo que de alguna manera también explicaría lo observado en este estudio. Como se dijo anteriormente en zonas y bordes de cultivo, luego de las cosechas hay un aumento en la abundancia y riqueza de las especies de roedores, lo que influye en la composición de la comunidad (Fraschina *et al.* 2012; Fischer y Schröder 2014). En hábitats mayormente degradados, un aumento de recursos alimenticios beneficiaría a los roedores generalistas. Mientras que en hábitats con poca o nula degradación, esto favorecería cierta competencia debido a una mayor diversidad presente en ellas. Independientemente de este análisis, es un hecho que el hábitat poco degradado no se ve afectado por el aumento de alimento, ya que es un hábitat de buena calidad.

Distancia máxima recorrida y área de acción. En trabajos experimentales, donde se manipuló la disponibilidad de alimento, se detectó una menor área de acción como también movimientos más cortos, mientras que ambas variables aumentaron cuando no se proporcionó alimento adicional (Taylor y Quy 1978; Desy *et al.* 1990). Esto coincide con los resultados obtenidos en este estudio, en las parcelas experimentales con hábitats menos degradados, en promedio las distancias recorridas y los tamaños del área de acción disminuyeron luego del aumento de recursos alimentarios. Sin embargo, ocurrió lo contrario en el hábitat más degradado.

En algunos estudios se ha observado que los roedores se mueven de forma limitada y restringida, viajando distancias cortas dentro de límites de las granjas (*e. g.*, granjas avícolas), y con mayor frecuencia en los bordes o áreas de zonas de cultivo, debido a la disponibilidad de recursos, refugio y agua que se encuentran concentrados (no ampliamente dispersos en el paisaje) y proporcionados independientemente de la temporada en esas zonas (Pocock *et al.* 2004; Gómez-Villafañe *et al.* 2008; Fraschina 2011; Muratore *et al.* 2019). Mientras en hábitats más pobres, el tamaño del área de acción tiende a ser mayor, pues el animal necesita explorar un área mayor en la búsqueda de recursos tales como alimento (Gentile *et al.* 1997; Ribble y Stanley 1998).

Otros factores que pueden determinar el área de acción son el sexo o la densidad poblacional. Cuando las densidades son altas, los individuos responden restringiendo el área de acción o aumentando la superposición espacial con los otros individuos o especies (Gentile *et al.* 1997; Ribble y Stanley 1998). En este trabajo, en las parcelas de degradación baja no experimentales (que no tuvieron aumento de recursos) disminuyó la densidad y aumentó el área de acción, y en las de degradación alta aumentó la densidad y disminuyó el área de acción. Por otro lado, en las parcelas experimentales con menor degradación y un aumento de recursos la densidad se mantuvo similar y disminuyó el área de acción, pero en las de degradación alta ambas características aumentaron. Estos resultados estarían mostrando que existe cierta interacción entre ambas variables.

Aparentemente en las parcelas con diferentes grados de degradación en un hábitat donde no se aumenten los recursos de manera artificial, las poblaciones de *A. montensis* se comportan como habría de esperarse en un hábitat cualquiera que depende de los recursos alimenticios que brinda de por sí el lugar.

Proporción de sexos y clases de edad. La disponibilidad de recursos es uno de los factores que determina la conformación y el comportamiento de las poblaciones, como las clases de edad, la proporción de sexos o el movimiento, relacionadas a las variaciones ambientales en las diferentes épocas del año (Desy y Batzli 1989; Batzli 1992). En este trabajo, se observó diferencia entre las capturas de adultos y subadultos ante el aumento de alimentos. En las parcelas antes del aumento de recursos, se capturó un mayor número de adultos, mientras que después del aumento de recursos hubo mayor captura de subadultos, y también mayor captura de estos en comparación con las parcelas sin el aumento de recursos, lo que indica que el cambio de proporciones fue efecto del aumento de recursos.

Interacción entre la calidad del hábitat y la disponibilidad de recursos. El tamaño del área de acción, el movimiento y la abundancia de las especies de pequeños mamíferos están determinados por varios factores; extrínsecos como la calidad del hábitat, la distribución y abundancia de alimento, e intrínsecos como la densidad poblacional, sexo, edad, y la actividad reproductiva; además de las necesidades energéticas de cada especie (Pires *et al.* 1999; Priotto y Steinmann 1999). Los factores extrínsecos a nivel de microhábitat afectan las tendencias en la coexistencia de cada especie. En la mayoría de los hábitats *A. montensis* presenta un comportamiento dominante, sin embargo, algunas especies con las cuales *A. montensis* se ha encontrado con mayor coincidencia, muestran mayor dominio en hábitats con ciertas características, evidenciando que las respuestas de los comportamientos interespecíficos (factores intrínsecos) son determinantes para la selección del hábitat por parte de las diferentes especies en una comunidad (Owen *et al.* 2019b). Muchas especies de roedores comparten corredores y cruzan sus recorridos en un mismo hábitat, sin embargo, las características del mismo determinan su presencia. Por otro lado, sus movimientos son determinados no solo por el tipo de ambiente sino también por el tamaño e incluso el sexo de la especie (Maroli 2014).

En este trabajo no se analizaron detenidamente como los factores intrínsecos de la especie explicarían su comportamiento, así como tampoco se analizó de manera detenida la posible superposición con otras especies, pero se puede suponer que hubo algún tipo de interacción entre las especies presentes en los hábitats estudiados. Queda pendiente ver como esos otros factores influyeron también en el movimiento de la especie para este trabajo en particular.

A pesar de que los resultados estadísticos no son definitivos, se observa interacción entre los dos factores extrínsecos estudiados: la calidad del hábitat y la disponibilidad de recursos. Ambos tuvieron un impacto en la población de *A. montensis* influyendo principalmente en la abundancia y densidad. Ambas características disminuyen en hábitats degradados, pero aumentan (en el mismo hábitat degradado) con la disponibilidad de recursos alimenticios, demostrando que cualquier cambio a una pequeña escala implica un cambio en las características poblacionales del roedor. Si bien no se puede definir la interacción entre ambas variables y como eso afecta a las diferentes características poblacionales de esta especie en particular, se puede inferir cuál es la tendencia de su comportamiento. Es necesaria una mejor comprensión de la relación entre el hospedero-hábitat para prevenir escenarios futuros donde la degradación del hábitat debido a actividades que promueven la disponibilidad de recursos alimenticios, como por ejemplo la agricultura, favorezca el contacto de las personas con hospederos de enfermedades zoonóticas, como *Akodon montensis*.

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Literatura Citada

Barreto Cáceres, M. B., y R. D. Owen. 2019. Relación de los pequeños mamíferos terrestres (Rodentia y Didelphimorphia) con la estructura de la vegetación en el Bosque Atlántico Interior – un análisis multivariado. Therya 10:359-369.

Batzli, G. O. 1992. Dynamics of small mammal populations: a review. Pp. 831-850, *en* Wildlife 2001: populations. (McCullough, D. R., y R. H. Barrett, eds). Springer. Países Bajos.

Calenge, C. 2011. Home Range Estimation in R: The AdehabitatHR Package. Office national de la classe et de la faune sauvage. Saint Benoist, Francia.

Cavia, R., I. E. Gómez Villafañe, E. A. Cittadino, D. N. Bilenca, M. H. Miño, y M. Busch. 2005. The effect of cereal harvest on abundance and spatial distribution of *Akodon azarae* (Rodentia, Muridae) in Central Argentina. Agriculture Ecosystems and Environment 107:95-99.

Cebollada-Pütz, C., M. Basso, E. Ruiz de los Llanos, y M. Kufner. 2012. La fauna chaqueña de Córdoba (Argentina) afectada por la transformación agrícola. Ecología Aplicada 11:77-87.

Corbalán, V. E., y R. A. Ojeda. 2005. Áreas de acción en un ensamble de roedores del desierto del Monte (Mendoza, Argentina). Mastozoología Neotropical 12:145-152.

D'Elía, G., y U. F. J. Pardiñas. 2015. Tribe Akodontini. Pp. 140-279 *en* Mammals of South America, Vol. 2, Rodents (Patton, J. L., U. F. J. Pardiñas y G. D'Elía, eds.). The University of Chicago Press. Chicago, EE.UU.

Dearing, M. D., y L. Dizney. 2010. Ecology of hantavirus in a changing world. Annals of the New York Academy of Sciences 1195:99-112.

Desy, E. A., y G. O. Batzli. 1989. Effects of food availability and predation on prairie vole demography: a field experiment. Ecology 70:411-421.

Desy, E. A., G. O. Batzli, y J. Liu. 1990. Effects of food and predation on behaviour of prairie voles: a field experiment. Oikos 58:159-168.

Eastwood, G., J. V. Camp, Y. K. Chu, A. M. Sawyer, R. D. Owen, X. Cao, M. K. Taylor, L. Valdivieso-Torres, R. D. Sage, A. Yu, D. G. Goodin, V. J. Martinez Bruyn, R. C. McAllister, L. Rodriguez, E. P. William, and C. B. Jonsson. 2018. Habitat, species richness and hantaviruses of sigmodontine rodents within the Interior Atlantic Forest, Paraguay. PLoS One 13:30201307.

Escudero, P., S. Ivana, J. Polop, y M. Provensal. 2014. Environmental variables and reproductive activity in small rodents of pampean agroecosystems. Mammalia 78:23-33.

Fischer, C., y B. Schröder. 2014. Predicting spatial and temporal habitat use of rodents in a highly intensive agricultural area. Agriculture, Ecosystems & Environment 189:145-153.

FM, and BBM. 2005. Reserva Natural del Bosque Mbaracayú. Plan de Manejo 2005-2010. Asunción – Paraguay: Fundación Moisés Bertoni para la Conservación de la Naturaleza (FMB), Banco Mundial (BM). Asunción, Paraguay.

Fraschina, J. 2011. Efectos de cambios en el uso de la tierra sobre ensambles de roedores en agroecosistemas pampeanos. Tesis Doctoral. Universidad de Buenos Aires, Buenos Aires, Argentina.

Fraschina, J., V. A. Leon, y M. Busch. 2012. Long-term variations in rodent abundance in a rural landscape of the Pampas, Argentina. Ecological Research 27:191-202.

García-Estrada, C., Y. A. Peña-Sánchez, y H. Colín-Martínez. 2015. Diversidad de mamíferos pequeños en dos sitios con diferente grado de alteración en la Sierra Sur, Oaxaca, México. Revista Mexicana de Biodiversidad 86:1014-1023.

Gentile, R., P. S. D'Andrea, y R. Cerqueira. 1997. Home ranges of *Philander frenata* and *Akodon cursor* in a Brazilian restinga (coastal shrubland). Mastozoología Neotropical 4:105-112.

Gómez-Villafañe, I. E., E. Muschetto, y M. Busch. 2008. Movements of Norway rats (*Rattus norvegicus*) in two poultry farms, Exaltación de la Cruz, Buenos Aires, Argentina. Mastozoología Neotropical 15:203-208.

González-Romero, A., L. Hernández, J. W. Laundré, E. Aragón, y J. López-Portillo. 2005. Monitoreo de dos comunidades de roedores en la reserva de la biosfera Mapimí, Durango, México. Pp 15-26, *en* Contribuciones Mastozoológicas en Homenaje a Bernardo Villa (Sánchez-Cordero V., y R. A. Medellín eds.). Instituto de Biología and Instituto de Ecología, Universidad Nacional Autónoma de México. Ciudad de México, México.

Goodin, D. G., D. E. Koch, R. D. Owen, Y. K. Chu, J. M. S. Hutchinson, y C. B. Jonsson. 2006. Land cover associated with hantavirus presence in Paraguay. Global Ecology and Biogeography 15:519-527.

Goodin, D. G., R. Paige, R. D. Owen, K. Ghimire, D. E. Koch, Y-K. Chu, y C. B. Jonsson. 2009. Microhabitat characteristics of *Akodon montensis,* a reservoir for hantavirus, and hantaviral seroprevalence in an Atlantic forest site in eastern Paraguay. Journal of Vector Ecology 34:104-113.

Hernández-Betancourt, S. F., R. López-Wilchis, J. A. Cimé-Pool, y S. Medina-Peralta. 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 90:77-91.

Infostat. 2008. InfoStat versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba. Córdoba, Argentina.

Klemann, N., y H. J. Pelz. 2006. The feeding pattern of the Norway rat (*Rattus norvegicus*) in two differently structured habitats on a farm. Applied Animal Behaviour Science 97:293-302.

Maroli, M. 2014. Determinación de los movimientos diarios y selección de microhábitats de pequeños roedores en la Reserva Natural Otamendi, Buenos Aires. Mastozoología Neotropical 2:186-187.

Melo, G. L., B. Miotto, B. Peres, y N. C. Cáceres. 2013. Microhabitat of small mammals at ground and understorey levels in a deciduous, southern Atlantic Forest. Anais da Academia Brasileira de Ciências 85:727-736.

Muratore, M., M. de la Reta, S. Perna, A. Oggero, S. Ferrero, J. J. Polop, y M. C. Provensal. 2019. Microhabitat use by sigmodontine rodents in crop-field borders of pampean agroecosystems. Mastozoología Neotropical 26:183-189.

National Research Council of the National Academies (NRC). 2011. Guide for the care and use of laboratory animals. The National Academies Press. Washington, EE.UU.

Oliveira, R. C., R. Gentile, A. Guterres, J. Fernandes, B. R. Teixeira, V. Vaz, F. Valdez, L. Vicente, S. Da Costa-Neto, C. Bonvicino, P. D'Andrea, y E. S. Lemos. 2014. Ecological study of hantavirus infection in wild rodents in an endemic area in Brazil. Acta Tropica 131:1-10.

Owen, R. D., H. Sánchez, L. Rodriguez, y C. B. Jonsson. 2018. Composition and characteristics of a diverse didelphid community (Mammalia: Didelphimorphia) in sub-tropical South America. Occasional Papers, Museum of Texas Tech University 358:1-18.

Owen, R. D., J. V. Camp, y C. B. Jonsson. 2019a. Sigmodontine community and species responses to El Niño and precipitation in different levels of forest degradation. Therya 10:255-265.

Owen, R. D., J. V. Camp, R. Sage, L. Rodríguez, V. J. Martínez Bruyn, R. C. McAllister, y C. B. Jonsson. 2019b. Sympatry and habitat associations of sigmodontine rodents in a neotropical forest-savanna interface. Mammalia 84:227–23.

Pires A. S., F. A. S. Fernandez, y D. Defreitas. 1999. Patterns of space use by *Micoureus demerarae* (Marsupialia: Didelphidae) in a fragment of Atlantic forest in Brazil. Mastozoología Neotropical 6:39-45.

Pocock M. J. O, J. B. Searle, y P. C. L. White. 2004. Adaptations of animals to commensal habitats: population dynamics of house mice *Mus musculus domesticus* on farms. Journal of Animal Ecology 73:878-888.

Prado, A. F. 2015. Abundância de roedores reservatorios de hantavirus no bioma de Mata Atlântica: efeitos da estructura da paisagem e da escala de análise. Tesis de Maestría, Universidade de São Paulo, Brasil.

Priotto J. W., y A. R. Steinmann. 1999. Factors affecting home range size and overlap in *Akodon azarae* (Muridae: Sigmodontinae) in natural pasture of Argentina. Acta Theriologica 44:37-44.

Prist, P. R. 2016. O risco de transmissão da Hantavirose em função do clima e da estructura da paisagem. Tesis de Doctorado, Universidade de São Paulo, Brasil.

Püttker, T., R. Pardini, Y. Meyer-Lucht, y S. Sommer. 2008. Responses of five small mammal species to micro-scale variations in vegetation structure in secondary Atlantic Forest remnants, Brazil. BMC Ecology 8:1-10.

Ribble, D. O., y S. Stanley. 1998. Home ranges and social organization of syntopic *Peromyscus boylii* and *P. truei*. Journal of Mammalogy 79:932-941.

Rohlf, F. J. 2018. NTSYSpc: Numerical Taxonomy System. ver. 2.21c. Applied Biostatistics, Inc. New York, EE.UU.

Rubio, A., R. Ávila-Flores, y G. Suzán. 2014. Responses of small mammals to habitat fragmentation: Epidemiological considerations for rodent-borne Hantaviruses in the Americas. Ecohealth 11:526-533.

Santos-Filho, M., C. A. Peres, D. J. da Silva, y T. M. Sanaiotti. 2012. Habitat patch and matrix effects on small-mammal persistence in Amazonian forest fragments. Biodiversity and Conservation 21:1127-1147.

Schnell, G. D., R. D. Owen, R. K. Chesser, y P. G. Risser. 1980. Populations of small mammals in north-central Oklahoma. The Southwestern Naturalist 25:67-80.

Sikes, R. S., W. H. Gannon y the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235–253.

Suzán, G., A. Armién, J. N. Mills, E. Marcé, G. Ceballos, M. Ávila, J. Salazar-Bravo, L. Ruedas, B. Armién, y T. L. Yates. 2008. Epidemiological considerations of rodent community composition in fragmented landscapes in Panama. Journal of Mammalogy 89:684-690.

Taylor K. D., y R. J. Quy. 1978. Long distance movements of a common rat (*Rattus norvegicus*) revealed by radio-tracking. Mammalia 42:63-71.

Vergara, P. M., A. Rivera-Hutinel, A. Farías, H. Cofré, H. Samaniego, y I. J. Hahn. 2014. ¿Cómo responden los animales del bosque a las perturbaciones antropogénicas? Pp 235-254 *en* Ecología Forestal: Bases para el Manejo Sustentable Vol. 8 (Donoso, C., M. E. González, A. Lara, y P. Donoso, eds.). Marisa Cuneo Ediciones, Chile. Ediciones Universidad Austral de Chile. Valdivia, Chile.

White, G. C. 2014. Program MARK. Warner College of Natural Resources at Colorado State University. Boulder, EE.UU.

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Conservation status and natural history of *Ctenomys,* tuco-tucos in Bolivia

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The genus *Ctenomys* consists of about 70 species and in addition to the Geomyidae of the Nearctic, Neotropical tuco-tucos represent a well-documented case of diversification in the subterranean biotype. Here we will: i) Provide an updated summary of the natural history of the 12 species of extant tuco-tucos from Bolivia; ii) Update information on distributions of each species; and iii) Using ecological niche modeling, evaluate recent and projected habitat transformation or habitat degradation within the known range of each species to provide a preliminary assessment of the preservation or conservation status of ctenomyids within Bolivia. We follow Gardner *et al.* (2014) and combine species summaries with both updated published and new data to compile a complete list of known extant species of tuco-tucos from Bolivia. Occurrence data for *Ctenomys* in Bolivia and surrounding areas were extracted from the database Arctos and GBIF. All individual specimen-based locality records were checked and georeferenced by referring to original museum collection records. We created species distribution models for the species with enough locality records using climate and soil data, while for the rest of the species we estimated the ranges based on the known occurrence localities. Finally, we quantified the amount of large-scale habitat conversion occurring within each species range, as well as the potential effect of climatic change on species distribution. Here we present information regarding the biology of tuco-tuco (*Ctenomys*) species known to occur in Bolivia, including unpublished natural history data such as habitat association, interactions and activity patterns gathered by the authors through extensive field work. Besides this, we estimated the current distribution of *Ctenomys* species, quantified large-scale habitat transformation within each species range and assessed the potential effect of climatic change on five tuco-tuco species. We found that the habitats within the ranges of *C. boliviensis* and *C. steinbachi* have experienced significant land-cover conversions in recent years. We also show that *C. opimus*, as well as the above mentioned species are expected to undergo range contractions resulting from climatic change by 2070. Our review shows that there is a dearth of information regarding natural history, taxonomy and distribution for many Bolivian tuco-tuco species. Nonetheless, the information presented here can be a tool for directing and focusing field studies of these species. This is of great importance if we take into account that most of the Bolivian tuco-tucos are subject to one or several conservation/preservation threats. These include: Habitat destruction via land use or climatic changes in conjunction with geographic ranges of *Ctenomys* that are small in areal extent and which in many cases are not adequately covered by protected areas.

El género *Ctenomys* cuenta con alrededor de 70 especies. Al igual que la familia Geomyidae en el Neártico, los tuco-tucos representan un claro caso de diversificación adaptativa al biotipo subterráneo en el Neotrópico. En este trabajo presentamos: i) una síntesis de la historia natural de las 12 especies actuales de *Ctenomys* registradas en Bolivia; ii) información actualizada de su distribución; y iii) una evaluación de la degradación del hábitat dentro del rango de cada especie con el fin de brindar una evaluación preliminar del estado de conservación de los tuco-tucos de Bolivia. A partir del trabajo de Gardner *et al.* (2014), información publicada y nuevos datos, compilamos una lista completa de las especies de tuco-tucos (*Ctenomys*) de Bolivia junto con la información disponible sobre su biología e historia natural. Las localidades de ocurrencia de las especies en Bolivia fueron obtenidas de las bases de datos GBIF y Arctos. Toda la información sobre las localidades de ocurrencia fue posteriormente depurada y georreferenciada, haciendo referencia a los registros originales de las colecciones de museos. Adicionalmente, creamos modelos de distribución para las especies con suficientes registros. Para las especies menos colectadas, estimamos la distribución a partir de las localidades de ocurrencia e información sobre su hábitat. Finalmente, cuantificamos la transformación del hábitat ocurrida dentro de los rangos de cada especie, al igual que el efecto potencial del cambio climático sobre su distribución. Presentamos un resumen del conocimiento de la biología de cada una de las especies que ocurren en Bolivia, incluyendo datos no publicados de historia natural colectados por los autores durante varias décadas de trabajo de campo. Entre estos nuevos aporte se encuentran descripciones de hábitat, interacciones y patrones de actividad. Adicionalmente, presentamos estimaciones de la distribución, cuantificamos la conversión a gran escala del hábitat y evaluamos el efecto potencial del cambio climático sobre las especies de tuco-tucos en Bolivia. Encontramos que los rangos de distribución de *C. boliviensis* y *C. steinbachi* han experimentado importantes cambios en las coberturas vegetales naturales y seminaturales en años recientes. Adicionalmente proyectamos que la distribución de estas dos especies, junto con la de *C. opimus* experimentarán contracciones importantes como consecuencia del cambio climático para el año 2070. La información revisada en este trabajo muestra que hay importantes vacíos de conocimiento con respecto a la historia natural, taxonomía y distribución en la mayoría de especies de tuco-tucos de Bolivia. Sin embargo, la información presentada en este trabajo puede servir como herramienta para guiar trabajos de campo sobre estas especies. Esto es de gran importancia si se tiene en cuenta que la mayoría de las especies de *Ctenomys* que ocurren en Bolivia presentan uno o varios factores que amenazan su conservación, como la pérdida de hábitat por transformaciones de las coberturas, el cambio climático, y los rangos de distribución pequeños y con mínima representación en áreas protegidas.

Keywords: Bolivia; Caviomorpha; climate change; Ctenomyidae; *Ctenomys;* Hystricomorpha; land use change; natural history; species distribution model; subterranean rodents.

Introduction

Rodents are the most speciose of all orders of mammals, having a cosmopolitan distribution with extant autochthonous species on all continents except Antarctica (Wilson and Reeder 2005). At the current time, even though new species of mammals are being described at a fairly high rate globally, many forms remain unknown and undescribed and the natural history of most known species is still obscure (Wilson *et al.* 2017). Over time, the subterranean niche has been repeatedly invaded by species from several independent lineages of rodents in disparate geographic areas (Lacey and Patton 2000) leading in many cases to extensive diversification and speciation with examples especially salient in species comprising the Geomyidae in the Nearctic and northern Neotropical regions, the Bathygeridae in the Ethiopian region, Spalacidae in the Palearctic region, and the Ctenomyidae in the southern neotropics (Lacey *et al.* 2000; Wallace 1876; Wilson *et al.* 2017).

One of the most diverse and speciose groups of subterranean rodents includes members of the genus *Ctenomys* Blainville, 1826 (Ctenomyidae). Species of *Ctenomys* occur in suitable habitats in South America with a known northernmost limit of distribution of around 12° S. latitude in western Brazil and about 14° S just to the west of Lake Titicaca on the Altiplano of Peru. Many other species occur south in Bolivia, Paraguay, Uruguay, Argentina, and Chile, even extending south to the island of the Tierra del Fuego (Wilson *et al.* 2017). The genus *Ctenomys* now includes an estimated 70 species and in addition to species in the Geomyidae that occur mostly in the Nearctic, the tuco-tucos represent a well-documented case of diversification in the subterranean biotype (Teta and D'Elia 2020; Gardner *et al.* 2014; Bidau 2015; Wilson *et al.* 2017).

The species in the genus *Ctenomys* are broadly known through their distributional range as tuco-tucos because of the vocalizations made by individuals in their burrows that are detectable by persons listening above ground (Darwin 1860). Field surveys have made it clear that the true species diversity in *Ctenomys* is unknown due to the cryptic nature of these rodents and the fact that many geographic areas that may support populations of tuco-tucos in the Neotropics have not yet been adequately surveyed (Gardner *et al.* 2014).

Tuco-tucos range in size from small to medium-sized rodents (Figure 1A) with a morphologically fusiform and compact body shape, loose skin that allows them to easily turn in a burrow, short legs, and long claws on their forefeet for tunneling. On their forefeet they have relatively thick, rapidly-growing nails that they use to dig their tunnels. On their rear feet, they have strong nails and they have well-developed ctenidia or combs (Figure 1 B) on the margins of their pes, and these hard bristle-like combs (*kteis* = comb in Greek - *mys* = mouse in Greek; thus the name comb-footed-mouse = *Ctenomys*) assist the animal in kicking soil out of the burrow. In contrast to species of Geomyoidea that have a behavior in which all species that have been observed, push soil through and out of their burrows with their front feet (Eisenberg 1962), all species of Ctenomyidae studied up to the current time push and kick the soil through and from their burrows using only their rear feet (S.L. Gardner, pers. obs). As far as is known, this rear-soil-kicking action appears to be a behavioral trait that is plesiomorphic in the ctenomyids and may be a behavioral synapomorphy when also considering the Octodontidae which contains the species considered as the sister group of the Ctenomyidae. Ctenomyids have large heads, small pinnae, and relatively well-haired but tactile tails. Variability in color of the pelage, even within populations, coupled with high levels of variation in both diploid and fundamental numbers of chromosomes in some species set the stage for the current state of affairs in the taxonomy of species in this genus where a relatively large number of species names are available (Gardner *et al.* 2014).

Although there are studies of their biogeography, phylogeny, evolution, taxonomy, ecology, sociality, and parasitology (*e.g.* Gardner 1991; Cook and Yates 1994; Lessa and Cook 1998; Cook and Lessa 1998; Mascheretti *et al.* 2000; Castillo *et al.* 2005; Freitas 2006; Parada *et al.* 2011; Gardner *et al.* 2014), basic information available on ecological aspects, local and regional distributions, biology, and reproduction are still relatively scarce, especially in Bolivia, a country with great, and probably underestimated, tuco-tuco species diversity. The great potential for discovery of new species and the concomitant need for research on all aspects of the biology of *Ctenomys* in Bolivia is exemplified by the description of four new species from collection efforts, initially started by Dr. Sydney Anderson, that took place from about 1984 up to the current time over many areas of the country (Anderson 1997; Gardner *et al.* 2014). Also, the restricted distribution of the known endemic species of *Ctenomys* in Bolivia, coupled with the rapid large-scale land use changes consisting mostly of industrial agriculture that is being established throughout the eastern lowlands of the country suggests the importance of the ctenomyids from a species conservation/preservation perspective (Caraballo *et al.* 2020). Furthermore, of the 12 tuco-tuco species documented to occur in Bolivia, little is known of their population structure, numerical density, and conservation status; and of these, only seven species have been formally evaluated by the IUCN (IUCN 2020).

In Bolivia, the 12 species of tuco-tucos, cujuchis (cuhuchi), topos, or tojos (Anderson 1997) that are known to occur there are distributed through the southern half of the country ranging from a westernmost distribution on the Altiplano near Lake Titicaca south through the Altiplano. They also occur through the Andes east into the Yungas through the lowland regions of eastern Bolivia. At least 3 of the known species each have scientific reports from only one collection locality. In Bolivia, besides basic data on species descriptions and a few spurious records (Anderson 1997; Gardner *et al.* 2014) there is little additional information available in the literature on these rodents. This paper reviews and summarizes published literature, museum records, and unpublished data from extensive field work by (SLG and JSB) in the country with several objectives. Here we will: i) Provide an updated account of the natural history of extant tuco-tucos from Bolivia; ii) Update information on species distributions in Bolivia; and iii) Assess recent and projected habitat transformation or habitat degradation within the known range of each species to provide a preliminary assessment of the preservation or conservation status of ctenomyids within Bolivia.

Materials and Methods

We follow the general systematic summary and taxonomy of Gardner *et al.* (2104) and we used the species descriptions found therein to compile a complete list of known extant species of tuco-tucos from Bolivia. To report general sizes of body (in millimeters), we used data directly from Gardner *et al.* (2104) and from unpublished data taken by SLG from museum specimen tags, weight is reported in grams. Number of individuals is given followed by the mean of the measurements.

To update occurrence data for *Ctenomys* in Bolivia, we used verified collection records from the Arctos database and the Global Biodiversity Information Facility (GBIF). We georeferenced and checked each individual specimen-based locality record by referring to original museum collection records. This double-checking was necessary because we discovered a significant number of errors in the GBIF data-set. These errors were traced primarily to improper reciprocal translation of the format of degree-minute-second to the format of decimal degrees by either automatic computer assisted operations or human error. We include the updated/corrected database as supplementary material. For the summary account for each species of tuco-tucos, citations of the literature describing relevant characters and other data are given at the beginning. Means are given for all measurement data. Colors regarding these animals generally follow Gardner *et al.* (2104) and these color definitions are all derived from Ridgeway (1912).

Species distribution estimation. To provide estimates of the distribution of species of *Ctenomys* in Bolivia, we created species distribution models (SDMs) for the taxa that have sufficient numbers of verified occurrence or collection records. Although SDM's are widely used in studies of biogeography and conservation biology and their predictive power and utility have been repeatedly shown (Phillips and Dudík 2008; Raxworthy *et al.* 2003), there are some factors that could introduce bias in the models and affect both robustness and predictive performance. Probably, the most important of these factors is that records of the presence of animals at a locality usually do not come from a random sampling of the study area and sometimes the data might be spatially clumped, due to any number of logistical reasons for collecting in one locality, resulting in models biased in some way, which in the worst cases are predicting sampling conditions rather than suitability for the studied species (Fourcade *et al.* 2014). However; when our team collects tuco-tucos, we go where they exist, and because of the burrow systems and soil that is thrown up at the mouth of each tunnel, it is relatively easy to see where these mammals are (and where they are not) over geographic space. In many cases, for the *Ctenomys* species in question, the absence of collection records means that no (or few) individuals exist in these areas. The potential missing information derives from the fact that we only collected tuco-tucos where roads existed at the time of our surveys in Bolivia. Finally, most of the collection localities cited in this paper are given in Anderson (1997) and Gardner *et al.* (2104. The other potential problem is the risk of creating very complex models overfitting the training data, but with little predictive or extrapolation power (Muscarella *et al.* 2014). Finally, when no true absence data are available, it is very important that the study area from which background environmental data is extracted, is in fact the area accessible to the species over relevant time periods (Barve *et al.* 2011).

In order to account for the above factors, we took the following modeling approach; first, we defined the study area for each species as the minimum convex polygon of all the recovered specimen collection records, plus an 80 km buffer, a value selected taking into account the potentially limited dispersal capability of these subterranean rodents; however, as pointed out by Busch *et al.* (2000) little work has been done on the ecology of dispersal in subterranean rodents and essentially none has been conducted in Bolivia. Second, we reduced spatial sampling bias by removing one of each pair of records that were closer than 5 km from each other using the R package SPTHIN (Aiello-Lammens *et al.* 2015), thus using only what we assume to be spatially independent records. Given that many of the Bolivian species of *Ctenomys* have few spatially independent sampling records (6 to 31), we employed the approach proposed by Breiner *et al.* (2015), of creating ensembles of small models (ESM) using the R package ecospat (Di Cola *et al.* 2017), in which models were developed for each pair of environmental prediction variables and using different algorithms, and a final consensus model created by averaging predicted suitability weighted by each simple model performance. With this approach, overfitting is reduced as the number of parameters in each model is small relative to the number of occurrences and has been shown to perform well for species with scarce presence data. Here, we created models employing all variable combinations and employing Maxent and GBM (gradient boosting machine) algorithms, as these were found to provide the most robust models which are transferable and the highest predictive performance respectively in an empirical evaluation of ESM (Breiner *et al.* 2018). Subsequently, we assessed the significance of each ensemble model using the area under the partial ROC, above an omission threshold of 10 % as implemented in the R package kuenm (Cobos *et al.* 2019). Finally, we estimated the distribution of suitable habitat by reclassifying the continuous suitability map to presence-absence using the threshold that retains 98 % of the species records.

This threshold was selected to predict all records of the species with low sample sizes and provide an inclusive estimation of suitable habitat.

To model habitat suitability, we used 15 bioclimatic variables that were obtained from WorldClim 2.0, which provides precipitation and temperature averages and variability (Fick *et al.* 2017). We did not include the bios variables 8, 9, 18, and 19 as they might show correlation artifacts resulting from their dependence on both precipitation and temperature (Campbell *et al.* 2015). Besides climatic information, and taking into account the subterranean life-habits of *Ctenomys*, we employed five variables describing soil properties, including: Bulk soil density, sand content, clay content, organic material content, and silt composition at a level of 15 cm below the surface - these soil data were derived from a set that was predicted at a global scale by Hengl *et al.* (2017). For our analysis, we reduced the number of variables and avoided including highly correlated ones by performing a principal component analysis (PCA) on separate climate and soil raster datasets. Of these resulting uncorrelated, orthogonal components generated by PCA, we used the first 6 PCs that in fact explained 99 % of the variation in climate. Relative to soil properties, we chose the first 4 components that actually accounted for 100 % of this variation. Finally, we evaluated the average importance of each of the types of data to assess the utility of soil properties to model species distributions of these subterranean mammals. To enhance reproducibility of these SDM, we use the ODMAP (Overview, Data, Model, Assessment and Prediction) protocol to provide a detailed report of our modelling process and data sources (Zurell *et al.* 2020).

For the species with insufficient independent collection locality records available that would enable us to create a robust model of their distribution using habitat variables, we provide minimum-distribution polygons derived from museum-based occurrence records, field work experience, field notes, and information on habitat associations. For species with only one locality record, we follow Caraballo *et al.* (2020) in which we assume the distribution area to be a circular buffer with a 38 km radius although we realize that true locality-occurrence of these mammals depends completely on the soil characteristics in and around the collection locality.

Recent and future habitat change. We assessed the potential effect of two important threats to the diversity of Bolivian tuco-tucos, including: (1) anthropogenic habitat conversion from natural/seminatural to agricultural and urban systems, and (2) global warming and associated climate change. To assess the magnitude of anthropogenic land cover change within the range of each species, we employed the land cover classification of the Copernicus Climate Change Service for 1993 and 2018 (https://cds.climate.copernicus.eu/cdsapp#!/dataset/satellite-land-cover?tab=overview). Given that detailed data on habitat use and anthropogenic tolerance is lacking for most of the tuco-tuco species, our aim was to quantify large scale transformation coinciding with the ranges of each species of *Ctenomys* considered here. To do this, we classified urban, rainfed and irrigated/post flooding agriculture categories from original classification as anthropogenic intensive land covers; and the reminder land cover types as natural/semi natural which beside natural covers included mosaics of cropland and natural vegetation and pastures. We included these categories as semi-natural because at least some species can survive in these habitats (*e.g. C. boliviensis* and *C. erikacuellarae*, see Gardner *et al.* 2014). In this way, we obtained a conservative estimate of large scale changes within the geographic range of the species of interest. We report the net percentage of natural/semi natural habitat within each species range in the year 2018, as well as the percentage of habitat loss between the years, 1993 to 2018 as a measure of more recent and/or ongoing loss of habitat.

To estimate the change in suitable habitat resulting from global warming we projected the distribution models into the climatic projections for 2070 from the 17 general circulation models (GCM) available at the WorldClim database under the contrasting 4.5 and 8.5 emission scenarios (RCPs) from the 5th Assessment Report of the United Nations Intergovernmental Panel on Climate Change (IPCC). The first scenario corresponds with a peak in emissions around 2040 and further emissions decline with stabilization around 2100; the second, more pessimistic scenario presents emissions rising throughout the 21st century (http://www.ipcc.ch/). We estimated suitability for each GCM RCP combination and predicted gain and loss of suitable habitat specifically for the ctenomyids of Bolivia for the median suitability of each scenario using the same threshold used in the current distribution model. We used the same soil variables.

Results

Species summaries -*Ctenomys* of bolivia:

Ctenomys opimus Wagner, 1848

(Figure 2, A, B, C, D; Map Figure 9)

Common name: highland tuco-tuco, tuco-tuco Andino.

Morphology: (n = 56) Body length 284; tail 83; hindfoot 42; ear 9; weight 306. When adults, these tuco-tucos are relatively large with a pale yellowish color. In some localities, individuals have darker fur with black intermixed with the pale yellow. Older individuals may have worn hair on top of the head, probably from moving through the burrow system and digging. Pelage is long and soft. The auditory canals are relatively short, associated with large auditory bullae. The upper incisors are slightly procumbent and the enamel on the teeth is orange in color. Gardner and Anderson (2001) noted that *C. opimus* has curious holes in the cranium, usually between the frontal and parietal bones and can be relatively common with 80 % of highland tuco-tucos in Bolivia showing this character. A great summary of the morphology of this species is given in Cook *et al.* (1990).

Distribution: *Ctenomys opimus* has a wide distribution with a documented geographic range from southern Peru (southwest of Lake Titicaca - see map, Figure 9) south through northeast Chile, the Altiplano of Bolivia, into extreme northwestern Argentina. In Bolivia, the highland tuco-tuco is found in the high-Andean puna habitat of the departments of La Paz, Oruro, and Potosi. The SDM (Table 1) we generated for this species predicts 100,733 km^2 of suitable habitat through the Altiplano except for the inhospitable Salar de Uyuni.

Natural history: Pearson (1959) studied *C. opimus* in southern Peru and provided some of the earliest detailed observations of the natural history of tuco-tucos. Anderson (1997) recorded that pregnant females were trapped in August, October, and December similar to observations made by Pearson in Peru, numbers of embryos ranged from 1 to 5 (mean 2.8), with a gestation period of approximately two months (Pearson 1959). Non-pregnant females were also recorded in August and September with a higher proportion of births evidently occurring from October through March each year (Anderson 1997).

From Puna habitat at 3,950 m., 7 km S and 4 km E, Cruce Ventilla (19° 08′ S; -66° 07′ W), a locality that our expedition visited on 30 September, 1986, we observed that individuals of *C. opimus* inhabited burrow systems that were also used by at least two other species of rodents. Collected from the same burrow systems (the specimens came out of the same burrow mouth and all were collected by SLG by shooting) were individuals of *C. opimus*, *Phyllotis xanthopygus* (Waterhouse, 1837), and *Galea musteloides* Meyen, 1832. During the time we spent at this site, we observed that the burrow systems of the tuco-tucos were not closed with more than about 5 cm of soil. Usually (for all individual burrow systems that we have studied throughout Bolivia) the entrance to the burrow of the tuco-tuco is almost always plugged with soil for more than 10 cm (sometimes much more), unless the animal is actively plugging the burrow mouth and was interrupted before it was finished with its work. At this site, during the day, these three species of rodents emerged from the same burrow systems at irregular intervals, sometimes with dozens of *P. xanthopygus* coming out of the burrow systems simultaneously, while at the same time *G. musteloides* and *C. opimus* were emerging. The individuals of *C. opimus* that were observed always stayed partially in their burrow, never leaving it completely, while the two other species of rodents strayed meters from the various mouths of the burrow systems. Individuals of all three species were collected easily with a shotgun at this site. Interestingly, when our expedition again visited the site in 1990, there remained no evidence of these rodents, the tuco-tuco colony evidently either suffering a local extinction or the colony and associated inquilines having moved on through the landscape (Gardner pers obs. 1993). Parasites - Studies of the parasites of *C. opimus* have yielded the description of four new species of *Eimeria* (Coccidia: Apicomplexa) including: *Eimeria opimi, E. oruroensis, E. granifera*, and *E. montuosi* (authority names all Lambert *et al.* 1988). Specific localities for these protistan parasites are given in Lambert *et al.* (1988) and Gardner and Duszynski (1990). In addition, two species of nematodes, *Litomosoides andersoni* and *L. ctenomyos* Brant and Gardner (1997) (Nemata: Filaroidea) were discovered and described from tuco-tucos at the Huancaroma locality (Oruro: Rancho Huancaroma, 17° 40′ S; -67° 27′ W, 3,720 m. altitude). Other nematodes collected at this locality included *Paraspidodera* (Nemata: Heterakoidea) and *Trichuris* (Nemata: Trichurata) both in very high prevalence and intensity of infection. In addition, from 32 highland tuco-tucos collected from the Huancaroma locality (17° 40′ S; -67° 27′ W) by members of our expedition in 1986, a single individual that was examined harbored one specimen of a large form of *Mathevotaenia* (Cestoda: Anoplocephalidae) which is now being described as a new species. Ectoparasites identified from our collecting throughout the range of *C. opimus* in Bolivia included fleas *Ectinorus galeanus* Jordan, 1939 (Siphonaptera: Rhopalopsyllidae) from the same Huancaroma locality listed above, chewing lice (*Phtheiropoios* sp.) currently being described as new, and beetles of the family Staphylinidae (*Edrabius weiseri* Seevers 1955). In addition, beetles of this species were reported from *C. opimus* from other localities in Bolivia including 7 km south, 4 km east, Cruce Ventilla, Dept. Potosi and 2 km east, ENDE camp, northeast shore of Laguna Colorado, 4,280 m. (Ashe *et al.* 1996).

The community of rodents at the collection locality near Cruce Ventilla was also interesting in that the only parasites that were shared among these rodents that lived in the same burrow systems were cestode larvae we discovered in

Table1. Results of the ensemble distribution models and projected changes in suitable habitat for 2070 under two carbon emission scenarios. *pROC ratio significantly different from 1, meaning that the model is statistically significant.

Species	N	Study area	Predicted suitable Area (km^2)	Predicted suitable Area Bolivia Area (km^2)	pROC ratio	AUC	Bioclimatic variables contribution	Soil variables contribution	%Suitable habitat change RCP4.5	%Suitable habitat change RCP4.5
Ctenomys opimus	31	445,644	187,163	107,733	1.6*	0.84	0.1	0.09	-34 %	-22 %
Ctenomys steinbachi	8	44,770	2,690	2,690	2*	0.86	0.11	0.09	-61 %	-58 %
Ctenomys frater	17	210,035	104,383	34,443	1.5*	0.71	0.09	0.12	-8 %	-9 %
Ctenomys boliviensis	25	97,941	14,347	14,347	1.8*	0.88	0.12	0.07	-78 %	-26 %
Ctenomys conoveri	15	202,966	62,437	28,936	1.7*	0.77	0.09	0.11	+12 %	+34 %

the mesenteries of the rodents. These were metacestodes of the genus *Taenia* and were identified by R. L. Rausch and SLG in 1988 (pers. obs.) as *Taenia talicei* Dollfus, 1960 which were multi-strobilate larval forms. Another paper describing these associations is in preparation.

Species Conservation/Preservation Status: *Ctenomys opimus* is globally categorized as a species of least concern (LC) by the IUCN (Dunnum *et al.* 2016) and is not mentioned in the Libro Rojo de la Fauna Silvestre de Vertebrados de Bolivia (Ministerio de Medio Ambiente y Agua 2009; LRVSB). The suitable conditions for this species are expected to contract by 34 % and 21 % under the GCM 4.5 and GCM 8.5 respectively, indicating that climatic change will have an important impact over this species (Figure 11; Table 1). Most of the estimated distribution of the species comprises natural, seminatural, and grassland/pastures vegetation covers (Table 2). Through the geographic area occupied by this species, around 21 % of its range is under protection. In Bolivia protected areas include: Reserva Natural y Deportiva Cerro Viscachchani, Refugio de Vida Silvestre Huancaroma, Parque Nacional Sajama, Reserva de Fauna Eduardo Avaroa, Parque Nacional Yura, and Patrimonio Nacional y Reserva Ecologica Poopo (Caraballo *et al.* 2020).

Figure 1. A. *Ctenomys conoveri* showing Orange, slightly grooved incisors. B. Left rear pes and claws of *Ctenomys conoveri* showing comb-like bristles on the margins of the toes.

Ctenomys andersoni Gardner *et al.* 2014

(Figure 2 A, B, C; Map, Figure 9).

This species was named by Gardner and colleagues in honor of Dr. Sydney Anderson. Syd was a major catalyst for the continuing work on the mammals of Bolivia and his encouragement and teaching to both students and professionals has had profound effects on the overall understanding of the biodiversity of Bolivia. His work in Bolivia, starting in 1964 accelerated through and beyond the completion of his monograph: "Mammals of Bolivia: Taxonomy and Distribution" in 1997 (Anderson 1997).

Common name: Anderson's tuco-tuco, Anderson's cujuchi.

Morphology: From the original description of *C. andersoni* by Gardner *et al.* (2014) (n = 27) Body length 253; tail 69.8; hindfoot 33.8; ear 6.8; weight 219.9. *Ctenomys andersoni* is a medium-sized tuco-tuco with brown dorsal coloration, an indistinct dorsal Olive Brown stripe, and no cap of dark hairs on its head (Figure 2 G). The ventral fur is much lighter and is well-differentiated from the dorsum. In contrast to many other species of ctenomyids, there is no obvious collar of differently colored fur in the gular region but there is a small patch of slightly lighter fur extending ventrad around and just below the pinnae. The most distinctive features of the skull and mandible of *C. andersoni* are the brightly colored Orange incisors (Figure 2 E, F). For a complete description of the cranium of this species, see Gardner *et al.* (2014).

Distribution: The type locality and only geographic location from where specimens of *C. andersoni* have been collected is Cerro Itahuaticua in the department of Santa Cruz at an elevation of 810 m (19° 48' S; -63° 31' W; Figure 9). No SDM was constructed for this species since there are not enough occurrence data points (specimens collected more than a few km apart) to enable this type of analysis.

Natural history: The habitat at the collection locality of *C. andersoni* comprises one area in the Andean dry valleys zone with a mixed vegetation of deciduous thorny trees and cacti as well as limestone outcrops on the hillsides. No other data are available on the ecological characteristics of the habitat of this species. The only other species of cajuchi that occurs in the general area from which *C. andersoni* was discovered are *C. erikacuellarae* Gardner *et al.* 2014 to the west (about 50 straightline km) and *C. conoveri* Osgood 1946 to the southeast (about 100 straightline km) see map in Figure 8 C, D). *Parasites* - Chewing lice (*Phtheiropoios* sp.) and nematodes of the genus *Paraspidodera* were collected from the type series of these mammals and these are being described as new species. New records of fleas have been reported (see Pucu *et al.* 2014 or a list).

Conservation/Preservation Status: The biological status relative to numerical density and viability of the populations of this species has not yet been evaluated by the IUCN. Little large-scale habitat conversion has occurred around the area where this tuco-tuco was collected (< 1 %) and 96 % of the habitat is under natural or semi-natural conditions (Table 2). However, because *C. andersoni* is known only from a very restricted geographic range at the type locality and only 3 % of the area near the type locality lies within the municipal protected area of Serrania Sararenda-Cuervo (Caraballo *et al.* 2020), we consider this species as susceptible and vulnerable to immediate extirpation by anthropogenic causes.

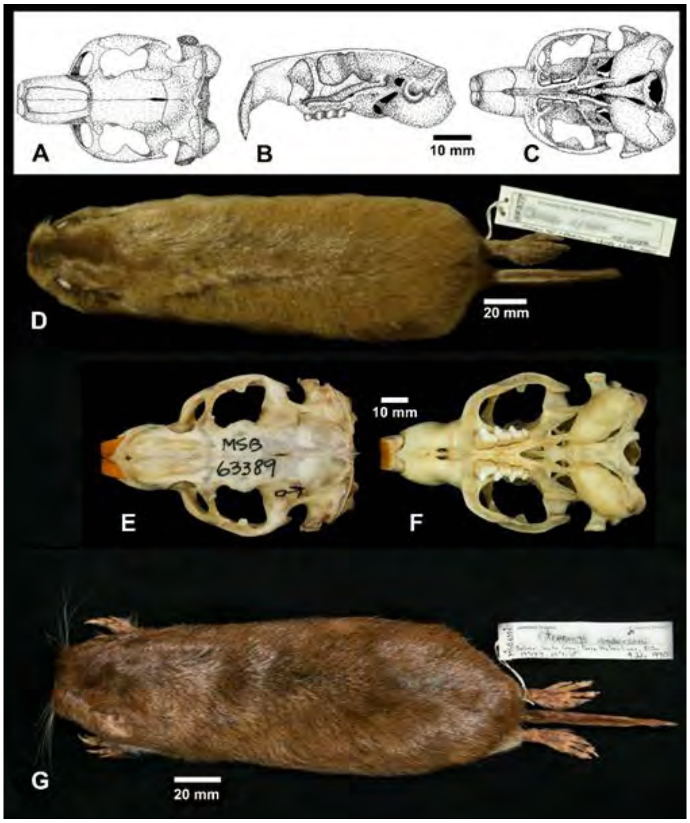

Figure 2. A-D. *Ctenomys opimus* Wagner 1848, Highland tuco-tuco or tuco-tuco Andino. A. Ink drawing of dorsal aspect of skull showing relatively inflated auditory canals and posteriorly protuberant auditory bullae. B. Left lateral view. C. Ventral view, Figure 2. E-G, *Ctenomys andersoni* Gardner *et al.* 2014, Anderson's tuco-tuco or Anderson's Cujuchi. E. Photo image of dorsal aspect. F. Photo of ventral aspect. G. Photo image of dorsal aspect of museum study skin.

Ctenomys boliviensis Waterhouse, 1848

(Figure 3 A, B, C, D; Map, Figure 9).

Common name: Bolivian tuco-tuco, cuhuchi, cujuchi.

Morphology: (n = 81) Body length 289; tail 77; hindfoot 42; ear 7; weight 329.

Individuals of this species are moderately large. The pelage is reddish brown, with darker areas mid-dorsally, especially on head and nape with a well-developed lighter colored collar extending ventrad from the pinnae. Regarding the skull, distinctive characters include the posterior position of the postorbital processes. A summary of the morphological characters for this species is given in Anderson *et al.* (1987), in addition, we follow Gardner *et al.* 2014 and regard *C. goodfellowi* Thomas 1921 as a synonym of *C. boliviensis.*

Distribution: *Ctenomys boliviensis* is found in the lowlands of the Department of Santa Cruz from 400 to 500 m (Anderson *et al.* 1987). The species has been recorded from approximately 25 separate localities in Bolivia, one cluster of records occurring around Santa Cruz de la Sierra and another in Santa Rosa de la Roca to the east. Our estimate using the SDM indicates there exists approximately 14,347 km^2 of suitable habitat through the species range in Bolivia, with a core area around Santa Cruz de la Sierra and smaller suitable patches east and north, among which are collection localities of Santa Rosa de la Roca (Figure 9 C; Table 1). On average, bioclimatic data contributed more to the final ensemble model of this species, although soil variables also provide important contributions to the estimate.

Natural history: Cujuchis commonly occur throughout agricultural areas in the lowlands sometimes causing damage to crops, especially yucca (*Manihot esculenta*), corn (*Zea mays*), and other crop plants (Gardner and Salazar-Bravo, pers. obs. 1990). Individuals of *C. boliviensis* were found in lower densities in grasslands and seldom occurred in the forests, although some specimens have been collected from wooded areas (Thomas 1921; Anderson *et al.* 1987). Near the western border of its distribution along the Andean foothills, *C. boliviensis* is replaced by *C. steinbachi* (Anderson *et al.* 1987) and in some areas, these two species occur in close proximity (separated by only 7.5 km). Individuals of *Ctenomys boliviensis* spend most of their time underground in burrows. Little scientific data are available on behavior and habits of this species, but observations by Gardner with Yates (pers obs. 1984, 1985) indicate that the Cajuchi is active throughout the 24 hour period with perhaps greater activity in early morning and late evening; at least one specimen was collected by hand around 11:00 am from sandy areas south of Santa Cruz de la Sierra. Anderson *et al.* (1987) also report that pregnant females have been recorded in May and June, with the number of embryos ranging from one to five (mean 1.7). Parasites - *Ctenomys boliviensis* is known to host the coccidian *Eimeria opimi* with a 36 % prevalence in a sample of 236 individuals examined from near Santa Cruz de la Sierra (see summary in Gardner and Duszynski 1990). Pucu *et al.* (2014) report the flea species *Gephyropsylla klagesi* Rothschild, 1904 and *Ectinorus galeanus* Jordan, 1939 from this tuco-tuco. Recently, a new species of hookworm, *Ancylostoma ctenomyos* Drabik and Gardner 2019*,* was described (Drabik and Gardner 2019) from cajuchi specimens collected during our work that extended from 1984 - 2000 (Also, see summary of this nematode from *C. steinbachi*, below). Both lice (*Phtheiropoios* sp.) and nematodes of the genus *Paraspidodera* have been recorded and many of these are being described as new species.

Species Conservation/Preservation Status: This tuco-tuco has been categorized as a species of least concern (LC) by the IUCN (Dunnum and Bernal 2016) and is not listed in any threat category within Bolivia by the LRFSV. However, the geographic range of this species is expected to be significantly reduced by climate change under both employed scenarios, with a loss of around 78 % of the suitable habitat area under the 4.5 RCP and a 26 % loss under the 8.5 RCP (see map, Figure 11; Table 1). The milder expected change of the 8.5 RCP projection results from an expansion of suitable habitat to the east of the current range. The suitable habitat of the species has also suffered important habitat transformation with 47 % of the natural and semi-natural areas of suitable habitat changing to agriculture or urban zones from 1993 to 2018, leaving only about 40 % of the suitable habitat under natural and semi-natural conditions (Table 2). Although it has been observed that this species can survive in or near agricultural land, it is likely that current and future land-cover/use changes will affect the long-term viability of *C. boliviensis* in Bolivia. Furthermore, less than 1 % of the area of distribution of the species lies in protected areas (Caraballo *et al.* 2020) which in Bolivia includes two municipal parks: Orquídeas del Encanto and Laguna Represa Sapocó, both in Santa Cruz Department. Land cover changes, as well as the projected impact of climatic change on suitable habitat for this species suggest the need of more in-depth studies of habitat use by this species as well as additional studies on populations and estimates of long-term viability.

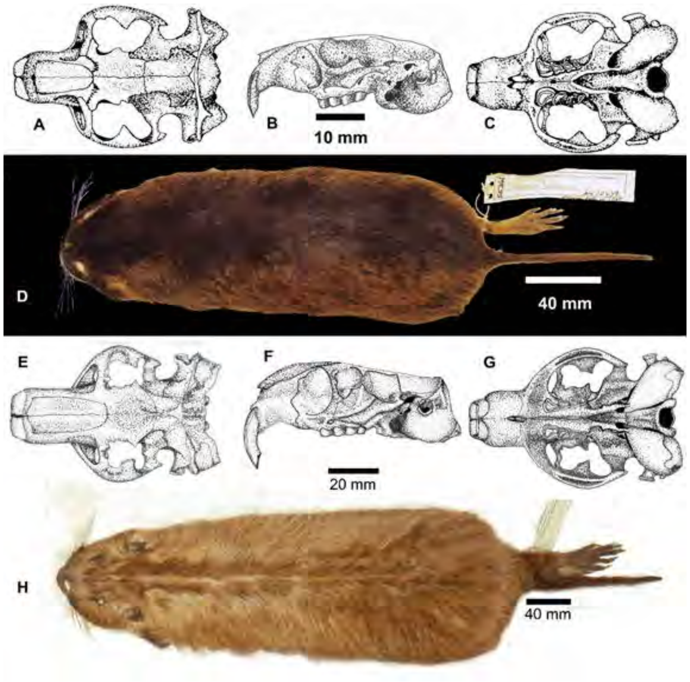

Figure 3. A-D *Ctenomys boliviensis* Waterhouse, 1848, Cuhuchi or Cujuchi. A. Ink drawing of dorsal aspect of skull showing robust bone architecture. B. Left lateral view of skull. C. Ventral view of skull. D. Dorsal view of skin. Figure 3. E-H, *Ctenomys conoveri* Osgood 1946, tuco-tuco del Chaco. E. Ink drawing of dorsal view of skull, F. Left lateral view of skull, G. Ventral view of skull.

Ctenomys conoveri Osgood, 1946

(Figures 1 A, B; 3 E, F, G; Map, Figure 9)

Common name: Conover's tuco-tuco, tuco-tuco del Chaco Morphology: (n = 4) Body length 474.75; tail 113; hindfoot 56; ear 14; weight 1178. Individuals of this species have the largest body size of any in the family Ctenomyidae with some adult males as heavy as 1,520 g (Figure 1 A, B). Pelage of several individuals examined by us is relatively coarse with long shaggy fur (many hairs are longer than 20 mm). The basal part of their dorsal hairs is pale grayish about 10 mm long, then there is a brownish part about 7 mm, ending in blackish tips. Some longer guard hairs have paler tips. Overall color of dorsum is blonde-brown, some individuals with fur darker mid-dorsally, especially on head and nape, and around the mouth (Figure 1 A; the venter is paler and more reddish. Hind foot is broad with well-developed combs (Figure 1 B). The upper incisors are light Orange in color with shallow longitudinal grooves evident in anterior view (Figure 1 A). A summary of skin and skull characters is given in Anderson *et al.* (1987).

Distribution: *Ctenomys conoveri* is distributed through the southern lowlands of Bolivia and where found is locally common in the Departments of Chuquisaca, Santa Cruz, and Tarija, as well as the Chaco region of Paraguay. This species has been reported from 15 separate collection localities in Bolivia, including southern Santa Cruz, eastern Tarija, and eastern Chuquisaca. The SDM predicts 29,000 km^2 of suitable habitat through the species range in Bolivia (Figure 9; Table 1). On average, soil variables show higher contribution to the final model, suggesting that soil conditions significantly influence the distribution of the species.

Natural history: *Ctenomys conoveri* occurs in friable soils that are mostly sandy in composition. This species inhabits the Gran Chaco ecosystem. The specimens we collected in 1985 were easy to catch with number 1 or 2 leghold traps placed in the burrow system. We noted dozens of neatly cut green stems approximately the same length (8 to 15 mm long) that had been trimmed from small stems of trees or bushes, piled in the burrow system of one of the individuals we caught. Parasites - Coccidia Apicomplexa: Eimeriidae) identified as *Eimeria opimi* Lambert *et al.* 1988 were reported from one of three individuals collected in 1985 and studied by Gardner and Duszynski (1990). Chewing lice (*Phtheiropoios* sp.) that were obtained from specimens we collected are being studied and described. Nematode parasites of the genus *Paraspidodera* were also found infecting the cecum of most of the individual tuco-tucos that were collected. These are currently being described as new species.

Species Conservation/Preservation Status: This species is listed as Least Concern (LC) globally by the IUCN as well as within Bolivia by the LRFSV. The distribution of suitable habitat for the species in Bolivia is expected to expand to the east and north of the current distribution, resulting in an increase of 12 % distribution area under the 4.5 RCP and 34 % under the 8.5 RCP scenario (Figure 11; Table 1). This is the only species of Ctenomys that has a geographic distribution that we project will expand under the expected global warming scenario. Less than 1 % of the natural and semi-natural areas within the species distribution in Bolivia have suffered significant transformations since 1993, resulting in about 98 % of the habitat remaining in at least semi-natural conditions or pasture land that is conducive to proliferation of *C. conoveri* (Table 2). Nonetheless, the area is dissected by several small roads, and many habitats of this

Table 2. Estimated percentage of natural and seminatural vegetation cover occurring in the ranges of *Ctenomys* in Bolivia for the years 1993 and 2018. The percentage of remaining natural and seminatural vegetation cover lost through this 15 year period is also displayed.

Species	Estimated range area (km^2)	%Natural-seminatural cover 1993	%Natural-seminatural cover 2018	% Natural-seminatural lost
Ctenomys opimus	100,733	99.6	100	0.04
Ctenomys andersoni	4,512	97.1	97	0.6
Ctenomys boliviensis	14,347	74.7	40	47.0
Ctenomys conoveri	28,936	98.7	98	0.5
Ctenomys erikacuellarae	7,461	97.8	98	0.3
Ctenomys frater	34,443	97.9	98	0.2
Ctenomys lessai	4,556	95.5	95	0.4
Ctenomys leucodon	8,382	98.4	98	0.2
Ctenomys lewisi	2,087	98.4	98	0.7
Ctenomys nattereri	43,495	99.3	97	1.8
Ctenomys steinbachi	2,690	73.9	58	21.1
Ctenomys yatesi	4,214	99.7	99	0.2

species in Paraguay have suffered severe transformations from industrial agriculture (Caldas et al. 2015). Across the area occupied by this species about 15 % of the range of *C. conoveri* overlaps with protected zones (Caraballo *et al.* 2020). In Bolivia, these areas include: Parque Nacional Aguarague, Area de Proteccion del Quebracho Colorado, and Reserva Natural El Corbalán, and the Monumento Natural - Muela del Diablo, near Boyuibe, Chuquisaca.

Ctenomys erikacuellarae Gardner *et al.* 2014

(Figure 4 A, B, C; Map, Figure 9).

Common name: Erika Cuellar's tuco-tuco, tuco-tuco de Erika Cuellar.

Morphology: (n = 69) Body length 259.2; tail 72.9; hindfoot 36.4; ear 7; weight 222.1. This is a medium-sized tuco-tuco with dorsal and ventral coloration well differentiated. The dorsum ranges from ochraceous orange to buckthorn brown except for the upper surface of the head and muzzle which is blackish brown. Most individuals with dark cap (fuscous black) on head, with dark fur running from just above the nose to at least neck. Mid-dorsal stripe of same dark color running posteriad mid-dorsally along back usually reaching rump.

The venter is drab brown or buffy brown and displays extensive white or light buff markings on inguinal, axillary and pectoral regions. The skull is strongly built, having strongly curved zygomatic arches. The rostrum is widest at the tip of premaxillaries giving a strongly robust look when observed from above. The upper incisors are large, robust, orange in color, and opisthodont; lower incisors are also orange in color.

Distribution: Erika Cuellar's tuco-tuco is known only from three localities situated on the eastern flanks in the Andes in the Cordillera Oriental. No species distribution model (SDM) was constructed for this species since not enough occurrence data to enable this type of analysis are yet available. The qualitatively estimated range of the species encompasses the valleys where the species occurs through an area of 7,461 km^2 (Figure 9; Table 2).

Natural history: These tuco-tucos occur in habitats consisting of friable soils, mostly in the floors of valleys ranging from a recorded low altitude of 810 m. in the area of Monteagudo, Department of Chuquisaca to a recorded maximum of around 1,800 m, near the Río Ciénega north east of Vallegrande, Santa Cruz Department in south-central Bolivia. From Gardner *et al.* (2014): "All three known localities are part of the ecological zone known as Bosques Secos Interandinos or Andean dry valleys (Ibisch *et al.* 2003; Lopez 2003). The locality designated "2 km SW of Monteagudo" was on an agricultural experimental station that was, at the time, relatively protected as the vegetation was less disturbed inside the fenced station and was typical of the lower, eastern escarpments of the Andes in southern Bolivia (see: Ibisch *et al.* 2003). From the field-expedition field notes our team indicates: At the collection locality "5.5 km NNE of Vallegrande" on the Río Ciénega, the hillsides were covered with mesquite (*Prosopis* L.), columnar cacti (*e. g., Trichocereus* sp.), acacia (*Acacia* sp.), and *Ximenia* sp. The Río Ciénega runs through the valley and is surrounded by dense stands of shrubs and trees (*Prosopis*, etc.). The area was mostly converted to cropland and was, at the time, grazed heavily by livestock. The collecting locality at "2 km E of Chuhuayaco" was similar in vegetation-type to that of Vallegrande. Parasites - Fleas identified by Pucu *et al.* (2014) as species of *Rhopalopsyllus* sp. and *Polygenis* sp. were collected from populations of *C. erikacuellarae*. Nematodes of the genus *Paraspidodera* were also obtained from Erika's tuco-tuco at time of collection; the nematodes are being described as new. In addition, lice (*Phtheiropoios* sp.) were also recovered as well as tapeworms. Tapeworms are rarely reported in the literature from any species of *Ctenomys* and we found cestodes in the small intestines of several individuals collected on the experiment station grounds near Monteagudo. These tapeworms, genus *Raillietina* are undescribed and we are currently finishing the descriptions of these worms.

Species Preservation/Conservation Status: *Ctenomys erikacuellarae* has not yet been evaluated by the IUCN or LRFSV. Few large-scale changes have occurred within the estimated distribution of the species during the period analyzed, with around 97 % of the species range under natural or seminatural conditions (Table 2). Across the range of this species in Bolivia, about 48 % of the area in which these tuco-tucos live is under some form of protection (Caraballo *et al.* 2020); these areas include: the Área de Protección Ambiental Serranía Cordillera de los Milagro, National park Iñao, area natural de manejo integrado Rio Grande Valles Crucenos, and the Zona de Inmovilizacion Forestal e Hídrica El Villar. Regardless, given its restricted distribution, the species conservation status requires close monitoring.

Ctenomys frater Thomas, 1902

(Figure 4 D, E, F; Map, Figure 9)

Common name: little Andean tuco-tuco, tuco-tuco pequeño de los Andes.

Morphology: (n = 7). Body length 250; tail 69; hindfoot 34; ear 8.4; weight 165 g. *Ctenomys frater* is a medium-sized tuco-tuco, the subspecies occurring in Bolivia present brown dorsal coloration and dull buffy underparts. Also, see the excellent summary-description of this species by Cook *et al.* (1990).

Distribution: *Ctenomys frater* occupies suitable habitat in the Andean valleys of south eastern Bolivia in the departments of Chuquisaca and Tarija extending south into NW Argentina, including the provinces of Salta and Jujuy. The species distribution model for *C. frater* predicts 34,443 km^2 of suitable habitat through Bolivia, with a large continuous suitable area in the eastern flanks of the Andes in Tarija and Chuquisaca and small patches of habitat further North up to Potosi and Oruro, where there are no records of the species at the moment (Figure 9; Table 1). On average, soil variables show greater contribution to the final model, suggesting that soil conditions appear to significantly impact the distribution of the species which can be expected for any subterranean rodent (Table 1). Variables that were investigated here included bulk density (fine earth) in kg / cubic-meter, clay content (0-2 micrometers) mass fraction in %, soil organic carbon content (fine earth fraction) in g per kg, silt content (2-50 micrometer size range) mass fraction in %, and sand content (50-2,000 micrometers) mass fraction in %.

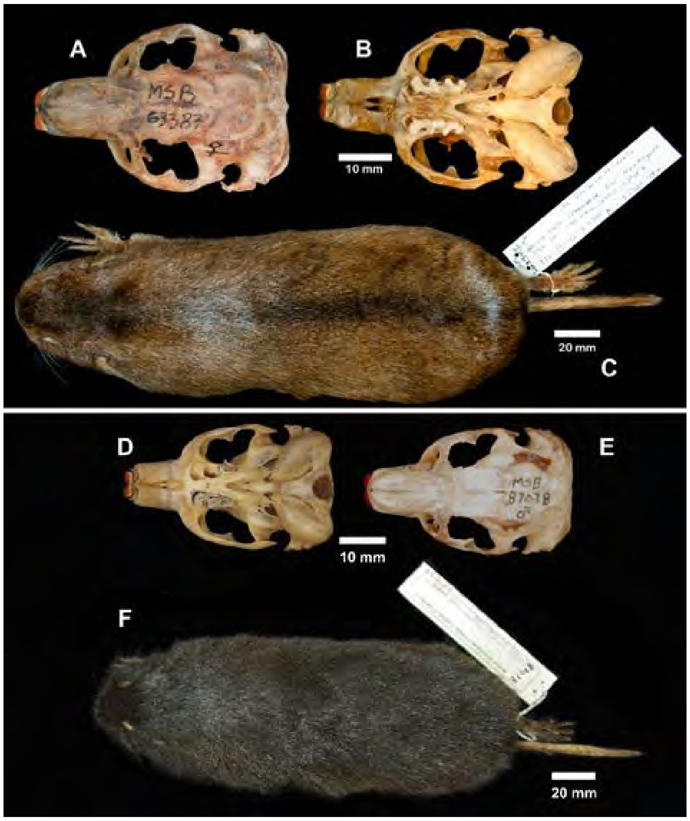

Figure 4. A-C *Ctenomys erikacuellarae* Gardner *et al.* 2014, Erika Cuellar's tuco-tuco or tuco-tuco de Erika Cuellar. A. Dorsal view of the skull showing the rostrum is widest at the tip of premaxillaries and the strongly curved zygomatic arches. B. Ventral view of the skull. C. Dorsal view of skin. Figure 4. D-F, *Ctenomys frater* Thomas 1902 Little Andean tuco-tuco or tuco-tuco de los Andes. D. Ventral view of the skull. E. Dorsal view of the skull. F. Dorsal view of the skin.

Natural history: *Ctenomys frater* occurs through a wide elevational range extending from 600 m in northwestern Argentina (Thomas 1919; Olrog and Lucero 1981) to approximately 4,300 m in central Bolivia (Anderson 1997). Specimens of *C. frater* were collected by our team from steep grassy hillsides (2,700 m) in the Andes at a locality called 8 km west, by road of Rancho Tambo (21° 27’ S; -64° 19’ W, estimated with map), while other specimens of the same species were found at elevations above 4,300 m in the department of Potosi. At the locality called Tapecua (21° 26’13.4” S; -63° 55’0.12” W, data from GPS), visited by our collection party in July 1991 and again in 1995, additional specimens of *Ctenomys frater* were collected. All of these specimens, collected on 4, June 1995 were extremely dark, their fur matching well the color of the soil where these mammals were found to be abundant. The animals were found to be living in friable, but not sandy soil on extremely steep hillsides under the canopy of trees. The habitat changed dramatically from the time we first visited Tapecua in 1991 compared to our subsequent visit in 1995. In 1995, we found that domestic goats had been introduced into the area and the vegetation was cut up to the level that a goat can reach. For much of the time when we were at both Rancho Tambo and Tapecua, Andean Condors were very common. Some condors landed within 5 meters of us as we worked our traplines on the steep hillsides at the locality designated 8 km west, by road of Rancho Tambo; the condors soared nearby during the middle of the day at Tapecua. At the Tapecua locality, tuco-tuco-trapping was difficult as moving up and down the hillsides required much effort. Parasites - Fleas (*Ectinorus galeanus* Jordan 1939) were collected by our field party in 1986 from 3 individual tuco-

tucos at 8 km west, by road from Rancho Tambo. Coccidia identified as *Eimeria opimi* were collected from this same locality and identified by Gardner and Duszynski (1990). Nematodes of the genus *Paraspidodera* were collected from specimens obtained from both the Rancho Tambo and Tapecua localities, these are being described as new. *Species Preservation/Conservation Status:* This tuco-tuco has been categorized as a species of least concern (LC) by the IUCN (Vivar 2017) and is listed as data deficient by the LRFSV. The distribution of suitable habitat for *C. frater* in Bolivia is expected to be reduced 8 % under the 4.5 RCP and 9 % reduction under the 8.5 RCP scenario (see Map 11; Table 1). Few large scale changes have occurred within the estimated distribution of the species during the period analyzed, with around 97 % of the species range under natural or semi-natural conditions (Table 2). About 15 % of the range of *C. frater* is protected in more than 20 areas in Bolivia (Caraballo *et al.* 2020).

Ctenomys lessai Gardner *et al.* 2014

(Figure 5 A, B, C; Map, Figure 10)

Common name: Lessa's tuco-tuco, tuco-tuco de Lessa.

Morphology: (n = 9), Body length 238; tail 64; hindfoot 32; ear 6.6; weight 176 g. *Ctenomys lessai* shares a common ancestor with *C. conover*i (see phylogeny in Gardner *et al.* 2014). Because this is such a unique and isolated species we directly quote Gardner *et al.* (2104) in the description of the skins and skulls: "*Pelage dense, fine, soft, about 5–20 mm long over back and rump. Color of dorsal pelage olive brown to buffy brown, ventral pelage cinnamon buff, some individuals with ventral fur olive buff. Dorsally, darkest fur a clove brown in central diffuse dorsal stripe, more prominent anteriorly on head and fading posteriad... ...Small light area of cinnamon buff fur, just posterior to and below the pinnae, evident in most individuals. Collar not evident in specimens examined.*" In contrast to the well developed collars of differently colored fur extending from the pinnae ventrad in both *C. boliviensis* and *C. steinbachi* there is no collar in the gular region of *C. lessai*. In describing the skull, Gardner *et al.* (2104) state: "*Upper incisors with enamel a light pale yellow ocher to ivory yellow color. Enamel initially white in unexposed roots, changing to yellow ocher with mottled yellowing slightly on frontal surfaces of exposed parts of incisors. Sometimes appearing mottled in frontal view with white showing through. Enamel of lower incisors yellow ocher in color. Mandible relatively delicate.*"

Distribution: This species is known only from the type locality near a place 0.5 km south of Lluthu Pampa, 17° 45'S; -64° 59'W, 2,700 m. elevation in the Andean portion of the department of Cochabamba (Figure 10).

Natural history: Specimens of this tuco-tuco were collected from burrow systems at elevations ranging from 2,500 to 2,750 m. The area from where specimens were secured was open grassland habitat near a running stream with remnant stands of *Polylepis* sp. These tuco-tucos appear to be diurnal as most of the captures occurred during daylight hours. Parasites - From the original collection material obtained on 30 and 31 May 1991, both ectoparasites and endoparasites were collected by our field crew. Lice (*Phtheiropoios* sp.) are currently being studied and the fleas have been identified and reported as *Ectinorus galeanus* Jordan, 1939 and *Sphinctopsylla inca* Rothschild, 1914 by Pucu *et al.* (2014). Nematodes of the genus *Paraspidodera* were collected from the cecum of these mammals and these are currently being described as a new species.

Species Conservation/Preservation Status: Individuals of this species have been collected only from the type locality and this species has not been evaluated by the IUCN or the LRFSV. The area surrounding the type locality has suffered less than 1 % large scale landscape changes in the period analyzed, but including previous changes, 95 % of the area remains as natural or semi-natural habitat (Table 2). Almost 50 % of the area surrounding the collection localities is currently listed as protected, including both Parque Nacional Amboró and Parque Nacional Carrasco (Caraballo *et al.* 2020).

Ctenomys leucodon Waterhouse, 1848

(Figure 5 D, E, F; Map, Figure 10)

Common name: white-toothed tuco-tuco, tuco-tuco de dientes blancos.

Morphology: **(**n = 3) body length 223; tail 67; hindfoot 31; ear 5; weight 160. White-Toothed tuco-tucos are small in size and very reddish in color (Figure 5 D, E, F). Their fur is a rich Buckthorn Brown and is thick over the body and the ears are well-furred with darkly colored skin on the pes (Figure 5 D). The pale, relatively narrow and proödont upper incisors are noteworthy. See the excellent summary of the morphology of this species in Cook *et al.* (1990).

Distribution: *Ctenomys leucodon* occurs in a relatively small area generally south of Lake Titicaca at elevations of about 3,850 m. in the department of La Paz, in Bolivia but also west of the southern part of the lake in Peru (two collection locality records; Figure 10). No SDM was constructed for this species since not enough occurrence data points have been recorded in museums to enable this type of analysis. The qualitatively estimated range of the species encompasses the valleys where the species occurs through an area of 8,382 km^2.

Natural history: Habitat of the tuco-tuco de dientes blancos consists of flat puna with compact soils that are friable only with difficulty as soil is held together by masses of grass roots that bind the soil well. In the areas where these were collected are dispersed bunch grasses that dot the landscape. Associated throughout the area and especially where the *C. leucodon* colonies were found were many common yellow toothed cavys (*Galea musteloides*), but, even though they occurred syntopically at the locality (11.5 km west of San Andres de Machaca, 17° 00' S; -69° 04' W; 3,800 m.) we did not observe these two species of mammals using the same burrow systems at this locality. The Tuquitos de Dientes Blancos were located in isolated colonies sometimes separated by distances as great as kilometers. No data on colony size could be determined from our work. First encountering these animals in 1986, some individuals of *C. leucodon* exhibited a high-pitched whistle vocalization on several occasions. Individuals of this species seemed to spend more time at burrow openings than did other species of Bolivian *Ctenomys* as several were observed with their heads out of the burrow systems for a relatively long period of time. These animals appear to occur in moving landscape patches or colonies called "tucales" that move through space and time and appear to operate as mini soil plows that enable higher diversity of plants to grow in the soil where the mammals turn over the soils. In the field, members of our expeditions observed higher diversity of both plants and mammals among and in tucales. In Peru, *C. leucodon* appears to be the least common species of *Ctenomys*, occurring in a restricted area west of Lake Titicaca (see map, Figure 10). In August, one female with one embryo and seven females with no embryos were recorded (Anderson 1997). Parasites - Three individuals were examined for the presence of coccidia (*Eimeria*) by Gardner and Duszynski (1990) but were found to be uninfected. Two species of fleas were identified from material collected by our team in 1993 including *Ectinorus galeanus* Jordan, 1939 and *Tetrapsyllus tristis* Johnson 1957 (see report by: Pucu *et al.* 2014). A nematode called *Pudica* sp. was identified by Gardner pers. obs. and lice (*Phtheiropoios* sp.) were collected along with beetles of the family Staphylinidae from individuals of *C. leucodon* by our field expeditions in 1986 and 1993 (Anderson 1997 and Pucu *et al.* 2014). *Species Conservation/Preservation Status:* The species is listed as Least Concern by the IUCN and as data deficient by the LRFSV. Although 98 % of the distribution area comprises pasture, natural or semi-natural covers (Table 2), almost none (0.1 %) of the protected areas that are established overlap the range of this species (Caraballo *et al.* 2020).

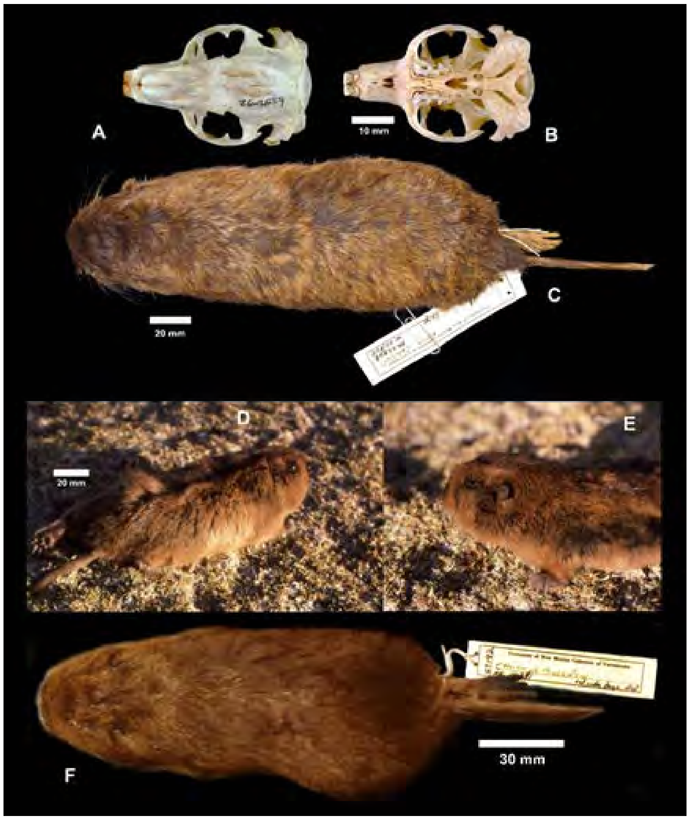

Figure 5. A-C *Ctenomys lessai* Gardner *et al.* 2014, Lessa's tuco-tuco or el tuco-tuco de Lessa. A. Photo image of dorsal aspect of skull, note particularly delicate rostrum. B. Photo image of ventral aspect of skull. C. Photo image of dorsal view of museum study skin of *C. lessai*. Figure 5. D-F. *Ctenomys leucodon* Waterhouse, 1848, White-Toothed tuco-tuco, el tuco-tuco de Dientes Blancos. D. Photo image of right lateral side of a living *C. leucodon*. E. Photo image of anterior left lateral side of same individual as in 5 D, F. Photo image of dorsal aspect of museum study skin.

Ctenomys lewisi Thomas, 1926

(Figure 6 A, B, C, D, E; Map, Figure 10)

Common Name: Lewis's tuco-tuco, tuco-tuco de Lewis. Morphology: (n = 15) Body length 268; tail 76; hindfoot 39; ear 8; weight 291 g. Thomas (1926) characterized *C. lewisi* as a large reddish-brown species with unusually proodont incisors. He stated: "*Ctenomys leucodon* has similarly proodont incisors, but these (*C. leucodon*) are comparatively slender, are not so strongly flattened in front, and are almost unpigmented." Because Thomas (1926) compared *C. leucodon* with *C. lewisi*, we will make some similar comparisons here. Individuals of *C. lewisi* that we trapped at the Iscayachi locality were of a rich dark black/brown color dorsally and lighter with grey ventrally. The dorsal coloration appears to closely match the color of the soil of the area when it is moist. The pinnae are sparsely furred and pink in color (Figure 6 D) in sharp contrast to the dense reddish-brown-black fur on the pinnae of *C. leucodon* (Figure 6 D, E). See also the excellent summary of the morphology of this species in Cook *et al.* (1990).

Distribution: *Ctenomys lewisi* is known only from the highlands of Tarija in the valley near Iscayachi (elevation of collecting localities in 1986 was 3,400 m) with the Serrania de Sama rising rapidly to the east to about 4,000 m over a distance of 6 km. from the collecting site (Figure 10).

Natural history: This species was first collected from burrows in wet stream banks, which led Thomas (1926) to suggest *C. lewisi* had semi-aquatic habits. No such habit has been suggested for any other species of tuco-tuco and some, but not all, of the specimens captured by members of our expedition in 1986 near Iscayachi were trapped from burrows in damp soil near the Rio Tomayapo. There was no indication that any of the 15 animals we collected at the Iscayachi locality lived in or even very near water. Parasites - From 6 of 8 individuals examined for coccidian parasites in the feces, oocysts representing *Eimeria opimi* were found (Gardner and Duszynski 1990). Nematodes of the genus *Paraspidodera* were also recovered from the cecum of several individuals and these are being described as new. In addition, chewing lice (*Phtheiropoios* sp.) were recovered from this species at the Iscayachi locality and further up the mountain near Sama. These are also being described. Fleas were not recovered during collection of the mammals.

Species Conservation/Preservation Status: *Ctenomys lewisi* was listed as a Species of Least Concern (SLC) by the IUCN (Roach and Naylor 2016a) and as data deficient by the LRFSV. Approximately, 98 % of the distribution range of this species comprises natural and semi-natural land cover (Table 2), but with heavy grazing by domestic animals and 21 % of its estimated distribution range is covered by protected areas including the Reserva Biológica Cordillera de Sama and Parque Natural y Área de Manejo Integrado El Cardón (Caraballo *et al.* 2020).

Ctenomys steinbachi Thomas, 1907

(Figure 6, F - I; Map, Figure 10)

Common names: Steinbach's tuco-tuco, tuco-tuco de Steinbach.

Morphology: (n = 46) Body length 293; tail 81; hindfoot 43; ear 7; weight 315 g. The skin of this species has a light Black pelage dorsally with grizzled or mixed whitish and blackish ventral pelage. Well developed light grey collar extending ventrally from each pinna. See Anderson (1997) for an excellent summary of the morphology of this species.

Distribution: *Ctenomys steinbachi* is found only in the department of Santa Cruz, west of the Rio Grande. Individuals of this species have been collected from 8 separate localities ranging from 300 to 500 m. in altitude. Steinbach's tuco-tuco suitable habitat comprises 2,690 km^2 (Figure 10; Table 1). The environmental requirements of this species are different from those of *C. boliviensis*, resulting in very little suitable habitat overlap between the two species despite their close geographical distance. Individuals of this species have been collected as close as 15 km in geographic proximity of populations of *C. boliviensis* at and at slightly higher elevations than is inhabited by *C. boliviensis* along the eastern foothills of the Andes near Parque Nacional Cerro Amboro (Anderson *et al.* 1987).

Natural history: Steinbach's tuco-tuco has been recorded from open savanna areas, closed canopy areas with yucca and banana and areas that have been disturbed by sugarcane plantations, cattle grazing and other agricultural activities. The soil where these animals occur is darker and contains much less sand than the areas occupied by *C. boliviensis* in the area where these two species occur in proximity. Parasites - Of 72 specimens of Steinbach's tuco-tuco examined for the presence of the protistan parasite *Eimeria* sp., none were found to be passing oocysts when they were collected in contrast to a relatively high prevalence of the parasites found in other species of *Ctenomys* (Gardner and Duszynski 1990). Drabik and Gardner (2019) described *Anycylostoma ctenomyos* from duodenum in the small intestine of *Ctenomys steinbachi* collected from Bolivia, Departamento de Santa Cruz: 2 km S. Caranda, by road, (17° 33′ 0″ S; -63° 31′ 48″W, 345 m) (note that this species of nematode was also recovered from *C. boliviensis*) on August 14, 1987. The prevalence of this nematode was 6/13 = 46 %. Nematodes of the genus *Paraspidodera* have also been collected from *C. steinbachi*, and chewing lice of the genus (*Phtheiropoios* sp.) were collected from individuals of this species and these are being described elsewhere as new.

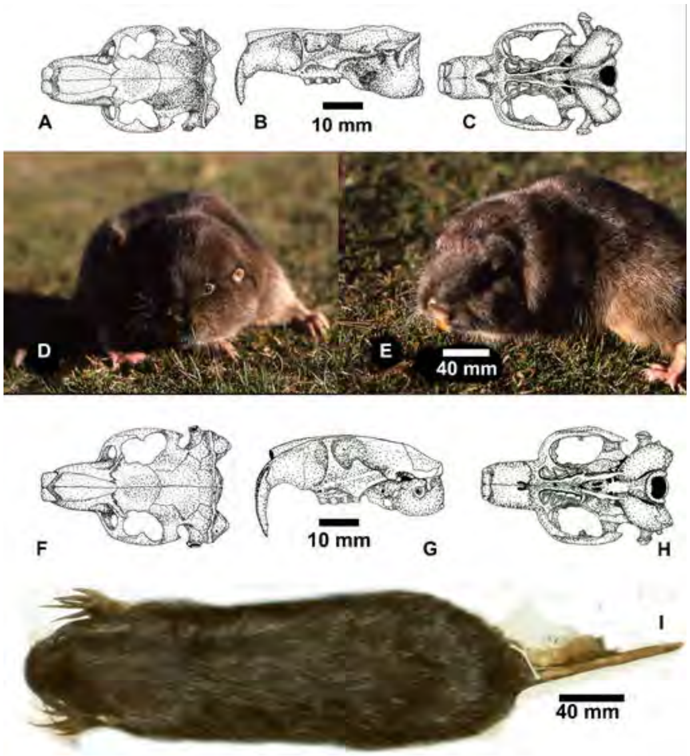

Figure 6. A-E. *Ctenomys lewisi* Thomas, 1926, Lewis's tuco-tuco, tuco-tuco de Lewis. A. Ink drawing of dorsal aspect of skull. B. Left lateral view. C. Ventral view, note extremely long auditory canals and rear-turned auditory bullae. Figure 6. F-I *Ctenomys steinbachi* Thomas, 1907, Steinbach's tuco-tuco or tuco-tuco de Steinbach. F. Ink drawing of dorsal aspect of skull, G. Left lateral view of skull. H. Ventral view of skull. I. Photo image of dorsal aspect of skull of *C. steinbachi*.

Species Conservation/Preservation Status: *Ctenomys steinbachi* is categorized as a SLC by the IUCN (Roach and Naylor 2016b). However, in Bolivia this species is categorized as near threatened (NT) by the LRVSB because of the lack of recent collection records and the coincidence of the species range with areas of intense habitat conversion into both urban and agricultural uses. Similar to the situation for *C. boliviensis,* this species is predicted to experience severe contraction in suitable habitat areas, with a 61 % and 58 % reduction under the GCM 4.5 and GCM 8.5, respectively (Table 1). In total, about 21 % of the remaining natural and semi-natural vegetation cover was lost through the range of the species since 1993, which leaves only 58 % of the species distribution area as natural or semi-natural conditions (Table 2). In addition, only 4 % of the species range is under protection. Given the endemic status, magnitude of observed habitat degradation, and expected impact of climate change; this species should be closely monitored, and we feel strongly that *Ctenomys steinbachi* should be assigned to a higher IUCN threat category. We recommend a higher threat category partly because of the problematical list of so-called protected areas in the department of Santa Cruz, Bolivia. Caraballo *et al.* (2020) shows that the area in which *C. steinbachi* occurs are overlapped by at least three areas with some degree of ecological protection. However, examining these areas shows that that they appear to provide minimal protection for native species, this is because, from aerial views, it can be seen that these areas are covered with urban sprawl or consist of intensive industrial agriculture with most of the natural areas being obliterated by humans. The areas that are listed as protected include: Parque de Protección Ecologica Río Piraí and the Área Protegida Municipal Curichi La Madre, Parque Regional Lomas de Arena see Caraballo *et al.* (2020).

Ctenomys nattereri Wagner 1848

(Figure 7 A - H; Map Figure 10)

Common name: Natterer's tuco-tuco, tuco-tuco de Natterer.

Morphology: (Taken from Wilson *et al.* 2017) Body length 245; tail 71; hindfoot 54; ear 11; No information on weight. This is a relatively large species of *Ctenomys* that presents a shiny brown dorsal coloration capped by a dark dorsal stripe comprised of black spots. The venter is black with white spots, especially in the axillary and inguinal regions. On the specimen examined, the claws of the forefeet are particularly long, reaching 16 mm. The specimen of *C. nattereri* that we present here is derived from the skull of (AMNH37121) collected on 24 Feb, 1914 by G. K. Cherrie on an expedition with Theodore Roosevelt and Cândido Mariano da Silva Rondon through the area of what would eventually become Rondonia, both upper and lower incisors are a dark orange, the skull is robust.

Distribution: The distribution of the species includes the eastern lowlands of Bolivia in the department of Santa Cruz east into Brazil (Figure 10). In Bolivia, tuco-tuco specimens from four localities in Santa Cruz have been assigned to *C. nattereri*. Based on an analysis of cyt-b phylogeny and genetic distances, Gardner *et al.* (2014) assigned the populations near Roboré, San Jose de Chiquitos, and San Ramón to *C. nattereri*. Besides Bolivia, the species has been recorded in the states of Mato Grosso and Rondônia in Brazil.

Natural history: Little is known of the habitats or behavior of this tuco-tuco. *Parasites* - Nematodes recorded by our collecting expeditions from this tuco-tuco in Bolivia include species of the genera *Paraspidodera* and *Trichuris.*

Species Conservation/Preservation Status: This species has not been evaluated by the IUCN or the LRFSV although IUCN treats *C. nattereri* as a synonym of *C. boliviensis* (see Dunnum and Bernal 2016). The range estimated from the abovementioned localities has lost around 2 % of its natural and semi-natural land cover since 1993 (Table 2). Approximately 32 % of the species range appears to be under some protection category (Caraballo *et al.* 2020) but at the current time, little information is known of the habits of these tuco-tucos. The areas in Bolivia in which some protection of this species is given include: Area Natural de Manejo Integrado San Matías and the Area Protegida Municipal San Rafael.

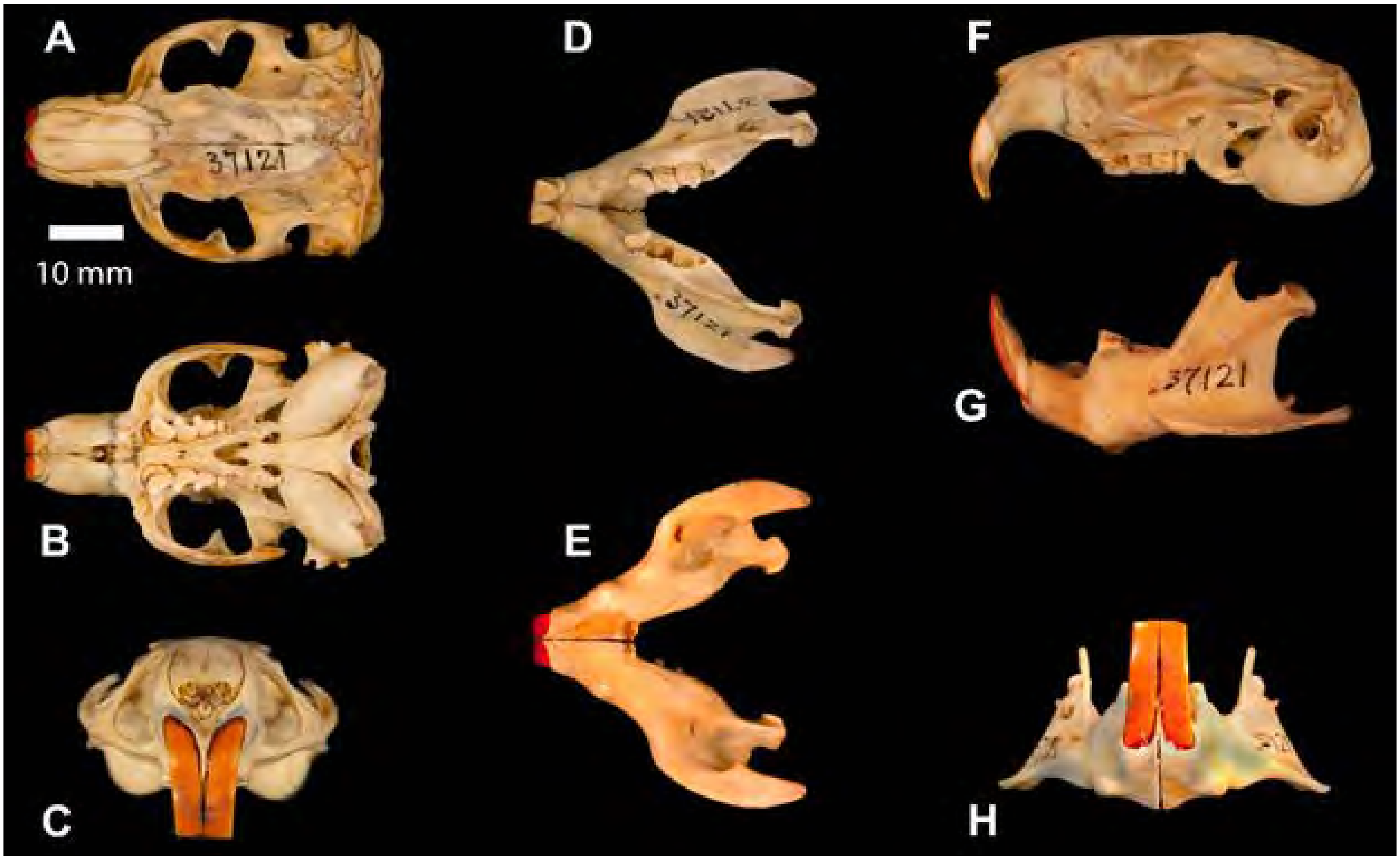

Figure 7. A-H. Photo images of the skull and jaw of *Ctenomys nattereri* Wagner 1848, Natterer's Tuco-tuco or tuco-tuco de Natterer. A. Dorsal view shows the robust skull. B. Ventral view of the skull. C. Frontal view of the skull, note dark colored upper incisors. D. Dorsal view of the jaw. E. Ventral view of the jaw. F. Left lateral view of the skull. G. Left lateral view of the jaw. H. Frontal view of the jaw displaying the dark incisors.

Ctenomys yatesi Gardner *et al.* 2014

(Figure 8 A, C, F - I, Map Figure 10)

Common Name: Yates' tuco-tuco, tuco-tuco de Yates.

Morphology: (n = 3) Body length 209 ; tail 58; hind foot 32; ear 4; weight 96 g. This species possesses a dense, fine, soft fur; dorsum with fur ranging from an ochraceous orange to buckthorn brown, hairs dark colored, except last 2.5 or 3 mm which are a distinctly lighter shade of the same color. Most individuals present a dark cap (Fuscous Black) on their heads. Color of ventral pelage is more sharply set off from dorsal in some individuals. Ears sparsely covered with short, brownish hairs, not contrasting conspicuously with color of head. Forefoot with a well-developed pollex, other four digits bearing long, basally closed claws. The skull is robust and wide. The interorbital region has squared margins and the frontals are widest at the level of connection of zygomatic arch, lateral to the posterior/anterior sutures of the frontals, nasals and maxillaries.

Distribution: *Ctenomys yatesi* is known only from the type locality, 7 kilometers north and 38 kilometers west of Roboré in southeastern Santa Cruz Department (Figure 10).

Natural history: Nothing is known of the natural history of Yates' tuco-tuco. The habitat was in fields of Yucca.

Species Conservation/Preservation Status: The species has not yet been evaluated by the IUCN or LRFSV. There have not been evident agriculturally based large-scale land-cover changes around the type locality of *C. yatesi* (Table 2), and only about 13 % of the surrounding area is protected and includes only the Reserva Departamental Valle de Tucavaca (Caraballo *et al.* 2020). Nonetheless, because it appears that this species has a very restricted distribution, close monitoring is warranted.

Results of species distribution modeling for 5 species of Bolivian Ctenomys *for changes in habitat suitability under greenhouse gas emission scenarios 4.5 and 8.5.* For the 5 species of tuco-tucos for which we had sufficient data (*i. e*., numbers of individuals collected throughout the range of the species) with which to produce SDMs including *C. opimus, C. boliviensis, C. conoveri, C. frater*, and *C. steinbachi*, the model results were robust and statistically significant as evaluated by the pROC metric (Table 1). The habitat suitability approximation out to year 2070 was made using the median projected suitability (MPS). The MPS enabled us to predict suitable habitat for the species with different levels of global greenhouse gas emission. Using this aspect of the model we evaluated the effect of climatic and soil-related variables on potential distribution of these species (see figures 11 and 12).

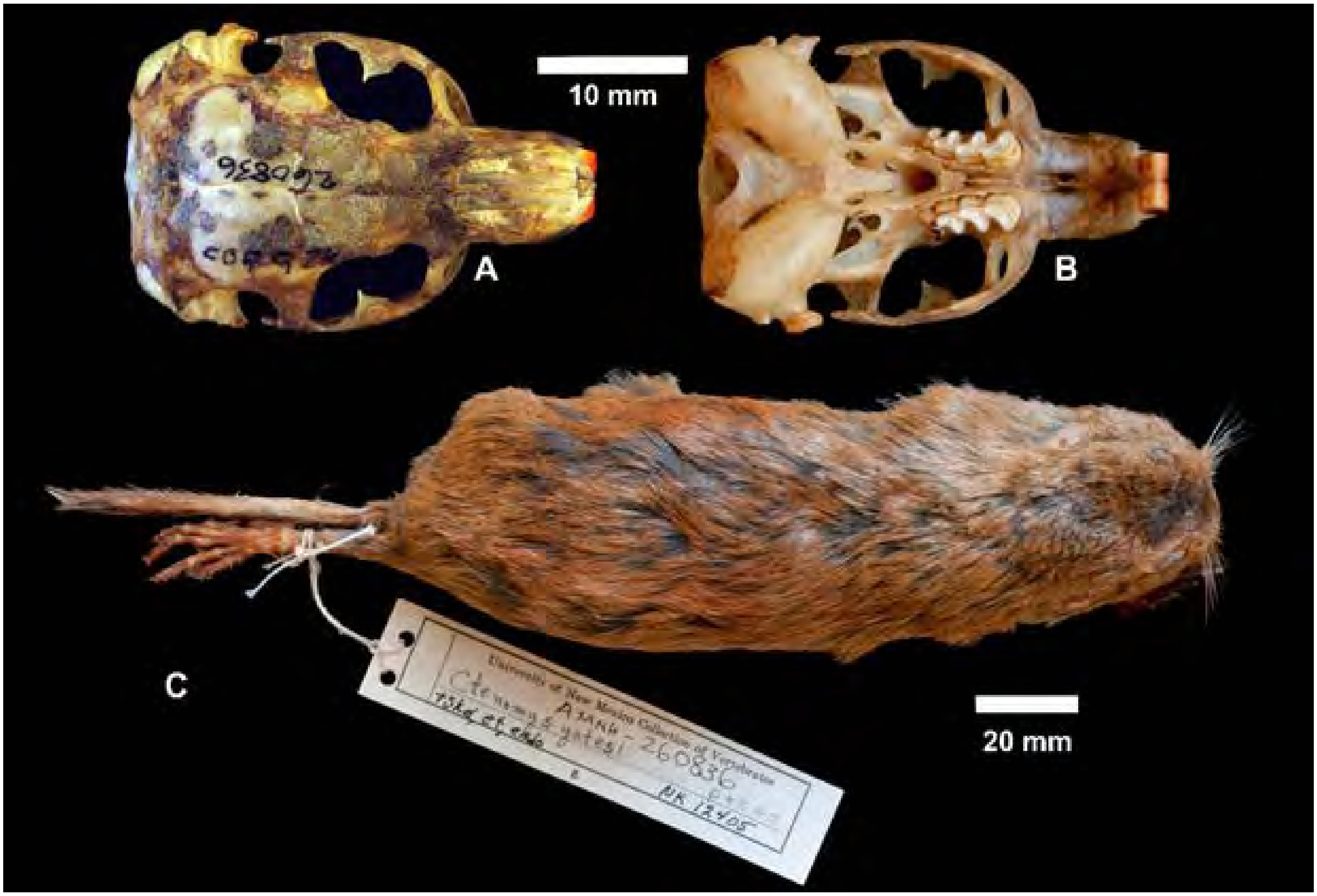

Figure 8. A-C. *Ctenomys yatesi* Gardner *et al.* 2014, Yates' tuco-tuco, el Tuquito de Yates or Yates' Tuquito. A. Photo image of dorsal aspect of skull. B. Photo image of ventral view of skull. C. Photo image of dorsal aspect of museum study skin.

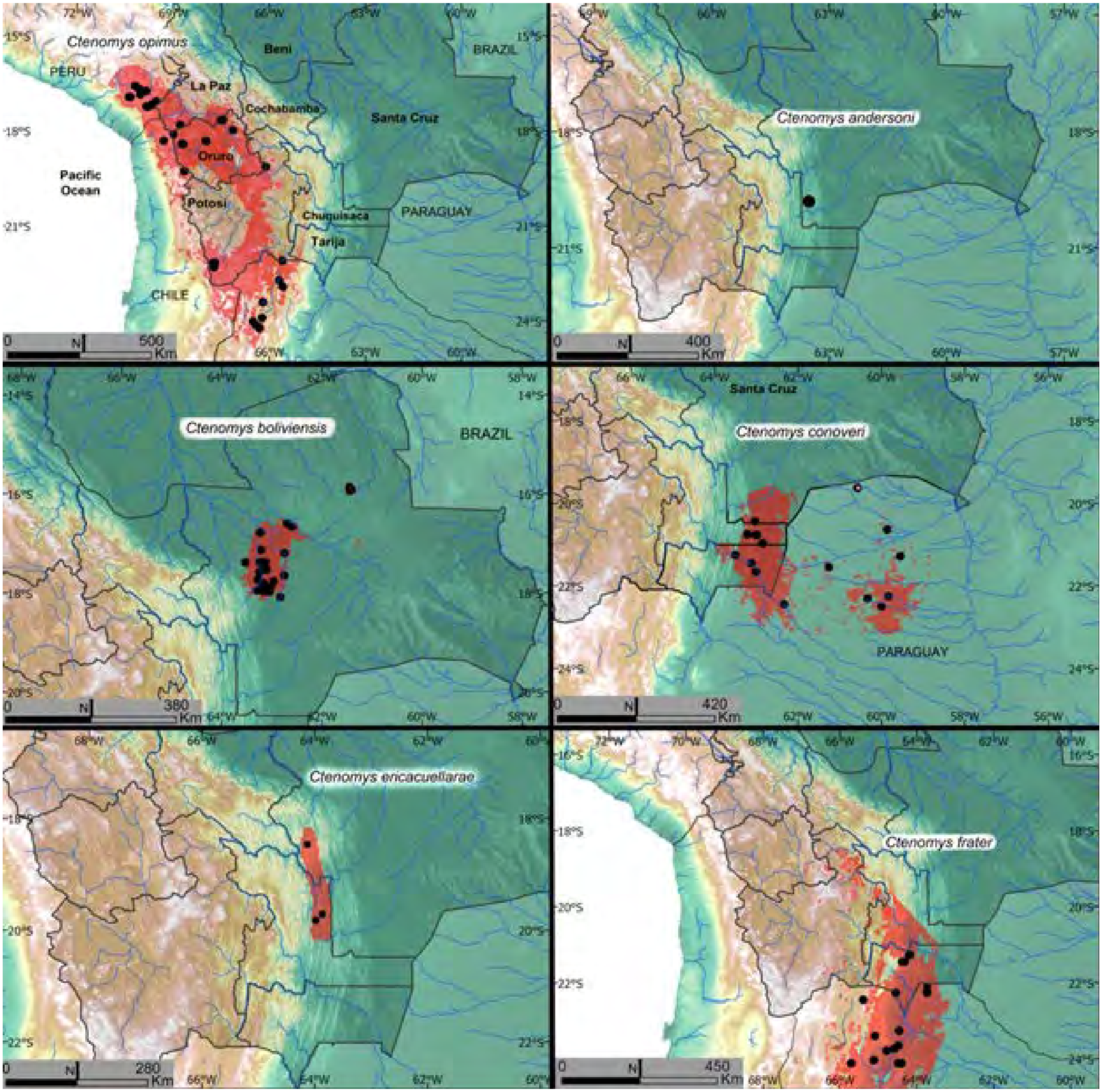

Figure 9. Estimated distribution of *Ctenomys* species of Bolivia. Black dots represent verified museum specimen collection locality records. Red-shaded areas represent the estimated distribution at the current time. Species names are included in each map. *Ctenomys opimus* is included first in the maps because this species has the largest geographic range, extending from southern Peru south through Bolivia, Chile, and northern Argentina. All departments in Bolivia are included on this map for comparison with the rest of the maps presented.

Discussion

An altitude-area optimization (not shown) onto the tree indicates that the probable common ancestor of the *Ctenomys* now occurring in Bolivia had an origin in high altitude areas of the Andes. It appears that tuco-tuco populations have been moving into open habitats, becoming isolated, re-united, and isolated again at geological time scales in the continually changing and evolving landscape of the central-eastern Andes of Bolivia. The greatest single factor that appears to be influencing the evolutionary trajectory of these rodents in the mountains is a component of the Andes called the Central Andean Backthrust belt (McQuarrie *et al.* 2005) that has created a dynamic erosional landscape resulting in incredibly diverse habitats ranging from cloud forest to cactus studded hillsides and valleys over only a few kilometers of straight-line geographic distance (Gardner *et al.* 2014; Unzueta 1975). Gardner and Ruedas (2019) showed, via optimization of occupied altitude of tuco-tuco species onto the phylogenetic tree of the ctenomyid species in Bolivia, that there was a striking correlation between the phylogeny and the age of origin of the eastern front-range mountain ridges of the Andes. The crustal folding from west to east combined with simultaneous and continuous erosion and riverine cutting of the north-south ridges appears to have resulted in episodes of repeated isolation and subsequent speciation in these rodents.

By applying species distribution models to predict suitable habitat for the known species of tuco-tucos in Bolivia within their range of distribution and evaluating the effect of climatic and soil-related variables on potential species distribution, we found that all the models resulted in robust predictions as indicated by the pROC ratios (Table 1). Not surprisingly, because of the fossorial/subterranean habit of these mammals, we found that soil variables have excellent predictive power and performed well in two of the five species for which their distributions were modeled, showing little difference in contribution to model performance with bioclimatic variables. We found higher average weight for the models when we including soil variables, indicating the utility of using these variable in the understanding of the habitat requirements of *Ctenomys* and probably other species of subterranean animals (see Figures 11 and 12).

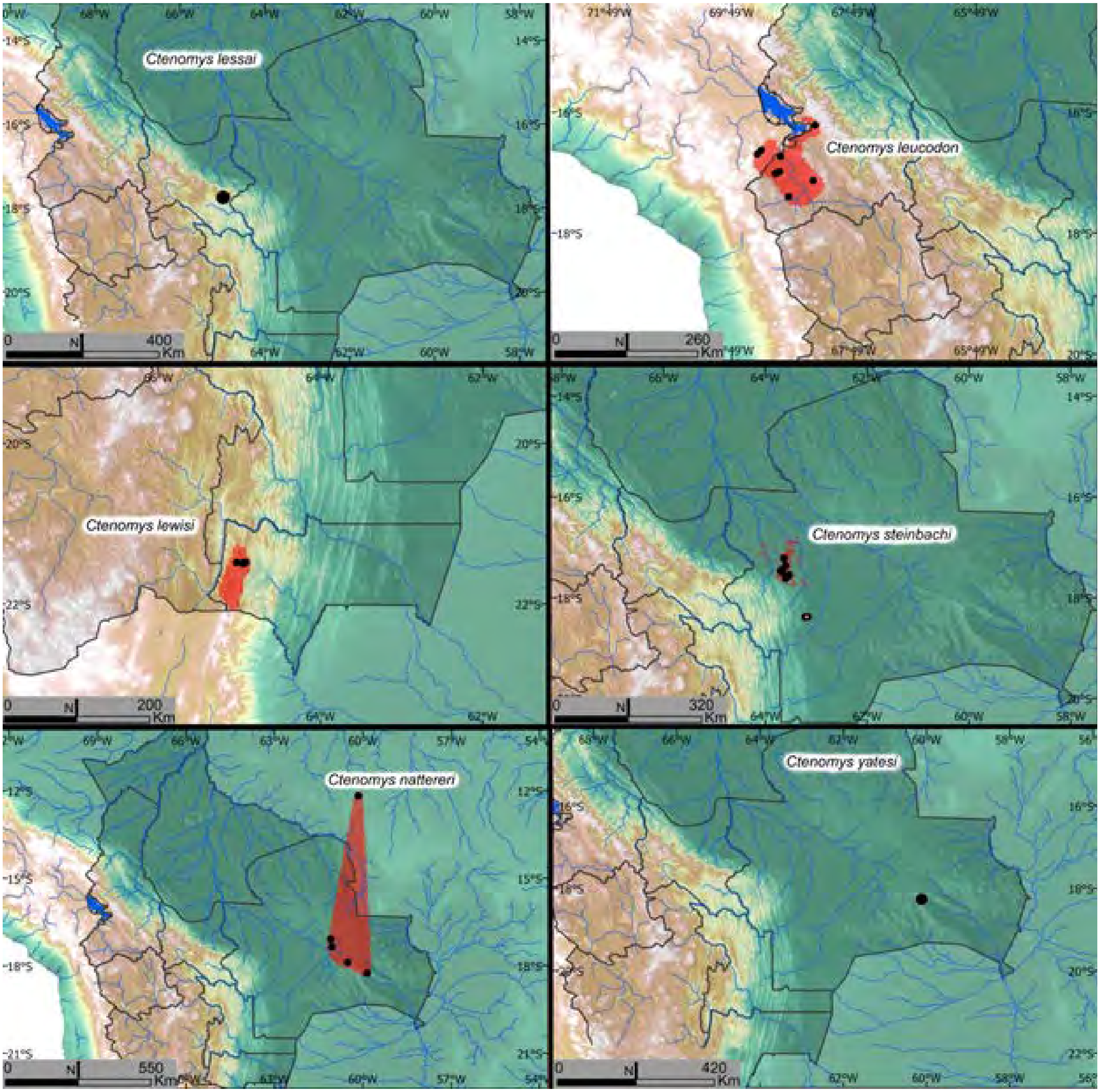

Figure 10. Estimated distribution of *Ctenomys* species of Bolivia. Black dots represent specimen collection localities. Red shaded areas represent the estimated distribution. Species names are included in each map.

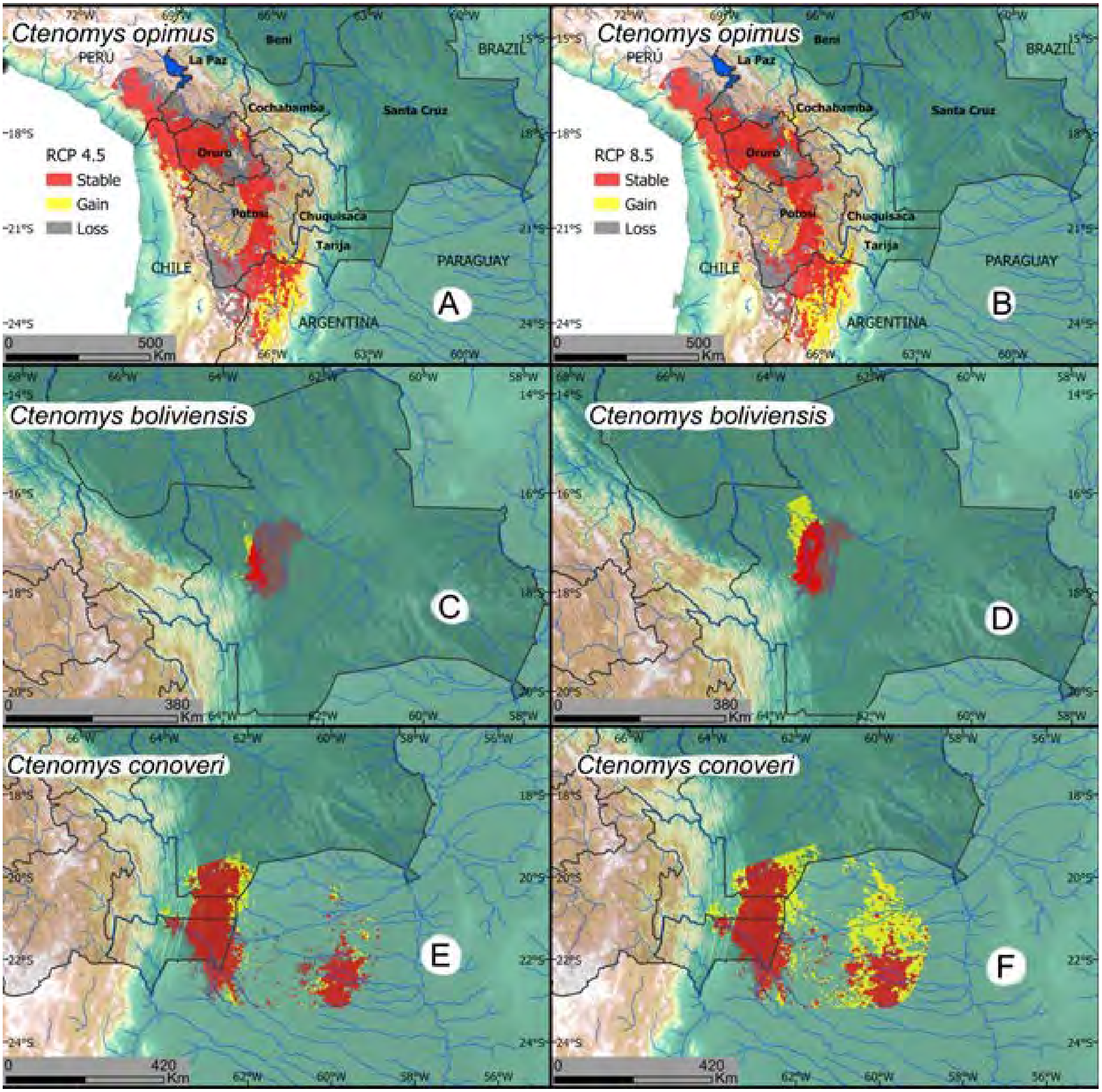

Figure 11. Projected changes in habitat suitability for 2070 under the 4.5 and 8.5 greenhouse gases emission scenarios. The 2070 suitable habitat is estimated for an assemble model employing the median projected suitability from 17 general circulation models. A. Predicted changes in habitat suitability for *C. opimus* under the 4.5 emission scenario. B. Predicted changes in habitat suitability for *C. opimus* under the 8.5 emission scenario. C. Predicted changes in habitat suitability for *C. boliviensis* under the 4.5 emission scenario. D. Predicted changes in habitat suitability for *C. boliviensis* under the 8.5 emission scenario. E. Predicted changes in habitat suitability for *C. conoveri* under the 4.5 emission scenario. F. Predicted changes in habitat suitability for *C. conoveri* under the 8.5 emission scenario.

Three of the five species that were model using ENM are expected to suffer significant range contractions by the year 2070 under both of the global carbon emission scenarios evaluated herein. Two of these species occur in the lowlands of the department of Santa Cruz (*C. steinbachi* and *C. boliviensis*) and one on the altiplano (*C. opimus*). Nonetheless, the extrapolations of suitable habitat distributions for the year 2070 should be considered with care as these estimates assume that the species will not adapt to new environments, perhaps because of complete niche conservatism, and that future environmental conditions nature are predicted to be analogous or comparable to current conditions (Elith and Leathwick 2009).

This study highlights the need for detailed conservation assessments and monitoring of tuco-tuco's in Bolivia as most of the species have already, or will soon experience, significant loss in habitat with concomitant fragmentation of their populations due to anthropogenic land cover change exacerbated by rapidly changing climatic conditions. In addition to these factors, the known geographic ranges of Bolivian tuco-tuco species (except for *C. opimus*) are small in spatial geographic extent, with three species known only from their type localities and in seven cases, a very small proportion of their estimated or actual distribution range is in some sort of protected area.

The analysis presented here suggests that *C. steinbachi* (because of its restricted range) and perhaps *C. boliviensis* should be moved into a higher IUCN threat category than their current IUCN allocation. This higher IUCN category is suggested because more than 20 % of their original geographic ranges have been significantly transformed by human activities. The anthropogenically mediated destruction of their habitats combined with predicted range contractions due to global warming portends the impending ecological and biodiversity catastrophe that is now taking place world-wide (Ceballos *et al.* 2020). In addition to these factors, the currently known geographic ranges of both *C. steinbachi* and *C. boliviensis* show very little overlap with any true protected areas (<5 %) and because much of the geographic ranges of these two species are near or in path of the rapidly expanding urban area of Santa Cruz de la Sierra, we expect additional pressure especially on these two species.

As presented here, the uses of ENMs and SDMs combined with broad-scale and intensive biological surveys of the mammalian fauna of Bolivia (Anderson 1997) will enable future students of biodiversity to have a basis for additional focussed studies. As the atmosphere of our earth continues to heat due to anthropogenically generated greenhouse gasses, unexpected effects on the species of mammals on the earth will be manifested (Ceballos *et al.* 2020). We hope that this work on documenting and eventually providing an

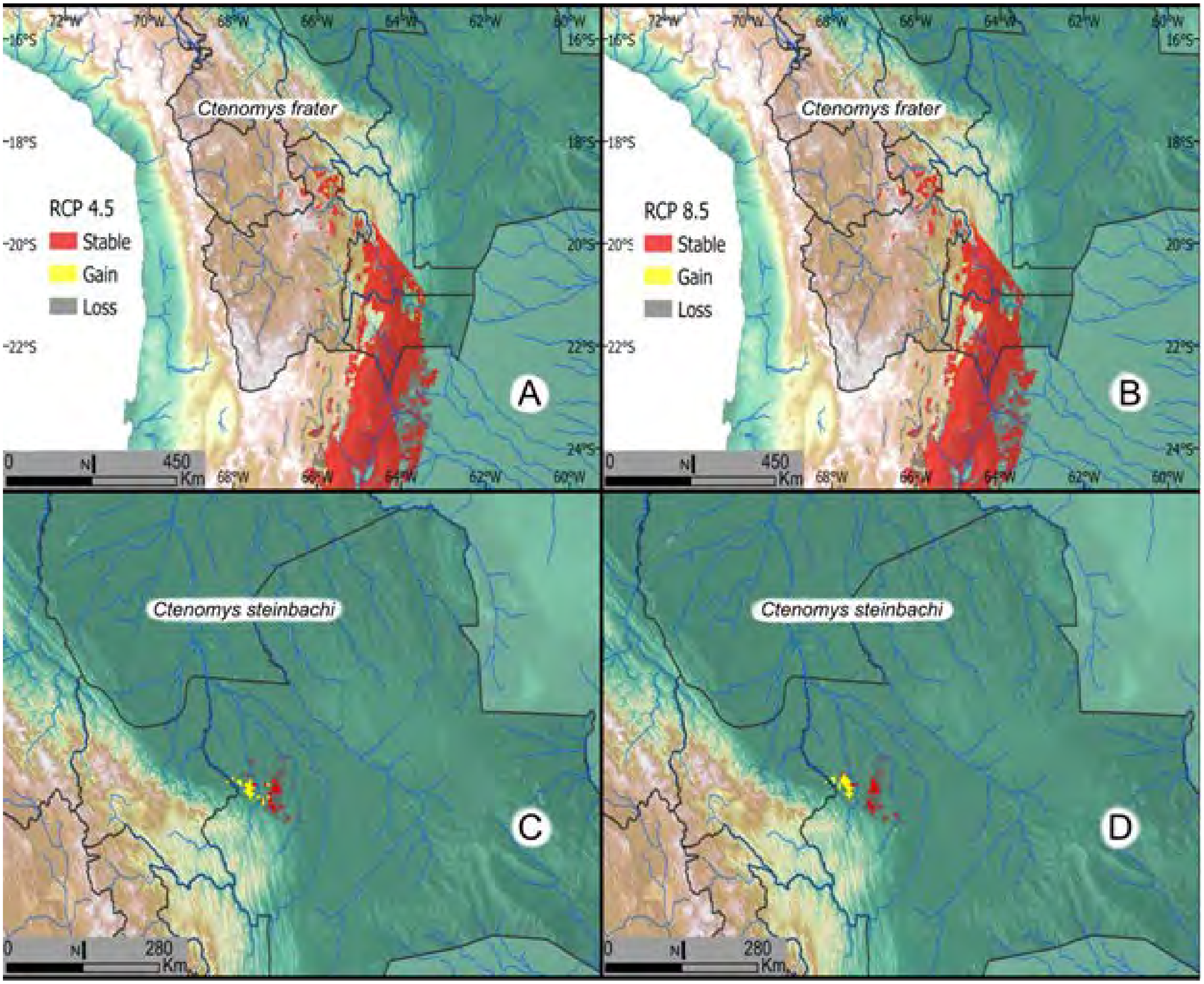

Figure 12. Projected changes in habitat suitability for 2070 under the 4.5 and 8.5 greenhouse gases emission scenarios. The 2070 suitable habitat is estimated for an assemble model employing the median projected suitability from 17 general circulation models. A. Predicted changes in habitat suitability for *C. frater* under the 4.5 emission scenario. B. Predicted changes in habitat suitability for *C. frater* under the 8.5 emission scenario. C. Predicted changes in habitat suitability for *C. steinbachi* under the 4.5 emission scenario. D. Predicted changes in habitat suitability for *C. steinbachi* under the 8.5 emission scenario.

understanding of the delicate balances of how mammals interact with their environment over geographic space will provide a model for future research. At the very least, this work will provide a baseline from which additional studies can be formulated, proposed, funded, and carried out. In his "Mammals of Bolivia," Syd Anderson (1997, p 3) stated about his book: "This work is dedicated to the hypothesis-testers of this world. Everything concluded here is subject to further testing. I expect much of this information to be substantiated, but not everything." We consider the current work to cohere to the way Syd approached his studies and our hypotheses, presented herein, and because they are based on museum specimens, will be available for testing far into the future. We feel that the prospects of viable biodiversity on our earth will be sustained if we are able to recruit new researchers walking the path of Syd and testing some of the hypotheses presented herein and generating new hypotheses along the way.

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Literature cited

Aiello-lammens, M. E., R. A. Boria, A. Radosavljevic, B. Vilela, and R.P. Anderson. 2015. spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. Ecography 38: 541–545.

Anderson, S., T. L. Yates, and J. A. Cook. 1987. Notes on Bolivian mammals 4: the genus *Ctenomys* (Rodentia: Ctenomyidae) in the eastern lowlands. American Museum Novitates 2891:1-20.

Anderson, S. 1997. Mammals of Bolivia: taxonomy and distribution. Bulletin of the AMNH 231 pp.

Ashe, J. S., R. M. Timm, and M. H. Gallardo. 1996. Systematics, distribution, and host specificity of *Edrabius fauvel* (Insecta: Coleoptera: Staphylinidae). Proceedings of the Biological Society of Washington 109:731-743.

Barve, N., V. Barve, A. Jiménez-valverde, A. Lira-noriega, S.P. Maher, A.T. Peterson, J. Soberón, and F. Villalobos. 2011. The crucial role of the accessible area in ecological niche modeling and species distribution modeling. Ecological Modelling 222:1810–1819.

Bidau, C. J. 2006. Familia Ctenomyidae. Pp. 212 -231, *in* Mamíferos de Argentina. Sistemática y distribución (Bárquez,R. J., M. M. Díaz, and R. A. Ojeda, eds.). Sociedad Argentina para el Estudio de los Mamíferos. Tucumán, Argentina.

Bidau, C. J. 2015. Family Ctenomyidae Lesson, 1842. In: Mammals of South America 2:818-877.

Brant, S. V., and S .L. Gardner. 1997. Two new species of *Litomosoides* (Nemata: Onchocercidae) from *Ctenomys opimus* (Rodentia: Ctenomyidae) on the Altiplano of Bolivia. Journal of Parasitology 83:700-705.

Breiner, F. T., A. Guisan, A. Bergamini, and M. P. Nobis. 2015. Overcoming limitations of modelling rare species by using ensembles of small models. Methods in Ecology and Evolution 6:1210– 1218.

Breiner, F. T., M. P. Nobis, A. Bergamini, and A. Guisan. 2018. Optimizing ensembles of small models for predicting the distribution of species with few occurrences. Methods in Ecology and Evolution 9:802-808.

Busch, C., C. D. Antinuchi, J. C. del Valle, M. J. Kittlein, A. I. Malizia, A. I. Vassallo, and R. R. Zenuto. 2000. Population ecology of subterranean rodents. Pp 183–226, *in* Life Underground: The Biology of Subterranean Rodents (Lacey E. A., J. L. Patton, and G. N. Cameron, eds). University of Chicago Press. Chicago, Illinois.

Caldas, M. M., D. Goodin, S. Sherwood, J. M. Campos Krauer, and S. M. Wisely. 2015. Land-cover change in the Paraguayan Chaco: 2000–2011. Journal of Land Use Science 10: 1-8.

Campbell, L.P., C. Luther, D. Moo-llanes, J. M. Ramsey, R. Danis-lozano, and A. T. Peterson. 2015. Climate change influences on global distributions of dengue and chikungunya virus vectors. Philosophical Transactions of the Royal Society B: Biological Sciences 370:20140135

Caraballo, D. A., S. L. López, A. A. Carmarán, and M. S. Rossi. 2020. Conservation status, protected area coverage of *Ctenomys* (Rodentia, Ctenomyidae) species and molecular identification of a population in a national park. Mammalian Biology 100:33-47.

Castillo, A., M. Cortinas, and E. P. Lessa. 2005. Rapid diversification of South American tuco-tucos (*Ctenomys*: Rodentia: Ctenomyidae): Contrasting mitochondrial and nuclear intron sequences. Journal of Mammalogy 86:170–179.

Ceballos, G., P. R. Ehrlich, and P. H. Raven. 2020. Vertebrates on the brink as indicators of biological annihilation and the sixth mass extinction. Proceedings of the National Academies of Sciences of the United States 117:13596-13602.

Cobos, M. E., A. T. Peterson, N. Barve, and L. Osorio-Olvera. 2019. kuenm: an R package for detailed development of ecological niche models using Maxent. PeerJ 7:e6281.

Cook, J. A., S. Anderson, and T. L. Yates. 1990. Notes on Bolivian mammals 6: the genus *Ctenomys* (Rodentia: Ctenomyidae) in the highlands. American Museum Novitates 2980:1–27.

Cook, J. A., and T. L. Yates. 1994. Systematic relationships of the tuco-tucos, genus *Ctenomys* (Rodentia: Octodontidae). Journal of Mammalogy 75: 583–599.

Cook, J. A., and E. P. Lessa. 1998. Are rates of diversification in subterranean South American tuco-tucos (genus *Ctено-*

mys, Rodentia: Octodontidae) unusually high? Evolution 52:1521–1527.

Darwin, C. R. 1860. Journal of researches into the natural history & geology of the countries visited during the voyage round the world of H.M.S. 'Beagle' under the command of captain Fitz Roy, R. N. John Murray. Albemarle St., London.

Di Cola, V., O. Broennimann, B. Petitpierre, F.T. Breiner, M. D'amen, C. Randin, R. Engler, J. Pottier, D. Pio, A. Dubuis, L. Pellissier, R. G. Mateo, W. Hordijk, N. Salamin, and A. Guisan. 2017. Ecospat: an R package to support spatial analyses and modeling of species niches and distributions. Ecography 40:774–787.

Drabik, G. O., and S. L. Gardner. 2019. A new species *Ancylostoma* (Nemata: Strongylida: Ancylostomatidae) from two species of *Ctenomys* in lowland Bolivia. Journal of Parasitology 105:904-912.

Dunnum, J., and N. Bernal. 2016. *Ctenomys boliviensis* (errata version published in 2017). The IUCN Red List of Threatened Species 2016: e.T5798A115079917. https://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T5798A22192171.en. Downloaded on 03 May 2020.

Dunnum, J., J. Vargas, N. Bernal, N. Zeballos, and E. Vivar. 2016. *Ctenomys opimus*. The IUCN Red List of Threatened Species 2016: e.T5818A22194246. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T5818A22194246.en. Downloaded on 06 June 2020.

Eisenberg, J. F. 1963. The behavior of heteromyid rodents. University of California Publications in Zoology 69: 1-114.

Elith, J., and J. R. Leathwick. 2009. Species distribution models: Ecological explanation and prediction across space and time. Annual Review of Ecology, Evolution, and Systematics 40:677–697.

Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37:4302-4315.

Fourcade, Y., J. O. Engler, D. Rödder, and J. Secondi. 2014. Mapping species distributions with MAXENT using a geographically biased sample of presence data: A performance assessment of methods for correcting sampling bias. PLoS One 9: e97122.

Freitas, T. R. O. 2006. Cytogenetics status of four *Ctenomys* species in the south of Brazil. Genetica 126:227–235.

Gardner, S. L. 1991. Phyletic coevolution between subterranean rodents of the genus *Ctenomys* (Rodentia: Hystricognathi) and nematodes of the genus *Paraspidodera* (Heterakoidea: Aspidoderidae) in the Neotropics: temporal and evolutionary implications. Zoological Journal of the Linnean Society 102:169-201.

Gardner, S. L, and D.W. Duszynski. 1990. Polymorphism of Eimerian Oocysts can be a problem in naturally infected hosts: An example from subterranean Rodents in Bolivia. Journal of Parasitology 76:805-811.

Gardner, S. L. and L. Ruedas. 2019. Origin and development of the South American montane mammal fauna by the Andean speciation engine. Abstract 197: 81. http://www.mammalsociety.org/uploads/2019%20ASM%20Abstracts-2019.06.18.pdf

Gardner, S. L. 1991. Phyletic coevolution between subterranean rodents of the genus *Ctenomys* (Rodentia: Hystricognathi) and nematodes of the genus *Paraspidodera* (Heterakoidea: Aspidoderidae) in the Neotropics: temporal and evolutionary implications. Zoological Journal of the Linnean Society 102:169-201.

Gardner, S. L., and S. Anderson. 2001. Persistent fontanelles in rodent skulls. American Museum Novitates 3327:1–15.

Gardner, S. L., J. Salazar-bravo, and J. A. Cook. 2014. New Species of *Ctenomys* Blainville 1826 (Rodentia: Ctenomyidae) from the lowlands and central valleys of Bolivia. Special Publications, Museum of Texas Tech University 62:1-34.

Hengl, T., G. B. Mendes de Jesus, M. Heuvelink, M. R. Gonzalez, M. Kilibarda, A. Blagotić, W. Shangguan, M. N. Wright, X. Geng, B. Bauer-Marschallinger, M. A. Guevara, R. Vargas, R. A. MacMillan, N. H. Batjes, J. G. B. Leenaars, E. Ribeiro, I. Wheeler, S.Mantel, and B. Kempen. 2017. SoilGrids250m: Global gridded soil information based on machine learning. Plos One 12:e0169748.

Ibisch, P., S. Beck, B. Gerkmann, and Y.A. Carretero. 2003. Ecoregiones y ecosistemas. Pp. 47–88, *in* Biodiversidad: La Riqueza de Bolivia (Ibisch, P., and G. Mérida, eds.). FAN. Santa Cruz, Bolivia.

IUCN. 2020. The IUCN Red List of Threatened Species. Version 2020-1. https://www.iucnredlist.org. Downloaded on 20 May 2020.

Lacey, E. A., J. L. Patton, and G. N. Cameron. 2000. Life underground: the biology of subterranean rodents. University of Chicago Press. Chicago, Illinois.

Lambert, C. R., S. L. Gardner, and D. W. Duszynski. 1988. Coccidia (Apicomplexa: Eimeriidae) from the subterranean rodent *Ctenomys opimus* Wagner (Ctenomyidae) from Bolivia, South America. Journal of Parasitology 74:1018-1022.

Lessa, E. P., and J. A. Cook. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Molecular Phylogenetics and Evolution 9:88–99.

López, R. P. 2003. Phytogeographical relations of the Andean dry valleys of Bolivia. Journal of Biogeography 30:1659–1668.

Mascheretti, S., P. Mirol, M. Giménez, C. Bidau, J. Contreras, and J. Searle. 2000. Phylogenetics of the speciose and chromosomally variable genus *Ctenomys* (Ctenomyidae: Octodontoidea), based on mitochondrial cytochrome-b sequences. Biological Journal of the Linnean Society 70: 361–376.

Mcquarrie, N., B. K. Horton, G. Zandt, S. Beck, and P. G. Decelles. 2005. Lithospheric evolution of the Andean fold-thrust belt, Bolivia, and the origin of the central Andean plateau. Tectonophysics 399:15–37.

Ministerio De Medio Ambiente y Agua. 2009. Libro rojo de la fauna silvestre de vertebrados de Bolivia. Ministerio de Medio Ambiente y Agua. La Paz, Bolivia.

Muscarella, R., P. J. Galante, M. Soley-guardia, R. A. Boria, J. M. Kass, M. Uriarte, and R. P. Anderson. 2014. ENM eval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for Maxent ecological niche models. Methods in Ecology and Evolution 5:1198-1205.

Olrog, C. C., and M. M. Lucero. 1981. Guía de los mamíferos argentinos. Ministerio de Cultura y Educación, Tucumán: Fundación Miguel Lillo.

Parada, A. S., G. D'Elía, C. J. Bidau, and E. P. Lessa. 2011. Species groups and the evolutionary diversification of tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). Journal of Mammalogy 92:671–682.

Pearson, O. P. 1959. Biology of the subterranean rodents, *Ctenomys*, in Peru. Memorias del Museo de Historia Natural Javier Prado 9:3-56.

Phillips, S. J., and M. Dudík. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31:161–175.

Pucu, E., M. Lareschi, and S. L. Gardner. 2014. Bolivian ectoparasites: a survey of the fleas of *Ctenomys* (Rodentia: Ctenomyidae). Comparative Parasitology 81:114-118.

Raxworthy, C. J., E. Martinez-meyer, N. Horning, R. A. Nussbaum, G. E. Schneider, M. A. Ortega-huerta, and A. T. Peterson. 2003. Predicting distributions of known and unknown reptile species in Madagascar. Nature 426:837-841.

Ridgway, R. 1912. Color standards and color nomenclature. Published by the author, A. Hoen (printer). Baltimore, Maryland. 53 colored plates.

Roach, N., and L. Naylor. 2016a. *Ctenomys lewisi*. The IUCN Red List of Threatened Species 2016: e.T5811A22193810. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T5811A22193810.en. Downloaded on 06 June 2020.

Roach, N., and L. Naylor. 2016b. *Ctenomys steinbachi*. The IUCN Red List of Threatened Species 2016: e.T5827A22194994. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T5827A22194994.en. Downloaded on 12 June 2020.

Teta, P., and G. D'Elía. 2020. Uncovering the species diversity of subterranean rodents at the end of the World: Three new species of Patagonian tuco-tucos (Rodentia, Hystricomorpha, *Ctenomys*). PeerJ. 2020;8:e9259. Published 2020 May 29. doi:10.7717/peerj.9259

Thomas, O. 1919. Two new rodents from Tartagal, Salta, N. Argentina. Annals and Magazine of Natural History, ser. 9, 4: 154-156.

Thomas, O. 1921. A new tuco-tuco from Bolivia. Annals and Magazine of Natural History, ser. 9, 7:136–137.

Thomas, O. 1926. The Spedan Lewis South American exploration, II: On mammals collected in the Tarija Department, southern Bolivia. Annals and Magazine of Natural History, ser. 9, 17:318–328.

Unzueta, Q. O. 1975. Mapa ecológico de Bolivia. 1:1,000,000. Ministerio de Asuntos Campesinos y Agropecuarios, Memoria explicativa by Unzueta, 11 pp. Color pl., 312 pp.

Vivar, E. 2017. *Ctenomys frater* (errata version published in 2018). The IUCN Red List of Threatened Species 2017: e.T115553730A123796865. https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T115553730A115553734.en. Downloaded on 04 June 2020.

Wallace, A. R. 1876. The geographical distribution of animals. With a study of the relations of living and extinct faunas as elucidating the past changes of the earth's surface. Volumes 1 and 2. Macmillan and Co. London, U.K.

Wilson, D. E., and D. M. Reeder (eds.). 2005. Mammal Species of the World, a Taxonomic and Geographic Reference, 3rd ed. Johns Hopkins Press, Baltimore, Maryland.

Wilson, D. E., T. E. Lacher Jr., and R. A. Mittermeier (eds.). 2017. The Mammals of the World-Vol. 7, Rodents II. Lynx Edicions. Barcelona, España.

Zeballos, H., and E. Vivar . 2016. *Ctenomys leucodon*. The IUCN Red List of Threatened Species 2016: e.T5810A22193526. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T5810A22193526.en. Downloaded on 05 June 2020.

Zurell, D., J. Franklin, C. König, P. J. Bouchet, C. F. Dormann, J. Elith, G. Fandos, X. Feng, G. Guillera-Arroita, A. Guisan, and J. J. Lahoz-Monfort. 2020. A standard protocol for reporting species distribution models. Ecography 43:1261-1277.

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Conservation status and natural history of *Ctenomys,* tuco-tucos in Bolivia

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The genus *Ctenomys* consists of about 70 species and in addition to the Geomyidae of the Nearctic, Neotropical tuco-tucos represent a well-documented case of diversification in the subterranean biotype. Here we will: i) Provide an updated summary of the natural history of the 12 species of extant tuco-tucos from Bolivia; ii) Update information on distributions of each species; and iii) Using ecological niche modeling, evaluate recent and projected habitat transformation or habitat degradation within the known range of each species to provide a preliminary assessment of the preservation or conservation status of ctenomyids within Bolivia. We follow Gardner *et al.* (2014) and combine species summaries with both updated published and new data to compile a complete list of known extant species of tuco-tucos from Bolivia. Occurrence data for *Ctenomys* in Bolivia and surrounding areas were extracted from the database Arctos and GBIF. All individual specimen-based locality records were checked and georeferenced by referring to original museum collection records. We created species distribution models for the species with enough locality records using climate and soil data, while for the rest of the species we estimated the ranges based on the known occurrence localities. Finally, we quantified the amount of large-scale habitat conversion occurring within each species range, as well as the potential effect of climatic change on species distribution. Here we present information regarding the biology of tuco-tuco (*Ctenomys*) species known to occur in Bolivia, including unpublished natural history data such as habitat association, interactions and activity patterns gathered by the authors through extensive field work. Besides this, we estimated the current distribution of *Ctenomys* species, quantified large-scale habitat transformation within each species range and assessed the potential effect of climatic change on five tuco-tuco species. We found that the habitats within the ranges of *C. boliviensis* and *C. steinbachi* have experienced significant land-cover conversions in recent years. We also show that *C. opimus*, as well as the above mentioned species are expected to undergo range contractions resulting from climatic change by 2070. Our review shows that there is a dearth of information regarding natural history, taxonomy and distribution for many Bolivian tuco-tuco species. Nonetheless, the information presented here can be a tool for directing and focusing field studies of these species. This is of great importance if we take into account that most of the Bolivian tuco-tucos are subject to one or several conservation/preservation threats. These include: Habitat destruction via land use or climatic changes in conjunction with geographic ranges of *Ctenomys* that are small in areal extent and which in many cases are not adequately covered by protected areas.

El género *Ctenomys* cuenta con alrededor de 70 especies. Al igual que la familia Geomyidae en el Neártico, los tuco-tucos representan un claro caso de diversificación adaptativa al biotipo subterráneo en el Neotrópico. En este trabajo presentamos: i) una síntesis de la historia natural de las 12 especies actuales de *Ctenomys* registradas en Bolivia; ii) información actualizada de su distribución; y iii) una evaluación de la degradación del hábitat dentro del rango de cada especie con el fin de brindar una evaluación preliminar del estado de conservación de los tuco-tucos de Bolivia. A partir del trabajo de Gardner *et al.* (2014), información publicada y nuevos datos, compilamos una lista completa de las especies de tuco-tucos (*Ctenomys*) de Bolivia junto con la información disponible sobre su biología e historia natural. Las localidades de ocurrencia de las especies en Bolivia fueron obtenidas de las bases de datos GBIF y Arctos. Toda la información sobre las localidades de ocurrencia fue posteriormente depurada y georreferenciada, haciendo referencia a los registros originales de las colecciones de museos. Adicionalmente, creamos modelos de distribución para las especies con suficientes registros. Para las especies menos colectadas, estimamos la distribución a partir de las localidades de ocurrencia e información sobre su hábitat. Finalmente, cuantificamos la transformación del hábitat ocurrida dentro de los rangos de cada especie, al igual que el efecto potencial del cambio climático sobre su distribución. Presentamos un resumen del conocimiento de la biología de cada una de las especies que ocurren en Bolivia, incluyendo datos no publicados de historia natural colectados por los autores durante varias décadas de trabajo de campo. Entre estos nuevos aporte se encuentran descripciones de hábitat, interacciones y patrones de actividad. Adicionalmente, presentamos estimaciones de la distribución, cuantificamos la conversión a gran escala del hábitat y evaluamos el efecto potencial del cambio climático sobre las especies de tuco-tucos en Bolivia. Encontramos que los rangos de distribución de *C. boliviensis* y *C. steinbachi* han experimentado importantes cambios en las coberturas vegetales naturales y seminaturales en años recientes. Adicionalmente proyectamos que la distribución de estas dos especies, junto con la de *C. opimus* experimentarán contracciones importantes como consecuencia del cambio climático para el año 2070. La información revisada en este trabajo muestra que hay importantes vacíos de conocimiento con respecto a la historia natural, taxonomía y distribución en la mayoría de especies de tuco-tucos de Bolivia. Sin embargo, la información presentada en este trabajo puede servir como herramienta para guiar trabajos de campo sobre estas especies. Esto es de gran importancia si se tiene en cuenta que la mayoría de las especies de *Ctenomys* que ocurren en Bolivia presentan uno o varios factores que amenazan su conservación, como la pérdida de hábitat por transformaciones de las coberturas, el cambio climático, y los rangos de distribución pequeños y con mínima representación en áreas protegidas.

Keywords: Bolivia; Caviomorpha; climate change; Ctenomyidae; *Ctenomys;* Hystricomorpha; land use change; natural history; species distribution model; subterranean rodents.

Introduction

Rodents are the most speciose of all orders of mammals, having a cosmopolitan distribution with extant autochthonous species on all continents except Antarctica (Wilson and Reeder 2005). At the current time, even though new species of mammals are being described at a fairly high rate globally, many forms remain unknown and undescribed and the natural history of most known species is still obscure (Wilson *et al.* 2017). Over time, the subterranean niche has been repeatedly invaded by species from several independent lineages of rodents in disparate geographic areas (Lacey and Patton 2000) leading in many cases to extensive diversification and speciation with examples especially salient in species comprising the Geomyidae in the Nearctic and northern Neotropical regions, the Bathygeridae in the Ethiopian region, Spalacidae in the Palearctic region, and the Ctenomyidae in the southern neotropics (Lacey *et al.* 2000; Wallace 1876; Wilson *et al.* 2017).

One of the most diverse and speciose groups of subterranean rodents includes members of the genus *Ctenomys* Blainville, 1826 (Ctenomyidae). Species of *Ctenomys* occur in suitable habitats in South America with a known northernmost limit of distribution of around 12° S. latitude in western Brazil and about 14° S just to the west of Lake Titicaca on the Altiplano of Peru. Many other species occur south in Bolivia, Paraguay, Uruguay, Argentina, and Chile, even extending south to the island of the Tierra del Fuego (Wilson *et al.* 2017). The genus *Ctenomys* now includes an estimated 70 species and in addition to species in the Geomyidae that occur mostly in the Nearctic, the tuco-tucos represent a well-documented case of diversification in the subterranean biotype (Teta and D'Elia 2020; Gardner *et al.* 2014; Bidau 2015; Wilson *et al.* 2017).

The species in the genus *Ctenomys* are broadly known through their distributional range as tuco-tucos because of the vocalizations made by individuals in their burrows that are detectable by persons listening above ground (Darwin 1860). Field surveys have made it clear that the true species diversity in *Ctenomys* is unknown due to the cryptic nature of these rodents and the fact that many geographic areas that may support populations of tuco-tucos in the Neotropics have not yet been adequately surveyed (Gardner *et al.* 2014).

Tuco-tucos range in size from small to medium-sized rodents (Figure 1A) with a morphologically fusiform and compact body shape, loose skin that allows them to easily turn in a burrow, short legs, and long claws on their forefeet for tunneling. On their forefeet they have relatively thick, rapidly-growing nails that they use to dig their tunnels. On their rear feet, they have strong nails and they have well-developed ctenidia or combs (Figure 1 B) on the margins of their pes, and these hard bristle-like combs (*kteis* = comb in Greek - *mys* = mouse in Greek; thus the name comb-footed-mouse = *Ctenomys*) assist the animal in kicking soil out of the burrow. In contrast to species of Geomyoidea that have a behavior in which all species that have been observed, push soil through and out of their burrows with their front feet (Eisenberg 1962), all species of Ctenomyidae studied up to the current time push and kick the soil through and from their burrows using only their rear feet (S.L. Gardner, pers. obs). As far as is known, this rear-soil-kicking action appears to be a behavioral trait that is plesiomorphic in the ctenomyids and may be a behavioral synapomorphy when also considering the Octodontidae which contains the species considered as the sister group of the Ctenomyidae. Ctenomyids have large heads, small pinnae, and relatively well-haired but tactile tails. Variability in color of the pelage, even within populations, coupled with high levels of variation in both diploid and fundamental numbers of chromosomes in some species set the stage for the current state of affairs in the taxonomy of species in this genus where a relatively large number of species names are available (Gardner *et al.* 2014).

Although there are studies of their biogeography, phylogeny, evolution, taxonomy, ecology, sociality, and parasitology (*e.g.* Gardner 1991; Cook and Yates 1994; Lessa and Cook 1998; Cook and Lessa 1998; Mascheretti *et al.* 2000; Castillo *et al.* 2005; Freitas 2006; Parada *et al.* 2011; Gardner *et al.* 2014), basic information available on ecological aspects, local and regional distributions, biology, and reproduction are still relatively scarce, especially in Bolivia, a country with great, and probably underestimated, tuco-tuco species diversity. The great potential for discovery of new species and the concomitant need for research on all aspects of the biology of *Ctenomys* in Bolivia is exemplified by the description of four new species from collection efforts, initially started by Dr. Sydney Anderson, that took place from about 1984 up to the current time over many areas of the country (Anderson 1997; Gardner *et al.* 2014). Also, the restricted distribution of the known endemic species of *Ctenomys* in Bolivia, coupled with the rapid large-scale land use changes consisting mostly of industrial agriculture that is being established throughout the eastern lowlands of the country suggests the importance of the ctenomyids from a species conservation/preservation perspective (Caraballo *et al.* 2020). Furthermore, of the 12 tuco-tuco species documented to occur in Bolivia, little is known of their population structure, numerical density, and conservation status; and of these, only seven species have been formally evaluated by the IUCN (IUCN 2020).

In Bolivia, the 12 species of tuco-tucos, cujuchis (cuhuchi), topos, or tojos (Anderson 1997) that are known to occur there are distributed through the southern half of the country ranging from a westernmost distribution on the Altiplano near Lake Titicaca south through the Altiplano. They also occur through the Andes east into the Yungas through the lowland regions of eastern Bolivia. At least 3 of the known species each have scientific reports from only one collection locality. In Bolivia, besides basic data on species descriptions and a few spurious records (Anderson 1997; Gardner *et al.* 2014) there is little additional information available in the literature on these rodents. This paper reviews and summarizes published literature, museum records, and unpublished data from extensive field work by (SLG and JSB) in the country with several objectives. Here we will: i) Provide an updated account of the natural history of extant tuco-tucos from Bolivia; ii) Update information on species distributions in Bolivia; and iii) Assess recent and projected habitat transformation or habitat degradation within the known range of each species to provide a preliminary assessment of the preservation or conservation status of ctenomyids within Bolivia.

Materials and Methods

We follow the general systematic summary and taxonomy of Gardner *et al.* (2104) and we used the species descriptions found therein to compile a complete list of known extant species of tuco-tucos from Bolivia. To report general sizes of body (in millimeters), we used data directly from Gardner *et al.* (2104) and from unpublished data taken by SLG from museum specimen tags, weight is reported in grams. Number of individuals is given followed by the mean of the measurements.

To update occurrence data for *Ctenomys* in Bolivia, we used verified collection records from the Arctos database and the Global Biodiversity Information Facility (GBIF). We georeferenced and checked each individual specimen-based locality record by referring to original museum collection records. This double-checking was necessary because we discovered a significant number of errors in the GBIF data-set. These errors were traced primarily to improper reciprocal translation of the format of degree-minute-second to the format of decimal degrees by either automatic computer assisted operations or human error. We include the updated/corrected database as supplementary material. For the summary account for each species of tuco-tucos, citations of the literature describing relevant characters and other data are given at the beginning. Means are given for all measurement data. Colors regarding these animals generally follow Gardner *et al.* (2104) and these color definitions are all derived from Ridgeway (1912).

Species distribution estimation. To provide estimates of the distribution of species of *Ctenomys* in Bolivia, we created species distribution models (SDMs) for the taxa that have sufficient numbers of verified occurrence or collection records. Although SDM's are widely used in studies of biogeography and conservation biology and their predictive power and utility have been repeatedly shown (Phillips and Dudík 2008; Raxworthy *et al.* 2003), there are some factors that could introduce bias in the models and affect both robustness and predictive performance. Probably, the most important of these factors is that records of the presence of animals at a locality usually do not come from a random sampling of the study area and sometimes the data might be spatially clumped, due to any number of logistical reasons for collecting in one locality, resulting in models biased in some way, which in the worst cases are predicting sampling conditions rather than suitability for the studied species (Fourcade *et al.* 2014). However; when our team collects tuco-tucos, we go where they exist, and because of the burrow systems and soil that is thrown up at the mouth of each tunnel, it is relatively easy to see where these mammals are (and where they are not) over geographic space. In many cases, for the *Ctenomys* species in question, the absence of collection records means that no (or few) individuals exist in these areas. The potential missing information derives from the fact that we only collected tuco-tucos where roads existed at the time of our surveys in Bolivia. Finally, most of the collection localities cited in this paper are given in Anderson (1997) and Gardner *et al.* (2104. The other potential problem is the risk of creating very complex models overfitting the training data, but with little predictive or extrapolation power (Muscarella *et al.* 2014). Finally, when no true absence data are available, it is very important that the study area from which background environmental data is extracted, is in fact the area accessible to the species over relevant time periods (Barve *et al.* 2011).

In order to account for the above factors, we took the following modeling approach; first, we defined the study area for each species as the minimum convex polygon of all the recovered specimen collection records, plus an 80 km buffer, a value selected taking into account the potentially limited dispersal capability of these subterranean rodents; however, as pointed out by Busch *et al.* (2000) little work has been done on the ecology of dispersal in subterranean rodents and essentially none has been conducted in Bolivia. Second, we reduced spatial sampling bias by removing one of each pair of records that were closer than 5 km from each other using the R package SPTHIN (Aiello-Lammens *et al.* 2015), thus using only what we assume to be spatially independent records. Given that many of the Bolivian species of *Ctenomys* have few spatially independent sampling records (6 to 31), we employed the approach proposed by Breiner *et al.* (2015), of creating ensembles of small models (ESM) using the R package ecospat (Di Cola *et al.* 2017), in which models were developed for each pair of environmental prediction variables and using different algorithms, and a final consensus model created by averaging predicted suitability weighted by each simple model performance. With this approach, overfitting is reduced as the number of parameters in each model is small relative to the number of occurrences and has been shown to perform well for species with scarce presence data. Here, we created models employing all variable combinations and employing Maxent and GBM (gradient boosting machine) algorithms, as these were found to provide the most robust models which are transferable and the highest predictive performance respectively in an empirical evaluation of ESM (Breiner *et al.* 2018). Subsequently, we assessed the significance of each ensemble model using the area under the partial ROC, above an omission threshold of 10 % as implemented in the R package kuenm (Cobos *et al.* 2019). Finally, we estimated the distribution of suitable habitat by reclassifying the continuous suitability map to presence-absence using the threshold that retains 98 % of the species records.

This threshold was selected to predict all records of the species with low sample sizes and provide an inclusive estimation of suitable habitat.

To model habitat suitability, we used 15 bioclimatic variables that were obtained from WorldClim 2.0, which provides precipitation and temperature averages and variability (Fick *et al.* 2017). We did not include the bios variables 8, 9, 18, and 19 as they might show correlation artifacts resulting from their dependence on both precipitation and temperature (Campbell *et al.* 2015). Besides climatic information, and taking into account the subterranean life-habits of *Ctenomys*, we employed five variables describing soil properties, including: Bulk soil density, sand content, clay content, organic material content, and silt composition at a level of 15 cm below the surface - these soil data were derived from a set that was predicted at a global scale by Hengl *et al.* (2017). For our analysis, we reduced the number of variables and avoided including highly correlated ones by performing a principal component analysis (PCA) on separate climate and soil raster datasets. Of these resulting uncorrelated, orthogonal components generated by PCA, we used the first 6 PCs that in fact explained 99 % of the variation in climate. Relative to soil properties, we chose the first 4 components that actually accounted for 100 % of this variation. Finally, we evaluated the average importance of each of the types of data to assess the utility of soil properties to model species distributions of these subterranean mammals. To enhance reproducibility of these SDM, we use the ODMAP (Overview, Data, Model, Assessment and Prediction) protocol to provide a detailed report of our modelling process and data sources (Zurell *et al.* 2020).

For the species with insufficient independent collection locality records available that would enable us to create a robust model of their distribution using habitat variables, we provide minimum-distribution polygons derived from museum-based occurrence records, field work experience, field notes, and information on habitat associations. For species with only one locality record, we follow Caraballo *et al.* (2020) in which we assume the distribution area to be a circular buffer with a 38 km radius although we realize that true locality-occurrence of these mammals depends completely on the soil characteristics in and around the collection locality.

Recent and future habitat change. We assessed the potential effect of two important threats to the diversity of Bolivian tuco-tucos, including: (1) anthropogenic habitat conversion from natural/seminatural to agricultural and urban systems, and (2) global warming and associated climate change. To assess the magnitude of anthropogenic land cover change within the range of each species, we employed the land cover classification of the Copernicus Climate Change Service for 1993 and 2018 (https://cds.climate.copernicus.eu/cdsapp#!/dataset/satellite-land-cover?tab=overview). Given that detailed data on habitat use and anthropogenic tolerance is lacking for most of the tuco-tuco species, our aim was to quantify large scale transformation coinciding with the ranges of each species of *Ctenomys* considered here. To do this, we classified urban, rainfed and irrigated/post flooding agriculture categories from original classification as anthropogenic intensive land covers; and the reminder land cover types as natural/semi natural which beside natural covers included mosaics of cropland and natural vegetation and pastures. We included these categories as semi-natural because at least some species can survive in these habitats (*e.g. C. boliviensis* and *C. erikacuellarae*, see Gardner *et al.* 2014). In this way, we obtained a conservative estimate of large scale changes within the geographic range of the species of interest. We report the net percentage of natural/semi natural habitat within each species range in the year 2018, as well as the percentage of habitat loss between the years, 1993 to 2018 as a measure of more recent and/or ongoing loss of habitat.

To estimate the change in suitable habitat resulting from global warming we projected the distribution models into the climatic projections for 2070 from the 17 general circulation models (GCM) available at the WorldClim database under the contrasting 4.5 and 8.5 emission scenarios (RCPs) from the 5th Assessment Report of the United Nations Intergovernmental Panel on Climate Change (IPCC). The first scenario corresponds with a peak in emissions around 2040 and further emissions decline with stabilization around 2100; the second, more pessimistic scenario presents emissions rising throughout the 21st century (http://www.ipcc.ch/). We estimated suitability for each GCM RCP combination and predicted gain and loss of suitable habitat specifically for the ctenomyids of Bolivia for the median suitability of each scenario using the same threshold used in the current distribution model. We used the same soil variables.

Results

Species summaries -*Ctenomys* of bolivia:

Ctenomys opimus Wagner, 1848

(Figure 2, A, B, C, D; Map Figure 9)

Common name: highland tuco-tuco, tuco-tuco Andino.

Morphology: (n = 56) Body length 284; tail 83; hindfoot 42; ear 9; weight 306. When adults, these tuco-tucos are relatively large with a pale yellowish color. In some localities, individuals have darker fur with black intermixed with the pale yellow. Older individuals may have worn hair on top of the head, probably from moving through the burrow system and digging. Pelage is long and soft. The auditory canals are relatively short, associated with large auditory bullae. The upper incisors are slightly procumbent and the enamel on the teeth is orange in color. Gardner and Anderson (2001) noted that *C. opimus* has curious holes in the cranium, usually between the frontal and parietal bones and can be relatively common with 80 % of highland tuco-tucos in Bolivia showing this character. A great summary of the morphology of this species is given in Cook *et al.* (1990).

Distribution: *Ctenomys opimus* has a wide distribution with a documented geographic range from southern Peru (southwest of Lake Titicaca - see map, Figure 9) south through northeast Chile, the Altiplano of Bolivia, into extreme northwestern Argentina. In Bolivia, the highland tuco-tuco is found in the high-Andean puna habitat of the departments of La Paz, Oruro, and Potosi. The SDM (Table 1) we generated for this species predicts 100,733 km^2 of suitable habitat through the Altiplano except for the inhospitable Salar de Uyuni.

Natural history: Pearson (1959) studied *C. opimus* in southern Peru and provided some of the earliest detailed observations of the natural history of tuco-tucos. Anderson (1997) recorded that pregnant females were trapped in August, October, and December similar to observations made by Pearson in Peru, numbers of embryos ranged from 1 to 5 (mean 2.8), with a gestation period of approximately two months (Pearson 1959). Non-pregnant females were also recorded in August and September with a higher proportion of births evidently occurring from October through March each year (Anderson 1997).

From Puna habitat at 3,950 m., 7 km S and 4 km E, Cruce Ventilla (19° 08' S; -66° 07' W), a locality that our expedition visited on 30 September, 1986, we observed that individuals of *C. opimus* inhabited burrow systems that were also used by at least two other species of rodents. Collected from the same burrow systems (the specimens came out of the same burrow mouth and all were collected by SLG by shooting) were individuals of *C. opimus*, *Phyllotis xanthopygus* (Waterhouse, 1837), and *Galea musteloides* Meyen, 1832. During the time we spent at this site, we observed that the burrow systems of the tuco-tucos were not closed with more than about 5 cm of soil. Usually (for all individual burrow systems that we have studied throughout Bolivia) the entrance to the burrow of the tuco-tuco is almost always plugged with soil for more than 10 cm (sometimes much more), unless the animal is actively plugging the burrow mouth and was interrupted before it was finished with its work. At this site, during the day, these three species of rodents emerged from the same burrow systems at irregular intervals, sometimes with dozens of *P. xanthopygus* coming out of the burrow systems simultaneously, while at the same time *G. musteloides* and *C. opimus* were emerging. The individuals of *C. opimus* that were observed always stayed partially in their burrow, never leaving it completely, while the two other species of rodents strayed meters from the various mouths of the burrow systems. Individuals of all three species were collected easily with a shotgun at this site. Interestingly, when our expedition again visited the site in 1990, there remained no evidence of these rodents, the tuco-tuco colony evidently either suffering a local extinction or the colony and associated inquilines having moved on through the landscape (Gardner pers obs. 1993). Parasites - Studies of the parasites of *C. opimus* have yielded the description of four new species of *Eimeria* (Coccidia: Apicomplexa) including: *Eimeria opimi, E. oruroensis, E. granifera*, and *E. montuosi* (authority names all Lambert *et al.* 1988). Specific localities for these protistan parasites are given in Lambert *et al.* (1988) and Gardner and Duszynski (1990). In addition, two species of nematodes, *Litomosoides andersoni* and *L. ctenomyos* Brant and Gardner (1997) (Nemata: Filaroidea) were discovered and described from tuco-tucos at the Huancaroma locality (Oruro: Rancho Huancaroma, 17° 40' S; -67° 27' W, 3,720 m. altitude). Other nematodes collected at this locality included *Paraspidodera* (Nemata: Heterakoidea) and *Trichuris* (Nemata: Trichurata) both in very high prevalence and intensity of infection. In addition, from 32 highland tuco-tucos collected from the Huancaroma locality (17° 40' S; -67° 27' W) by members of our expedition in 1986, a single individual that was examined harbored one specimen of a large form of *Mathevotaenia* (Cestoda: Anoplocephalidae) which is now being described as a new species. Ectoparasites identified from our collecting throughout the range of *C. opimus* in Bolivia included fleas *Ectinorus galeanus* Jordan, 1939 (Siphonaptera: Rhopalopsyllidae) from the same Huancaroma locality listed above, chewing lice (*Phtheiropoios* sp.) currently being described as new, and beetles of the family Staphylinidae (*Edrabius weiseri* Seevers 1955). In addition, beetles of this species were reported from *C. opimus* from other localities in Bolivia including 7 km south, 4 km east, Cruce Ventilla, Dept. Potosi and 2 km east, ENDE camp, northeast shore of Laguna Colorado, 4,280 m. (Ashe *et al.* 1996).

The community of rodents at the collection locality near Cruce Ventilla was also interesting in that the only parasites that were shared among these rodents that lived in the same burrow systems were cestode larvae we discovered in

Table1. Results of the ensemble distribution models and projected changes in suitable habitat for 2070 under two carbon emission scenarios. *pROC ratio significantly different from 1, meaning that the model is statistically significant.

Species	N	Study area	Predicted suitable Area (km^2)	Predicted suitable Area Bolivia Area (km^2)	pROC ratio	AUC	Bioclimatic variables contribution	Soil variables contribution	%Suitable habitat change RCP4.5	%Suitable habitat change RCP4.5
Ctenomys opimus	31	445,644	187,163	107,733	1.6*	0.84	0.1	0.09	-34 %	-22 %
Ctenomys steinbachi	8	44,770	2,690	2,690	2*	0.86	0.11	0.09	-61 %	-58 %
Ctenomys frater	17	210,035	104,383	34,443	1.5*	0.71	0.09	0.12	-8 %	-9 %
Ctenomys boliviensis	25	97,941	14,347	14,347	1.8*	0.88	0.12	0.07	-78 %	-26 %
Ctenomys conoveri	15	202,966	62,437	28,936	1.7*	0.77	0.09	0.11	+12 %	+34 %

the mesenteries of the rodents. These were metacestodes of the genus *Taenia* and were identified by R. L. Rausch and SLG in 1988 (pers. obs.) as *Taenia talicei* Dollfus, 1960 which were multi-strobilate larval forms. Another paper describing these associations is in preparation.

Species Conservation/Preservation Status: *Ctenomys opimus* is globally categorized as a species of least concern (LC) by the IUCN (Dunnum *et al.* 2016) and is not mentioned in the Libro Rojo de la Fauna Silvestre de Vertebrados de Bolivia (Ministerio de Medio Ambiente y Agua 2009; LRVSB). The suitable conditions for this species are expected to contract by 34 % and 21 % under the GCM 4.5 and GCM 8.5 respectively, indicating that climatic change will have an important impact over this species (Figure 11; Table 1). Most of the estimated distribution of the species comprises natural, seminatural, and grassland/pastures vegetation covers (Table 2). Through the geographic area occupied by this species, around 21 % of its range is under protection. In Bolivia protected areas include: Reserva Natural y Deportiva Cerro Viscachchani, Refugio de Vida Silvestre Huancaroma, Parque Nacional Sajama, Reserva de Fauna Eduardo Avaroa, Parque Nacional Yura, and Patrimonio Nacional y Reserva Ecologica Poopo (Caraballo *et al.* 2020).

Figure 1. A. *Ctenomys conoveri* showing Orange, slightly grooved incisors. B. Left rear pes and claws of *Ctenomys conoveri* showing comb-like bristles on the margins of the toes.

Ctenomys andersoni Gardner *et al.* 2014

(Figure 2 A, B, C; Map, Figure 9).

This species was named by Gardner and colleagues in honor of Dr. Sydney Anderson. Syd was a major catalyst for the continuing work on the mammals of Bolivia and his encouragement and teaching to both students and professionals has had profound effects on the overall understanding of the biodiversity of Bolivia. His work in Bolivia, starting in 1964 accelerated through and beyond the completion of his monograph: "Mammals of Bolivia: Taxonomy and Distribution" in 1997 (Anderson 1997).

Common name: Anderson's tuco-tuco, Anderson's cujuchi.

Morphology: From the original description of *C. andersoni* by Gardner *et al.* (2014) (n = 27) Body length 253; tail 69.8; hindfoot 33.8; ear 6.8; weight 219.9. *Ctenomys andersoni* is a medium-sized tuco-tuco with brown dorsal coloration, an indistinct dorsal Olive Brown stripe, and no cap of dark hairs on its head (Figure 2 G). The ventral fur is much lighter and is well-differentiated from the dorsum. In contrast to many other species of ctenomyids, there is no obvious collar of differently colored fur in the gular region but there is a small patch of slightly lighter fur extending ventrad around and just below the pinnae. The most distinctive features of the skull and mandible of *C. andersoni* are the brightly colored Orange incisors (Figure 2 E, F). For a complete description of the cranium of this species, see Gardner *et al.* (2014).

Distribution: The type locality and only geographic location from where specimens of *C. andersoni* have been collected is Cerro Itahuaticua in the department of Santa Cruz at an elevation of 810 m (19° 48′ S; -63° 31′ W; Figure 9). No SDM was constructed for this species since there are not enough occurrence data points (specimens collected more than a few km apart) to enable this type of analysis.

Natural history: The habitat at the collection locality of *C. andersoni* comprises one area in the Andean dry valleys zone with a mixed vegetation of deciduous thorny trees and cacti as well as limestone outcrops on the hillsides. No other data are available on the ecological characteristics of the habitat of this species. The only other species of cajuchi that occurs in the general area from which *C. andersoni* was discovered are *C. erikacuellarae* Gardner *et al.* 2014 to the west (about 50 straightline km) and *C. conoveri* Osgood 1946 to the southeast (about 100 straightline km) see map in Figure 8 C, D). *Parasites* - Chewing lice (*Phtheiropoios* sp.) and nematodes of the genus *Paraspidodera* were collected from the type series of these mammals and these are being described as new species. New records of fleas have been reported (see Pucu *et al.* 2014 or a list).

Conservation/Preservation Status: The biological status relative to numerical density and viability of the populations of this species has not yet been evaluated by the IUCN. Little large-scale habitat conversion has occurred around the area where this tuco-tuco was collected (< 1 %) and 96 % of the habitat is under natural or semi-natural conditions (Table 2). However, because *C. andersoni* is known only from a very restricted geographic range at the type locality and only 3 % of the area near the type locality lies within the municipal protected area of Serrania Sararenda-Cuervo (Caraballo *et al.* 2020), we consider this species as susceptible and vulnerable to immediate extirpation by anthropogenic causes.

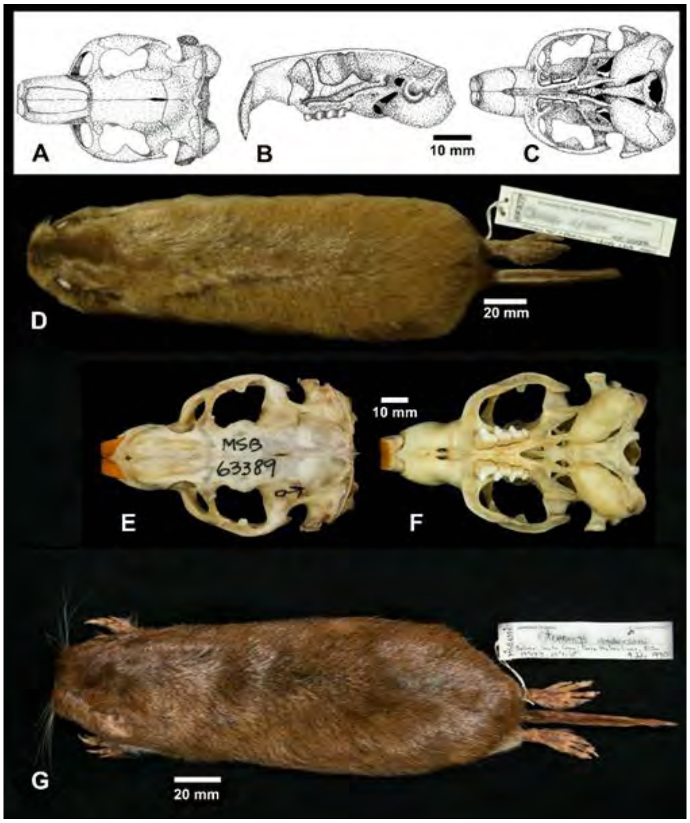

Figure 2. A-D. *Ctenomys opimus* Wagner 1848, Highland tuco-tuco or tuco-tuco Andino. A. Ink drawing of dorsal aspect of skull showing relatively inflated auditory canals and posteriorly protuberant auditory bullae. B. Left lateral view. C. Ventral view, Figure 2. E-G, *Ctenomys andersoni* Gardner *et al.* 2014, Anderson's tuco-tuco or Anderson's Cujuchi. E. Photo image of dorsal aspect. F. Photo of ventral aspect. G. Photo image of dorsal aspect of museum study skin.

Ctenomys boliviensis Waterhouse, 1848

(Figure 3 A, B, C, D; Map, Figure 9).

Common name: Bolivian tuco-tuco, cuhuchi, cujuchi.

Morphology: (n = 81) Body length 289; tail 77; hindfoot 42; ear 7; weight 329.

Individuals of this species are moderately large. The pelage is reddish brown, with darker areas mid-dorsally, especially on head and nape with a well-developed lighter colored collar extending ventrad from the pinnae. Regarding the skull, distinctive characters include the posterior position of the postorbital processes. A summary of the morphological characters for this species is given in Anderson *et al.* (1987), in addition, we follow Gardner *et al.* 2014 and regard *C. goodfellowi* Thomas 1921 as a synonym of *C. boliviensis.*

Distribution: *Ctenomys boliviensis* is found in the lowlands of the Department of Santa Cruz from 400 to 500 m (Anderson *et al.* 1987). The species has been recorded from approximately 25 separate localities in Bolivia, one cluster of records occurring around Santa Cruz de la Sierra and another in Santa Rosa de la Roca to the east. Our estimate using the SDM indicates there exists approximately 14,347 km^2 of suitable habitat through the species range in Bolivia, with a core area around Santa Cruz de la Sierra and smaller suitable patches east and north, among which are collection localities of Santa Rosa de la Roca (Figure 9 C; Table 1). On average, bioclimatic data contributed more to the final ensemble model of this species, although soil variables also provide important contributions to the estimate.

Natural history: Cujuchis commonly occur throughout agricultural areas in the lowlands sometimes causing damage to crops, especially yucca (*Manihot esculenta*), corn (*Zea mays*), and other crop plants (Gardner and Salazar-Bravo, pers. obs. 1990). Individuals of *C. boliviensis* were found in lower densities in grasslands and seldom occurred in the forests, although some specimens have been collected from wooded areas (Thomas 1921; Anderson *et al.* 1987). Near the western border of its distribution along the Andean foothills, *C. boliviensis* is replaced by *C. steinbachi* (Anderson *et al.* 1987) and in some areas, these two species occur in close proximity (separated by only 7.5 km). Individuals of *Ctenomys boliviensis* spend most of their time underground in burrows. Little scientific data are available on behavior and habits of this species, but observations by Gardner with Yates (pers obs. 1984, 1985) indicate that the Cajuchi is active throughout the 24 hour period with perhaps greater activity in early morning and late evening; at least one specimen was collected by hand around 11:00 am from sandy areas south of Santa Cruz de la Sierra. Anderson *et al.* (1987) also report that pregnant females have been recorded in May and June, with the number of embryos ranging from one to five (mean 1.7). Parasites - *Ctenomys boliviensis* is known to host the coccidian *Eimeria opimi* with a 36 % prevalence in a sample of 236 individuals examined from near Santa Cruz de la Sierra (see summary in Gardner and Duszynski 1990). Pucu *et al.* (2014) report the flea species *Gephyropsylla klagesi* Rothschild, 1904 and *Ectinorus galeanus* Jordan, 1939 from this tuco-tuco. Recently, a new species of hookworm, *Ancylostoma ctenomyos* Drabik and Gardner 2019*,* was described (Drabik and Gardner 2019) from cajuchi specimens collected during our work that extended from 1984 - 2000 (Also, see summary of this nematode from *C. steinbachi*, below). Both lice (*Phtheiropoios* sp.) and nematodes of the genus *Paraspidodera* have been recorded and many of these are being described as new species.

Species Conservation/Preservation Status: This tuco-tuco has been categorized as a species of least concern (LC) by the IUCN (Dunnum and Bernal 2016) and is not listed in any threat category within Bolivia by the LRFSV. However, the geographic range of this species is expected to be significantly reduced by climate change under both employed scenarios, with a loss of around 78 % of the suitable habitat area under the 4.5 RCP and a 26 % loss under the 8.5 RCP (see map, Figure 11; Table 1). The milder expected change of the 8.5 RCP projection results from an expansion of suitable habitat to the east of the current range. The suitable habitat of the species has also suffered important habitat transformation with 47 % of the natural and semi-natural areas of suitable habitat changing to agriculture or urban zones from 1993 to 2018, leaving only about 40 % of the suitable habitat under natural and semi-natural conditions (Table 2). Although it has been observed that this species can survive in or near agricultural land, it is likely that current and future land-cover/use changes will affect the long-term viability of *C. boliviensis* in Bolivia. Furthermore, less than 1 % of the area of distribution of the species lies in protected areas (Caraballo *et al.* 2020) which in Bolivia includes two municipal parks: Orquídeas del Encanto and Laguna Represa Sapocó, both in Santa Cruz Department. Land cover changes, as well as the projected impact of climatic change on suitable habitat for this species suggest the need of more in-depth studies of habitat use by this species as well as additional studies on populations and estimates of long-term viability.

Ctenomys conoveri Osgood, 1946

(Figures 1 A, B; 3 E, F, G; Map, Figure 9)

Common name: Conover's tuco-tuco, tuco-tuco del Chaco

Morphology: (n = 4) Body length 474.75; tail 113; hindfoot 56; ear 14; weight 1178. Individuals of this species have the largest body size of any in the family Ctenomyidae with some adult males as heavy as 1,520 g (Figure 1 A, B). Pelage of several individuals examined by us is relatively coarse with long shaggy fur (many hairs are longer than 20 mm). The basal part of their dorsal hairs is pale grayish about 10 mm long, then there is a brownish part about 7 mm, ending in blackish tips. Some longer guard hairs have paler tips. Overall color of dorsum is blonde-brown, some individuals with fur darker mid-dorsally, especially on head and nape, and around the mouth (Figure 1 A; the venter is paler and more reddish. Hind foot is broad with well-developed combs (Figure 1 B). The upper incisors are light Orange in color with shallow longitudinal grooves evident in anterior view (Figure 1 A). A summary of skin and skull characters is given in Anderson *et al.* (1987).

Distribution: *Ctenomys conoveri* is distributed through the southern lowlands of Bolivia and where found is locally common in the Departments of Chuquisaca, Santa Cruz, and Tarija, as well as the Chaco region of Paraguay. This species has been reported from 15 separate collection localities in Bolivia, including southern Santa Cruz, eastern Tarija, and eastern Chuquisaca. The SDM predicts 29,000 km^2 of suitable habitat through the species range in Bolivia (Figure 9; Table 1). On average, soil variables show higher contribution to the final model, suggesting that soil conditions significantly influence the distribution of the species.

Natural history: *Ctenomys conoveri* occurs in friable soils that are mostly sandy in composition. This species inhabits the Gran Chaco ecosystem. The specimens we collected in 1985 were easy to catch with number 1 or 2 leghold traps placed in the burrow system. We noted dozens of neatly cut green stems approximately the same length (8 to 15 mm long) that had been trimmed from small stems of trees or bushes, piled in the burrow system of one of the individuals we caught. Parasites - Coccidia Apicomplexa: Eimeriidae) identified as *Eimeria opimi* Lambert *et al.* 1988 were reported from one of three individuals collected in 1985 and studied by Gardner and Duszynski (1990). Chewing lice (*Phtheiropoios* sp.) that were obtained from specimens we collected are being studied and described. Nematode parasites of the genus *Paraspidodera* were also found infecting the cecum of most of the individual tuco-tucos that were collected. These are currently being described as new species.

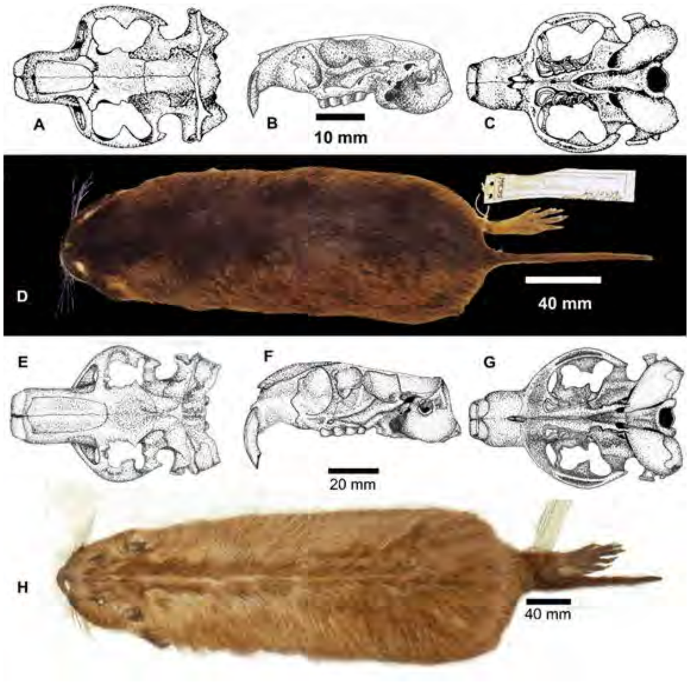

Figure 3. A-D *Ctenomys boliviensis* Waterhouse, 1848, Cuhuchi or Cujuchi. A. Ink drawing of dorsal aspect of skull showing robust bone architecture. B. Left lateral view of skull. C. Ventral view of skull. D. Dorsal view of skin. Figure 3. E-H, *Ctenomys conoveri* Osgood 1946, tuco-tuco del Chaco. E. Ink drawing of dorsal view of skull, F. Left lateral view of skull, G. Ventral view of skull.

Species Conservation/Preservation Status: This species is listed as Least Concern (LC) globally by the IUCN as well as within Bolivia by the LRFSV. The distribution of suitable habitat for the species in Bolivia is expected to expand to the east and north of the current distribution, resulting in an increase of 12 % distribution area under the 4.5 RCP and 34 % under the 8.5 RCP scenario (Figure 11; Table 1). This is the only species of Ctenomys that has a geographic distribution that we project will expand under the expected global warming scenario. Less than 1 % of the natural and semi-natural areas within the species distribution in Bolivia have suffered significant transformations since 1993, resulting in about 98 % of the habitat remaining in at least semi-natural conditions or pasture land that is conducive to proliferation of *C. conoveri* (Table 2). Nonetheless, the area is dissected by several small roads, and many habitats of this

Table 2. Estimated percentage of natural and seminatural vegetation cover occurring in the ranges of *Ctenomys* in Bolivia for the years 1993 and 2018. The percentage of remaining natural and seminatural vegetation cover lost through this 15 year period is also displayed.

Species	Estimated range area (km²)	%Natural-seminatural cover 1993	%Natural-seminatural cover 2018	% Natural-seminatural lost
Ctenomys opimus	100,733	99.6	100	0.04
Ctenomys andersoni	4,512	97.1	97	0.6
Ctenomys boliviensis	14,347	74.7	40	47.0
Ctenomys conoveri	28,936	98.7	98	0.5
Ctenomys erikacuellarae	7,461	97.8	98	0.3
Ctenomys frater	34,443	97.9	98	0.2
Ctenomys lessai	4,556	95.5	95	0.4
Ctenomys leucodon	8,382	98.4	98	0.2
Ctenomys lewisi	2,087	98.4	98	0.7
Ctenomys nattereri	43,495	99.3	97	1.8
Ctenomys steinbachi	2,690	73.9	58	21.1
Ctenomys yatesi	4,214	99.7	99	0.2

species in Paraguay have suffered severe transformations from industrial agriculture (Caldas et al. 2015). Across the area occupied by this species about 15 % of the range of *C. conoveri* overlaps with protected zones (Caraballo *et al.* 2020). In Bolivia, these areas include: Parque Nacional Aguarague, Area de Proteccion del Quebracho Colorado, and Reserva Natural El Corbalán, and the Monumento Natural - Muela del Diablo, near Boyuibe, Chuquisaca.

Ctenomys erikacuellarae Gardner *et al.* 2014

(Figure 4 A, B, C; Map, Figure 9).

Common name: Erika Cuellar's tuco-tuco, tuco-tuco de Erika Cuellar.

Morphology: (n = 69) Body length 259.2; tail 72.9; hindfoot 36.4; ear 7; weight 222.1. This is a medium-sized tuco-tuco with dorsal and ventral coloration well differentiated. The dorsum ranges from ochraceous orange to buckthorn brown except for the upper surface of the head and muzzle which is blackish brown. Most individuals with dark cap (fuscous black) on head, with dark fur running from just above the nose to at least neck. Mid-dorsal stripe of same dark color running posteriad mid-dorsally along back usually reaching rump.

The venter is drab brown or buffy brown and displays extensive white or light buff markings on inguinal, axillary and pectoral regions. The skull is strongly built, having strongly curved zygomatic arches. The rostrum is widest at the tip of premaxillaries giving a strongly robust look when observed from above. The upper incisors are large, robust, orange in color, and opisthodont; lower incisors are also orange in color.

Distribution: Erika Cuellar's tuco-tuco is known only from three localities situated on the eastern flanks in the Andes in the Cordillera Oriental. No species distribution model (SDM) was constructed for this species since not enough occurrence data to enable this type of analysis are yet available. The qualitatively estimated range of the species encompasses the valleys where the species occurs through an area of 7,461 km² (Figure 9; Table 2).

Natural history: These tuco-tucos occur in habitats consisting of friable soils, mostly in the floors of valleys ranging from a recorded low altitude of 810 m. in the area of Monteagudo, Department of Chuquisaca to a recorded maximum of around 1,800 m, near the Río Ciénega north east of Vallegrande, Santa Cruz Department in south-central Bolivia. From Gardner *et al.* (2014): "All three known localities are part of the ecological zone known as Bosques Secos Interandinos or Andean dry valleys (Ibisch *et al.* 2003; Lopez 2003). The locality designated "2 km SW of Monteagudo" was on an agricultural experimental station that was, at the time, relatively protected as the vegetation was less disturbed inside the fenced station and was typical of the lower, eastern escarpments of the Andes in southern Bolivia (see: Ibisch *et al.* 2003). From the field-expedition field notes our team indicates: At the collection locality "5.5 km NNE of Vallegrande" on the Río Ciénega, the hillsides were covered with mesquite (*Prosopis* L.), columnar cacti (*e. g., Trichocereus* sp.), acacia (*Acacia* sp.), and *Ximenia* sp. The Río Ciénega runs through the valley and is surrounded by dense stands of shrubs and trees (*Prosopis*, etc.). The area was mostly converted to cropland and was, at the time, grazed heavily by livestock. The collecting locality at "2 km E of Chuhuayaco" was similar in vegetation-type to that of Vallegrande. Parasites - Fleas identified by Pucu *et al.* (2014) as species of *Rhopalopsyllus* sp. and *Polygenis* sp. were collected from populations of *C. erikacuellarae*. Nematodes of the genus *Paraspidodera* were also obtained from Erika's tuco-tuco at time of collection; the nematodes are being described as new. In addition, lice (*Phtheiropoios* sp.) were also recovered as well as tapeworms. Tapeworms are rarely reported in the literature from any species of *Ctenomys* and we found cestodes in the small intestines of several individuals collected on the experiment station grounds near Monteagudo. These tapeworms, genus *Raillietina* are undescribed and we are currently finishing the descriptions of these worms.

Species Preservation/Conservation Status: *Ctenomys erikacuellarae* has not yet been evaluated by the IUCN or LRFSV. Few large-scale changes have occurred within the estimated distribution of the species during the period analyzed, with around 97 % of the species range under natural or seminatural conditions (Table 2). Across the range of this species in Bolivia, about 48 % of the area in which these tuco-tucos live is under some form of protection (Caraballo *et al.* 2020); these areas include: the Área de Protección Ambiental Serranía Cordillera de los Milagro, National park Iñao, area natural de manejo integrado Rio Grande Valles Crucenos, and the Zona de Inmovilizacion Forestal e Hídrica El Villar. Regardless, given its restricted distribution, the species conservation status requires close monitoring.

Ctenomys frater Thomas, 1902

(Figure 4 D, E, F; Map, Figure 9)

Common name: little Andean tuco-tuco, tuco-tuco pequeño de los Andes.

Morphology: (n = 7). Body length 250; tail 69; hindfoot 34; ear 8.4; weight 165 g. *Ctenomys frater* is a medium-sized tuco-tuco, the subspecies occurring in Bolivia present brown dorsal coloration and dull buffy underparts. Also, see the excellent summary-description of this species by Cook *et al.* (1990).

Distribution: *Ctenomys frater* occupies suitable habitat in the Andean valleys of south eastern Bolivia in the departments of Chuquisaca and Tarija extending south into NW Argentina, including the provinces of Salta and Jujuy. The species distribution model for *C. frater* predicts 34,443 km^2 of suitable habitat through Bolivia, with a large continuous suitable area in the eastern flanks of the Andes in Tarija and Chuquisaca and small patches of habitat further North up to Potosi and Oruro, where there are no records of the species at the moment (Figure 9; Table 1). On average, soil variables show greater contribution to the final model, suggesting that soil conditions appear to significantly impact the distribution of the species which can be expected for any subterranean rodent (Table 1). Variables that were investigated here included bulk density (fine earth) in kg / cubic-meter, clay content (0-2 micrometers) mass fraction in %, soil organic carbon content (fine earth fraction) in g per kg, silt content (2-50 micrometer size range) mass fraction in %, and sand content (50-2,000 micrometers) mass fraction in %.

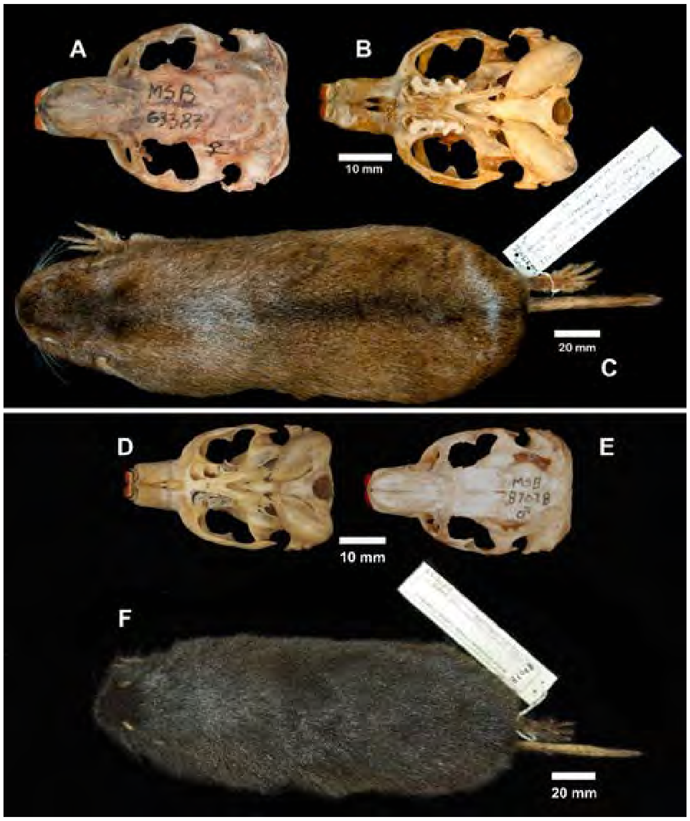

Figure 4. A-C *Ctenomys erikacuellarae* Gardner *et al.* 2014, Erika Cuellar's tuco-tuco or tuco-tuco de Erika Cuellar. A. Dorsal view of the skull showing the rostrum is widest at the tip of premaxillaries and the strongly curved zygomatic arches. B. Ventral view of the skull. C. Dorsal view of skin. Figure 4. D-F, *Ctenomys frater* Thomas 1902 Little Andean tuco-tuco or tuco-tuco de los Andes. D. Ventral view of the skull. E. Dorsal view of the skull. F. Dorsal view of the skin.

Natural history: *Ctenomys frater* occurs through a wide elevational range extending from 600 m in northwestern Argentina (Thomas 1919; Olrog and Lucero 1981) to approximately 4,300 m in central Bolivia (Anderson 1997). Specimens of *C. frater* were collected by our team from steep grassy hillsides (2,700 m) in the Andes at a locality called 8 km west, by road of Rancho Tambo (21° 27′ S; -64° 19′ W, estimated with map), while other specimens of the same species were found at elevations above 4,300 m in the department of Potosi. At the locality called Tapecua (21° 26′13.4″ S; -63° 55′0.12″ W, data from GPS), visited by our collection party in July 1991 and again in 1995, additional specimens of *Ctenomys frater* were collected. All of these specimens, collected on 4, June 1995 were extremely dark, their fur matching well the color of the soil where these mammals were found to be abundant. The animals were found to be living in friable, but not sandy soil on extremely steep hillsides under the canopy of trees. The habitat changed dramatically from the time we first visited Tapecua in 1991 compared to our subsequent visit in 1995. In 1995, we found that domestic goats had been introduced into the area and the vegetation was cut up to the level that a goat can reach. For much of the time when we were at both Rancho Tambo and Tapecua, Andean Condors were very common. Some condors landed within 5 meters of us as we worked our traplines on the steep hillsides at the locality designated 8 km west, by road of Rancho Tambo; the condors soared nearby during the middle of the day at Tapecua. At the Tapecua locality, tuco-tuco-trapping was difficult as moving up and down the hillsides required much effort. Parasites - Fleas (*Ectinorus galeanus* Jordan 1939) were collected by our field party in 1986 from 3 individual tuco-

tucos at 8 km west, by road from Rancho Tambo. Coccidia identified as *Eimeria opimi* were collected from this same locality and identified by Gardner and Duszynski (1990). Nematodes of the genus *Paraspidodera* were collected from specimens obtained from both the Rancho Tambo and Tapecua localities, these are being described as new. *Species Preservation/Conservation Status:* This tuco-tuco has been categorized as a species of least concern (LC) by the IUCN (Vivar 2017) and is listed as data deficient by the LRFSV. The distribution of suitable habitat for *C. frater* in Bolivia is expected to be reduced 8 % under the 4.5 RCP and 9 % reduction under the 8.5 RCP scenario (see Map 11; Table 1). Few large scale changes have occurred within the estimated distribution of the species during the period analyzed, with around 97 % of the species range under natural or semi-natural conditions (Table 2). About 15 % of the range of *C. frater* is protected in more than 20 areas in Bolivia (Caraballo *et al.* 2020).

Ctenomys lessai Gardner *et al.* 2014

(Figure 5 A, B, C; Map, Figure 10)

Common name: Lessa's tuco-tuco, tuco-tuco de Lessa.

Morphology: (n = 9), Body length 238; tail 64; hindfoot 32; ear 6.6; weight 176 g. *Ctenomys lessai* shares a common ancestor with *C. conoveri* (see phylogeny in Gardner *et al.* 2014). Because this is such a unique and isolated species we directly quote Gardner *et al.* (2104) in the description of the skins and skulls: "*Pelage dense, fine, soft, about 5–20 mm long over back and rump. Color of dorsal pelage olive brown to buffy brown, ventral pelage cinnamon buff, some individuals with ventral fur olive buff. Dorsally, darkest fur a clove brown in central diffuse dorsal stripe, more prominent anteriorly on head and fading posteriad... ...Small light area of cinnamon buff fur, just posterior to and below the pinnae, evident in most individuals. Collar not evident in specimens examined.*" In contrast to the well developed collars of differently colored fur extending from the pinnae ventrad in both *C. boliviensis* and *C. steinbachi* there is no collar in the gular region of *C. lessai*. In describing the skull, Gardner et al. (2104) state: "*Upper incisors with enamel a light pale yellow ocher to ivory yellow color. Enamel initially white in unexposed roots, changing to yellow ocher with mottled yellowing slightly on frontal surfaces of exposed parts of incisors. Sometimes appearing mottled in frontal view with white showing through. Enamel of lower incisors yellow ocher in color. Mandible relatively delicate.*"

Distribution: This species is known only from the type locality near a place 0.5 km south of Lluthu Pampa, 17° 45'S; -64° 59'W, 2,700 m. elevation in the Andean portion of the department of Cochabamba (Figure 10).

Natural history: Specimens of this tuco-tuco were collected from burrow systems at elevations ranging from 2,500 to 2,750 m. The area from where specimens were secured was open grassland habitat near a running stream with remnant stands of *Polylepis* sp. These tuco-tucos appear to be diurnal as most of the captures occurred during daylight hours. Parasites - From the original collection material obtained on 30 and 31 May 1991, both ectoparasites and endoparasites were collected by our field crew. Lice (*Phtheiropoios* sp.) are currently being studied and the fleas have been identified and reported as *Ectinorus galeanus* Jordan, 1939 and *Sphinctopsylla inca* Rothschild, 1914 by Pucu *et al.* (2014). Nematodes of the genus *Paraspidodera* were collected from the cecum of these mammals and these are currently being described as a new species.

Species Conservation/Preservation Status: Individuals of this species have been collected only from the type locality and this species has not been evaluated by the IUCN or the LRFSV. The area surrounding the type locality has suffered less than 1 % large scale landscape changes in the period analyzed, but including previous changes, 95 % of the area remains as natural or semi-natural habitat (Table 2). Almost 50 % of the area surrounding the collection localities is currently listed as protected, including both Parque Nacional Amboró and Parque Nacional Carrasco (Caraballo *et al.* 2020).

Ctenomys leucodon Waterhouse, 1848

(Figure 5 D, E, F; Map, Figure 10)

Common name: white-toothed tuco-tuco, tuco-tuco de dientes blancos.

Morphology: **(**n = 3) body length 223; tail 67; hindfoot 31; ear 5; weight 160. White-Toothed tuco-tucos are small in size and very reddish in color (Figure 5 D, E, F). Their fur is a rich Buckthorn Brown and is thick over the body and the ears are well-furred with darkly colored skin on the pes (Figure 5 D). The pale, relatively narrow and proödont upper incisors are noteworthy. See the excellent summary of the morphology of this species in Cook *et al.* (1990).

Distribution: *Ctenomys leucodon* occurs in a relatively small area generally south of Lake Titicaca at elevations of about 3,850 m. in the department of La Paz, in Bolivia but also west of the southern part of the lake in Peru (two collection locality records; Figure 10). No SDM was constructed for this species since not enough occurrence data points have been recorded in museums to enable this type of analysis. The qualitatively estimated range of the species encompasses the valleys where the species occurs through an area of 8,382 km^2.

Natural history: Habitat of the tuco-tuco de dientes blancos consists of flat puna with compact soils that are friable only with difficulty as soil is held together by masses of grass roots that bind the soil well. In the areas where these were collected are dispersed bunch grasses that dot the landscape. Associated throughout the area and especially where the *C. leucodon* colonies were found were many common yellow toothed cavys (*Galea musteloides*), but, even though they occurred syntopically at the locality (11.5 km west of San Andres de Machaca, 17° 00' S; -69° 04' W; 3,800 m.) we did not observe these two species of mammals using the same burrow systems at this locality. The Tuquitos de Dientes Blancos were located in isolated colonies sometimes separated by distances as great as kilometers. No data on colony size could be determined from our work. First encountering these animals in 1986, some individuals of *C. leucodon* exhibited a high-pitched whistle vocalization on several occasions. Individuals of this species seemed to spend more time at burrow openings than did other species of Bolivian *Ctenomys* as several were observed with their heads out of the burrow systems for a relatively long period of time. These animals appear to occur in moving landscape patches or colonies called "tucales" that move through space and time and appear to operate as mini soil plows that enable higher diversity of plants to grow in the soil where the mammals turn over the soils. In the field, members of our expeditions observed higher diversity of both plants and mammals among and in tucales. In Peru, *C. leucodon* appears to be the least common species of *Ctenomys*, occurring in a restricted area west of Lake Titicaca (see map, Figure 10). In August, one female with one embryo and seven females with no embryos were recorded (Anderson 1997). Parasites - Three individuals were examined for the presence of coccidia (*Eimeria*) by Gardner and Duszynski (1990) but were found to be uninfected. Two species of fleas were identified from material collected by our team in 1993 including *Ectinorus galeanus* Jordan, 1939 and *Tetrapsyllus tristis* Johnson 1957 (see report by: Pucu *et al.* 2014). A nematode called *Pudica* sp. was identified by Gardner pers. obs. and lice (*Phtheiropoios* sp.) were collected along with beetles of the family Staphylinidae from individuals of *C. leucodon* by our field expeditions in 1986 and 1993 (Anderson 1997 and Pucu *et al.* 2014). *Species Conservation/Preservation Status:* The species is listed as Least Concern by the IUCN and as data deficient by the LRFSV. Although 98 % of the distribution area comprises pasture, natural or semi-natural covers (Table 2), almost none (0.1 %) of the protected areas that are established overlap the range of this species (Caraballo *et al.* 2020).

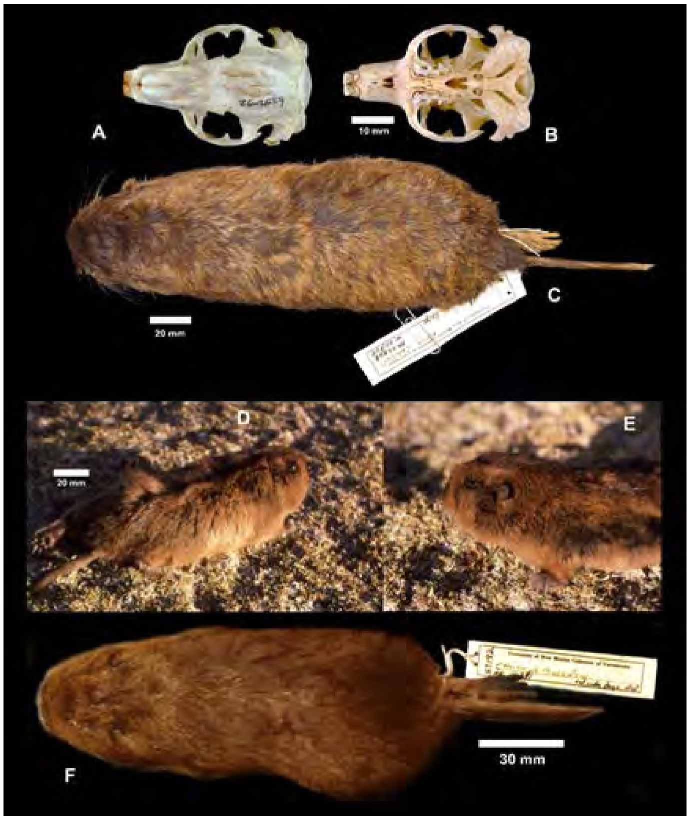

Figure 5. A-C *Ctenomys lessai* Gardner *et al.* 2014, Lessa's tuco-tuco or el tuco-tuco de Lessa. A. Photo image of dorsal aspect of skull, note particularly delicate rostrum. B. Photo image of ventral aspect of skull. C. Photo image of dorsal view of museum study skin of *C. lessai*. Figure 5. D-F. *Ctenomys leucodon* Waterhouse, 1848, White-Toothed tuco-tuco, el tuco-tuco de Dientes Blancos. D. Photo image of right lateral side of a living *C. leucodon*. E. Photo image of anterior left lateral side of same individual as in 5 D, F. Photo image of dorsal aspect of museum study skin.

Ctenomys lewisi Thomas, 1926

(Figure 6 A, B, C, D, E; Map, Figure 10)

Common Name: Lewis's tuco-tuco, tuco-tuco de Lewis. Morphology: (n = 15) Body length 268; tail 76; hindfoot 39; ear 8; weight 291 g. Thomas (1926) characterized *C. lewisi* as a large reddish-brown species with unusually proodont incisors. He stated: "*Ctenomys leucodon* has similarly proodont incisors, but these (*C. leucodon*) are comparatively slender, are not so strongly flattened in front, and are almost unpigmented." Because Thomas (1926) compared *C. leucodon* with *C. lewisi*, we will make some similar comparisons here. Individuals of *C. lewisi* that we trapped at the Iscayachi locality were of a rich dark black/brown color dorsally and lighter with grey ventrally. The dorsal coloration appears to closely match the color of the soil of the area when it is moist. The pinnae are sparsely furred and pink in color (Figure 6 D) in sharp contrast to the dense reddish-brown-black fur on the pinnae of *C. leucodon* (Figure 6 D, E). See also the excellent summary of the morphology of this species in Cook *et al.* (1990).

Distribution: *Ctenomys lewisi* is known only from the highlands of Tarija in the valley near Iscayachi (elevation of collecting localities in 1986 was 3,400 m) with the Serrania de Sama rising rapidly to the east to about 4,000 m over a distance of 6 km. from the collecting site (Figure 10).

Natural history: This species was first collected from burrows in wet stream banks, which led Thomas (1926) to suggest *C. lewisi* had semi-aquatic habits. No such habit has been suggested for any other species of tuco-tuco and some, but not all, of the specimens captured by members of our expedition in 1986 near Iscayachi were trapped from burrows in damp soil near the Rio Tomayapo. There was no indication that any of the 15 animals we collected at the Iscayachi locality lived in or even very near water. Parasites - From 6 of 8 individuals examined for coccidian parasites in the feces, oocysts representing *Eimeria opimi* were found (Gardner and Duszynski 1990). Nematodes of the genus *Paraspidodera* were also recovered from the cecum of several individuals and these are being described as new. In addition, chewing lice (*Phtheiropoios* sp.) were recovered from this species at the Iscayachi locality and further up the mountain near Sama. These are also being described. Fleas were not recovered during collection of the mammals.

Species Conservation/Preservation Status: *Ctenomys lewisi* was listed as a Species of Least Concern (SLC) by the IUCN (Roach and Naylor 2016a) and as data deficient by the LRFSV. Approximately, 98 % of the distribution range of this species comprises natural and semi-natural land cover (Table 2), but with heavy grazing by domestic animals and 21 % of its estimated distribution range is covered by protected areas including the Reserva Biológica Cordillera de Sama and Parque Natural y Área de Manejo Integrado El Cardón (Caraballo *et al.* 2020).

Ctenomys steinbachi Thomas, 1907

(Figure 6, F - I; Map, Figure 10)

Common names: Steinbach's tuco-tuco, tuco-tuco de Steinbach.

Morphology: (n = 46) Body length 293; tail 81; hindfoot 43; ear 7; weight 315 g. The skin of this species has a light Black pelage dorsally with grizzled or mixed whitish and blackish ventral pelage. Well developed light grey collar extending ventrally from each pinna. See Anderson (1997) for an excellent summary of the morphology of this species.

Distribution: *Ctenomys steinbachi* is found only in the department of Santa Cruz, west of the Rio Grande. Individuals of this species have been collected from 8 separate localities ranging from 300 to 500 m. in altitude. Steinbach's tuco-tuco suitable habitat comprises 2,690 km^2 (Figure 10; Table 1). The environmental requirements of this species are different from those of *C. boliviensis*, resulting in very little suitable habitat overlap between the two species despite their close geographical distance. Individuals of this species have been collected as close as 15 km in geographic proximity of populations of *C. boliviensis* at and at slightly higher elevations than is inhabited by *C. boliviensis* along the eastern foothills of the Andes near Parque Nacional Cerro Amboro (Anderson *et al.* 1987).

Natural history: Steinbach's tuco-tuco has been recorded from open savanna areas, closed canopy areas with yucca and banana and areas that have been disturbed by sugarcane plantations, cattle grazing and other agricultural activities. The soil where these animals occur is darker and contains much less sand than the areas occupied by *C. boliviensis* in the area where these two species occur in proximity. Parasites - Of 72 specimens of Steinbach's tuco-tuco examined for the presence of the protistan parasite *Eimeria* sp., none were found to be passing oocysts when they were collected in contrast to a relatively high prevalence of the parasites found in other species of *Ctenomys* (Gardner and Duszynski 1990). Drabik and Gardner (2019) described *Anyclostoma ctenomyos* from duodenum in the small intestine of *Ctenomys steinbachi* collected from Bolivia, Departamento de Santa Cruz: 2 km S. Caranda, by road, (17° 33′ 0″ S; -63° 31′ 48″W, 345 m) (note that this species of nematode was also recovered from *C. boliviensis*) on August 14, 1987. The prevalence of this nematode was 6/13 = 46 %. Nematodes of the genus *Paraspidodera* have also been collected from *C. steinbachi*, and chewing lice of the genus (*Phtheiropoios* sp.) were collected from individuals of this species and these are being described elsewhere as new.

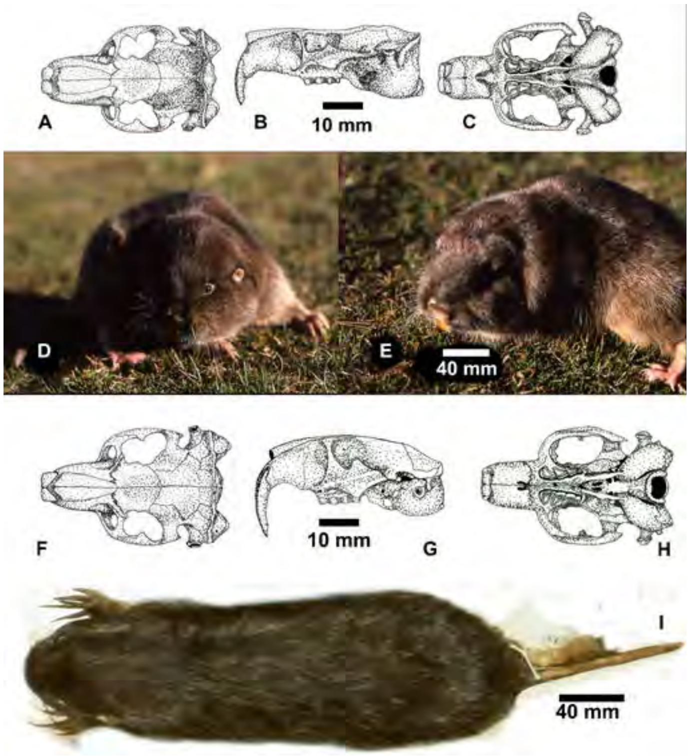

Figure 6. A-E. *Ctenomys lewisi* Thomas, 1926, Lewis's tuco-tuco, tuco-tuco de Lewis. A. Ink drawing of dorsal aspect of skull. B. Left lateral view. C. Ventral view, note extremely long auditory canals and rear-turned auditory bullae. Figure 6. F-I *Ctenomys steinbachi* Thomas, 1907, Steinbach's tuco-tuco or tuco-tuco de Steinbach. F. Ink drawing of dorsal aspect of skull, G. Left lateral view of skull. H. Ventral view of skull. I. Photo image of dorsal aspect of skull of *C. steinbachi*.

Species Conservation/Preservation Status: *Ctenomys steinbachi* is categorized as a SLC by the IUCN (Roach and Naylor 2016b). However, in Bolivia this species is categorized as near threatened (NT) by the LRVSB because of the lack of recent collection records and the coincidence of the species range with areas of intense habitat conversion into both urban and agricultural uses. Similar to the situation for *C. boliviensis,* this species is predicted to experience severe contraction in suitable habitat areas, with a 61 % and 58 % reduction under the GCM 4.5 and GCM 8.5, respectively (Table 1). In total, about 21 % of the remaining natural and semi-natural vegetation cover was lost through the range of the species since 1993, which leaves only 58 % of the species distribution area as natural or semi-natural conditions (Table 2). In addition, only 4 % of the species range is under protection. Given the endemic status, magnitude of observed habitat degradation, and expected impact of climate change; this species should be closely monitored, and we feel strongly that *Ctenomys steinbachi* should be assigned to a higher IUCN threat category. We recommend a higher threat category partly because of the problematical list of so-called protected areas in the department of Santa Cruz, Bolivia. Caraballo *et al.* (2020) shows that the area in which *C. steinbachi* occurs are overlapped by at least three areas with some degree of ecological protection. However, examining these areas shows that that they appear to provide minimal protection for native species, this is because, from aerial views, it can be seen that these areas are covered with urban sprawl or consist of intensive industrial agriculture with most of the natural areas being obliterated by humans. The areas that are listed as protected include: Parque de Protección Ecologica Río Piraí and the Área Protegida Municipal Curichi La Madre, Parque Regional Lomas de Arena see Caraballo *et al.* (2020).

Ctenomys nattereri Wagner 1848

(Figure 7 A - H; Map Figure 10)

Common name: Natterer's tuco-tuco, tuco-tuco de Natterer.

Morphology: (Taken from Wilson *et al.* 2017) Body length 245; tail 71; hindfoot 54; ear 11; No information on weight. This is a relatively large species of *Ctenomys* that presents a shiny brown dorsal coloration capped by a dark dorsal stripe comprised of black spots. The venter is black with white spots, especially in the axillary and inguinal regions. On the specimen examined, the claws of the forefeet are particularly long, reaching 16 mm. The specimen of *C. nattereri* that we present here is derived from the skull of (AMNH37121) collected on 24 Feb, 1914 by G. K. Cherrie on an expedition with Theodore Roosevelt and Cândido Mariano da Silva Rondon through the area of what would eventually become Rondonia, both upper and lower incisors are a dark orange, the skull is robust.

Distribution: The distribution of the species includes the eastern lowlands of Bolivia in the department of Santa Cruz east into Brazil (Figure 10). In Bolivia, tuco-tuco specimens from four localities in Santa Cruz have been assigned to *C. nattereri*. Based on an analysis of cyt-b phylogeny and genetic distances, Gardner *et al.* (2014) assigned the populations near Roboré, San Jose de Chiquitos, and San Ramón to *C. nattereri*. Besides Bolivia, the species has been recorded in the states of Mato Grosso and Rondônia in Brazil.

Natural history: Little is known of the habitats or behavior of this tuco-tuco. *Parasites* - Nematodes recorded by our collecting expeditions from this tuco-tuco in Bolivia include species of the genera *Paraspidodera* and *Trichuris.*

Species Conservation/Preservation Status: This species has not been evaluated by the IUCN or the LRFSV although IUCN treats *C. nattereri* as a synonym of *C. boliviensis* (see Dunnum and Bernal 2016). The range estimated from the abovementioned localities has lost around 2 % of its natural and semi-natural land cover since 1993 (Table 2). Approximately 32 % of the species range appears to be under some protection category (Caraballo *et al.* 2020) but at the current time, little information is known of the habits of these tuco-tucos. The areas in Bolivia in which some protection of this species is given include: Area Natural de Manejo Integrado San Matías and the Area Protegida Municipal San Rafael.

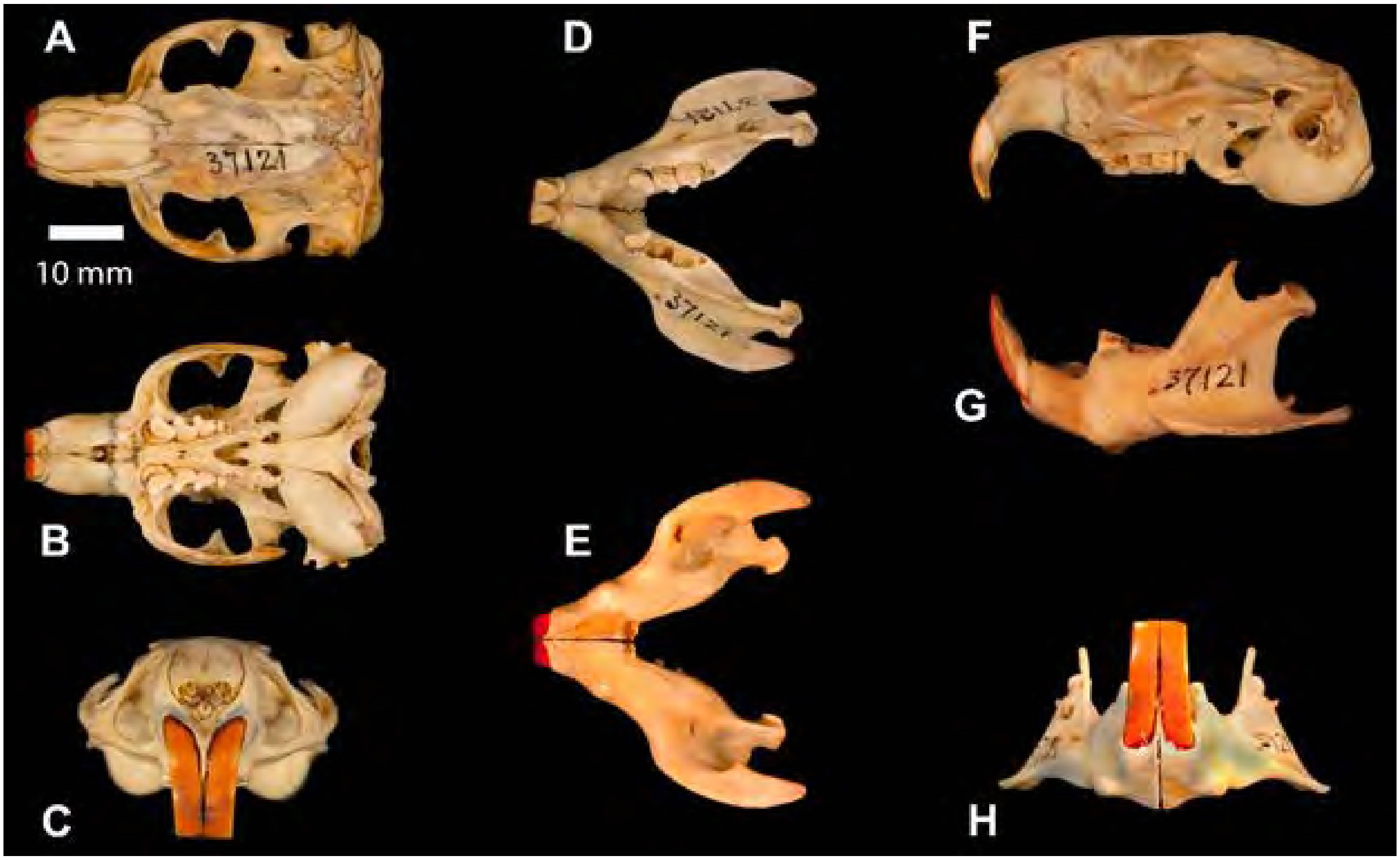

Figure 7. A-H. Photo images of the skull and jaw of *Ctenomys nattereri* Wagner 1848, Natterer's Tuco-tuco or tuco-tuco de Natterer. A. Dorsal view shows the robust skull. B. Ventral view of the skull. C. Frontal view of the skull, note dark colored upper incisors. D. Dorsal view of the jaw. E. Ventral view of the jaw. F. Left lateral view of the skull. G. Left lateral view of the jaw. H. Frontal view of the jaw displaying the dark incisors.

Ctenomys yatesi Gardner *et al.* 2014

(Figure 8 A, C, F - I, Map Figure 10)

Common Name: Yates' tuco-tuco, tuco-tuco de Yates.

Morphology: (n = 3) Body length 209 ; tail 58; hind foot 32; ear 4; weight 96 g. This species possesses a dense, fine, soft fur; dorsum with fur ranging from an ochraceous orange to buckthorn brown, hairs dark colored, except last 2.5 or 3 mm which are a distinctly lighter shade of the same color. Most individuals present a dark cap (Fuscous Black) on their heads. Color of ventral pelage is more sharply set off from dorsal in some individuals. Ears sparsely covered with short, brownish hairs, not contrasting conspicuously with color of head. Forefoot with a well-developed pollex, other four digits bearing long, basally closed claws. The skull is robust and wide. The interorbital region has squared margins and the frontals are widest at the level of connection of zygomatic arch, lateral to the posterior/anterior sutures of the frontals, nasals and maxillaries.

Distribution: *Ctenomys yatesi* is known only from the type locality, 7 kilometers north and 38 kilometers west of Roboré in southeastern Santa Cruz Department (Figure 10).

Natural history: Nothing is known of the natural history of Yates' tuco-tuco. The habitat was in fields of Yucca.

Species Conservation/Preservation Status: The species has not yet been evaluated by the IUCN or LRFSV. There have not been evident agriculturally based large-scale land-cover changes around the type locality of *C. yatesi* (Table 2), and only about 13 % of the surrounding area is protected and includes only the Reserva Departamental Valle de Tucavaca (Caraballo *et al.* 2020). Nonetheless, because it appears that this species has a very restricted distribution, close monitoring is warranted.

Results of species distribution modeling for 5 species of Bolivian Ctenomys *for changes in habitat suitability under greenhouse gas emission scenarios 4.5 and 8.5.* For the 5 species of tuco-tucos for which we had sufficient data (*i. e.*, numbers of individuals collected throughout the range of the species) with which to produce SDMs including *C. opimus, C. boliviensis, C. conoveri, C. frater*, and *C. steinbachi*, the model results were robust and statistically significant as evaluated by the pROC metric (Table 1). The habitat suitability approximation out to year 2070 was made using the median projected suitability (MPS). The MPS enabled us to predict suitable habitat for the species with different levels of global greenhouse gas emission. Using this aspect of the model we evaluated the effect of climatic and soil-related variables on potential distribution of these species (see figures 11 and 12).

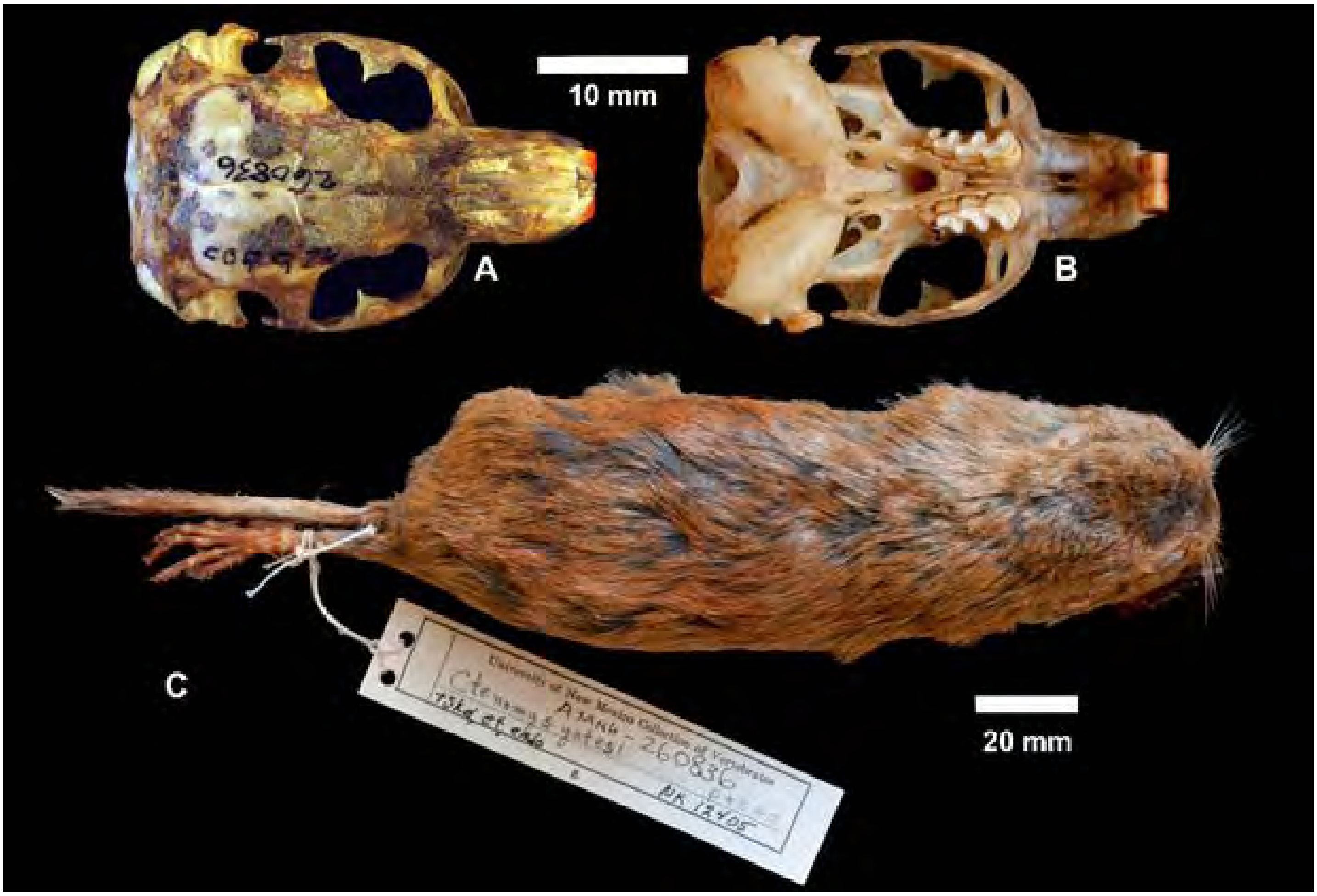

Figure 8. A-C. *Ctenomys yatesi* Gardner *et al.* 2014, Yates' tuco-tuco, el Tuquito de Yates or Yates' Tuquito. A. Photo image of dorsal aspect of skull. B. Photo image of ventral view of skull. C. Photo image of dorsal aspect of museum study skin.

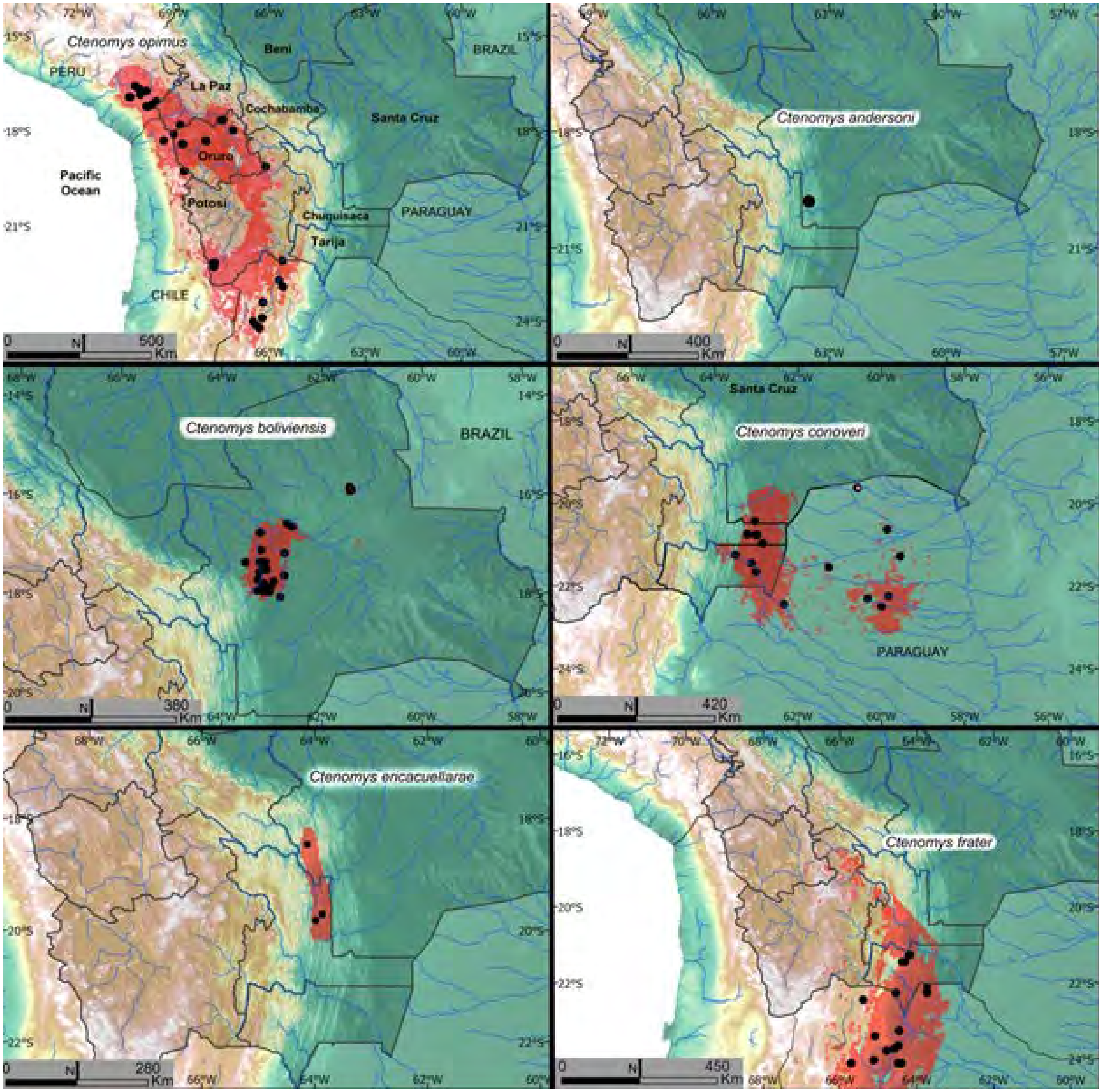

Figure 9. Estimated distribution of *Ctenomys* species of Bolivia. Black dots represent verified museum specimen collection locality records. Red-shaded areas represent the estimated distribution at the current time. Species names are included in each map. *Ctenomys opimus* is included first in the maps because this species has the largest geographic range, extending from southern Peru south through Bolivia, Chile, and northern Argentina. All departments in Bolivia are included on this map for comparison with the rest of the maps presented.

Discussion

An altitude-area optimization (not shown) onto the tree indicates that the probable common ancestor of the *Ctenomys* now occurring in Bolivia had an origin in high altitude areas of the Andes. It appears that tuco-tuco populations have been moving into open habitats, becoming isolated, re-united, and isolated again at geological time scales in the continually changing and evolving landscape of the central-eastern Andes of Bolivia. The greatest single factor that appears to be influencing the evolutionary trajectory of these rodents in the mountains is a component of the Andes called the Central Andean Backthrust belt (McQuarrie *et al.* 2005) that has created a dynamic erosional landscape resulting in incredibly diverse habitats ranging from cloud forest to cactus studded hillsides and valleys over only a few kilometers of straight-line geographic distance (Gardner *et al.* 2014; Unzueta 1975). Gardner and Ruedas (2019) showed, via optimization of occupied altitude of tuco-tuco species onto the phylogenetic tree of the ctenomyid species in Bolivia, that there was a striking correlation between the phylogeny and the age of origin of the eastern front-range mountain ridges of the Andes. The crustal folding from west to east combined with simultaneous and continuous erosion and riverine cutting of the north-south ridges appears to have resulted in episodes of repeated isolation and subsequent speciation in these rodents.

By applying species distribution models to predict suitable habitat for the known species of tuco-tucos in Bolivia within their range of distribution and evaluating the effect of climatic and soil-related variables on potential species distribution, we found that all the models resulted in robust predictions as indicated by the pROC ratios (Table 1). Not surprisingly, because of the fossorial/subterranean habit of these mammals, we found that soil variables have excellent predictive power and performed well in two of the five species for which their distributions were modeled, showing little difference in contribution to model performance with bioclimatic variables. We found higher average weight for the models when we including soil variables, indicating the utility of using these variable in the understanding of the habitat requirements of *Ctenomys* and probably other species of subterranean animals (see Figures 11 and 12).

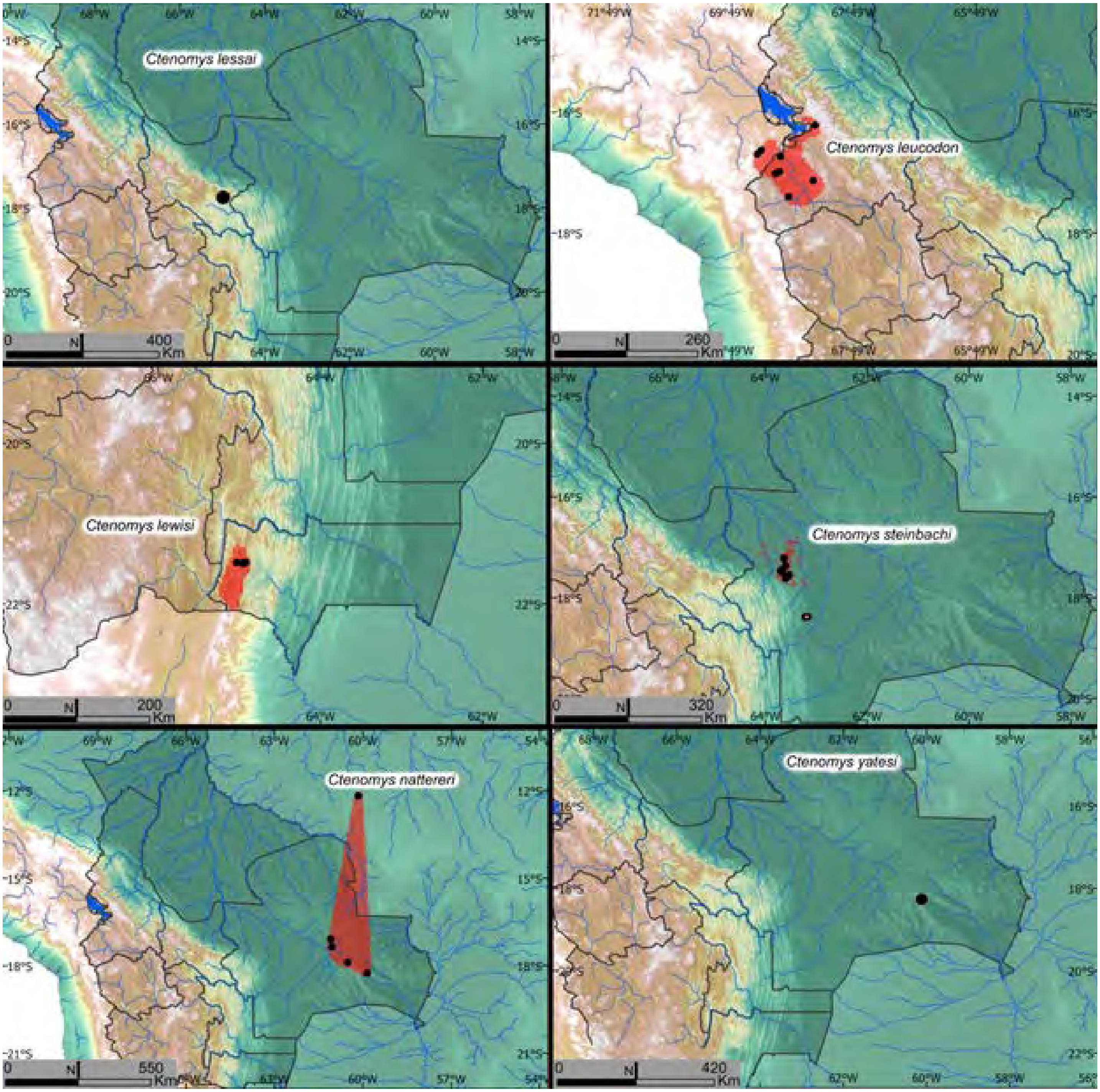

Figure 10. Estimated distribution of *Ctenomys* species of Bolivia. Black dots represent specimen collection localities. Red shaded areas represent the estimated distribution. Species names are included in each map.

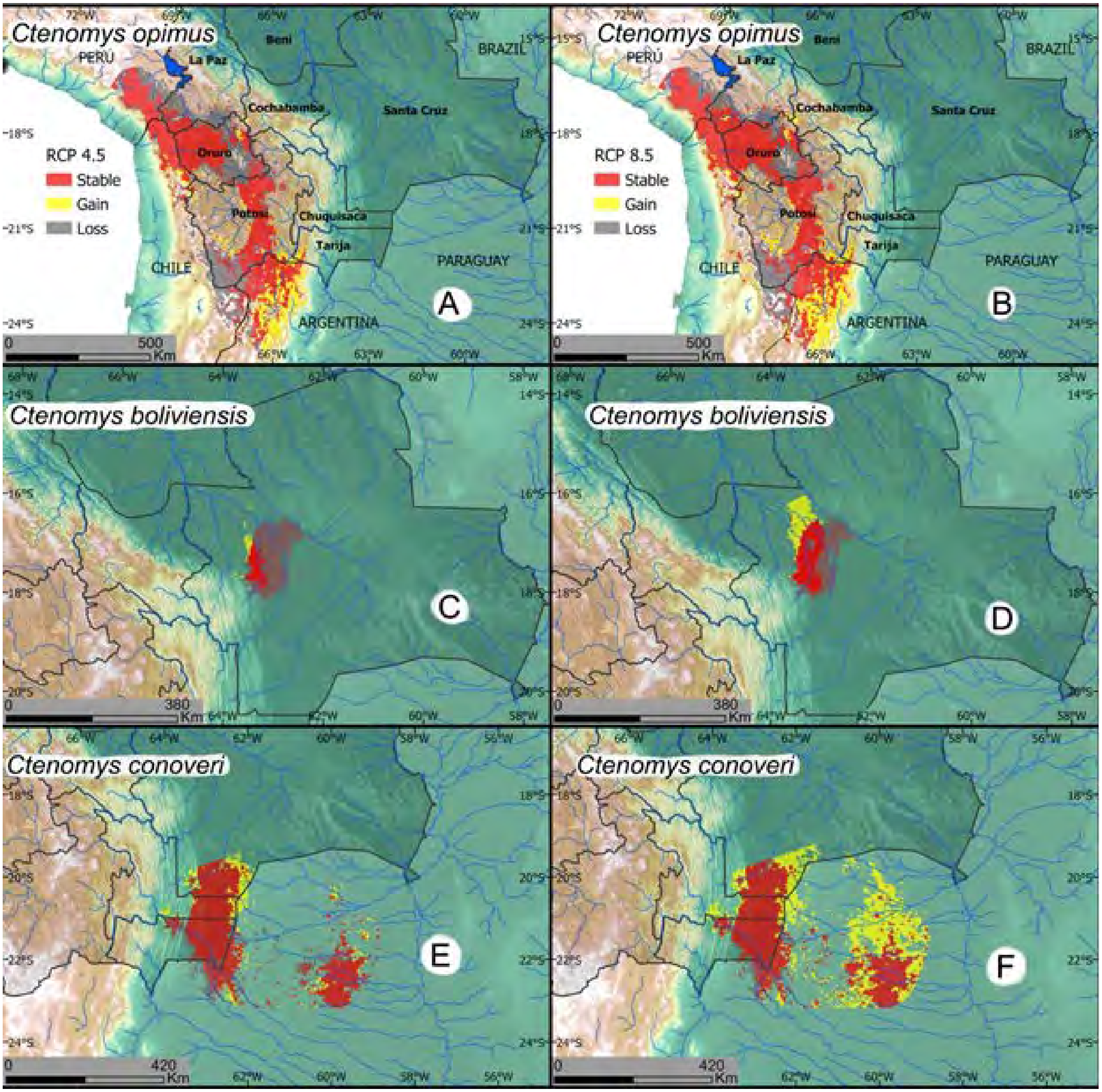

Figure 11. Projected changes in habitat suitability for 2070 under the 4.5 and 8.5 greenhouse gases emission scenarios. The 2070 suitable habitat is estimated for an assemble model employing the median projected suitability from 17 general circulation models. A. Predicted changes in habitat suitability for *C. opimus* under the 4.5 emission scenario. B. Predicted changes in habitat suitability for *C. opimus* under the 8.5 emission scenario. C. Predicted changes in habitat suitability for *C. boliviensis* under the 4.5 emission scenario. D. Predicted changes in habitat suitability for *C. boliviensis* under the 8.5 emission scenario. E. Predicted changes in habitat suitability for *C. conoveri* under the 4.5 emission scenario. F. Predicted changes in habitat suitability for *C. conoveri* under the 8.5 emission scenario.

Three of the five species that were model using ENM are expected to suffer significant range contractions by the year 2070 under both of the global carbon emission scenarios evaluated herein. Two of these species occur in the lowlands of the department of Santa Cruz (*C. steinbachi* and *C. boliviensis*) and one on the altiplano (*C. opimus*). Nonetheless, the extrapolations of suitable habitat distributions for the year 2070 should be considered with care as these estimates assume that the species will not adapt to new environments, perhaps because of complete niche conservatism, and that future environmental conditions nature are predicted to be analogous or comparable to current conditions (Elith and Leathwick 2009).

This study highlights the need for detailed conservation assessments and monitoring of tuco-tuco's in Bolivia as most of the species have already, or will soon experience, significant loss in habitat with concomitant fragmentation of their populations due to anthropogenic land cover change exacerbated by rapidly changing climatic conditions. In addition to these factors, the known geographic ranges of Bolivian tuco-tuco species (except for *C. opimus*) are small in spatial geographic extent, with three species known only from their type localities and in seven cases, a very small proportion of their estimated or actual distribution range is in some sort of protected area.

The analysis presented here suggests that *C. steinbachi* (because of its restricted range) and perhaps *C. boliviensis* should be moved into a higher IUCN threat category than their current IUCN allocation. This higher IUCN category is suggested because more than 20 % of their original geographic ranges have been significantly transformed by human activities. The anthropogenically mediated destruction of their habitats combined with predicted range contractions due to global warming portends the impending ecological and biodiversity catastrophe that is now taking place world-wide (Ceballos *et al.* 2020). In addition to these factors, the currently known geographic ranges of both *C. steinbachi* and *C. boliviensis* show very little overlap with any true protected areas (<5 %) and because much of the geographic ranges of these two species are near or in path of the rapidly expanding urban area of Santa Cruz de la Sierra, we expect additional pressure especially on these two species.

As presented here, the uses of ENMs and SDMs combined with broad-scale and intensive biological surveys of the mammalian fauna of Bolivia (Anderson 1997) will enable future students of biodiversity to have a basis for additional focussed studies. As the atmosphere of our earth continues to heat due to anthropogenically generated greenhouse gasses, unexpected effects on the species of mammals on the earth will be manifested (Ceballos *et al.* 2020). We hope that this work on documenting and eventually providing an

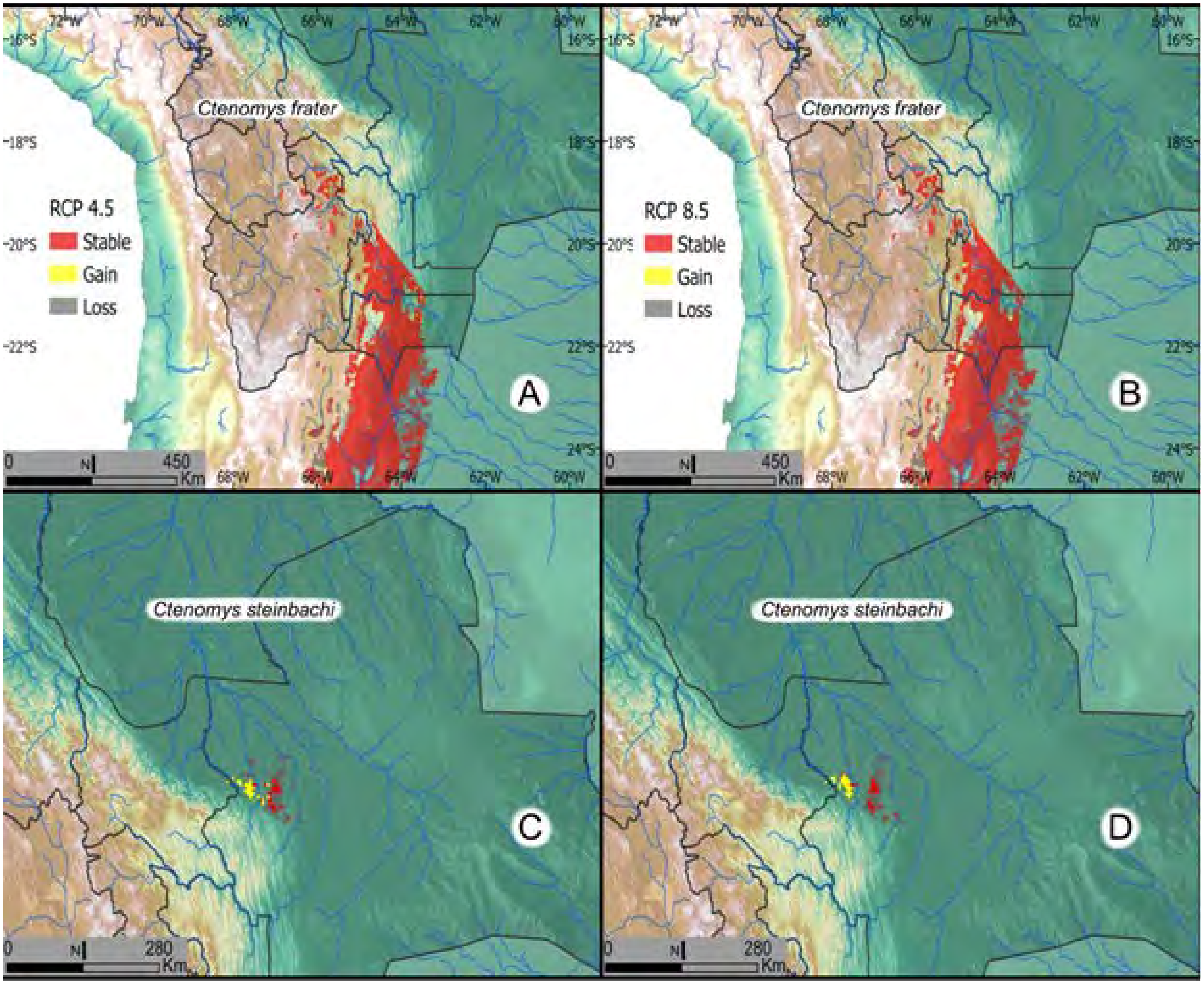

Figure 12. Projected changes in habitat suitability for 2070 under the 4.5 and 8.5 greenhouse gases emission scenarios. The 2070 suitable habitat is estimated for an assemble model employing the median projected suitability from 17 general circulation models. A. Predicted changes in habitat suitability for *C. frater* under the 4.5 emission scenario. B. Predicted changes in habitat suitability for *C. frater* under the 8.5 emission scenario. C. Predicted changes in habitat suitability for *C. steinbachi* under the 4.5 emission scenario. D. Predicted changes in habitat suitability for *C. steinbachi* under the 8.5 emission scenario.

understanding of the delicate balances of how mammals interact with their environment over geographic space will provide a model for future research. At the very least, this work will provide a baseline from which additional studies can be formulated, proposed, funded, and carried out. In his "Mammals of Bolivia," Syd Anderson (1997, p 3) stated about his book: "This work is dedicated to the hypothesis-testers of this world. Everything concluded here is subject to further testing. I expect much of this information to be substantiated, but not everything." We consider the current work to cohere to the way Syd approached his studies and our hypotheses, presented herein, and because they are based on museum specimens, will be available for testing far into the future. We feel that the prospects of viable biodiversity on our earth will be sustained if we are able to recruit new researchers walking the path of Syd and testing some of the hypotheses presented herein and generating new hypotheses along the way.

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Literature cited

Aiello-lammens, M. E., R. A. Boria, A. Radosavljevic, B. Vilela, and R.P. Anderson. 2015. spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. Ecography 38: 541–545.

Anderson, S., T. L. Yates, and J. A. Cook. 1987. Notes on Bolivian mammals 4: the genus *Ctenomys* (Rodentia: Ctenomyidae) in the eastern lowlands. American Museum Novitates 2891:1-20.

Anderson, S. 1997. Mammals of Bolivia: taxonomy and distribution. Bulletin of the AMNH 231 pp.

Ashe, J. S., R. M. Timm, and M. H. Gallardo. 1996. Systematics, distribution, and host specificity of *Edrabius fauvel* (Insecta: Coleoptera: Staphylinidae). Proceedings of the Biological Society of Washington 109:731-743.

Barve, N., V. Barve, A. Jiménez-valverde, A. Lira-noriega, S.P. Maher, A.T. Peterson, J. Soberón, and F. Villalobos. 2011. The crucial role of the accessible area in ecological niche modeling and species distribution modeling. Ecological Modelling 222:1810–1819.

Bidau, C. J. 2006. Familia Ctenomyidae. Pp. 212 -231, *in* Mamíferos de Argentina. Sistemática y distribución (Bárquez,R. J., M. M. Díaz, and R. A. Ojeda, eds.). Sociedad Argentina para el Estudio de los Mamíferos. Tucumán, Argentina.

Bidau, C. J. 2015. Family Ctenomyidae Lesson, 1842. In: Mammals of South America 2:818-877.

Brant, S. V., and S .L. Gardner. 1997. Two new species of *Litomosoides* (Nemata: Onchocercidae) from *Ctenomys opimus* (Rodentia: Ctenomyidae) on the Altiplano of Bolivia. Journal of Parasitology 83:700-705.

Breiner, F. T., A. Guisan, A. Bergamini, and M. P. Nobis. 2015. Overcoming limitations of modelling rare species by using ensembles of small models. Methods in Ecology and Evolution 6:1210– 1218.

Breiner, F. T., M. P. Nobis, A. Bergamini, and A. Guisan. 2018. Optimizing ensembles of small models for predicting the distribution of species with few occurrences. Methods in Ecology and Evolution 9:802-808.

Busch, C., C. D. Antinuchi, J. C. del Valle, M. J. Kittlein, A. I. Malizia, A. I. Vassallo, and R. R. Zenuto. 2000. Population ecology of subterranean rodents. Pp 183–226, *in* Life Underground: The Biology of Subterranean Rodents (Lacey E. A., J. L. Patton, and G. N. Cameron, eds). University of Chicago Press. Chicago, Illinois.

Caldas, M. M., D. Goodin, S. Sherwood, J. M. Campos Krauer, and S. M. Wisely. 2015. Land-cover change in the Paraguayan Chaco: 2000–2011. Journal of Land Use Science 10: 1-8.

Campbell, L.P., C. Luther, D. Moo-llanes, J. M. Ramsey, R. Danis-lozano, and A. T. Peterson. 2015. Climate change influences on global distributions of dengue and chikungunya virus vectors. Philosophical Transactions of the Royal Society B: Biological Sciences 370:20140135

Caraballo, D. A., S. L. López, A. A. Carmarán, and M. S. Rossi. 2020. Conservation status, protected area coverage of *Ctenomys* (Rodentia, Ctenomyidae) species and molecular identification of a population in a national park. Mammalian Biology 100:33-47.

Castillo, A., M. Cortinas, and E. P. Lessa. 2005. Rapid diversification of South American tuco-tucos (*Ctenomys*: Rodentia: Ctenomyidae): Contrasting mitochondrial and nuclear intron sequences. Journal of Mammalogy 86:170–179.

Ceballos, G., P. R. Ehrlich, and P. H. Raven. 2020. Vertebrates on the brink as indicators of biological annihilation and the sixth mass extinction. Proceedings of the National Academies of Sciences of the United States 117:13596-13602.

Cobos, M. E., A. T. Peterson, N. Barve, and L. Osorio-Olvera. 2019. kuenm: an R package for detailed development of ecological niche models using Maxent. PeerJ 7:e6281.

Cook, J. A., S. Anderson, and T. L. Yates. 1990. Notes on Bolivian mammals 6: the genus *Ctenomys* (Rodentia: Ctenomyidae) in the highlands. American Museum Novitates 2980:1–27.

Cook, J. A., and T. L. Yates. 1994. Systematic relationships of the tuco-tucos, genus *Ctenomys* (Rodentia: Octodontidae). Journal of Mammalogy 75: 583–599.

Cook, J. A., and E. P. Lessa. 1998. Are rates of diversification in subterranean South American tuco-tucos (genus *Cteno-*

mys, Rodentia: Octodontidae) unusually high? Evolution 52:1521–1527.

Darwin, C. R. 1860. Journal of researches into the natural history & geology of the countries visited during the voyage round the world of H.M.S. 'Beagle' under the command of captain Fitz Roy, R. N. John Murray. Albemarle St., London.

Di Cola, V., O. Broennimann, B. Petitpierre, F.T. Breiner, M. D'amen, C. Randin, R. Engler, J. Pottier, D. Pio, A. Dubuis, L. Pellissier, R. G. Mateo, W. Hordijk, N. Salamin, and A. Guisan. 2017. Ecospat: an R package to support spatial analyses and modeling of species niches and distributions. Ecography 40:774–787.

Drabik, G. O., and S. L. Gardner. 2019. A new species *Ancylostoma* (Nemata: Strongylida: Ancylostomatidae) from two species of *Ctenomys* in lowland Bolivia. Journal of Parasitology 105:904-912.

Dunnum, J., and N. Bernal. 2016. *Ctenomys boliviensis* (errata version published in 2017). The IUCN Red List of Threatened Species 2016: e.T5798A115079917. https://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T5798A22192171.en. Downloaded on 03 May 2020.

Dunnum, J., J. Vargas, N. Bernal, N. Zeballos, and E. Vivar. 2016. *Ctenomys opimus*. The IUCN Red List of Threatened Species 2016: e.T5818A22194246. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T5818A22194246.en. Downloaded on 06 June 2020.

Eisenberg, J. F. 1963. The behavior of heteromyid rodents. University of California Publications in Zoology 69: 1-114.

Elith, J., and J. R. Leathwick. 2009. Species distribution models: Ecological explanation and prediction across space and time. Annual Review of Ecology, Evolution, and Systematics 40:677–697.

Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37:4302-4315.

Fourcade, Y., J. O. Engler, D. Rödder, and J. Secondi. 2014. Mapping species distributions with MAXENT using a geographically biased sample of presence data: A performance assessment of methods for correcting sampling bias. PLoS One 9: e97122.

Freitas, T. R. O. 2006. Cytogenetics status of four *Ctenomys* species in the south of Brazil. Genetica 126:227–235.

Gardner, S. L. 1991. Phyletic coevolution between subterranean rodents of the genus *Ctenomys* (Rodentia: Hystricognathi) and nematodes of the genus *Paraspidodera* (Heterakoidea: Aspidoderidae) in the Neotropics: temporal and evolutionary implications. Zoological Journal of the Linnean Society 102:169-201.

Gardner, S. L, and D.W. Duszynski. 1990. Polymorphism of Eimerian Oocysts can be a problem in naturally infected hosts: An example from subterranean Rodents in Bolivia. Journal of Parasitology 76:805-811.

Gardner, S. L. and L. Ruedas. 2019. Origin and development of the South American montane mammal fauna by the Andean speciation engine. Abstract 197: 81. http://www.mammalsociety.org/uploads/2019%20ASM%20Abstracts-2019.06.18.pdf

Gardner, S. L. 1991. Phyletic coevolution between subterranean rodents of the genus *Ctenomys* (Rodentia: Hystricognathi) and nematodes of the genus *Paraspidodera* (Heterakoidea: Aspidoderidae) in the Neotropics: temporal and evolutionary implications. Zoological Journal of the Linnean Society 102:169-201.

Gardner, S. L., and S. Anderson. 2001. Persistent fontanelles in rodent skulls. American Museum Novitates 3327:1–15.

Gardner, S. L., J. Salazar-bravo, and J. A. Cook. 2014. New Species of *Ctenomys* Blainville 1826 (Rodentia: Ctenomyidae) from the lowlands and central valleys of Bolivia. Special Publications, Museum of Texas Tech University 62:1-34.

Hengl, T., G. B. Mendes de Jesus, M. Heuvelink, M. R. Gonzalez, M. Kilibarda, A. Blagotić, W. Shangguan, M. N. Wright, X. Geng, B. Bauer-Marschallinger, M. A. Guevara, R. Vargas, R. A. MacMillan, N. H. Batjes, J. G. B. Leenaars, E. Ribeiro, I. Wheeler, S.Mantel, and B. Kempen. 2017. SoilGrids250m: Global gridded soil information based on machine learning. Plos One 12:e0169748.

Ibisch, P., S. Beck, B. Gerkmann, and Y.A. Carretero. 2003. Ecoregiones y ecosistemas. Pp. 47–88, *in* Biodiversidad: La Riqueza de Bolivia (Ibisch, P., and G. Mérida, eds.). FAN. Santa Cruz, Bolivia.

IUCN. 2020. The IUCN Red List of Threatened Species. Version 2020-1. https://www.iucnredlist.org. Downloaded on 20 May 2020.

Lacey, E. A., J. L. Patton, and G. N. Cameron. 2000. Life underground: the biology of subterranean rodents. University of Chicago Press. Chicago, Illinois.

Lambert, C. R., S. L. Gardner, and D. W. Duszynski. 1988. Coccidia (Apicomplexa: Eimeriidae) from the subterranean rodent *Ctenomys opimus* Wagner (Ctenomyidae) from Bolivia, South America. Journal of Parasitology 74:1018-1022.

Lessa, E. P., and J. A. Cook. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Molecular Phylogenetics and Evolution 9:88–99.

López, R. P. 2003. Phytogeographical relations of the Andean dry valleys of Bolivia. Journal of Biogeography 30:1659–1668.

Mascheretti, S., P. Mirol, M. Giménez, C. Bidau, J. Contreras, and J. Searle. 2000. Phylogenetics of the speciose and chromosomally variable genus *Ctenomys* (Ctenomyidae: Octodontoidea), based on mitochondrial cytochrome-b sequences. Biological Journal of the Linnean Society 70: 361–376.

Mcquarrie, N., B. K. Horton, G. Zandt, S. Beck, and P. G. Decelles. 2005. Lithospheric evolution of the Andean fold-thrust belt, Bolivia, and the origin of the central Andean plateau. Tectonophysics 399:15–37.

Ministerio De Medio Ambiente y Agua. 2009. Libro rojo de la fauna silvestre de vertebrados de Bolivia. Ministerio de Medio Ambiente y Agua. La Paz, Bolivia.

Muscarella, R., P. J. Galante, M. Soley-guardia, R. A. Boria, J. M. Kass, M. Uriarte, and R. P. Anderson. 2014. ENM eval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for Maxent ecological niche models. Methods in Ecology and Evolution 5:1198-1205.

Olrog, C. C., and M. M. Lucero. 1981. Guía de los mamíferos argentinos. Ministerio de Cultura y Educación, Tucumán: Fundación Miguel Lillo.

Parada, A. S., G. D'Elía, C. J. Bidau, and E. P. Lessa. 2011. Species groups and the evolutionary diversification of tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). Journal of Mammalogy 92:671–682.

Pearson, O. P. 1959. Biology of the subterranean rodents, *Ctenomys*, in Peru. Memorias del Museo de Historia Natural Javier Prado 9:3-56.

Phillips, S. J., and M. Dudík. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31:161–175.

Pucu, E., M. Lareschi, and S. L. Gardner. 2014. Bolivian ectoparasites: a survey of the fleas of *Ctenomys* (Rodentia: Ctenomyidae). Comparative Parasitology 81:114-118.

Raxworthy, C. J., E. Martinez-meyer, N. Horning, R. A. Nussbaum, G. E. Schneider, M. A. Ortega-huerta, and A. T. Peterson. 2003. Predicting distributions of known and unknown reptile species in Madagascar. Nature 426:837-841.

Ridgway, R. 1912. Color standards and color nomenclature. Published by the author, A. Hoen (printer). Baltimore, Maryland. 53 colored plates.

Roach, N., and L. Naylor. 2016a. *Ctenomys lewisi*. The IUCN Red List of Threatened Species 2016: e.T5811A22193810. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T5811A22193810.en. Downloaded on 06 June 2020.

Roach, N., and L. Naylor. 2016b. *Ctenomys steinbachi*. The IUCN Red List of Threatened Species 2016: e.T5827A22194994. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T5827A22194994.en. Downloaded on 12 June 2020.

Teta, P., and G. D'Elía. 2020. Uncovering the species diversity of subterranean rodents at the end of the World: Three new species of Patagonian tuco-tucos (Rodentia, Hystricomorpha, *Ctenomys*). PeerJ. 2020;8:e9259. Published 2020 May 29. doi:10.7717/peerj.9259

Thomas, O. 1919. Two new rodents from Tartagal, Salta, N. Argentina. Annals and Magazine of Natural History, ser. 9, 4: 154-156.

Thomas, O. 1921. A new tuco-tuco from Bolivia. Annals and Magazine of Natural History, ser. 9, 7:136–137.

Thomas, O. 1926. The Spedan Lewis South American exploration, II: On mammals collected in the Tarija Department, southern Bolivia. Annals and Magazine of Natural History, ser. 9, 17:318–328.

Unzueta, Q. O. 1975. Mapa ecológico de Bolivia. 1:1,000,000. Ministerio de Asuntos Campesinos y Agropecuarios, Memoria explicativa by Unzueta, 11 pp. Color pl., 312 pp.

Vivar, E. 2017. *Ctenomys frater* (errata version published in 2018). The IUCN Red List of Threatened Species 2017: e.T115553730A123796865. https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T115553730A115553734.en. Downloaded on 04 June 2020.

Wallace, A. R. 1876. The geographical distribution of animals. With a study of the relations of living and extinct faunas as elucidating the past changes of the earth's surface. Volumes 1 and 2. Macmillan and Co. London, U.K.

Wilson, D. E., and D. M. Reeder (eds.). 2005. Mammal Species of the World, a Taxonomic and Geographic Reference, 3rd ed. Johns Hopkins Press, Baltimore, Maryland.

Wilson, D. E., T. E. Lacher Jr., and R. A. Mittermeier (eds.). 2017. The Mammals of the World-Vol. 7, Rodents II. Lynx Edicions. Barcelona, España.

Zeballos, H., and E. Vivar . 2016. *Ctenomys leucodon*. The IUCN Red List of Threatened Species 2016: e.T5810A22193526. https://dx.doi.org/10.2305/IUCN.UK.2016-2.RLTS.T5810A22193526.en. Downloaded on 05 June 2020.

Zurell, D., J. Franklin, C. König, P. J. Bouchet, C. F. Dormann, J. Elith, G. Fandos, X. Feng, G. Guillera-Arroita, A. Guisan, and J. J. Lahoz-Monfort. 2020. A standard protocol for reporting species distribution models. Ecography 43:1261-1277.

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Occupancy rate and observations of Baird's tapir (*Tapirella bairdii*) near waterholes in the Maya forest corridor, Belize

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It is estimated that 25 % of the mammal species in the world are at risk of extinction due to habitat loss, fragmentation, farming, and overexploitation. Baird's tapir (*Tapirella bairdii*), an ungulate with part of its distribution within the Maya Forest, is at risk of extinction. Waterholes, a primary source for water and an essential element for the survival of tapirs, were the target of this study design. We used a camera-trap survey to determine tapir occupancy and detection rate probability models at seven survey stations at Runaway Creek Nature Reserve which is located within the Maya Forest Corridor in central Belize. The survey was carried out from March to September 2015 and from January 2017 to October 2019 with a total sampling effort of 8,932 camera-trap nights. Our results indicated a cumulative naïve occupancy estimate of 85.7 %, a rate probability of occupancy of 0.97 +/- 0.15 (SE), and a probability of detection of 0.14 +/- 0.01 for all sites and years. The results of occupancy models with human infrastructure covariate showed that the distance to roads model had the highest influence on tapir occurrence (β = -0.95 +/- 0.87), followed by the village covariate model (β = 0.77 +/- 0.99). The topographic covariate of river model (β = 0.47 +/- 0.85) had minimal support. This study highlights the critical importance of waterholes for survival of tapirs, and the influence of roads on tapir occurrence at Runaway Creek Nature Reserve.

Se estima que el 25 % de las especies de mamíferos del planeta se encuentran en riesgo de extinción debido a perdida de hábitat, fragmentación y sobreexplotación. El tapir (*Tapirella bairdii*), un ungulado con parte de su distribución en la Selva Maya está en peligro de extinción. Cuerpos de aguas, una fuente esencial para la sobrevivencia de los tapires fue el enfoque de este estudio. Usamos cámaras-trampa como unidades de muestreo para determinar índice y modelos de ocupación en siete sitios en Runaway Creek situado en el corredor del Bosque Maya en Belice central. El estudio se llevó a cabo de marzo a septiembre 2015, y de enero 2017 a octubre 2019 con un esfuerzo de muestreo de 8,932 cámaras-trampa/noche. Nuestros resultados indican una estimación acumulativa (naive) de ocupación de 85.7 %, con un índice de probabilidad de ocupación de 0.97 +/- 0.15 (SE), probabilidad de detección de 0.14 +/- 0.01 de todo los sitios y años. Los modelos de ocupación de los variables de infraestructura indica que el modelo de carreteras (β = -0.95 +/- 0.87) tiene la mayor influencia seguido por el modelo de poblados (β = 0.77 +/- 0.99). El modelo de variable de rio (β = 0.47 +/- 0.85) tiene menos apoyo. El estudio resalta la importancia de cuerpos de agua para la sobrevivencia de los tapires y la influencia de las carreteras en la ocurrencia de tapires en la reserva natural Runaway Creek.

Keywords: camera trap; central american tapir; distance to road; occupancy rate; Selva Maya.

Introduction

It is estimated that approximately 25 % of the world's mammal species and 44 out of 77 large herbivores are threatened with extinction, mainly because of land use change and overexploitation (Baillie *et al.* 2010; IUCN 2013). Despite great efforts being made on their conservation, the extinction threat prevails as anthropogenic pressures (*e. g.,* destruction of suitable habitat) increase. Efforts such as the creation of protected areas, enhanced connectivity, reduction of human footprints and reintroduction of species are a few of the mitigation initiatives being implemented to curve the demise of endangered mammals (Margules and Sarkar 2007; Bodin and Norberg 2007; Kadoya 2009).

Drought, an extreme natural event, can have devastating impacts on animal populations and it is a potential factor in species extinction (Duncan *et al.* 2012). The Intergovernmental Panel on Climate Change (IPCC) reported a likely increase in droughts over the 21st century in various regions of the world, including southern Europe and the Mediterranean, central Europe, central North America, Central America and Mexico, northeast Brazil, and southern Africa (IPCC 2012). The Maya Forest in the Mesoamerican region is experiencing below average rainfall and more intense droughts are predicted to occur in many areas due to climate change (Mardero *et al.* 2012). Drought poses an additional threat to mammals, especially those that are water dependent such as some ungulates (Terwilliger 1978; Naranjo 1995; Algers *et al.* 1998; Foerster and Vaughan 2002; Reyna-Hurtado *et al.* 2009).

The focal species of this study was Baird's tapir (*Tapirella bairdii*) the largest land mammal in Central America. Historically, its geographic distribution extended from southern Veracruz, México, to northeastern Ecuador (Reid 2009; Garcia *et al.* 2016; Hernandez *et al.* 2020). Its elevational range extends from sea level to the mountains (3,620 masl; Naranjo 2009). Baird's tapir exhibits an affinity for lowlands and it is highly dependent on water (Wainwright 2007). Tapirs play a unique role in forest regeneration due to their feeding ecology as a herbivore and seed disperser (Fragoso *et al.* 2003).

Central America has experienced a 70 % reduction of forest cover in the last 40 years, and Baird's tapir has suffered a dramatic reduction in both distribution and population size over the past several decades. It is now estimated that Baird's tapir populations are reduced in size by 50 % (Garcia *et al.* 2016). They were considered vulnerable by the International Union for the Conservation of Nature (IUCN) in 1996, but were subsequently uplisted to endangered in 2002 (IUCN 2013). Moreover, Baird's tapir was ranked 34th in urgency for conservation among more than 4,000 mammal species assessed by experts from the Institute of Zoology of London due to its level of evolutionary distinctiveness and level of threat (Isaac *et al.* 2007). Tapirs were considered the 10th rarest forest mammal in the Neotropics (Dobson and Yu 1993). This alarming status is due to the combined effects of tapirs' natural rarity, habitat loss, habitat fragmentation, overhunting, and their vulnerability to cattle-borne disease (Castellanos *et al.* 2008).

The Maya Forest, where the tapir thrives, is the largest continuous block of tropical forest in Mesoamerica and it is a site of global importance for biodiversity. It extends through parts of northwestern Belize, northern Guatemala, and southern Mexico. The Maya Forest is well known for its rich biodiversity, a substantial array of endemic species, and several ancient civilizations (Garcia-Gil and Pat 2001). This area is also highly important for the conservation of wide-ranging species that require extensive tracts of intact forest to sustain viable populations, including Baird's tapir (Naranjo 2009) and the white-lipped peccary (*Tayassu pecari*; Reyna-Hurtado *et al.* 2009). The Maya Forest has been categorized as one of the strongholds for tapir populations due to its suitable habitats with large continuous areas that allow potential connectivity across the Yucatan Peninsula with northeastern Guatemala and Belize (Mendoza *et al.* 2013; *Schank et al.* 2015). Tobler (2002) showed that tapirs in southern Costa Rica were more abundant in areas with limited human presence. These requirements indicate that tapirs thrive in large and undisturbed protected areas as opposed to human dominated landscapes.

Despite tapirs' level of extinction threat and their ecological and evolutionary relevance, there are critical gaps in our understanding of their basic ecology such as habitat use, habitat selection, spatial ecology and population demography (García *et al.* 2012). The availability of water resources in the Maya Forest has increasingly become indispensable for ungulates of concern such as Baird's tapir (Reyna-Hurtado *et al.* 2019) and white-lipped peccary (Moreira-Ramirez *et al.* 2016). Due to the fact that water can be a limiting factor to Baird's tapir populations (Krausman and Etchberger 1995; Cain *et al.* 2006; Krausman *et al.* 2006), and that the dry season (December to May) is becoming more intense and anthropogenic pressures (*e. g.,* habitat fragmentation and road modernization) are increasing, we focused our study on assessing tapir occupancy and detection at waterholes at Runaway Creek Nature Reserve (RCNR) in Belize. Waterholes at this study site are depressions filled with water during the peak of the wet season.

Our primary objective was to estimate site-specific variables that influence the occurrence of tapirs by integrating the data into an occupancy analysis (MacKenzie *et al.* 2006). We used camera-trap data from waterholes at RCNR in Belize to test the hypothesis that the probability of tapir occupancy and detection were higher when further away from human infrastructure (*e. g.,* villages, roads) and closer to topographic covariates (*e.g.,* river, elevation).

Methods and Materials

Study site. The Maya Forest is a tri-national and continuous tropical forest in Mesoamerica that encompasses the southern Mexican states of Campeche, Chiapas, Quintana Roo and Yucatan, the northern Petén department in Guatemala, and northwestern Belize. Our study site at Runaway Creek Nature Reserve (RCNR) is located in central Belize within the Belize district (Figure 1). Its location is at the southernmost region of the Maya Forest. RCNR is a core section of the Maya Forest Corridor within central Belize connecting the tri-national Maya Forest with the Chiquibul Maya Mountains massif of southern Belize. The corridor is considered the last viable region for wildlife species to persist. RCNR is a private nature reserve established in 1999 with an extension of 2,500 hectares at the geographical coordinates 17° 22' N, -88° 35' W. The elevation ranges from 20 to 120 m above sea level and the area has an annual precipitation of 2,000 to 2,200 mm. The region has two main seasons: wet (June to November) and dry (December to May). At the peak of the wet season, the river floods and supplies the seasonal waterholes with water and at the peak of dry season water becomes a limiting factor. Topographically, RCNR has a range of karst hills and falls within the watershed of the Sibun River. Vegetation types include pine savanna forest, broad leaf forest, riparian forest, and tropical lowland forest (Meerman 1999).

Methods. We conducted camera trap surveys at seven sites at RCNR. Camera-trap stations were deployed along trails leading to waterholes (*aguadas*). We selected waterholes based on their availability within the reserve and their capacity to retain water during the peak of the dry season. The average size of waterholes was 50 m2 and 2 m in depth at the peak of the wet season. The remote digital camera-traps (Bushnell 12MP Trophy camera HD, Brown) were attached to trees at a height of 40 to 50 cm above ground, and were set with a delayed trigger time of 0.8 seconds. The cameras were interspersed 1.0 to 3.7 km (mean = 2.5 km) apart to permit independence of capture events. This distance fits well with the most common estimate of Baird's tapir home range of 1.0 to 3.0 km2 (Foerster and Vaughan 2002), but there are exceptions that demonstrate that tapirs could move up to 23 km2 (Reyna-Hurtado *et al.* 2016). Following Sandoval-Seres

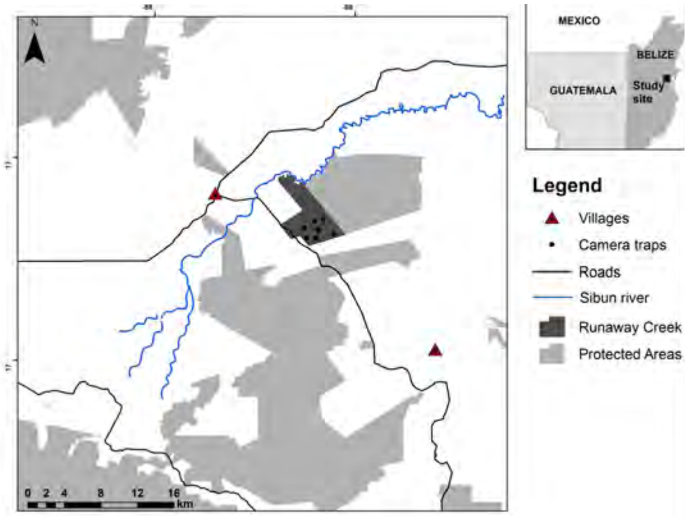

Figure 1. Map depicts Runaway Creek Nature Reserve study site in Belize, showing the network of protected areas, camera trap site locations, roads, and villages.

et al. (2016), we used a selection criterion of less than 24 hour intervals between camera capture events for considering independence. Camera-traps were checked every three weeks to download data and service the cameras.

We used the occupancy modeling methodology to infer the probability of tapir occupancy and detection with standard of error (SE) estimates. Occupancy is an alternative state variable to abundance, that uses the proportion when detection is lower than 1 (MacKenzie *et al.* 2006). We partitioned the detection history into 176 blocks of 7 days each and incorporated them in a single-season occupancy model using the program Presence 12.37 (Hines 2009). Although the data were collected over several seasons and years, we did not resample any sites in a multi-season framework. We do not believe that grouping the field seasons violates any of the assumptions of the modelling process because this analysis refers to occupancy as site use (Cove *et al.* 2013).

Seven candidate models were evaluated using five site-specific covariates. The covariates were selected based on known ecology of tapirs and possible factors that influence occurrence and detectability (Wainwright 2007; Padilla *et al.* 2010; Licona *et al.* 2011;Cove *et al.* 2013). We measured the nearest distances in km to three human disturbance areas or infrastructure (village, road, and forest edge) and two topographic factors (river and elevation) with the use of a Garmin GPS and Google Earth imagery (City and Country). All continuous covariate data were standardized to z scores for further analysis (MacKenzie *et al.* 2002) and the models were averaged using the maximum likelihood of occupancy (Burnham and Anderson 2002).

In this study we assumed that all survey sites had an equal probability of tapir detection (p) and occurrence (psi), so we allowed occupancy (Ψ) to vary with the covariates. The models were evaluated using Akaike Information Criterion corrected for small sample size (AICc) and Akaike weights (w). All models considered were within 95 % CI (Confidence Intervals). The global model which includes all covariates was run to ensure that there were no covariate interactions. A Pearson correlation test was run on all covariates, and correlated covariates were not included in the analysis. The detection history together with the covariate were fitted in a single season occupancy model in PRESENCE 12.37 software (Hines 2009).

Results

The total sampling effort at RCNR was 8,932 camera-trap nights in two separate periods from March to December 2015, and January 2017 to October 2019, accumulating a total of 3.6 years of camera-trap sampling. No sampling was con ducted during the year 2016. The cumulative naïve occupancy estimate of both periods was 85.7 %; the probability of occupancy (psi) was 0.97 +/-0.15 (SE), and the probability of detection (p) was 0.14 +/- 0.01 for all sites and years. Using photo data from each camera site and the cumulative of all the years, the model that kept both occupancy and detection constant had the highest support with an AIC weight value of 0.49 (Table 1). However, models of distance to roads and villages had the highest influence in occupancy with AIC weight of 0.14 and 0.11, respectively. The river covariate was at the mid-section of models ranking with 0.09 of AIC weights. The global model and series of model combinations with road permutation and all covariates had the lowest support for occupancy model ranking.

The untransformed coefficient of covariates for occupancy models showed that villages (β = 0.77 +/- 0.99), and rivers (β = 0.47 +/- 0.86) had a positive relation, while roads (β = -0.95 +/- 0.87) had a negative relation for occupancy models (Table 2). Occupancy modeling was also conducted for each independent year with an increase of occupancy and detection probabilities from 2015 to 2019 (Table 3). Naïve occupancy estimate was 0.71, 0.51, 0.86, and 1.0, respectively for each year (Table 3).

Table 1. Occurrence probability models of Baird's tapir based on camera traps at Runaway Creek Nature Reserve, Belize, showing the covariates with Akaike ranking and weights.

Model	QAIC	Delta QAIC	AIC wgtModel	Likelihood	no.Par.	-2*LogLike
psi(.),p(.)	488.18	0	0.49	1.00	2.00	726.27
psi(Road),p(.)	490.65	2.47	0.14	0.29	2.00	729.97
psi(Village),p(.)	491.18	3.00	0.11	0.22	2.00	730.77
psi(River),p(.)	491.41	3.23	0.09	0.19	2.00	731.12
psi(Road and River),p(.)	492.47	4.29	0.05	0.11	3.00	729.71
psi(Road and village),p(.)	492.56	4.38	0.05	0.11	3.00	729.84
psi(Global),p(.)	493.29	5.11	0.03	0.0777	6.00	721.93

Table 2. Model selection statistics and occurrence probabilities models with all covariates for Baird's tapir based on camera traps at Runaway Creek Nature Reserve, Belize with untransformed coefficient.

				Untransformed coefficients of ovariates (SE)[1]			
Model	**Δ[2]**	**ω[3]**	**K[4]**	**(.)[5]**	**Road**	**Village**	**River**
Ψ(.) p(.)	0	0.32	2	3.32 (+4.47)			
Ψ(Road) p(.)	2.47	0.09	2		-0.95 (+0.87)		
Ψ(Village) p(.)	3	0.07	2			0.77 (+0.99)	
Ψ(River) p(.)	3.23	0.06	2				0.47 (+0.86)
Ψ(Road and River) p(.)	4.29	0.05	3		-1.44 (+1.39)		-0.71 (+1.44)
Ψ(Road and Village) p(.)	4.38	0.05	3		-0.85 (+0.93)	0.38 (+1.05)	

[1] Coefficients are in logit space and corresponds to each model covariate

[2] AIC difference

[3] Akaike weights

[4] Number of model parameters

[5] Model constant

Discussion

Our study of tapir occupancy at RCNR is unique because it is located at the core of the Maya Forest Corridor which is currently subject to the modernization of the Coastal road, and real estate development is rapidly encroaching on the last remaining forest in the area (Belize Press Office 2017). Despite the small sample size of camera sites, this study was reinforced with an extended period of camera trap sampling of the waterholes with 8,932 camera-trap nights over 3.6 years.

This study represents the first attempt in Belize's Maya Forest Corridor to evaluate the occurrence of Baird's tapirs at waterholes using human infrastructure and topographic covariates. The rate of tapir occupancy appears to be stable at RCNR with a probability of occupancy (psi) of 0.97 +/- 0.15 and probability of detection (p) of 0.14 +/- 0.01 for all sites and years. Slight increases in occupancy and detections rates were seen from 2015 and 2019, which can be attributed to unusually extended dry seasons in central Belize (Meteorological Service of Belize 2020). This study is similar to a 11-year study conducted at another site in the Maya Forest, the Calakmul Biosphere Reserve in Mexico (Reyna-Hurtado *et al.* 2019), where tapir occupancy rate was relatively constant despite huge variations on water availability on the forest floor. However, caution should be taken when applying occupancy results as an alternate for tapir abundance. Sampling at waterholes tends to overestimate tapir abundance due to waterholes acting as lures for tapirs. A potential explanation could be that tapirs use waterholes more frequently in dry season due to the lack of water in the landscape (O'Farrill *et al.* 2014). Another reason for overall high occupancy probability at our study site could be related to tapir movement patterns. *T. bairdii* tends to reduce the size of their core activity areas during the dry season and remain near sources of water (Foerster and Vaughan 2002; Noss *et al.* 2003; Trolle *et al.* 2008). This cluster in tapir movement patterns near water sources probably increases the capture rate at cameras (Reyna-Hurtado *et al.* 2016). Nevertheless, our study results should serve as baseline information for further and systematic sampling of this rare and endangered species.

Human disturbance, especially villages, influenced tapir occurrence, as shown by our top ranked models where tapir occurrence increased with distance to human infrastructures. This finding concurred with Cove *et al.* (2013), who found that increasing distance from villages led to higher tapir abundance in Costa Rica, and with Tobler (2002), who also found that tapirs in southern Costa Rica were more abundant in areas with limited human presence. Interestingly, the covariate of roads had the highest ranking in the occupancy model but with a negative relation (β = -0.95 (0.87 SE)) with tapir occurrence. This implies that tapirs at RCNR are thriving despite nearby roads. A potential explanation for this phenomenon is the underdeveloped status of the Coastal road which is located on the southwestern boundaries of RCNR. The 60 km-long Coastal road connects the George Price Highway in central Belize to the Hummingbird Highway in the south, and it is unpaved with only two villages that each have a population of around 400 inhabitants. Hence, the Coastal road is practically isolated with very low traffic. On the contrary, the Burrell Boom Highway in central Belize is paved and has higher traffic flow. Poot and Clevenger (2018), documented a total of 14 tapir-vehicle collisions from 2008 to 2012 along the Burrell Boom Highway. This collision documentation corroborates our road model findings that tapirs thrive near roads, but with very alarming mortality results. Furthermore, observations from September 2018 to September 2019 of two radio collared tapirs present in the study site showed that both tapirs had an established home range only 20 m away from the Coastal road (unpublished data). Apparently, the nearest road at RCNR is not a deterrent for tapirs.

The topographic covariate river received little support from the occupancy models. However, observation on the two radio collared tapirs revealed an interesting behavior towards the river and waterholes. Baird's tapir has recently been documented to have larger home ranges than previously reported. Jordan *et al.* (2019) documented a home range of 18.72 km^2, Reyna-Hurtado *et al.* (2016) with 23.9

Table 3. Occurrence and detection probabilities rates with Standard Error (SE), Naïve occupancy estimates per year of Baird's tapir based on camera traps at Runaway Creek Nature Reserve, Belize.

Year	Occurrence probability	Detection probability	Naïve occupancy estimate
2015	0.76 (0.18SE)	0.13 (0.02SE)	0.71
2017	0.57 (0.18SE)	0.10 (0.02SE)	0.51
2018	0.86 (0.13SE)	0.16 (0.2SE)	0.86
2019	1.00 (0SE)	0.18 (0.02SE)	1.00

km^2, and Naranjo (2009) documented a tapir moving 10 km to visit ponds. Observations on the radio collared tapirs at RCNR yielded a home range of 8 km^2 and they did not use free flowing water bodies (river or creek) despite these being an average distance of 3.5 km away from the tapirs' home ranges (unpublished data). Instead, they relied exclusively on waterholes for water requirements. These observations reinforce the importance of waterholes for tapirs at RCNR.

Our results suggest that Baird's tapir occupancy probability rate at waterholes appears to be stable when further away from human infrastructure (villages) and thriving near the Coastal road. The topographic covariate (river) had minimal support. Apart from undisturbed areas being ideal habitat for tapirs, this study highlights: 1) the critical importance of waterholes to tapirs and thus the need for higher protection efforts of habitat with suitable waterholes which is necessary for the survival of tapirs, and 2) tapirs are thriving at close distance to undeveloped roads and the need for careful planning of the modernization of the Coastal road which may increase tapir vehicle collisions.

Conservation implications. This study reports the current status of Baird's tapir on waterholes at RCNR. Apart from hunting, land use changes and increased infrastructure as main threats to the species, severe and prolonged dry seasons and their impact on the availability of water at ponds are also imminent threats (Magrin *et al.* 2007). More collaborative efforts on systematic research designs are essential not only for the conservation of tapir habitat but also for an understanding of waterhole usage dynamics. We propose a collaborative effort with the Belize Forest Department and Government of Belize in the installation of water containers on strategic waterholes like the Calakmul, Mexico model (Reyna-Hurtado *et al.* 2019). The continued monitoring of tapirs at waterholes on a long-term basis and with an increased sample size of waterholes and non-waterholes is also important. This initiative will identify which waterholes are of more importance to tapirs, and determine the rate of abundance, as well as find trends of usage by tapirs for management and conservation purposes. In addition, it is imperative to conduct more studies on movement ecology of tapirs. This type of study will shed light on the usage dynamics and movement patterns among the network of waterholes and within the Maya Forest Corridor. This study sets the path for future and similar studies in the Maya Forest where strategic research is needed to enhance survival and viability of the Baird's tapir population which is estimated to include 1,000 to 1,500 individuals in the region (Garcia *et al.* 2016).

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Literature cited

Algers, S. J., C. Vaughan, and C. Foerster. 1998. Resting site microhabitat selection by *Tapirus bairdii* during the dry season in Corcovado nationa park, Costa Rica. Vida Silvestre Neotropical 7:36–138.

Baillie, J. E. M., G. Janine, S.T. Turvey, J. Loh, and B. Collen. 2010. Evolution Lost: Status and trends of the world's vertebrates. Zoological Society of London. London, U. K..

Belize Press Office. 2017. Government signs contract for the updating of a feasibility study and the preparation of detailed designs for the upgrading of the Coastal Highway. Retrieved from www.belize.gov.bz. Accessed on June 3, 2020.

Bodin, O., and J. Norberg. 2007. A network approach for analyzing spatially structured populations in fragmented landscape. Landnscape Ecology 22:31–44.

Burnham, K. P., and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. New York, U.S.A.

Cain, J. W. I., P. R. Krausman, S. S. Rosenstock, and C. J. Turner. 2006. Mechanisms of thermoregulation and water balance in desert ungulates. Wildlife Society Bulletin 34:570–581.

Castellanos, A., C. Foerester, D. J. Lizcano, E. Naranjo, E. Cruz-Aldana, I. Lira Torres, and J. Gonzalez-Maya. 2008. Baird's Tapir, The IUCN red list of threaten species. http://doi.org/http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T21471A9284327 Accessed June 30, 2017.

Cove, M. V., L. E. P. Vargas, J. C. Cruz, R. M. Spinola, V. L. Jackson, J. C. Saenz, and O. Chassot. 2013. Factors influencing the occurrence of the endangered Baird's tapir *Tapirus bairdii*: potential flagship species for a Costa Rican biological corridor. Oryx 48:402–409.

Dobson, F. S., and J. Yu. 1993. Rarity in Neotropical forest mammals revisited. Conservation Biology 7:586–591.

Duncan, C., A. L. M. Chauvenet, L. M. McRae, and N. Pettorelli. 2012. Predicting the Future Impact of Droughts on Ungulate Populations in Arid and Semi-Arid Environments. PloS One 7:e51490.

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

Fragoso, J. M., K. Silvius, and J. A. Correa. 2003. Long distance seed dispersal by tapirs increase seed survival and aggregates tropical trees. Ecology 84:1998–2006.

Garcia-Gil, G., and J. Pat. 2001. Apropiación del espacio y colonización de la selva en la reserva de la biosfera Calakmul, Campeche. Revista Mexicana del Caribe 10:212–231.

García, M. J., E. P. Medici, E. J. Naranjo, W. Novarino, and R.S. Leonardo. 2012. Distribution, habitat and adaptability of the genus *Tapirus*. Integrative Zoology 7:346–55.

García, M., C. Jordan, G. O'Farril, C. Poot, N. Meyer, N. Estrada, R. Leonardo, E. Naranjo, A. Simons, A. Herrera, C. Urgilés, C. Schank, L. Boshoff, and M. Ruiz-Galeano. 2016. *Tapirus bairdii*. The IUCN Red List of Threatened Species 2016: e.T21471A45173340. http://dx.doi.org/10.2305/IUCN.UK.20161.RLTS.T21471A45173340.en Accessed on July 3, 2020.

Hernández-Ordonez, O., V. H. Reynoso and R. S. Lagunes. 2020. Recent records of *Tapirella bairdii* and *Panthera onca* in a region highly transformed by human activities in southern Veracruz, México. Therya 11:151-156

Hines, J. E. 2009. PRESENCE 6.9 Software to estimate patch occupancy and related parameters: USGS-PWRC. www.mbr-pwrc.usgs.gov/software/presence.html Accessed on July 3, 2020.

Intergovernmental Panel on Climate Change. 2012. Managing the risks of extreme events and disasters to advance climate change adaptation. A special report of Working Groups I and II of the Intergovernmental Panel on Climate Change. Cambridge University Press. Cambridge, U. K., and New York, U.S.A.

Isaac, N. J. B., S.T. Turvey, B. Collen, C. Waterman, and J. E. M. Baillie. 2007. Mammals on the EDGE: Conservation priorities based on threats and phylogeny. PloS One 2:296.

IUCN. 2013. IUCN red list of threatened species. Version 2013.2. Retrieved from www.iucnredlist.org Accessed on July 3, 2020.

Jordan, C. A., B. Hoover, A. J. Dans, C. Schank, and J. A. Miller. 2019. The Impact of Hurricane Otto on Baird's Tapir Movement in Nicaragua's Indio Maíz Biological Reserve. Pp. 5-19 in Movement Ecology of Neotropical Forest Mammals (Reyna-Hurtado, R., and C. Chapman, eds.) Springer International Publishing. Gewerbestrasse, Switzerland.

Kadoya, T. 2009. Assessing functional connectivity using empirical data. Population Ecology 51:5–15.

Krausman, P., and R. C. Etchberger. 1995. Response of desert ungulates to a water project in Arizona. Journal of Wildlife Management 59:292–300.

Krausman, P., S. S. Rosenstock, and J. W. I. Cain. 2006. Developed waters for wildlife: Science perception, Values and Controversy. Wildlife Society Bulletin 34:563–569.

Licona, M., R. McCleery, B. Collier, D. J. Brighsmith, and R. Lopez. 2011. Using ungulate occurrence to evaluate community-based conservation within a biosphere reserve model. Animal Conservation 14:206–214.

MacKenzie, D. L., J. D. Nichols, J. D. Royle, K. H. Pollock, L. L. Bailey, and J. E. Hines. 2006. Occupancy estimation and modeling. Academic Press. Burlington, U.S.A.

MacKenzie, D. L., J. D. Nichols, G. B. Lachman, S. Droege, J. R. Andrew, and C. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83:2248–2255.

Magrin, G. C., D. Gay-Garcia, J. C. Cruz-Choque, A. R. Gimenez, J. C. Moreno, C. Nagy, and A. Villamizar. 2007. Latin America. Climate change 2007: Impacts, adaptability and vulnerability. Contribution of working groupd II to the fourth assessment report of the Intergovernmental panel on climate change. Cambridge, U. K..

Mardero, S., E. Nickl, B. Shmook, L. Schnieder, and J. Rogan. 2012. Sequias en el sur de la península de Yucatán: Analisis de la variabilidad anual y estacional de la precipitacion. Boletin Del Intituto de Geografia 78:19–33.

Margules, C. R., and S. Sarkar. 2007. Systematic conservation planning. Cambridge University Press. Cambridge, U. K.

Meerman, J. C. 1999. Rapid ecological assessment Runaway Creek Belize. Belmopan, Belize.

Mendoza, E., T. L. Fuller, H. A. Thomassen, W. Buermann, D. Ramírez-Mejía, and T. B. Smith. 2013. A preliminary assessment of the effectiveness of the Mesoamerican Biological Corridor for protecting potential Baird's tapir *(Tapirus bairdii)* habitat in southern Mexico. Integrative Zoology 8:35–47.

Meteorological Service of Belize. 2020. www.hydromet.gov.bz. Accessed on May 21, 2020.

Moreira-Ramírez, J. F., R. Reyna-Hurtado, M. Hidalgo-Mihart, E. Naranjo, M. Ribeiro, R. Garcia-Anleu, G. Ponce-Santizo. 2016. Importance of waterholes for white-lipped peccary (*Tayassu pecari*) in the Selva Maya, Guatemala. Therya 7:51–64.

Naranjo, E. J. 1995. Abundancia y uso de habitat del tapir *(Tapirus bairdii)* en un bosque tropical humedo de Costa Rica. Vida Silvestre Neotropical 4:20–31.

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

Noss, A. J., R. L. Cuellar, J. Barrientos, L. Maffei, E. Cuellar, R. Arispe, K. Rivero. 2003. A Camera Trapping and Radio Telemetry Study of Lowland Tapir *(Tapirus terrestris)* in Bolivian Dry Forests. Newsletter of the IUCN/SSC Tapir Specialist Group 12:24–32.

O'Farrill, G., K.G. Schampaert, B. Rayfield, O. Bodin, S. Calmé, R. Sengupta, and A. Gonzalez. 2014. The potential connectivity of waterhole networks and the effectiveness of a protected area under various drought scenarios. PloS one 9:1–10.

Padilla, M., R. C. Dowler, and C. C. Downer. 2010. *Tapirus pinchaque* (Perissodactyla: Tapiridae). Mammalian Species 42:166–182.

Poot, C., and A. P. Clevenger. 2018. Reducing Vehicle Collisions with the Central American Tapir in Central Belize District, Belize. Tropical Conservation Science 11:1–4.

Reid, A. F. 2009. A Field Guide to the Mammals of Central America and Southeast Mexico. Oxford University press. New York, 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., G. O´Farrill, D. Sima, M. Andrade, A. Padilla, and L. Sosa. 2010. Las aguadas de Calakmul. Conabio. Biodiversitas 93:1–5.

Reyna-Hurtado, R., M. Sanvicente-López, J. Pérez-Flores, N. Carrillo-Reyna, and S. Calmé. 2016. Insights into the multiannual home range of a Baird's tapir *(Tapirus bairdii)* in the Maya Forest. Therya 7:271–276.

Reyna-Hurtado, R., D. Sima-Pantí, M. Andrade, A. Padilla, O. Retana-Guaiscon, K. Sanchez-Pinzón, W. Martinez, N. Meyer, J. F. Moreira-Ramírez, N. Carrillo-Reyna, C. M. Rivero-Hernández, I. Serrano Mac-Gregor, S. Calme and N. Arias-Dominguez. 2019. Tapir population patterns under the disappearance of free-standing water. Therya 10:353.

Sandoval-Seres, E., R. Reyna-Hurtado, M. Briceño-Mendes, R. De La Cerda-Vega. 2016. Pond use and relative abundance of *Tapirus bairdii* in the Calakmul region, Campeche, Mexico. Therya, 7:39–50.

Schank, C., E. Mendoza, M. J. G. Vettorazzi, M. V. Cove, C. A. Jordan, O. Farrill, R. Leonardo. 2015. Integrating current range-wide occurrence data with species distribution models to map the Potential distribution of Baird's Tapir. IUCN/SSC Tapir Specialist Group 15–30.

Terwilliger, V. J. 1978. Natural history of Baird's Tapir on Barro Colorado Island, Panama canal zone. Biotropica 10:211–220.

Tobler, M. W. 2002. Habitat Use and Diet of Baird's Tapirs *(Tapirus bairdii)* in a Montane Cloud Forest of the Cordillera de Talamanca, Costa Rica1. Biotropica 34:468–474.

Trolle, M., A. J. Noss, J. L. P. Cordeiro, and L. F. B. Oliveira. 2008. Brazilian tapir density in the pantanal: A comparison of systematic camera-trapping and line-transect surveys. Biotropica 40:211–217.

Wainwright, M. 2007. The Mammals of Costa Rica: A natural history and field guide. Cornell University Press. London, U.K.

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Visitation patterns of jaguars *Panthera onca* (Carnivora: Felidae) to isolated water ponds in a tropical forest landscape

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Jaguar populations have declined dramatically in the last century. The Maya Biosphere Reserve (MBR) within the Selva Maya (SM) is a priority area for jaguar conservation. Influential factors in the jaguar seasonal distribution include the availability of surface water in wetlands such as the "aguadas" and the availability of prey. Aguadas are formed by isolated depressions in the landscape and serve as a water supply for wildlife during dry periods. The goal of this study was to describe the visitation patterns of jaguars to aguadas in a Tropical forest at Dos Lagunas Protected Biotopo, a core zone of the MBR, Guatemala. We used camera-trap data from seven aguadas during the dry seasons 2014-2017. We determined visitation rates (VR, records for 1,000 trap-days) and activity patterns (AP) of jaguars for all years, aguadas, and sexes. We tested for significant differences in AP between sexes, and we estimated the coefficient of overlap (D) for female and male jaguar activity. We recorded 14 individuals (five females, eight males, and one unsexed) in 60 visit events. Jaguars have significant more diurnal activity at aguadas, and showed a VR = 13.1 for 1,000 trap-days. VR varied between aguadas (VR = 9.5 – 19.4), years (VR = 1.9 – 39.2) and sexes (VR = 6.1 for females and 5.5 for males). We did not find significant differences in the AP of female and male jaguars ($P > 0.05$). The coefficient of overlap between activity of females and males was D = 0.77 (95 % confidence interval: 0.70 to 0.84; $P > 0.05$). Aguadas may represent regular sites of jaguar home-ranges overlap for the important resources they provide for wildlife during the dry season. Aguadas could play an important role in the conservation and management of jaguar populations since the spatial distribution of these bodies of water is scattered, but ecologically important for jaguars within the MBR and the SM. Aguadas also, are important landscape features that could influence the spatial interactions of individuals. We encourage jaguar researchers to increase investigation on jaguars visiting aguadas and other wetlands in the SM to better understand the jaguar activity patterns and sex-specific habitat requirements.

Las poblaciones de jaguares disminuyeron drásticamente en el último siglo. La Reserva de la Biosfera Maya (RBM), en la Selva Maya (SM) es un área prioritaria para su conservación. Entre los factores que afectan su distribución estacional están la disponibilidad de agua en humedales como las aguadas, y la disponibilidad de presas. Las aguadas son formadas por depresiones en el paisaje y sirven de abastecimiento de agua para la fauna silvestre. El objetivo de este estudio fue describir los patrones de visita de jaguares a las aguadas en un bosque tropical en el Biotopo Protegido Dos Lagunas, una zona núcleo de la RBM, Guatemala. Se emplearon datos de trampas-cámara en siete aguadas durante las épocas secas 2014-2017. Se determinaron tasas de visita (TV, registros por 1,000 días-trampa) y patrones de actividad (PA) de jaguares por todos los años, aguadas y sexos. Se evaluaron diferencias significativas en los PA de ambos sexos, y se estimó el coeficiente de traslape (D) en los patrones de actividad de hembras y machos. Se registraron 14 individuos (cinco hembras, ocho machos, uno indeterminado) en 60 eventos independientes. Los jaguares se catalogaron con mayor actividad diurna, presentando una TV = 13.1 eventos por 1,000 días-trampa. Las TV variaron entre aguadas (TV = 9.5 – 19.4), años (TV = 1.9 – 39.2) y sexos. Las hembras presentaron una mayor TV en comparación a los machos (TV = 6.1 y 5.5, respectivamente). No se encontraron diferencias significativas en la actividad de ambos sexos, con un nivel de traslape de D = 0.77 (95 % de intervalo de confianza: 0.70 a 0.84; $P > 0.05$). Las aguadas podrían representar sitios de traslape en los ámbitos hogareños de los jaguares, debido a su importancia como recurso vital durante la época seca. Además, las aguadas pueden ejercer un importante rol en la conservación y manejo de poblaciones de jaguares debido a su distribución aislada, pero ecológicamente importante, que influye en las interacciones espaciales de los individuos. Recomendamos incrementar la investigación en jaguares y sus visitas a las aguadas y otros humedales para una mejor comprensión de sus patrones de actividad y requerimientos del hábitat específicos para ambos sexos.

Keywords: Aguadas; camera-traps; habitat use; overlap; Selva Maya.

Introduction

Jaguars *Panthera onca* (Linnaeus, 1758) are the largest cats in the Americas. In the northern hemisphere, their main populations are distributed in the north and south of Mexico (for example in Sonora and in the Sierra Madre de Chiapas), as well as in the north of Guatemala and Belize. In South America, the most important populations are located in the Brazilian Pantanal and in the Amazon rainforest of Colombia, Ecuador, Peru, Venezuela, Bolivia, and Brazil (Isasi-Catalá 2013; Payán *et al.* 2013; Espinosa *et al.* 2016; Maffei *et al.* 2016; De la Torre *et al.* 2017, 2019). Jaguars are top predators and therefore play a fundamental role in the dynamics of the ecosystems in which they live (Seymour 1989; Currier 1983). Specifically, jaguars contribute significantly in the top-down regulation of Neotropical food chains, because they regulate the abundance of secondary and primary consumers, which, in turn, regulates the abundance of producers, modifying the complete species assemblage in the ecosystems where they occur (Estes *et al.* 2011).

Because the jaguar is a species of Neotropical affinity that prefers dense forest environments with high levels of humidity, its populations have decreased dramatically in some of the driest areas of its distribution (southeastern United States, eastern Brazil and the plains of Argentina; De Azevedo *et al.* 2016; Di Bitetti *et al.* 2016; Pereira-Garbero and Sappa 2016). Currently, the Selva Maya (SM), located in the north of Mesoamerica (southeast of Mexico, north of Guatemala and northwest of Belize) is one of the highest priority jaguar conservation areas since it harbors the second largest population in the continent. In Guatemala, The Maya Biosphere Reserve (MBR) is the highest priority area for conservation of jaguars. The SM which includes the MBR has been identified as a Jaguar Conservation Unit (JCU), which means is one of the most important areas for jaguar conservation and that includes strict protection zones (Zeller 2007; García-Anleu *et al.* 2016; De la Torre *et al.* 2016, 2017; Jędrzejewski *et al.* 2018).

In the MBR, the aguadas are water ponds supplied with rainwater which are formed by isolated depressions on the land in which the soil is clayey and compact (Reyes 2009; Reyna-Hurtado *et al.* 2010; García *et al.* 2018). The aguadas provide a valuable resource for a variety of wildlife species, particularly during the dry season (Reyna-Hurtado *et al.* 2010; García *et al.* 2018). For predators such as jaguars, the aguadas represent a determining factor for home-range delineation, because in addition to providing drinking water, they favors the seasonal aggregation of prey that also visit these bodies of water (Muckenhirm and Eisenberg 1973; Núñez *et al.* 2002; Simá *et al.* 2008).

Home-ranges of female and male jaguars are different (Sunquist and Sunquist 2002). Females generally occupy home-ranges which include a sufficient number of prey for themselves and their cubs, while males occupy larger home-ranges, maximizing access to females and maintaining sufficient prey for individual survival (Schaller and Crawshaw 1980). As a consequence, the home-range of a single male jaguar is occupied by the home-ranges of several females (Astate *et al.* 2008; Morato *et al.* 2016; McBride and Thompson 2018).

The availability of surface water and the abundance and availability of prey are factors that largely determine the patterns of habitat use by jaguars (Sunquist 1981; Mizutani and Jewell 1998; Scognamillo *et al.* 2002; Figel *et al.* 2019; Rabelo *et al.* 2019). In most of the MBR and the SM, the availability of surface water during the dry season is mostly restricted to the aguadas. In this study, we described the patterns of visitation to aguadas by jaguars in the Dos Lagunas Protected Biotopo (DLPB), a core zone of the MBR, through visitation rates (VR) and activity patterns (AP) during the dry season.

Material and Methods

Study site. The DLPB, located in the extreme north of Guatemala, has an extension of 307 km2. It borders the Mirador-Río Azul National Park in the east and west, the Calakmul Biosphere Reserve in Mexico to the north, and the Multiple-use Zone of the MBR to the south. It is one of the protected areas with least human impact in Guatemala and is considered part of the "Heart of the Selva Maya" (CONAP 2015; Veras 2009). The DLPB is characterized, like the rest of the MBR and the SM, by karstic and permeable soils which result in an underground drainage. The lower areas of the forest have the highest percentage of clayey soil and less permeability, favoring the formation of water ponds that store rainwater such as the aguadas (Araujo 2014; Veras 2009; Reyna-Hurtado *et al.* 2010; García *et al.* 2018).

Aguadas are usually found in low densities; they are scattered in the landscape and can vary in size, ranging from 236 m^2 to several hectares (González 2015; Reyna-Hurtado *et al.* 2010). Since the aguadas are formed by the accumulation of rainwater, their dynamics is strongly associated with the intensity and duration of the dry and rainy season of each year. Some aguadas can remain filled through the year, and some tend to dry out completely during the dry season (González 2015). Due to the low availability of surface water in the region, the aguadas are one of the main sources of water for wildlife during dry periods, especially where large bodies of water are non-existent and humidity is low (Simá *et al.* 2008; Reyna-Hurtado *et al.* 2010; García *et al.* 2018).

Camera-trapping. Photographs obtained from the San Carlos University of Guatemala (Usac), Centro de Estudios Conservacionistas (Cecon) and the Central American Tapir Monitoring Program of the Defensores de la Naturaleza Foundation (FDN) were used to register jaguar activity in the aguadas of the DLPB. We monitored seven aguadas by setting up Bushnell® *Trophycam* camera-traps during the 2014 and 2015 dry seasons, programed to take three photographs per capture event and a 15 seconds interval between capture events; and setting up Bushnell® *Trophycam Agressor No Glow* camera-traps during dry season 2016 and 2017, programmed to take one photograph per capture event with a one second interval between capture events. The number of aguadas sampled and camera-traps used varied according to the year of sampling: 2 aguadas and 6 cameras in 2014; 3 aguadas and 9 cameras in 2015; six aguadas and 17 cameras in 2016; and, six aguadas and 10 cameras in 2017. Total sampling effort was 4,587 trap-days (Tables 1 and 2; Figure 1).

Table 1. Characteristics of aguadas from Dos Lagunas Protected Biotopo, Maya Biosphere Reserve, Guatemala.

Aguada	Seasonality	Forest type	Distance to nearest aguada (km)
A1	Seasonal	Tall	4.9 (A6)
A2	Seasonal	Tall	1.7 (A7)
A3	Permanent	Tall	3.2 (A4)
A4	Permanent	Tall	3.2 (A3)
A5	Permanent	Short	5.3 (A1)
A6	Permanent	Short	4.9 (A1)
A7	Seasonal	Short	1.7 (A2)

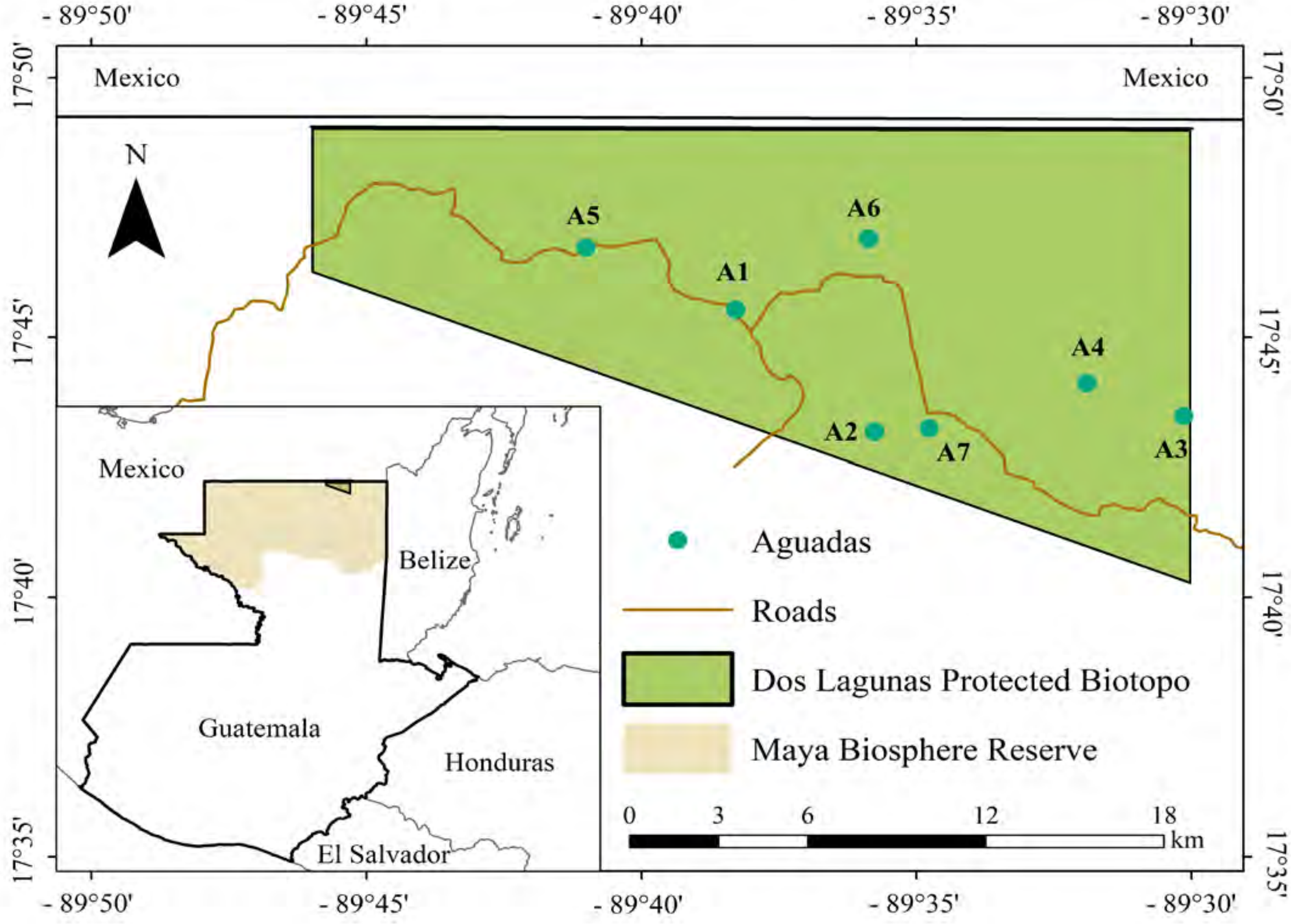

Figure 1. Location of aguadas of the Dos Lagunas Protected Biotopo, Maya Biosphere Reserve, Guatemala.

We used Camera Base version 1.7 software (Tobler 2015) to process photographs and organize them in a database with the following information: site, date, time, camera-trap station (aguada), sex and age of individuals. We identified individuals by comparing rosette patterns of the coat (Karanth and Nichols 1998). Each capture event was considered independent of another capture event if 1 hour elapsed between capture events of the same individual in the same aguada.

Visitation rates (VR). To account for differences in sampling effort, we determined visitation rates (VR) of jaguars (for females, for males, and both) using the following equation: VR = (N/SE) x 1,000 trap-days [N = number of independent captures, and SE = sampling effort measured as the number of days multiplied by the number of camera-traps in each aguada]. We calculated VR for each aguada and for all aguadas combined.

Activity patterns (AP). The activity pattern of each jaguar was classified as diurnal, nocturnal or crepuscular activity. The initial time of each visit event was used to determine these visiting patterns. Capture events recorded from one hour after dawn (sunrise) to one hour before dusk (sunset) were considered as diurnal; events recorded between one hour after dusk to one hour before dawn were considered as nocturnal; and, events recorded between one hour before dawn and one hour after dawn, and between one hour before dusk and one hour after dusk were classified as crepuscular (Monroy-Vilchis *et al.* 2011; Jiménez *et al.* 2010). The time of sunrise and sunset for each day was determined using the solar calculator from the Global Monitoring Division of the Eastern System Research Laboratory of the National Oceanic and Atmospheric Administration of the U. S. Department of Commerce (NOAA 2014).

We used the methodology of Jiménez *et al.* (2010) to determine the AP of jaguars in aguadas: "diurnal" when < 10 % of events were recorded at night; nocturnal when > 90 % of the visit events were recorded at night; "mostly diurnal" when night time events ranged between 10 to 30 %; "mostly nocturnal" when 70 to 90 % of the events were recorded at night; "crepuscular" when 50 % or more of the events were during twilight periods; and "catameral" when events occurred sporadically during the day and night.

Activity and overlap models. We tested for significant differences ($P < 0.05$) in AP between sexes fitting kernel density models to estimate and compare female and male jaguar activity distributions using a Wald test (tested on chi square distribution with one degree of freedom), and quantified and compared levels of activity for both sexes

Table 2. Sampling effort in aguadas. *1 Camera deactivated from March 15 to May 31 2015; *2 Camera deactivated from May 7 to May 31 2015.

Year	Aguada	Cameras	First	Last	Days	No Cameras	Effort	Total
2014	A3	All	10/04/2014	20/06/2014	71	3	213	420
	A6	All	11/04/2014	19/06/2014	69	3	207	
2015	A3	All	26/04/2015	06/07/2015	71	3	213	567
	A6	All	25/04/2015	01/07/2015	67	3	201	
	A2	Camera 1	27/04/2015	01/07/2015	65	1	65	
		Camera 2*1	27/04/2015	01/07/2015	48	1	48	
		Camera 3*2	27/04/2015	01/07/2015	40	1	40	
2016	A3	All	5/04/2016	30/08/2016	147	4	588	2,319
	A6	Cameras 1-3	06/04/2016	27/08/2016	143	3	429	
		Camera 4	12/05/2016	10/08/2016	90	1	90	
	A5	Cameras 1-2	06/04/2016	23/08/2016	139	2	278	
		Camera 3	11/05/2016	23/08/2016	104	1	104	
	A4	All	5/04/2016	29/08/2016	146	3	438	
	A1	Camera 1	11/05/2016	23/08/2016	104	1	104	
	A7	Cameras 1-2	12/05/2016	27/08/2016	107	2	214	
		Camera 3	12/05/2016	25/07/2016	74	1	74	
2017	A3	Camera 1	30/03/2017	19/06/2017	81	1	81	1,281
		Camera 2	30/03/2017	10/08/2017	133	1	133	
		Camera 3	30/03/2017	12/08/2017	135	1	135	
	A6	All	29/03/2017	10/08/2017	134	3	402	
	A5	Camera 1	29/03/2017	10/08/2017	134	1	134	
	A4	Camera 1	30/03/2017	5/08/2017	128	1	128	
	A1	Camera 1	29/03/2017	10/08/2017	134	1	134	
	A7	Camera 1	29/03/2017	10/08/2017	134	1	134	
Total								4,587

through bootstrapping (10,000 replicates) employing the package 'Activity' (Rowcliffe 2019) in software R (R Core Team 2020, R version 3.6.3). We estimated the coefficient of overlap (D = level of overlap) for female and male jaguar activity, and we fitted kernel density models to compare AP between sexes by using the capture events in each aguada employing the package 'Overlap' (Meredith and Ridout 2020) in software R (R Core Team 2020, R version 3.6.3). We estimated the confidence intervals (CI) between lower and upper 95 % limits in estimates of female and male activity overlap, calculated as percentile intervals from 10,000 bootstrap samples (Ridout and Linkie 2009). We plotted models not extended (extend = NULL, Meredith and Ridout 2020).

Results

We obtained 409 photographs of jaguars corresponding to 60 independent visit events in the seven sampled aguadas (A1 to A7). Aguadas A3, A4 and A6, were the most frequented by jaguars (Table 3). We identified 14 individuals (eight males, five females and one of unknown sex) in 54 events (90 %) across five aguadas. At two aguadas we recorded six events (10 %) of individuals who could not be identified or sexed (Table 4).

Table 3. Visitation rates (VR) of jaguars by aguada, year and sex. F: Females, M: Males.

Aguada	2014 (F , M)	2015 (F , M)	2016 (F , M)	2017 (F , M)	All years (F , M)
A1			9.6 (0, 0)	0 (0, 0)	4.2 (0, 0)
A2		39.2 (0, 39.2)			39.2 (0, 39.2)
A3	9.4 (4.7, 4.7)	18.8 (0, 18.8)	6.8 (5.1, 0)	17.2 (14.3, 0)	11.7 (6.6, 3.7)
A4			27.4 (20.5, 6.8)	23.4 (15.6, 0)	26.5 (19.4, 5.3)
A5			0 (0, 0)	7.5 (0, 0)	1.9 (0, 0)
A6	19.3 (9.7, 4.8)	5.0 (5.0, 0)	3.9 (1.9, 1.9)	22.4 (5.0, 17.4)	12.0 (4.5, 6.8)
A7			10.4 (0, 6.9)	14.9 (14.9, 0)	11.8 (4.7, 4.7)
All aguadas	14.3 (7.1, 4.8)	19.4 (1.8, 17.6)	9.5 (5.6, 2.6)	16.4 (8.6, 19.5)	13.1 (6.1, 5.5)

From the 54 visit events with identified individuals, 17 (31.5 %) belonged to a single female; the rest of individuals were observed on one (2 individuals), two (3 individuals), three (4 individuals) or four (4 individuals) visit events (Table 4). The aguadas with the highest number of visit events with identified individuals were A6 with 16 (19.6 %), A3 and A4 with 13 each (48.1 %), A2 with six (11.1 %) and A7 with four visit events (7.4 %; Table 4). The aguadas with the highest number of visit events in a single year of study (of the 60 total events) were A4 (2016) and A6 (2017), with 12 and 9 events respectively, which represent 35 % of visit events from all the study (Table 3). The aguadas visited by the highest number of individuals were A6 (3 females, 4 males, 1 of unknown sex), A4 (5 females, 2 males, 1 of unknown sex), and A3 (3 females, 3 males, 2 of unknown sex), registering 8 individuals each.

Visitation rates (VR). We estimated a VR of 13.1 records for 1,000 trap-days for jaguars in all aguadas and all years (Table 4). However, VR varied among individual aguadas (VR = 1.9 to 39.2 records for 1,000 trap-days) and years (VR = 9.5 to 19.4 records for 1,000 trap-days), both for all jaguars combined and separately for females and males. We did not record visit events in aguadas A1 in 2017 and A5 in 2016 (VR = 0 records for 1,000 trap-days), while in aguadas A2 in 2015 and A4 in 2016 we determined the highest values, VR = 39.2 and VR = 27.4 records for 1,000 trap-days, respectively. Females showed higher VR in aguadas than males (VR = 6.1 and 5.5 records for 1,000 trap-days, respectively), with variation according to the years and the aguadas sampled (Table 3).

Activity patterns (AP). Jaguars presented a bimodal activity pattern, showing two peaks that occurred at mid-morning, and before dusk. Across all years, jaguars showed more frequency of visits (independent events) from 18:00 to 18:59 h. However, we recorded the highest density of events between 04:00 to 11:59 h (before dawn to before noon), which represented the highest peak of activity of jaguars in aguadas (Figure 2a).

From the 60 total visit events, 30 (50 %) corresponded to diurnal activity (19 for females, 8 for males and 3 of unknown sex and identity), 10 (16.7 %) to nocturnal activity (2 for a female, 7 for males and 1 of unknown sex and identity) and 20 (33.3 %) to crepuscular activity (7 for females, 10 for males and 3 of unknown sex and identity). Females showed greater diurnal activity (19 events) compared to nocturnal (2 events) and crepuscular activity (7 events); while males showed 8, 7 and 10 events for diurnal, nocturnal and crepuscular activity events, respectively (Figure 2b). Females showed a unimodal distribution, visiting aguadas during the day (after dawn and before dusk), while males showed a trimodal distribution, with their maximum peak at mid-morning, and two smaller peaks after dusk, and at mid-afternoon (Figure 3a). During night (after dusk and before dawn), females showed a bimodal distribution with similar peaks after 20:00 h and before midnight, while nocturnal activity of males was increasing until reaching their peak before dawn twilight (Figure 3b). In the case of twilight periods (crepuscular activity), females and males showed different and similar activity through dawn and dusk, respectively. During dawn twilight, females showed a bimodal

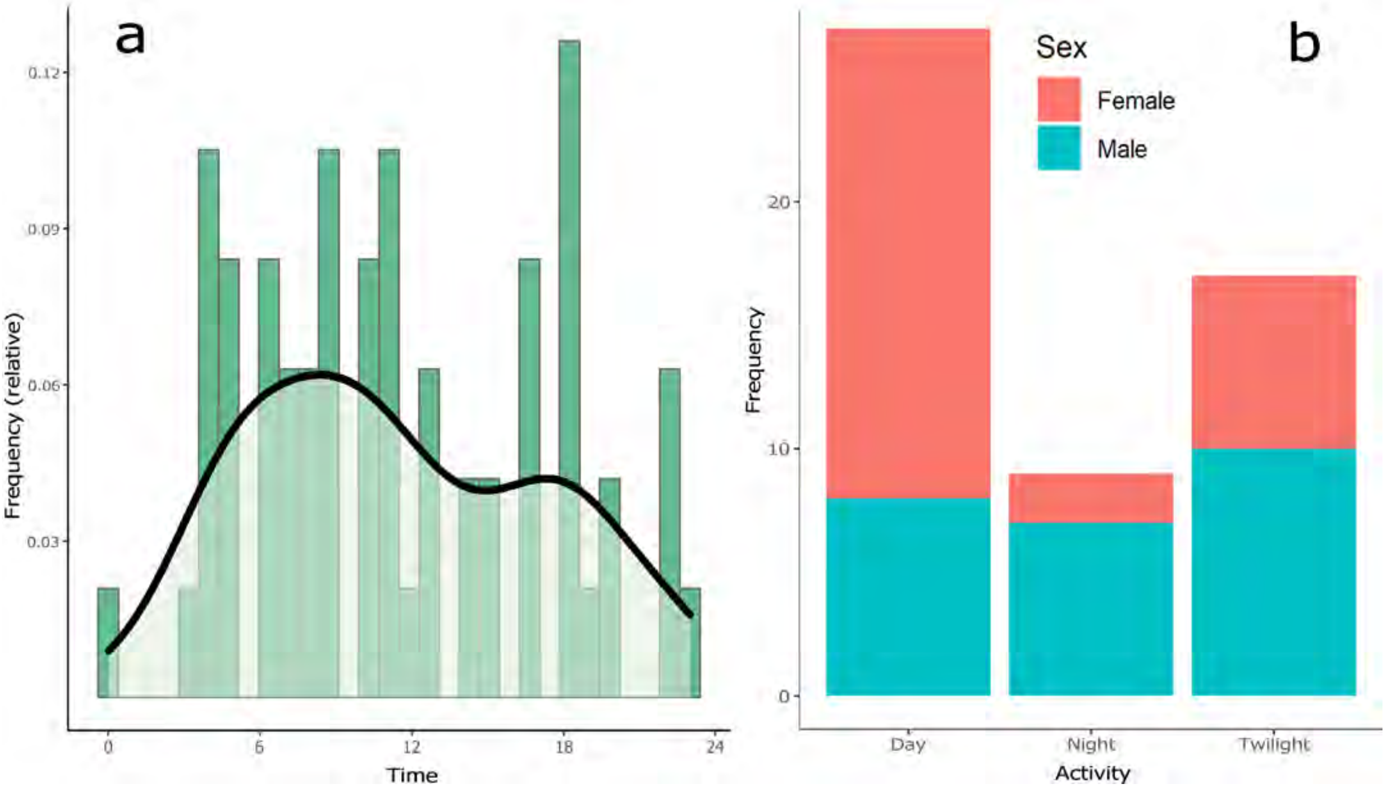

Figure 2. a: Relative frequency of records and daily activity patterns of jaguars in aguadas (*n* = 60), black line: bimodal distribution curve. b: Frequency of records and daily activity pattern of female and male jaguars in aguadas (*n* = 53). Relative frequency and bimodal distribution curve (a) were calculated and plotted using '*geom_histogram(aes(y = ..density..))*' function and '*geom_density ()*' function of package 'Ggplot2' in software R.

Table 4. Jaguar individuals visit events in aguadas of the Dos Lagunas Protected Biotopo, Maya Biosphere Reserve, Guatemala. F: Female; M: Male; U: Unknown sex.

Year/Aguada		F1	F2	F3	F4	F5	U6	M7	M8	M9	M10	M11	M12	M13	M14	Total
2014	A3	1							1							
	A6	2					1	1								
		3					1	1	1							6
2015	A2								3	2	1					
	A3							2			2					
	A6		1													
			1					2	3	2	3					11
2016	A3	2			1											
	A4	4	2	2		1				2		1				
	A6			1								1				
	A7									1			1			
		6	2	3	1	1				3		2	1			19
2017	A3	4				1										
	A4			1	1											
	A6	2												3	4	
	A7	2														
		8		1	1	1								3	4	18
Total		17	3	4	2	2	1	3	4	5	3	2	1	3	4	54

distribution in a very short period of time (two events at 05:37 and 05:39 h, respectively), while males increased their activity throughout dawn twilight (Figure 3c). During dusk twilight, females showed a trimodal distribution, with their maximum peak at 18:00 to 18:10 h, while males showed a bimodal distribution with their maximum peak at 18:30 to 18:45 h (Figure 3d). Only 16 % of all visit events were recorded at night hours, while 50 % were recorded at daytime, classifying jaguar activity as diurnal in aguadas.

Activity and overlap models. Despite the observed differences in peaks, overall female and male jaguars did not show significant differences in their activity ($P > 0.05$; standard error = 0.13; Wald statistics estimate = 0.019). The estimates for observed coefficient of overlap (D = 0.65) and the mean coefficient of overlap between female and male jaguar activity (D = 0.77, 95 % confidence interval: 0.70 to 0.84), showed no significant difference between sexes in their activity ($P > 0.05$, Figure 4).

Discussion

The observed patterns suggest that female and male home-ranges overlap in the aguadas, with variation in the number of individuals and visit events among sites and years. For females, the recurrent use of aguadas in different years suggests a certain degree of site fidelity as reported by Cavalcanti and Gese (2009) in Brazil, where home-ranges were maintained up to 90 % for the second season, but changed the areas used more intensively. The five female individuals identified in this study were recorded in three of the seven aguadas sampled, exhibiting a high degree of home-range overlap, similar to Cavalcanti and Gese (2009) where 25 to 47 % home-ranges of female jaguars overlapped during the dry season.

For males, individuals M7, M8 and M9, were recorded in different aguadas in each of two consecutive years, which seems to suggest less fidelity to home-ranges than females, as found by Cavalcanti and Gese (2009). Our study also documented records of multiple males visiting the same aguada during the same year, which may suggest home-range overlap between males, a phenomenon that has been reported in other studies (De Azevedo and Murray 2007; Cavalcanti and Gese 2009; Harmsen *et al.* 2009).

Aguadas may represent regular sites of home-range overlap for jaguars considering the important resource they provide during the dry season, when they may become the only source of water used to cool off and to hunt down potential prey. In addition, Figel *et al.* (2019) have demonstrated that wetlands are keystone habitats for jaguars that increase their occupancy and detection probabilities. In 2016, the driest year in this study, aguada A4 was visited by six individuals (four females and two males), which may be related with the fact that A4 was the aguada that best maintained water levels across 4 study aguadas during that sampling season in the DLPB (García *et al.* 2018). Jaguar distribution is strongly influenced by availability of key resources (Sandell 1989), so the spatial distribution of aguadas may be an important landscape feature that influences the movements and interactions of individuals. Home-range overlap between conspecifics of the same sex has been reported to occur mainly outside core, high-quality areas, especially between females (De Azevedo and Murray 2007; Cavalcanti and Gese 2009); and the critical importance of aguadas in the JCU of the SM may rely in that these isolated water ponds are often the only surface water source available in this landscape, resulting that several individuals that use larger areas necessarily include these aguadas in their home-range.

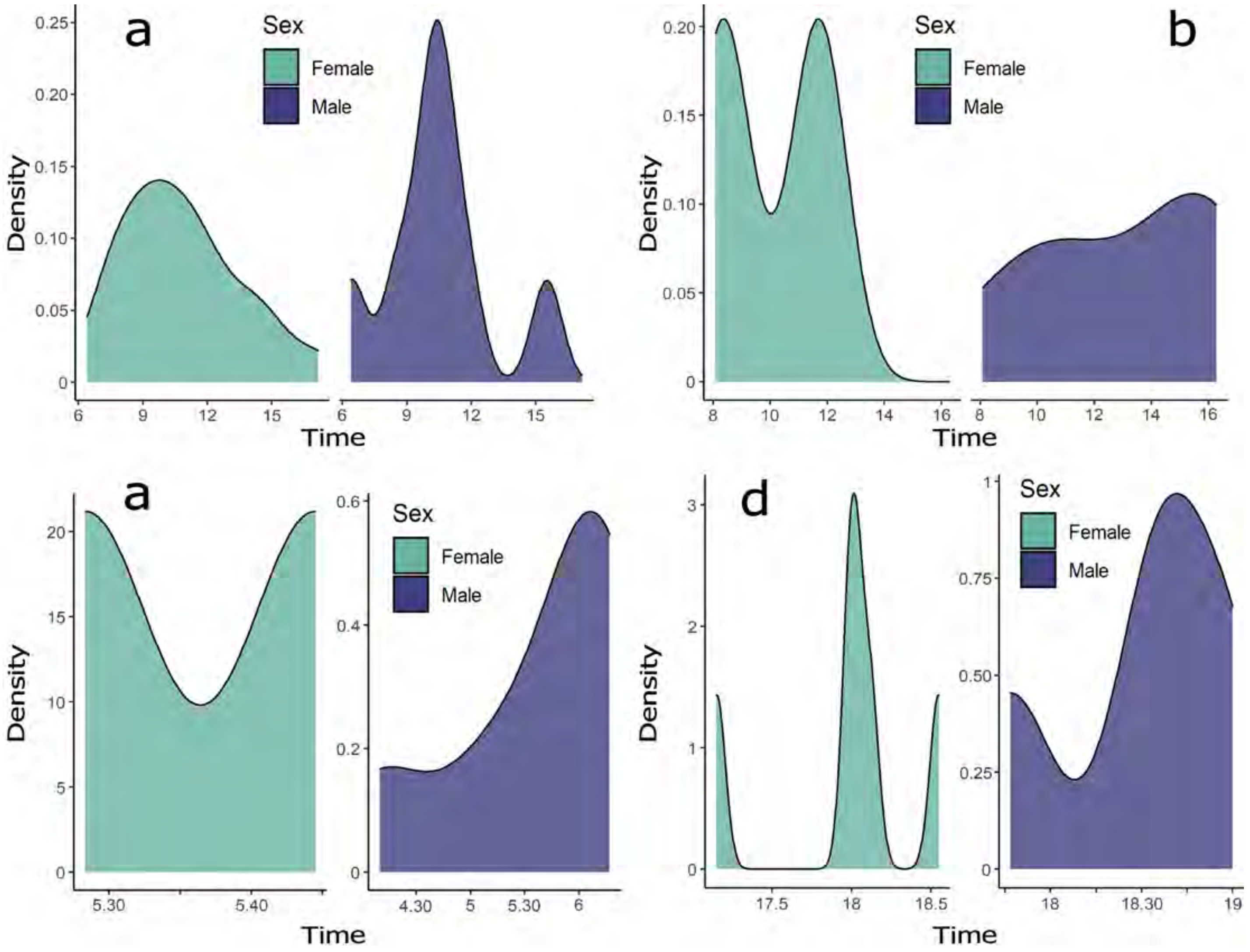

Figure 3. Activity patterns of female and male jaguars in aguadas. a: Diurnal activity. b: Nocturnal activity. c: Crepuscular activity during dawn. d: Crepuscular activity during dusk. Curves represent activity distribution density of jaguars at each data set of visit events ordered by sex. Curves were plotted using density function '*geom_density()*' of package 'Ggplot2' in software R.

The number of jaguars identified in our study (14 individuals) is higher than previous studies within the MBR that used camera-traps installed on trails and roads (range 7 to 10 individuals), which may indicate the ecological importance of aguadas to jaguars and which has not been studied until now (Moreira *et al.* 2008; Moreira *et al.* 2009a; Moreira *et al.* 2009b; García-Anleu *et al.* 2015a; García-Anleu *et al.* 2015b).

Visitation rates (VR). According to Salom-Pérez *et al.* (2007), Conde *et al.* (2010) and Sollmann et al. (2011), female jaguars occupy smaller home-ranges, move less, and show different habitat use than males. Female jaguars do not move frequently on roads, trails, and open areas, and in previous studies, they have had lower camera-trap capture rates than males when cameras are installed near these spatial features (Salom-Pérez *et al.* 2007; Harmsen *et al.* 2010a). In contrast to this, our results showed that females in aguadas have higher capture rates compared to males, despite being represented by a smaller number of individuals (55.5 % of the males recorded). This suggests the use of aguadas is more frequent by females compared to males.

Srbek-Araujo (2018) suggests that the difference in capture rates between females and males reported in literature (higher for males and smaller for females) is probably due to the fact that females tend to avoid encounters with camera-traps deliberately. This behavior was not observed in this study. In fact, the individual with the highest number of detections was a female (F1) recorded in four different aguadas across three of the four years of study (Table 4). This indicates that instead of avoiding camera-traps specifically, female jaguars in other studies may be avoiding the habitat features where camera-traps are usually installed.

Activity patterns (AP). Jaguars exhibited primarily diurnal activity at aguadas; only 16 % of records occurred at night (Jiménez *et al.* 2010). This data coincides with a report by González (2015) for jaguars and pumas *Puma concolor* (Linnaeus, 1771) in aguadas of the DLPB during the dry season 2013. According to Fedriana *et al.* (1999), Gliwicz and Dabrowski (2008), and Harmsen *et al.* (2010b), activity patterns of predators such as jaguars are associated with times of the day when their prey species are most vulnerable to preda-

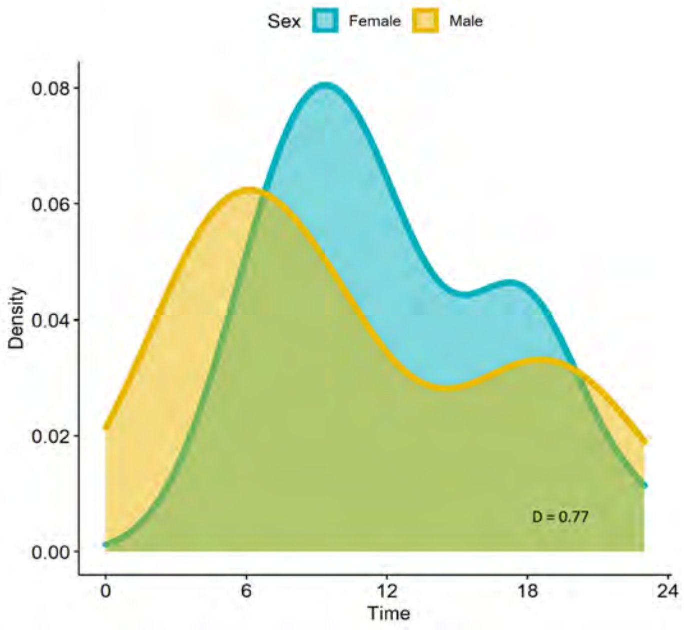

Figure 4. Proportion of overlap (D = 0.77, green area) in activity patterns of female and male jaguars in aguadas. Curves represent the bimodal distribution curves of activity density of female and male jaguars, calculated and plotted using packages 'Overlap' and 'Ggplot2' in software R.

tion. González (2015) reported that many potential prey species for jaguars that visit the aguadas of the DLPB are diurnal, including the great curassow *Crax rubra* Linnaeus, 1758, the crested guan *Penelope purpurascens* Wagler, 1830, the white-lipped peccary *Tayassu pecari* (Link, 1795), the collared peccary *Pecari tajacu* (Linnaeus, 1758), and the white-tailed deer *Odocoileus virginianus* (Zimmermann, 1780).

Although we did not find significant differences between female and male activity (Rowcliffe 2019), and that the level of overlap in activity revealed no significant differences (Meredith and Ridout 2020), sexes do differ in their peak hours of activity. Males visit the aguadas over a longer range of hours, as they have three peaks in activity. Activity of females in aguadas is concentrated primarily from mid-morning to mid-afternoon. Males showed the most activity just before and after dawn, while females showed the most activity just before and after noon; males had another peak of activity after dusk and a small peak around midday (Figure 3).

In a recent study, Sánchez-Pinzón *et al.* (2020) found that jaguar visitation to aguadas is significantly associated with the activity on these water ponds of the white-lipped peccary, one of its main prey (Aranda 1994; Estrada 2008). In this suggestion, sex of jaguars was not considered (Sánchez-Pinzón *et al.* 2020); so if female and male jaguar visitation patterns are also different as we suggest based on their different peak hours of activity in aguadas, preferred prey species may differ between males and females, as peak hours of activity of predators are strongly associated with peak hours of activity of their main prey (Fedriana *et al.* 1999; Gliwicz and Dabrowski 2008; Harmsen *et al.* 2010b).

Body mass in jaguars largely impacts their hunting decisions (Sunquist and Sunquist 2002), and it has been demonstrated that body mass among jaguar populations reflect the body mass of preferred prey species (Hoogesteijn and Mondolfi 1996). For Central American jaguars, which exhibit mean body mass differences between sexes [41.1 and 56.1 kg for females and males, respectively] (Hoogesteijn and Mondolfi 1996), differences in peak hours of activity among female and male jaguars from the same population may suggest that both sexes are preying on different prey, at different times. However, further investigation is required to identify sex-specific preferences of prey species within jaguar populations.

Sandoval-Lemus (2020) characterized the AP of Baird's tapir *Tapirella bairdii* (Gill, 1865) in aguadas of the DLPB, and found that tapirs increase their activity when temperature is high and tend to reduce it when humidity rises. He also found that tapirs tend to visit the aguadas more often during the dry season compared to the rainy season, and that use of the aguadas is negatively correlated with precipitation patterns, meaning that the number of days between visits tend to increase as rain increases. Sandoval-Lemus (2020) also found that tapirs are mainly nocturnal in aguadas (70 %), and its peaks of activity are during night at 21:00 and 02:00 h. We only have sparse detections of female jaguars around these times and no detections of male jaguars but until before dawn (Figure 3b). Given this, it is possible that tapirs may be avoiding jaguars at aguadas of the DLPB, and additional research on the interaction of these two largest terrestrial mammals of the Neotropics, based on sex-specific and climatic variables at aguadas could yield interesting results (Sandoval-Lemus 2020).

This study suggests that aguadas are a limiting feature for jaguar home-ranges, and could elucidate unknown information about the habitat use of females. Despite the fact that this study did not contemplate a specific experimental design, given that its objectives were clearly descriptive, the information gathered and described reveals the frequency with which jaguars visit the bodies of water in a landscape where surface water is seasonally available, that the dynamics of use by females and males is also different, and the fact that many jaguars visit specific aguadas across multiple years. Additional research on this topic will provide invaluable information for efforts focused on the conservation and management of jaguar populations in the MBR and the SM.

Our data cannot be used for population estimates due to the design bias, as the aguadas are specific elements in the landscape that were not sampled randomly. Nonetheless, given that aguadas are a resource visited frequently by jaguars, the monitoring of aguadas and other bodies of water should be an important part of any population monitoring study and will prove useful to related research, such as jaguar collaring projects. For aguadas specific research, we recommend larger scale studies to extend this research to be able to compare visitation rates and activity patterns of jaguars in both the dry and rainy seasons, and in different landscapes features as roads and trails. Our results provide a baseline for such studies and provide data on capture rates and activity patterns that can be compared to more typical jaguar studies that tend to be done on trails and roads. In this sense, this study contributes to our overall understanding of jaguar behavior in different habitat types, information that can be useful for conservations purposes.

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Literature cited

Aranda, M. 1994. Importancia de los pecaríes (*Tayassu* spp.) en la alimentación del jaguar (*Panthera onca*). Acta Zoológica Mexicana 62:11-22.

Araujo, R. 2014. Programa municipal de desarrollo de gran visión para el municipio de Calakmul, Campeche (2013-2040). Guatemala: Deutsche Gesellschaft für Internationale Zusammenarbeit. Santa Elena, Guatemala.

Astate, S., R. Sollman, and L. Silvira. 2008. Comparative Ecology of Jaguars in Brazil. CAT News Special Issue 4:9-14.

Cavalcanti, S. M. C., and E. M. Gese. 2009. Spatial ecology and social interactions of jaguars (*Panthera onca*) in the southern Pantanal, Brazil. Journal of Mammalogy 90:935–945.

Consejo Nacional de Áreas Protegidas (Conap). 2015. Plan Maestro de la Reserva de la Biosfera Maya. Segunda Actualización. Guatemala, Guatemala.

Conde, D. A., F. Colchero, H. Zarza, N. L. Christensen, J. O. Sexton, C. Manterola, C. Chávez, A. Rivera, D. Azuara, and G. Ceballos. 2010. Sex matters: Modeling male and female habitat differences for jaguar conservation. Biological Conservation 143:1980–1988.

Currier, M. 1983. *Felis concolor*. Mammalian Species 200:1-7.

De Azevedo, F. C. C., T. Gomes-De-Oliveira, C. R. De Paula, C. Bueno De Campos, E. A. Morales Jr, S. M. C. Cavalcanti, W. Moraes, R. L. Pires, P. G. Crawshaw, B. de Mello, D. Alessio, K. M. Paschoaleeto, M. de Barros, and J. Polisar . 2016. Estatus del jaguar (*Panthera onca*) en Brasil. Pp. 366-433, *in* El jaguar en el siglo XXI: La perspectiva Continental (Medellín, R. A., J. A De la Torre, H. Zarza, C. Chávez, and G. Ceballos, eds.). Fondo de Cultura Económica, Universidad Nacional Autónoma de México. Ciudad de México, México.

De Azevedo, F. C. C., and D. L. Murray. 2007. Spatial organization and food habits of jaguars (*Panthera onca*) in a floodplain forest. Biological Conservation 137:391-402.

De La Torre, J. A., G. Ceballos, C. Chávez, H. Zarza, and R. A. Medellín. 2016. Prioridades y recomendaciones, ¿Cuáles son las necesidades para el jaguar en los próximos años? Pp. 495-505 *in* El jaguar en el siglo XXI: La perspectiva Continental (Medellín, R. A., J. A. De la Torre, H. Zarza, C. Chávez, and G. Ceballos, eds.). Fondo de Cultura Económica, Universidad Nacional Autónoma de México. Ciudad de México, México.

De La Torre, J. A., J. F. González-Maya, H. Zarza, G. Ceballos, and R. A. Medellín. 2017. The jaguar's spots are darker than they appear: assessing the global conservation status of the jaguar *Panthera onca*. Oryx 52:300-315.

De La Torre, J. A., M. Rivero, G. Camacho, L. A. Álvarez-Márquez, and R. A. Medellín. 2019. First assessment of the conservation status of the jaguar *Panthera onca* in the Sierra Madre de Chiapas, Mexico. Oryx 53:192-195.

Di Bitetti, M. S., C. De Angelo, V. Quiroga, M. Altrichter, A. Paviolo, G. A. E. Cuyckens, and P. G. Perovic. 2016. Estado de conservación del jaguar en Argentina. Pp. 447–478, *in* El jaguar en el siglo XXI: La perspectiva Continental (Medellín, R. A., J. A. De la Torre, H. Zarza, C. Chávez, and G. Ceballos, eds.). Fondo de Cultura Económica, Universidad Nacional Autónoma de México. Ciudad de México, México.

Espinosa, S., L. Albuja, D. Tirira, G. Zapata-Ríos, E. Araguillin, V. Uteras, and A. Noss. 2016. Análisis del estado de conservación del jaguar en el Ecuador. Pp. 320-339, *in* El jaguar en el siglo XXI: La perspectiva Continental (Medellín, R. A., J. A. De la Torre, H. Zarza, C. Chávez, and G. Ceballos, eds.). Fondo de Cultura Económica, Universidad Nacional Autónoma de México. Ciudad de México, México.

Estes, J. A., J. Terborgh, J. S. Brashares, M. E. Power, J. Berger, and A. D. Wardle. 2011. Trophic Downgrading of Planet Earth. Science 333:301-306.

Estrada, C. 2008. Dieta, uso de hábitat y patrones de actividad del Puma (*Puma concolor*) y el jaguar (*Panthera onca*) en la Selva Maya, Centroamérica. Revista Mexicana de Mastozoología 12:113-130.

Fedriana, J., F. Palomares, and M. Delibes. 1999. Niche relations among three sympatric Mediterranean carnivores. Oecologia 121:138-148.

Figel, J. J., S. Botero-Cañola, G. Forero-Medina, J. D. Sánchez-Londoño, L. Valenzuela, and R. F. Noss. 2019. Wetlands are keystone habitats for jaguars in an intercontinental biodiversity hotspot. PLos One 14:e0221705.

García, M. J., A. L. Aguilera, G. D. Guzmán-Flores, D. I. Rodríguez, and V. R. González. 2018. Dinámica temporal de la disponibilidad de agua y microclima en aguadas del biotopo protegido Dos Lagunas, Petén, Guatemala. Ciencia, Tecnología y Salud 5:99-110.

García-Anleu, R. A., G. Ponce-Santizo, A. Rodas, O. Cabrera, R. B. McNab, J. Polisar, and M. Lepe. 2015a. Jaguares y productores agropecuarios en la Zona de Amortiguamiento de la Reserva de Biosfera Maya, Guatemala: herramientas para mejorar la coexistencia. Pp. 139-149, *in* Conflictos entre felinos y humanos en América Latina (Castaño-Uribe, C., C. A. Lasso, R. Hoogesteijn, A. Diaz-Pulido, and E. Payán, eds.). Instituto Humboldt. Bogotá, Colombia.

García-Anleu, R. A., G. Ponce-Santizo, R. B. Mcnab, J. Polisar, A. Noss, J. Moreira, and G. Ruano. 2015b. The queen of Tikal and her suitors. CAT News 62 Spring 2015.

García-Anleu, R. A., R. B. McNab, J. Polisar, V. Ramos, J. Moreira, G. Ponce-Santizo, K. Duchez, R. Escobar, and A. Santos. 2016. Estado del Jaguar en Guatemala. Informe del 2013. Pp. 336-443, *in* El jaguar en el siglo XXI: La perspectiva Continental (Medellín, R. A., J. A. De la Torre, H. Zarza, C. Chávez, and G. Ceballos, eds.). Fondo de Cultura Económica, Universidad Nacional Autónoma de México. Ciudad de México, México.

Gliwicz, J., and M. Dabrowski. 2008. Ecological factors affecting the diel activity of voles in a multi-species community. Annales Zoologici Fennici 45:242-247.

González, V. R. 2015. Vertebrados medianos y mayores asociados a las aguadas del Biotopo Protegido Naachtún Dos Lagunas, Petén. Licentiate Thesis, San Carlos University of Guatemala, Guatemala, Guatemala.

Harmsen, B. J., R. Foster, S. C. Silver, L. E. Ostro, and C. P. Doncaster. 2009. Spatial and temporal interactions of sympatric jaguars (*Panthera onca*) and pumas (*Puma concolor*) in a neotropical forest. Journal of Mammalogy 90:612-620.

Harmsen, B. J., R. Foster, E. Sanchez, C. E. Gutiérrez-González, S. C. Silver, L. E. Ostro, M. Kelly, and H. Quigley. 2010a. Long term monitoring of jaguars in the Cockscomb Basin Wildlife Sanctuary, Belize; Implications for camera trap studies of carnivores. PLoS One 12:e0179505.

Harmsen, B.J., R. Foster, S. Silver, L. Ostro, and C. P. Doncaster. 2010b. Differential use of trails by forest mammals and the implications for camera-trap studies: a case study from Belize. Biotropica 42:126–133.

Hoogesteijn, R., and E. Mondolfi. 1996. Body mass and skull measurements in four jaguar populations and observations on their prey base. Bulletin Journal of the Florida Museum of Natural History 39:1,952-1,219.

Isasi-Catalá, E. 2013. Estado de conservación del jaguar (*Panthera onca*) en el Parque Nacional Guatopo, Venezuela: unidad prioritaria para su conservación. Pp. 95-102, *in* Grandes Felinos de Colombia. Vol. 1 (Payán, E., and C. Castaño-Uribe, eds.), Panthera Colombia, Fundación Herencia Ambiental Caribe and Cat Specialist Group IUCN/SSC. Bogotá, Colombia.

Jędrzejewski, W., H. S. Robinson, M. Abarca, K. Zeller, G. Velasquez, E. A. D. Paemeleare, J. F. Goldberg., E. Payan, R. Hoogesteijn, E. O. Boede., K. Schmidt, M. Lampo., A. L. Viloria, R. Carreño, N. Robinson, P. M. Lukacs, J. J. Nowak., R. Salom-Pérez, F. Castañeda, V. Boron, and H. Quigley. 2018. Estimating large carnivore populations at global scale based on spatial predictions of density and distribution – Application to the jaguar (*Panthera onca*). PLoS One 13:e0194719.

Jiménez, C., H. Quintana, V. Pacheco, D. Melton, J. Torrealva, and G. Tello. 2010. Camera trap survey of medium and large mammals in a montane rainforest of northern Peru. Revista Peruana de Biología 17:191-196.

Karanth, K., and J. Nichols. 1998. Estimation of tiger densities in India using photographic captures and recaptures. Ecology 79:2852-2862.

Maffei, L., D. Rumiz, R. Arispe, E. Cuéllar, and A. Noss. 2016. Situación del jaguar en Bolivia. Pp. 353-366, *in* El jaguar en el siglo XXI: La perspectiva Continental (Medellín, R. A., De la Torre, J. A, Zarza, H, Chávez, C, and G. Ceballos, eds.). Fondo de Cultura Económica, Universidad Nacional Autónoma de México. Ciudad de México, México.

McBride, R. T. Jr., and J. J. Thompson. 2018. Space use and movement of jaguar (*Panthera onca*) in western Paraguay. Mammalia 82:540-549.

Meredith, M., and M. Ridout. 2020. Package 'Overlap'. R package version 0.3.3. 1-20.

Mizutani, F., and P. Jewell. 1998. Home-range and movements of leopards (*Panthera pardus*) on a livestock ranch in Kenya. Journal of Zoology 244:269–286.

Monroy-Vilchis, O., M. Zarco-González, C. Rodríguez- Soto, L. Soria-Díaz, and V. Urios. 2011. Fototrampeo de mamíferos en la Sierra Nanchititla, México: Abundancia relativa y patrón de actividad. Revista de Biología Tropical 59:373-383

Morato, R. G., J. A. Stabach, C. H. Fleming, J. M. Calabrese, R. C. De Paula, K. M. P. Ferraz, D. L. Z. Kantek, S. S. Miyazaki, T. D. C. Pereira, G. R. Araujo, A. Paviolo, C. De Angelo, M. Di Bitetti, P. Cruz, F. Lima, L. Cullen, D. A. Sana, E. E. Ramalho, M. M. Carvalho, F. H. S. Soares, B. Zimbres, M. X. Silva, M. D. F. Moraes, A. Vogliotti, J. A. May Jr, M. Haberfeld, L. Rampim, L. Sartorello, M. C. Ribeiro, and P. Leimgruber. 2016. Space use and Movement of a Neotropical Top Predator: The Endangered Jaguar. PLoS One 11:e0168176.

Moreira, J., R. McNab, R. García, and G. Ponce-Santizo. 2008. Densidad de jaguares en el Biotopo Protegido Dos Lagunas, Parque Nacional Mirador Rio Azul, Petén, Guatemala. Wildlife Conservation Society. Flores, Guatemala.

Moreira, J., R. García, R. McNab, G. Ponce-Santizo, M. Mérida, adn G. Ruano. 2009a. Abundancia de jaguares y evaluación de presas asociadas al fototrampeo en las Concesiones Comunitarias del Bloque de Melchor de Mencos, Reserva de la Biósfera Maya, Petén, Guatemala. Wildlife Conservation Society. Flores, Guatemala.

Moreira, J., R. McNab, R. García, G. Ponce, M. Mérida, V. Méndez, M. Córdova, G. Ruano, K. Tut, H. Tut, F. Córdova, E. Muñoz, E. González, J. Cholom, and A. Xol. 2009b. Abundancia y densidad de jaguares en el Parque Nacional Laguna del Tigre-Corredor Biológico Central, Reserva de la Biósfera Maya. Wildlife Conservation Society. Flores, Guatemala.

Moreira, J., R. Garcia, R. McNab, G. Ruano, G. Ponce, M. Merida, K. Tut, P. Diaz, E. Gonzalez, M. Cordova, E. Centeno, C. Lopez, A. Vanegas, Y. Vanegas, F. Cordova, J. Kay, G. Polanco, and M. Barnes. 2011. Abundancia de jaguares y presas asociadas al fototrampeo en el sector oeste del Parque Nacional Mirador-Rio Azul, Reserva de Biosfera Maya. Wildlife Conservation Society. Flores, Guatemala.

Moreira, J., R. Garcia, R. McNab, G. Ruano, G. Ponce, M. Merida, K. Tut, P. Diaz, E. Gonzalez, M. Cordova, E. Centeno, C. Lopez, A. Vanegas, Y. Vanegas, F. Cordova, J. Kay, G. Polanco, and M. Barnes. 2011. Abundancia de jaguares y presas asociadas al fototrampeo en el sector oeste del Parque Nacional Mirador-Rio Azul, Reserva de Biosfera Maya. Wildlife Conservation Society. Flores, Guatemala.

Muckenhirn, N., and L. Eisenberg. 1973. Home ranges and predation of the Ceylon leopard. Pp. 142–173, *in* The world's cats (Eaton, R. L., ed.). World Wildlife Safari. Winston, USA.

National Oceanic and Atmospheric Administration (NOAA). 2014. Solar Calculator of the National Oceanic & Atmospheric Administration in Department of Commerce of the United States. http://www.esrl.noaa.gov/gmd/grad/solcalc/. Acceced on July 08 2019.

Núñez, R., B. Miller, and F. Lindzey. 2002. Ecología del jaguar en la Reserva de la Biosfera Chamela-Cuixmala, Jalisco, México. Pp.107–126, *in* El jaguar en el Nuevo Mileno. Una evaluación de su estado, detección de prioridades y recomendaciones para la conservación de los jaguares en América (Medellín, R. A., C. Equihua, C. L. Chetkiewicz, P. G. Crawshaw, A. Rabinowitz, K. H. Redford, J. G. Robinson, E. W. Sanderson, and A. Taber, eds.). Universidad Nacional Autónoma de México, and Wildlife Conservation Society. México City, México.

Payán, E., C. Castaño-Uribe, J. F. González-Maya, C. Soto, C. Valderrama, M. Ruiz-García, and C. Soto. 2013. Distribución y estado de conservación del jaguar en Colombia. Pp. 23-36, *in* Grandes felinos de Colombia (Payán, E., and C. Castaño-Uribe, eds.). Panthera Colombia, Fundación Herencia Ambiental Caribe, Conservación Internacional Colombia, CAT Specialist Group IUCN-SSC. Bogotá, Colombia.

Pereira-Garbero, R., and A. Sappa. 2016. Historia del jaguar en Uruguay y la banda oriental. Pp. 336–443, *in* El jaguar en el siglo XXI: La perspectiva Continental (Medellín, R. A., De la Torre, J. A, Zarza, H, Chávez, C, and G. Ceballos, eds.). Fondo de Cultura Económica, Universidad Nacional Autónoma de México. Ciudad de México, México.

Rabelo, R. M., S. Aragón, and J. C. Bicca-Márquez. 2019. Prey abundance drives habitat occupancy by jaguars in Amazonian floodplain river islands. Acta Oecologica 97:28-33.

R Core Team. 2020. R: A Language and Environment for Statistical Computing. R Foundation 503 for Statistical Computing. Vienna, Austria.

Reyes, F. 2009. Los cuerpos de agua de la región Maya Tikal-Yaxhá: Importancia de la vegetación acuática asociada, su conservación y el valor desde el uso humano. Centro de Datos para la Conservación, Centro de Estudios Conservacionistas, San Carlos University of Guatemala, Consejo Nacional de Ciencia y Tecnología. Guatemala, Guatemala.

Reyna-Hurtado, R., G. O´Farril, D. Simá, M. Andrade, A. Padilla, and L. Sosa. 2010. Las aguadas de Calakmul: Reservorios de vida silvestre y de la riqueza natural de México. Biodiversitas 93:1-6.

Ridout, M. S., and M. Linkie. 2009. Estimating overlap of daily activity patterns from camera trap data. Journal of Agricultural, Biological and Environmental Statistics 14:32-337.

Rowcliffe, M. 2019. Package 'Activity': Animal Activity Statistics. R package version 1.3. 1-22.

Salom-Pérez, R., E. Carrillo, J. C. Sáenz, and J. M. Mora. 2007. Critical condition of the jaguar *Panthera onca* population in Corcovado National Park, Costa Rica. Oryx 41:51-56.

Sánchez-Pinzón, K., R. Reyna-Hurtado, E. Naranjo, and A. Keuroghlian. 2020. Peccaries and their relation with water availability and their predators in Calakmul, México. Therya 11:213-220.

Sandell, M. 1989. The mating tactics and spacing patterns of solitary carnivores. Pp. 164-182, *in* Carnivore behavior, ecology, and evolution (Gittleman J. L., ed.). Springer. Boston, U.S.A.

Sandoval-Lemus, M. A. 2020. Caracterización del patrón de actividad del tapir centroamericano (*Tapirus bairdii*) en aguadas del Biotopo Protegido Dos Lagunas, Reserva de Biosfera Maya. Licentiate Thesis, San Carlos University of Guatemala. Guatemala, Guatemala.

Schaller, G. B., and P. Crawshaw Jr. 1980. Movement patterns of jaguar. Biotropica 12:161–168.

Scognamillo, D., I. Maxit, M. Sunquist, and L. Farrell. 2002. Ecología del jaguar y el problema de la depredación de ganado en un hato de los Llanos venezolanos. Pp. 139-150, *in* El jaguar en el nuevo mileno. Una evaluación de su estado, detección de prioridades y recomendaciones para la conservación de los jaguares en América (Medellín, R. A., C. Equihua, C. L. Chetkiewicz, P. G. Crawshaw, A. Rabinowitz, K. H. Redford, J. G. Robinson, E. W. Sanderson, and A. Taber, eds.). Universidad Nacional Autónoma de México, and Wildlife Conservation Society. México City, México.

Seymour, K. L. 1989. *Panthera onca*. Mammalian Species 340:1-9.

Simá, D., O. Retana, R. Reyna, and J. Miranda. 2008. Detección de fauna silvestre mediante el sistema de fototrampeo en dos ampliaciones forestales de la reserva de la Biosfera de Calakmul, Campeche, México. Reserva de Biósfera Calakmul, ProNatura Península de Yucatán, The Nature Conservancy, Universidad Autónoma de Campeche, and Florida University. Ciudad de México, México.

Sollmann, R., M. M. Furtado, B. Gardner, H. Hofer, A. Jacomo, N. M. Tôrres, N, and L. Silveira. 2011. Improving density estimates for elusive carnivores: accounting for sex-specific detection and movements using spatial capture-recapture models for jaguars in central Brazil. Biological Conservation 144:1017-1024.

Srbeck-araujo, A. C. 2018. Do female jaguars (*Panthera onca* Linneus, 1758) deliberately avoid camera traps? Mammalian Biology 88:26-30.

Sunquist, M. E. 1981. The social organization of tigers in Royal Chitawan National Park, Nepal. Smithsonian Contributions to Zoology 336:1–98.

Sunquist, M., and F. Sunquist. 2002. The essence of cats. Pp. 11–13, *in* Wild cats of the world (Sunquist, M., and F. Sunquist, eds.). University of Chicago Press. Chicago, U.S.A.

Tobler, M. 2015. Camera Base, User Guide. Version 1.7 On line. http://www.atrium-biodiversity.org/tools/camerabase/files/CameraBaseDoc1.7.pdf. Acceced on August 25 2019.

Veras, E. 2009. Diagnóstico de la situación actual de las áreas protegidas que co-administra la Universidad de San Carlos de Guatemala y propuesta de lineamientos estratégicos para su sostenibilidad. Dirección General de Investigación, San Carlos University of Guatemala. Guatemala, Guatemala.

Zeller, K. 2007. Jaguars in the New Millennium Data Base Update: The State of the Jaguar in 2006. Wildlife Conservation Society-Jaguar Conservation Program. New York, U.S.A.

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65 years of museum-based mammal research in México: from taxonomy to worldwide information networks

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Biological collections have become a key tool for biodiversity research. They are repositories of germplasm and data on modified or extinct natural populations, providing valuable information for understanding anthropogenic impacts on the natural world. We appraised the scientific value of the three mammal collections maintained by the Instituto Politécnico Nacional of Mexico (IPN): Escuela Nacional de Ciencias Biológicas (ENCB), CIIDIR Durango (CRD), and CIIDIR Oaxaca (OAXMA). We evaluated their specimen inventory, geographic coverage, and scientific importance for mammalogy. We assessed their physical conditions and provided insights into their future as data sources for understanding natural changes in the 21st century. We measured the scientific importance of the collections in terms of the number of specimens and taxa held and their geographic coverage. We also quantified research projects, publications, theses, and dissertations that have used at least one specimen, associated data, or materials deposited in these collections. We assessed their physical conditions by comparison with the standards set by the American Society of Mammalogists. As of 2018, these collections held a total of 61,560 cataloged specimens (ENCB 44,275; CRD 12,163; OAXMA 5,122), the largest repository of Mexican mammals in the Americas. All the orders, 86 % of the genera, and 69% (342) of the species of terrestrial mammals known to inhabit Mexico are represented in these collections. Specimens from all the Mexican States are included, with CRD focusing mostly on the northern part of the country, ENCB on central and southeastern Mexico, and OAXMA on the southeast. Materials from these collections have been used for at least 285 peer-reviewed publications, 91 research projects, and 107 theses and dissertations. CRD keeps >9,000 tissue samples, while OAXMA keeps >300 tissue samples plus 1,139 echolocation recordings of 24 bat species. All collections meet most curatorial standards of specimen preservation and availability and are being actively used in research projects. Nonetheless, we believe a sound institutional policy is necessary for the IPN mammal collections to successfully face the 21st-century challenges, particularly for ensuring the availability of usable data for the scientific community, digitizing the holdings, and designing and implementing a long-term preservation strategy.

Las colecciones biológicas han adquirido un nuevo valor como recurso para la investigación en biodiversidad por ser depositarias de germoplasma e información sobre poblaciones naturales modificadas o extintas, lo que las convierte en invaluables fuentes de datos para comprender las modificaciones de origen antropogénico sobre el mundo natural. El presente trabajo documenta el valor científico de las colecciones del Instituto Politécnico Nacional (IPN): Escuela Nacional de Ciencias Biológicas (ENCB), Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Durango (CRD) y CIIDIR Oaxaca (OAXMA), y su impacto en la mastozoología; se contrasta, además, su estado de conservación con respecto a los estándares mínimos de la American Society of Mammalogists. Finalmente, se discute su potencial como fuente de información para comprender los cambios en el mundo natural en el Siglo XXI. La importancia científica se midió como número de ejemplares y taxones contenidos en los acervos y su representatividad geográfica, y colecciones asociadas. Su impacto en la mastozoología se determinó mediante el número de publicaciones, tesis y proyectos que hayan utilizado por lo menos un ejemplar o material asociado de alguna de las colecciones. Su estado de conservación se estimó por contraste con los estándares mínimos para colecciones mastozoológicas de la American Society of Mammalogists. Hasta 2018 las colecciones incluían 61,560 ejemplares catalogados (ENCB 44,275, CRD 12,163, OAXMA 5,122), que representa el mayor acervo de mamíferos mexicanos de América. Incluyen todos los órdenes terrestres mexicanos, 86 % de los géneros y 69 % de las especies. Todos los estados del país están representados, en CRD principalmente los del norte, en ENCB el centro y sureste, y en OAXMA el sureste. Se documentaron 285 publicaciones arbitradas, 91 proyectos y 107 tesis. CRD alberga >9,000 muestras de tejido, OAXMA >300 (Tabla 3) y una colección de 1,139 sonidos de ecolocalización de 24 especies de murciélagos. Aunque todas las colecciones cubren la mayor parte de los estándares curatoriales en lo que a preservación y disponibilidad de los ejemplares para la comunidad científica se refiere, y se encuentran activamente en crecimiento y generando investigación, se concluye que se requiere una clara política institucional que permita a las colecciones mastozoológicas del IPN enfrentar los retos del siglo XXI. Esto incluye reconocer su valor y diseñar las estrategias para lograr la digitalización de los acervos y documentación asociada, la disponibilidad de la información en formatos y plataformas digitales utilizables para la comunidad científica internacional, y la preservación del acervo físico y digital a largo plazo.

Keywords: anthropogenic change; biological collections; collection management; Mammalia; natural history.

Introduction

During the second half of the 20th century, many persons, including some biologists, regarded biological collections as "dinosaurs" or "white elephants" whose original function as taxonomic repositories had been surpassed and had no place in modern science (Shetler 1969; Lavoie 2013). This perception often led to budget cuts, reluctance to hire qualified personnel for handling and curatorial work, reduction of facilities, and little or no support for further growth, all of which threatened the existence of numerous collections. Lacking proper institutional support, some collections, especially small ones, were put at the brink of disappearance or disappeared completely over the past 30 to 40 years. For instance, some collections in the United States had to be merged with larger collections having better maintenance capability (Kemp 2015).

Paradoxically, in parallel with this period of apparent decline, other scientific community members were increasingly valuing the irreplaceable role of biological collections in documenting the changes that have been occurring on the planet as a consequence of human activities. Thirty years ago, Danks (1991) claimed that scientific collections were valuable mainly as sources of materials for reference, identification, and description of species, and as means for understanding the diversity and evolutionary relationships between current and past fauna and the ecosystems where they thrive. He also stated that scientific collections play a key role in scientific training, cultural outreach, and raising public awareness of environmental matters. Although all these are still essential functions of scientific collections, the recent development of molecular analysis tools, geographic information systems, and bioinformatics, together with the unprecedented degradation of the biota, have given renewed prominence to biological collections for studying the environmental modification processes that threaten the very survival of human populations (Vázquez-Domínguez and Hafner 2006). Specimens deposited in collections make it possible to address many environmental and health issues since they provide information on the condition of populations, species, and communities of organisms prior to anthropogenic modification (Lister *et al.* 2011).

Geist (1992) pointed out that "the dismantling of museum collections is not only tragic, but irresponsible, now that specimens have gained legal significance", in reference to the fact that once a country puts an animal or plant species under legal protection by the State, its accurate identification becomes of paramount importance in deciding whether or not hunting, using, exploiting, trading, exporting, or destroying one or more specimens constitutes an offense. The proper application of biodiversity-related laws requires having scientific collections and scientific experts that can issue science-based recommendations.

Given the usefulness of molecular data for taxonomic identification and discovery of new taxa (Borisenko *et al.* 2008), the current collection of specimens usually includes sampling and preservation of tissues, turning scientific collections into repositories of genetic material from populations that may become extinct in the short- or medium-term. This practice also opens endless possibilities by making entire genomes of plants and animals available to the scientific and technological community for probing their potential for use as food, in pharmaceutical applications, or in the development of new technologies. For these reasons, biological collections in Mexico are deemed a matter of national security that bear strategic value (CONABIO 2019).

The Instituto Politécnico Nacional (IPN) is a higher-education and research institution that hosts several biological collections. Given their size and scientific value, some of these collections are amongst the most important both in Mexico and at an international level (*e. g.*, Yates *et al.* 1987; Guzmán 1994; Hafner *et al.* 1997; Ramírez-Pulido and González-Ruiz 2006; Dunnum *et al.* 2018; Thiers 2018). However, no study documenting the scientific value of the IPN collections and their impact throughout their history has been conducted to date. IPN hosts three major mammal collections: the oldest one was founded in 1955 at the Escuela Nacional de Ciencias Biológicas (ENCB); a second collection was set in 1984 at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-Unidad Durango (CRD); and the third was established in 1985 at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-Unidad Oaxaca (OAXMA). This study aimed at appraising the scientific value of the IPN mammal collections and their impact on scientific research and assessing their physical conditions relative to the minimum standards set by the American Society of Mammalogists (2004). Finally, the potential of the IPN mammal collections as a source of information for understanding the changes in the natural world that are taking place in the 21st century is discussed.

Materials and Methods

The scientific value of each collection was assessed in terms of the number of specimens it holds; number of orders, families, genera, and species represented both in each collection and overall (taxonomic scope); number of type specimens hosted; and collections associated with them. The nomenclature follows Ramírez-Pulido *et al.* (2014), with some exceptions and updates (as of 2019, see Appendix 1). Appendix 1 also indicates the endemism and conservation status of each species, as listed in the official Mexican standard (SEMARNAT 2019). The geographic representation of these collections was assessed in terms of the number of specimens from each Mexican State; ancillary collections were also identified.

The indicators most used in Mexico to evaluate scientific research performance are number and funding resources of research projects, number of peer-reviewed publications, and number of students trained (CONACYT 2020). To estimate the number of scholarly publications produced, we carried out a search in the major literature databases, including, but not limited to: REDALCYC, SCOPUS, SCIELO, Google Scholar, Research Gate, Journal Citation Reports, and CONRICYT, and in library catalogs of the institutions hosting the collections. We used the following search terms: "Mammalia", "mammals", "mamíferos", "ENCB", "CRD", "OAXMA", "Colección", "Collection", and the names of the curators and their collaborators. All publications in peer-reviewed journals, books, and book chapters that made use of materials or data from any of the three collections studied were gathered. The inclusion criteria were that at least one collection specimen had been used; at least one bat call has been recorded and organized in a reference sound library; at least one photograph has been captured either with a camera trap or digital camera and mounted as a science photo library sheet (Botello *et al.* 2007); at least one tissue sample or data has been deposited in any of the collections; or the curators or the collection have been acknowledged for granting access to specimens or information deposited in the collection. Some publications met more than one of these criteria; these were recorded in the numbers of the respective collections but were counted only once for computing total figures. The publications thus identified were then classified into 1) publications indexed in the Journal Citation Report or CONACYT index; 2) non-indexed publications; and 3) book chapters. "Gray" literature or popular science articles were not included. Finally, the average number of publications produced per year from each collection was calculated.

Student training was evaluated in terms of the total number of students that carried out social service (unpaid training work), internships, or short-term summer internships (short stays for undergraduate students sponsored by the Mexican Academy of Sciences) at each collection. Terminal projects, bachelor's and master's theses, and doctoral dissertations were identified by searching in the institutional theses archives of IPN (https://www.repositoriodigital.ipn.mx/handle/123456789/144), Universidad Nacional Autónoma de México (UNAM, http://oreon.dgbiblio.unam.mx/F?RN=656817172), and Universidad Autónoma Metropolitana (UAM, http://tesiuami.izt.uam.mx/uam/default.php), and in the library of each collection; the inclusion criteria were the same used for publications. Other contributions to student training that did not produce theses or dissertations were also identified and included.

The number and, whenever possible, the amount (in Mexican pesos) granted to research projects carried out by scientists affiliated to each collection (either as principal investigators or collaborators) were quantified. The amount of funding that IPN has received from collection-based research projects, consultancy work, or external services was estimated as well. Because funding data for older projects were not available and the figures are not comparable over time due to inflation, these data necessarily underestimate the real amount; nevertheless, they provide a rough idea of the income generated by collections. Those projects (2) that were carried out jointly by two or more collections were assigned to the collection where the leading investigator was affiliated. Finally, the infrastructure and physical conditions of each collection were described and compared with the minimum standards set by the American Society of Mammalogists (American Society of Mammalogists Systematics Collections Committee 2004).

Results

Scientific Value. As of the end of 2018, the IPN mammal collections jointly housed a total of 61,560 cataloged voucher specimens (ENCB = 44,275, CRD = 12,163, OAXMA = 5,122) representing 11 orders, 141 genera (84 % of all genera recorded in Mexico), and 348 species of Mexican terrestrial mammals. These species amount to 69.4 % of the 496 reported by Ramírez-Pulido *et al.* (2014) plus five additional species (*Tlacuatzin balsasensis*, *T. sinaloae* [Arcangeli *et al.* 2018], *Peromyscus leucurus*, *P. micropus*, and *P. zamorae* [López-González *et al.* 2019]) that were described or elevated to species after their publication; 116 of these species are endemic to the country (Appendix 1, Figure 1). The ENCB collection houses four type specimens of valid taxa: *Notocitellus adocetus infernatus* Álvarez and Ramírez-Pulido 1968 (ENCB 1019), *Microtus mexicanus ocotensis* Álvarez and Hernández-Chávez, 1993 (ENCB 27603); *Molossus alvarezi* González-Ruíz, Ramírez-Pulido and Arroyo-Cabrales, 2011 (ENCB 34208), and *Tlacuatzin balsasensis* Arcangeli, Light and Cervantes, 2018 (ENCB 26195). In the 1980s, ENCB carried out a project aiming at collecting topotypical samples from central Mexico (encompassing the States of Aguascalientes, Colima, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Puebla, Querétaro, San Luis Potosí, Tlaxcala, and Zacatecas). As a result, the size of the ENCB collection increased considerably and currently includes topotypes from 115 of the 792 type localities of mammal species or subspecies that had been described in Mexico up to that time (Álvarez *et al.* 1997).

OAXMA and CRD also host ancillary collections of tissue samples (OAXMA: 350 cataloged samples preserved in 95% ethanol and frozen at -18 °C; CRD: 9,003 samples preserved in 95 % ethanol and frozen at -20 °C), most of which are linked to voucher specimens in the main collection. OAXMA also maintains a sound library including 1,139 echolocation calls of 24 insectivorous bat species from the State of Oaxaca; 48 of these recordings are part of the national echolocation sound library of the Sonozotz project led by the Asociación Mexicana de Mastozoología A. C. (Briones-Salas *et al.* 2013; Sosa-Escalante 2018; Zamora-Gutiérrez *et al.* 2020).

All the 32 Mexican states are represented in at least one of the IPN collections (Table 1; Figure 2). Due to their particular history and geographic location, the three collections supplement each other in both species composition and geographic representation. ENCB is mainly focused on the north-central (San Luis Potosí, Durango, and Zacatecas), central (State of Mexico, Michoacán, Jalisco, Aguascalientes,

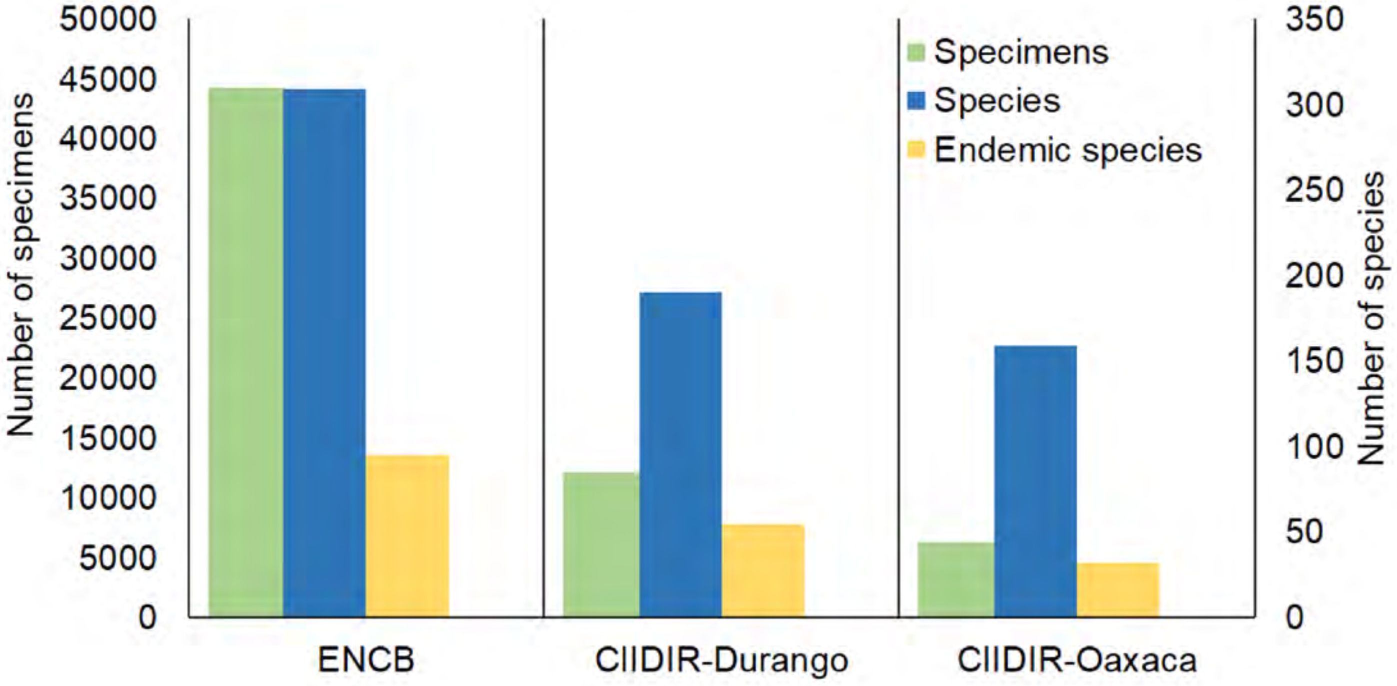

Figure 1. Number of voucher specimens, species, and endemic species deposited at the IPN mammal collections.

Hidalgo, Puebla, Tlaxcala, Veracruz, Guerrero, and Morelos) and southeast (Yucatán, Chiapas, and Oaxaca) parts of the country. CRD focuses on the Western Sierra Madre (Nayarit, Zacatecas, Durango, and Chihuahua States) and northern Mexico (Coahuila, Nuevo León, and Tamaulipas). OAXMA is mostly restricted to the State of Oaxaca (Table 1; Figure 2).

Indicators. Publications. A total of 285 peer-reviewed publications meeting the inclusion criteria were gathered (Table 2); specimens from ENCB were mentioned in 171 of these publications, from CRD in 61, and from OAXMA in 62; specimens from two or more of these collections were mentioned in nine publications. An average of 2.7 publications per year have been produced using material from the ENCB collection since its foundation, 1.8 publications per year using CRD materials, and 1.9 using OAXMA materials. Some 37 % of the publications based on the ENCB collection were published in indexed journals; 60 % of those produced based on the other two collections are indexed. Data from the ENCB collection have been used in ten books; no books have been published based on OAXMA or CRD materials. Book chapters account for 20 % of the publications produced using materials from ENCB, 13 % from CRD, and 17 % from OAXMA (Table 2).

Student training. We identified a total of 95 theses and dissertations that were supervised or co-supervised by researchers affiliated to the collections and that used materials housed therein: 34 for ENCB, 11 for CRD, and 50 for OAXMA. Two additional theses from ENCB were supervised by researchers not affiliated to the collection, for an overall total of 97. We found 30 additional theses/dissertations from other institutions that have used data or materials from the IPN collections (Table 2). A total of 73 college or junior college students carried out social service or short stays at the IPN collections; OAXMA has been the most active collection in this regard, followed by CRD and ENCB, only CRD has hosted post-doctorates (Table 2). The ENCB collection has also been used for delivering customized training courses for staff from PEMEX (the national oil company), SEMARNAT (the ministry of the environment), CONANP (the national commission for protected areas), and other government agencies.

Research Projects. We identified a total of 91 research or external service projects, 52 of which were directly funded by the IPN research and postgraduate secretariat (SIP), and 39 by external bodies. The main sources of external funding for the three IPN collections were CONABIO (the national commission for biodiversity) and CONACYT (the national council for science and technology), followed by Mexican and foreign government agencies, private companies, and non-governmental organizations. We estimated a total investment of at least 11,560,090 Mexican pesos; funding from external sources more than doubled the amount received from IPN (Table 2).

Other Products. Specimens from the ENCB collection have been used as a reference to identify material collected or recovered, through various projects, by the Comisión Nacional de *Á*reas Naturales Protegidas CONANP), the Instituto Nacional de Antropología e Historia (INAH), and other government agencies. As of 2018, all the voucher specimens of the ENCB collection, approximately 60 % of CRD, and 17 % of OAXMA have been incorporated into the Sistema Nacional de Información sobre Biodiversidad (SNIB) maintained by CONABIO, which provides access to domestic and international users to georeferenced information through its online database. Photographs of specimens of many of the species included in the SNIB database are also accessible to the general public through the CONABIO website.

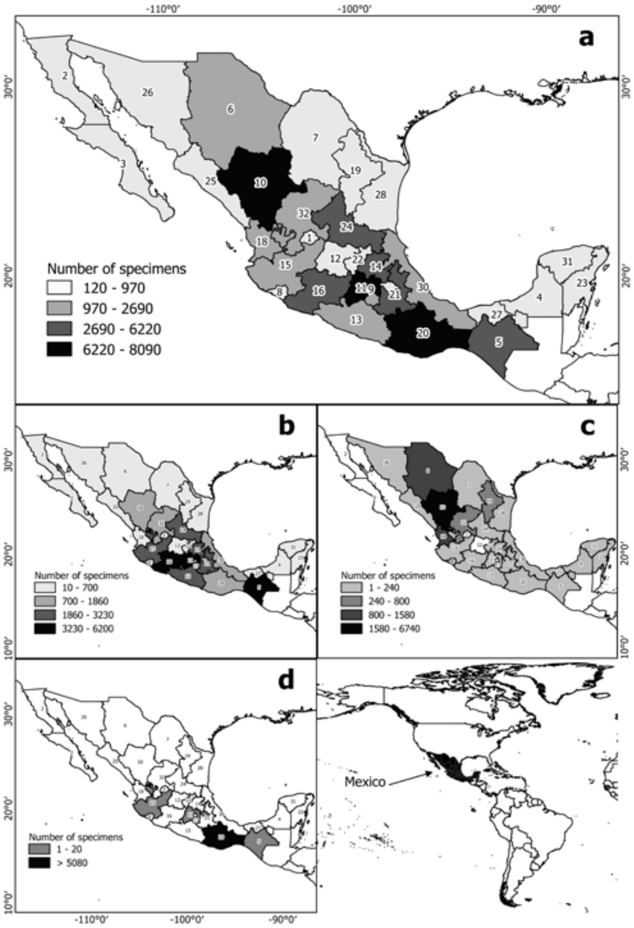

Figure 2. Number of voucher specimens of terrestrial mammals, per Mexican State, deposited in the IPN mammal collections: (a) total, (b) ENCB, (c) CRD, and (d) OAXMA. Numbers denote the State as listed in Table 1.

Physical Conditions: The ENCB Collection. The ENCB collection is housed in a room of approximately 70.0 m^2 furnished with two work tables, 91 *ad hoc* wooden boxes for dried skins and skulls, two metal cabinets for tanned skins, a metal shelf for fluid-preserved specimens, and a wooden shelf for skeletons or skulls of large mammals. Three additional metal cabinets, each with five sections, mounted on compactor rails are also available. Controlled environmental conditions (relative humidity and temperature, separation of dried and fluid-preserved materials) are not available in this facility for preserving the specimens, thus fumigation with para-dichloro-benzene is carried out every six months. Given the toxicity and carcinogenicity hazard of this chemical, funds are currently being raised to install temperature and humidity controls and discontinue this fumigation procedure to avoid potential health risks. A small (10 m^2) dermestarium is also available.

Most specimens (40,855) are preserved as dried skin and skull; complete or partial skeletons, and dried skins (3,020) are also included, as well as fluid-preserved whole-body specimens (400). The majority (99 %) of the specimens are labeled with information on the collecting locality and date, collector and preparator names, and are georeferenced and identified to species. The collection is managed by a curator and an assistant, both part-time permanent staff of IPN.

Physical Conditions: Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Durango. The CRD collection is housed in a 49 m^2 area, in addition to a library and office space (49 m^2) where collection documents are kept, a small (8 m^2) storage unit, a separate (19 m^2) preparation room where fluid-preserved specimens (including the amphibian and reptile collections) are also kept, and a small (2 m^2) dermestarium. The collection is currently supplied with 29 collection boxes, a cabinet for skins and large specimens, and two stereomicroscopes. The dried collection is kept in a temperature-and-humidity-controlled room separately from the fluid-preserved

Table 1. Geographic representativeness, per Mexican State, of the IPN mammal collections. The total number of cataloged voucher specimens deposited in each collection is given below the acronym.

	State	Representativeness		
		ENCB	CRD	OAXMA
		44,275	12,163	5,122
1	Aguascalientes	1.25	0	0
2	Baja California	0.88	0	0
3	Baja California Sur	0.91	0	0
4	Campeche	0.15	0.60	0
5	Chiapas	9.74	0.09	0.08
6	Chihuahua	0.29	12.75	0
7	Coahuila	0.02	2	0
8	Colima	0.66	0	0
9	Ciudad de México	2.86	0	0
10	Durango	2.84	55.35	0
11	Estado de México	13.99	0.04	0.35
12	Guanajuato	1.33	0	0
13	Guerrero	5.29	0.01	0
14	Hidalgo	6.89	0.11	0
15	Jalisco	5.31	0.83	0.06
16	Michoacán	11.37	0.35	0
17	Morelos	3.60	0	0
18	Nayarit	0.36	10.75	0
19	Nuevo León	0.03	6.55	0
20	Oaxaca	4.21	0.01	99.35
21	Puebla	5.93	0.53	0
22	Querétaro	0.76	0.79	0
23	Quintana Roo	1.59	0.01	0
24	San Luis Potosí	7.30	0.09	0
25	Sinaloa	0.20	1.67	0
26	Sonora	0.24	0.09	0
27	Tabasco	0.36	0.01	0
28	Tamaulipas	1.40	1.40	0
29	Tlaxcala	2.09	0.31	0.06
30	Veracruz	4.10	0.46	0
31	Yucatán	1.48	0.16	0
32	Zacatecas	2.60	4.07	0
	Other countries	0	0.67	0

collection. The collection is regularly fumigated with paradichloro-benzene, and there is a double door control to prevent the entry of insects.

Most specimens (10,451) are preserved as dried skin and skull or partial skeleton, in addition to complete or partial skeletons (902), dried skins without skeletal material (252), fluid-preserved whole-body specimens (15), and fluid-preserved specimens with no skull (543). The majority (99%) of these specimens are labeled with information on the collecting locality and date, collector and preparator names, and is georeferenced and identified to species. The collection is managed by a curator and a manager, both full-time permanent staff of IPN.

Physical Conditions: Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca. The OAXMA collection is housed in an approximately 36 m^2 facility split into three sections: office space, preparation room, and specimen storage room. The preparation room is furnished with a freezer, materials, and four worktables. The specimen storage room is furnished with 18 wooden cabinets and two metal shelves where all the biological material (skulls, skeletons, and dried skins) is kept, as well as footprint casts, tissue samples, and photographs. The office and the specimen storage rooms are both supplied with air conditioning and humidity extraction systems.

Most specimens (3,019) are preserved as dried skin and skull or partial skeleton, followed by fluid-preserved whole-body specimens (546), complete or partial skeletons (438), and skulls (62). All specimens are labeled with information on the collecting locality and date, and collector name; 94.3 % have been identified to species. The collection is managed by a curator and a technician, both full-time staff of IPN.

As per the standards issued by the American Society of Mammalogists (American Society of Mammalogists Systematics Collections Committee, 2004), the IPN collections meet 90 % of the minimum conditions for the proper operation of a mammal collection.

Discussion

Scientific Value. With a grand total of 61,560 cataloged specimens, the IPN mammal collection is the largest in Mexico. For comparison, the second-largest institutional collection is the mammal collection of UNAM, with a grand total of 57,081 specimens, distributed among the Colección Nacional de Mamíferos of the Instituto de Biología (CNMA), the Alfonso L. Herrera Museum of the Facultad de Ciencias (MZFC), and the Paleontology laboratory of the Instituto de Geología (https://datosabificios.unam.mx/biodiversidad/). However, since the IPN collection does not include fossils, the specimens in the Paleontology laboratory of the Instituto de Geología should, strictly speaking, be excluded to make the total number of specimens comparable. Taken separately, the ENCB collection is the second-largest mammal collection in Mexico after the CNMA, which housed a total of 48,526 specimens as of 2015 (Rivera León *et al.* 2018). CRD is the fifth-largest collection, after the CNMA, ENCB, Centro de Investigaciones Biológicas del Noroeste in Baja California Sur (CIBNOR), and Universidad Autónoma Metropolitana Iztapalapa (UAM-I) collections.

The IPN collection houses 14.6 % of the 421,466 voucher specimens of Mexican mammals deposited in scientific collections in North America (Lorenzo *et al.* 2012) and 32.7 % of those (188,350 specimens) in Mexican collections. Only the CNMA collection holds a larger representation ("close to 90 %", Cervantes *et al.* 2016) of Mexican mammal species. The ENCB collection used to be among the 20 largest mammal collections in the Western Hemisphere (Choate and Genoways 1975; Genoways *et al.* 1976; Hafner *et al.* 1997; Lorenzo *et al.* 2012) and, although it has now been surpassed by other collections (Dunnum *et al.* 2018), it is still the third largest collection of Mexican mammals in North America, after the CNMA and KU (University of Kansas) collections (Lorenzo *et al.* 2012).

Lorenzo *et al.* (2012) pointed out that Mexican collections house a relatively low number of specimens from northern Mexico (Chihuahua = 2,619, Nayarit = 2,561, Sinaloa

Table 2. Productivity of each IPN collection since its foundation to 2018. Level: L = Bachelor's degree, M = Master's degree, D = Doctorate, SS = Social Service, PR = Internship. Funding sources: SIP = IPN Secretaría de Investigación y Posgrado. Funding figures have not been adjusted for inflation. Data used for this table are available from the first author upon request. PRA = peer-reviewed articles, IA= articles indexed by ISI; JCR or CONACYT, BC = Book chapters, OI = other institutions (L, M, D).

Collection	Number of grants/funding amount in x 10^3 Mexican pesos		Number of peer-reviewed publications				Theses				Stays	Postdocs
	External funding	SIP	PRA	IA	Books	BC	L	M	D	OI (L, M, D)	SS, PR	
ENCB	10/4,749.01	10/139.49	62	64	10	35	36	0	0	11, 5, 9	3, 1	0
CRD	12/1,814.34	29/1,220.6	12	41	0	8	4	7	0	1, 2, 3	5, 5*	2
OAXMA	15/2,565.84	13/1,070.8	10	41	0	11	26	20	4	0, 0, 1	38, 20*	0
Total	37/9,129.19	52/2,430.9	84/82**	146/139**	10	54	66	27	4	12, 7, 12/11**	46, 27	2

* Includes summer stays; ** Total minus publications involving more than one collection.

Notes: The funding figures do not include 1,999 943.50 Mexican pesos from the "Compilación de una biblioteca de referencia de murciélagos insectívoros de México: Fase 1" research project funded by CONABIO through grant M004 and led by MBS (2016 to date). Part of these funds was distributed among eight working groups, including OAXMA, CRD, and ENCB. Also not included are 6,628,488.00 Mexican pesos from the "Actualización del equipamiento de Laboratorios en el área de Biología Molecular, CIIDIR Durango" project funded by CONACYT through grant 122786 and led by CLG (2010-2011); 50% of those funds were used to purchase laboratory equipment, including equipment used by the personnel of CRD.

= 1,487, Nuevo León = 1,112, Coahuila = 1,107), most of them deposited in IPN collections, including 1,729 voucher specimens from Chihuahua (66 %), 1,529 from Nayarit (59.7 %), and 815 from Nuevo León (73.31 %). Lorenzo *et al.* (2012) found a total of 2,450 specimens from San Luis Potosí State, 1,039 from Zacatecas State, and 7,882 from Durango State deposited in Mexican collections; however, the IPN collection currently holds 4,506, 2,092, and 8,481of such specimens, respectively. Thus, as of 2018, most of the mammalian voucher specimens from northern Mexico (not including the Baja California peninsula and Sonora) are deposited in the ENCB and CRD collections. The OAXMA collection currently houses the largest number of mammalian specimens from Oaxaca in Mexico (Briones-Salas *et al.* 2015). These findings evidence the complementary nature of the three IPN collections in terms of both species composition and geographic representation.

Tissue Collections. No inventory of the tissue collections in Mexico has been compiled to date. However, Lorenzo *et al*. (2006) reviewed the mammal collections existing in Mexico and found that eight of the 36 collections also included tissue samples; the largest collections were those of CNMA, MZFC, and CIBNOR, which in 2006 housed a total of 1,750, 2,556 and 4,945 individual samples, respectively. The CIBNOR collection is currently the largest tissue collection in Latin America, with a total of about 24,000 samples (Álvarez-Castañeda *et al.* 2019); the CRD collection is still one of the largest in Mexico with 9,003 samples, and OAXMA has increased its size to 350 samples. Both CRD and OAXMA meet most of the curation standards for genetic material collections issued by the American Society of Mammalogists (Phillips *et al.* 2019).

The development of these ancillary collections has created new demands from the biological community; the IPN collections are now regarded as a major repository of reference and verification (voucher specimens) material for studies using tissue samples conducted both in Mexico and elsewhere. In fact, most of the recent theses and publications that made use of these collections involved molecular analyses. As the DNA or RNA sequences elucidated in those studies are then archived in publicly accessible databases (*e. g.,* GenBank, https://www.ncbi.nlm.nih.gov/genbank/), their scientific value will continue increasing as they are used in future studies or as identification tools by users in Mexico and other countries.

Publications and Theses. An objective appraisal of the relevance of the publications, in particular those from ENCB, is a complex task. The publication standards by which the quality of a publication was judged in its first 30 years are not the same as in the latest 30 years, when publication citation indices and impact measures were created and widely adopted. Only 37 % of the publications based on the ENCB collection were published in currently indexed journals. By contrast, the other two collections, CRD and OAXMA, were founded 30 years later and, thus, have been subjected to modern productivity evaluation standards almost since their inception. Thus, 60 % of the publications based on the CRD and OAXMA collections were published in indexed journals.

Also consistent with this change in the standards and information dissemination modalities, ten books have been produced using data from the ENCB collection, with 20 % of its production in the form of book chapters. In contrast, no books associated with the other two collections have yet been published, and less than 20 % of their publications are book chapters (Table 2). Thus, although the overall publication productivity of the ENCB collection is almost one publication per year higher than that of the other two collections, their rates are about the same when only indexed journals are considered (1.1 per year versus 1.2).

The first curators of the ENCB (Ticul Álvarez Solórzano), CNMA (Bernardo Villa Ramírez), and UAM-Iztapalapa (José Ramírez Pulido) collections are recognized as the pioneers of mammalogy in Mexico. Up to the early 1970s, Mexican mammalogy was led by work conducted in or associated with these three collections (Ríos-Muñoz *et al.* 2014), where the next generation of Mexican mammalogists was trained.

Most publications and theses produced in that early period were descriptive, mainly focusing on taxonomy, anatomy, physiology, or reproductive aspects (Ríos-Muñoz *et al.* 2014). Over time, and as research priorities and technologies evolved, material deposited in the collections has been increasingly used for other types of studies. The earliest non-descriptive study that we were able to document was the one by Genoways (1973), who used ENCB specimens to examine evolutionary relationships in the genus *Liomys* (currently *Heteromys*). The first molecular analyses conducted using material from IPN collections were those by Hafner *et al.* (2001), Bell *et al.* (2001), and Guevara-Chumacero *et al.* (2010), who used materials from the ENCB, CRD, and OAXMA collections, respectively. Ecological studies began with Álvarez and Arroyo-Cabrales (1990), Muñiz-Martínez (1997), and Prieto-Bosch and Sánchez-Cordero (1993). The latter is also the first publication in which geographic information systems were used, with data from the OAXMA collection. Álvarez-Castañeda (1993, ENCB), Illoldi-Rangel *et al.* (2004, OAXMA), and López-González *et al.* (2015, CRD) were the first studies that used information from IPN collections to address biogeographic questions. More recently, OAXMA added an echolocation sound library that has been used for taxonomic studies (Briones-Salas *et al*. 2013).

The examples above reflect the global trend towards diversifying the uses of scientific collections (Schindel and Cook 2018). Technological innovations enable novel uses for traditional materials such as dried skins and skulls, which acquire higher value when also supplied with newly available information, such as georeference. When non-traditional material such as tissues, sound recordings, photographs, or videos are also collected and incorporated into scientific collections, these become repositories of verifiable evidence valuable not only for taxonomic but also for ecological and behavioral studies, to name a few (Pyke and Ehrlich 2010; Schindel and Cook 2018; Winker 2004).

Research Projects. Despite being the oldest one, the ENCB collection has contributed the fewest research projects. This may be partly due to an artifact caused by the scarce information available on the operation of this collection prior to 1995, but also reflects the fact that during the first half of its existence, prior to the foundation of CONABIO in 1992, financing for the development and operation of biological collections in Mexico was far more limited. In contrast, the CRD and OAXMA collections started growing actively after 1990 and enjoyed better access to funds from both the IPN and external agencies such as CONABIO and CONACYT. Funding from CONABIO in the 2000-2010 period strongly promoted the digitization of the IPN collections and financed inventory projects in specific areas of interest. In line with the historical trends in scientific publication described above, those research projects have mainly addressed mammal inventories and descriptive studies; projects focused on more analytical topics such as population structure and organism-environment relationships started in the late 1990s.

Student Training. The role of IPN collections on student training has been quite different for the CRD and OAXMA than for the ENCB and other major institutional collections because CIIDIRs were not associated with higher education institutions in their early years (Figure 3). The ENCB mammal collection includes a small section entirely dedicated to college teaching purposes, which has been extensively used over the past 55 years by students (ten per semester on average) enrolled in the elective Mammalogy course as part of the B.Sc. Biology program.

Figure 3 lists the IPN staff who have worked for the collection. In addition, many other persons have collaborated as students doing social service and theses or dissertations, temporarily hired to work on research projects, or as volunteers collecting, preparing, and curating specimens, sometimes for periods up to five years or longer. This group includes, in alphabetical order: Martín Aguilar Cervantes, Sergio Ticul Álvarez-Castañeda, Joaquín Arroyo-Cabrales, Tania Berrocal Espino, Pablo Domínguez, Salvador Gaona Ramírez, Manuel González Escamilla, Noé González-Ruíz, José Juan Hernández-Chávez, Karina Lagunes Serrano, Nansy López, Consuelo Psiabini López Valentín, Nisa Victoria López Valentín, Isaac M. Cruz Márquez Benítez, Eliecer Martin, Matías Martínez-Coronel, Noemí Matías Ferrer, Raúl Muñiz-Martínez, Sergio Murillo Jiménez, Francisco Javier Navarro Frías, Oscar J. Polaco, Enrique Quiroz Uhart, Claudia Yeyetzi Salas Rodriguez, Carolina Salazar Motolinía, Norma Valentín Maldonado, Jorge Villalpando, and Ángel Vega López, all of which worked at the collections for over a year. Many of these individuals eventually became prominent professional mammalogists.

For its first 20 years, the CRD collection was located in Vicente Guerrero City, Durango, 80 km from the state capital. It was relocated to the state capital, Durango City, in 2004, shortly after the local university, Instituto Tecnológico de Valle de Guadiana, launched its B.Sc. Biology program in 2003. However, it was not until 2016 that a cooperation agreement was signed allowing B.Sc. Biology students from Instituto Tecnológico to carry out social service, study stays, and thesis at CIIDIR Durango. CIIDIR Durango launched its M. Sc. program in Environmental Management in 2005 and was included by CONACYT in its quality postgraduate program (PNPC, for its acronym in Spanish) in 2007. CIIDIR Durango became one of the campuses of the Ph.D. program in Conservation of Landscape Heritage, which was also included in the PNPC in 2014, thus allowing the participation of local out-of-state, and foreign students. In this way, a number of students have collaborated in collecting activities over the years, either as students, interns, or as temporary workers (for at least one year), including Nancy Enríquez, Ángel García Rojas, Alejandra J. Guerrero González, Nancy Lemus Medina, Abraham Lozano, Teresa Salas-H., Gloria Tapia-Ramírez, and Alí I. Villanueva-Hernández.

Similarly, few students collaborated with the OAXMA collection in its early years. The local university, Instituto Tecnológico de los Valles de Oaxaca (ITVO) has been collaborating actively with OAXMA since 2008; 86 % ($n = 26$) of the B.Sc. theses associated with OAXMA were carried out by ITVO undergraduate students. CIIDIR Oaxaca launched its M.Sc. program in use and conservation of natural resources in 2003 and the corresponding PhD program in 2005; both postgraduate programs belong to PNPC and opened the possibility for postgraduate students to participate in OAXMA activities. The establishment of PNPC-listed postgraduate programs at both CIIDIRs and the ensuing possibility of accessing scholarships from CONACYT have enabled using both collections in student training at the regional and country-wide level, as does the ENCB collection.

Physical Conditions. All three collections meet most of the ASM minimum conservation standards (American Society of Mammalogists Systematics Collections Committee 2004). All of them have specialized trained staff dedicated to preservation and curatorial work, and voucher specimens have the proper curation level to be used for scientific work. Their primary weakness is the lack of sufficient, suitable infrastructure (buildings and insect-resistant, fire- and water-proof furniture) to prevent the long-term deterioration of specimens, and the slow implementation of online electronic catalogs to make the information easily accessible to users. Although the three collections enjoy implicit institutional recognition, no explicit policy for their continued maintenance and long-term conservation is in place.

The three collections are well on their way to meeting the minimum conservation standards issued by the ASM in 2004 (American Society of Mammalogists Systematics Collections Committee 2004). However, such standards were intended for traditional collections of dried skin and skeletal materials. The increasing use of specimens collected including tissue and hair samples, echolocation recordings,

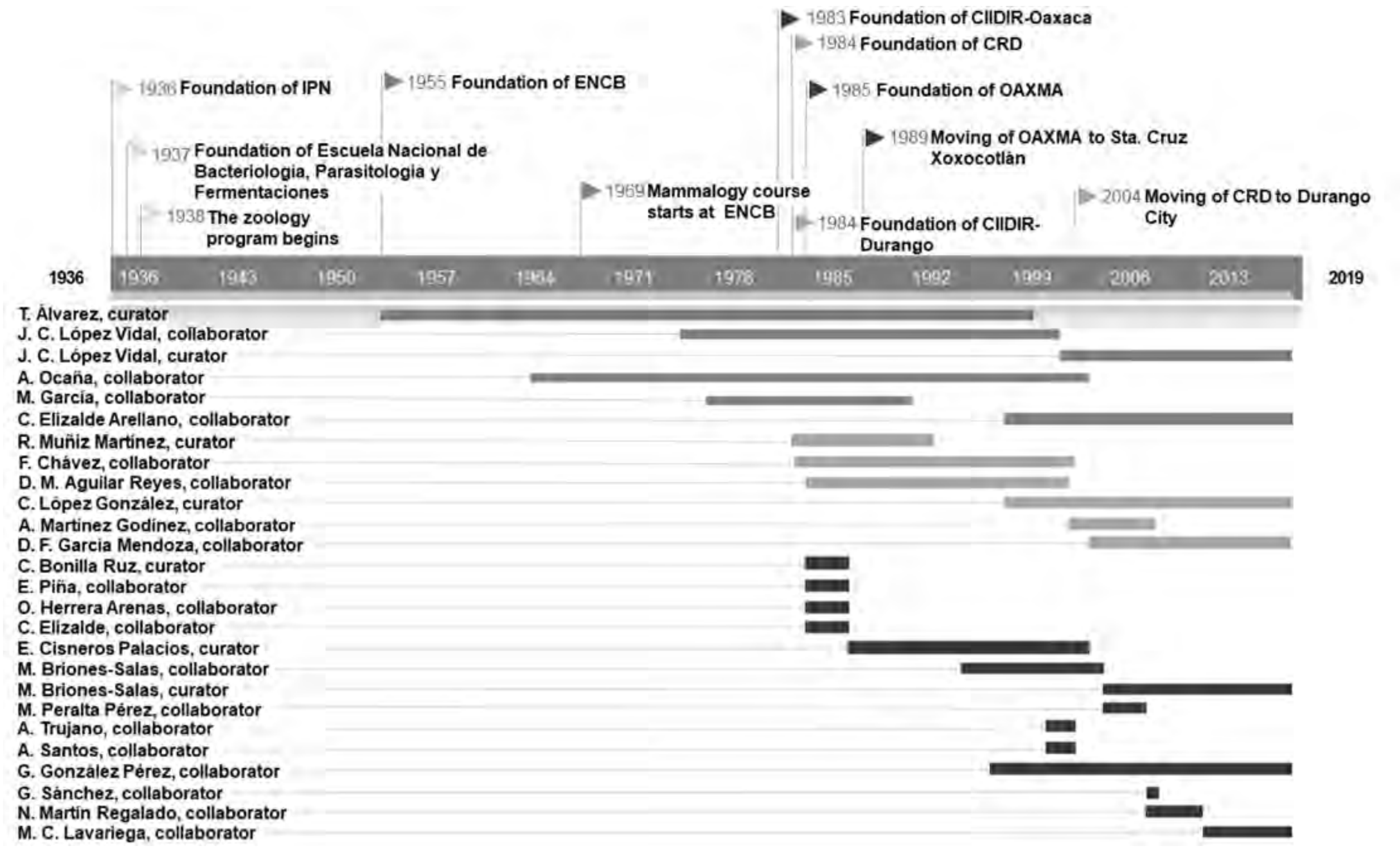

Figure 3. Timeline and major milestones of the IPN mammal collections. ENCB, gray line; CRD, light-gray line; OAXMA, dark-gray line. Only IPN staff are indicated; others are listed in the text.

ectoparasites, or other ancillary materials, and the trend towards the digitization of collections to make their information available online, has necessarily given rise to novel curatorial techniques (*e. g.*, Phillips *et al.* 2019) and methods for the storage and handling of materials and information. All these demand new types of infrastructure, equipment, and staff, and entail increased maintenance costs.

The economic value of the IPN mammal collections has not been estimated; an accurate annual maintenance cost is unavailable, and they are not insured. Because the IPN collections do not have a dedicated budget assigned, these costs are hard to estimate. Uribe (1997) pointed out that the economic value of an object is set with reference to its market value. Because collection specimens rarely have market value (except for feline skins and similar items), their replacement value (*i. e.*, capturing, processing, and maintenance costs) can be used instead to assign a monetary value to scientific collections. Their cultural and scientific value (for example, species richness, rarity of specimens, quality of the information associated with them, among others) should be considered as well.

Baker *et al.* (2014) estimated the "door to drawer" cost of voucher specimens, *i. e.*, the cost of processing a specimen from its arrival from the field until it can be used, at US$17.51 (around 332.69 Mexican pesos of 2019), plus US$0.25 (4.75 Mexican pesos) per year per specimen for long-term maintenance. This estimate includes the costs of curating, installing, and databasing, as well as curator and student fees, for traditional specimens (dried skin plus skeleton) and tissue samples. Digitization costs in Europe have been estimated between 0.50 and 73.44 euros (10.45 to 1,535.63 Mexican pesos of 2019) per item (Blagoderov *et al.* 2012), depending on the collection type and preservation method used. As more ancillary materials are collected and the information and voucher specimens themselves are digitized, these costs would increase with the type and amount of material and the information being preserved. However, even if replacement cost estimates were available, the institution and curators of the IPN collections are fully aware that many of the materials housed in these collections are irreplaceable as the populations from which they were extracted may no longer exist due to natural or anthropogenic changes.

Future outlook. The Mexican government explicitly recognized the strategic importance of scientific collections since 1992 with the creation of CONABIO. Funding provided by CONABIO through grants for biological inventories and collection computerization supported the continued operation and expansion of many collections. Much of the information deposited in Mexican collections has now been incorporated into the National System of Biodiversity Information (SNIB, for its acronym in Spanish), which, in turn, is part of global biological database networks, such as the Global Biodiversity Information Facility (GBIF, https://www.gbif.org).

McLean *et al.* (2016) highlighted the importance of mammal collections as research instruments. They documented that 25 % of all 1,403 articles published between 2005 and 2014 in just a single journal specialized in the study of mammals (the Journal of Mammalogy) used material deposited or eventually deposited in collections. The main subjects addressed in those articles included systematics and biogeography, genomics, morphometrics, parasitology and pathogens, stable isotope analysis, and teaching. These authors also pointed out that a significantly higher proportion of those studies used a combination of historical material and recently collected specimens, thus evidencing the importance of continue incorporating new material to collections.

Mexican mammal collections are expected continue growing; we are still far from having explored the entire territory and fully described the diversity it harbors. Mammals are one of the best-sampled zoological groups and, nevertheless, eight new species were added to the known Mexican fauna between 2005 and 2014 (Ramírez-Pulido *et al.* 2014). Two of these recently described species were determined from newly collected specimens, but the others were determined based on materials already deposited in a collection. The use of new study techniques has allowed describing numerous species based on already existing specimens that had not been previously examined or that were re-examined in the light of new knowledge.

Fontaine *et al.* (2012) pointed out that, in developed countries that have large scientific collections, more new species are being described based on existing material than on newly collected specimens. The opposite holds for developing countries with no or only limited collections. Mexico is midway between these two extremes; vast parts of the Mexican territory remain practically unexplored and numerous zoological groups have been scarcely studied. Collecting efforts in those areas and zoological groups would likely lead to the discovery of new species. In addition, examining newly collected materials in relation to the materials already available might also lead to discoveries in our own cabinets.

The coverage and quality of voucher specimens, as well as the information associated with them, are key for developing a more accurate perception of scientific collections as essential resources for understanding the consequences of human development on our planet. Information deposited in scientific collections can provide a scientifically sound baseline for understanding modification processes, by supplying data on the former condition of the biota (Lister *et al.* 2011). Properly georeferenced data are the base for studying distributional changes driven by urban or agricultural development and for predicting future distribution scenarios. These data are also useful for documenting the decline of natural populations (Shaffer *et al.* 1998), changes in the morphology (MacLean *et al*. 2018) or life history of plants and animals, measuring changes in nutrient availability or heavy metal concentrations, or detecting emerging diseases, among other uses (Meineke *et al.* 2018). Combining geographic information with genetic information derived from the same specimens offers a powerful tool for studying anthropogenic effects on the genetic health of populations (Cook and Light 2019).

The past 50 years have brought about rapid changes in the approach and techniques used collect, analyze, preserve, and document specimens of biological collections. The IPN mammal collections have been under continued activity since their foundation; their specimens have been successfully used for research and student training. Although the IPN collections meet most of the minimum curatorial standards, the process of digitizing the collections has not yet started, and their catalogs have not yet been fully incorporated into global databases; only part of their data have been incorporated into the SNIB. The challenge for the IPN collections to become even more useful in the contemporary world lies, in addition to ensuring their long-term preservation, in attaining the standardization, management, and availability of information and voucher specimens in digital formats (iDigBio 2019; DiSSCo 2019; Sunderland 2013). Digitizing the IPN collections and making them accessible over the internet will add another dimension to their potential use (Beaman and Cellinese 2012; McLean *et al.* 2016) and lead to research results that would not be possible otherwise (*e.g.*, Redding *et al.* 2016).

CONABIO has made a significant effort in this direction through the SNIB, which includes a standardized database, an inventory of Mexican collections, their affiliated staff, and their taxonomic scope and geographic representativeness (CONABIO 2019). Nevertheless, a national policy for the reliable and continuously updated management, conservation, and digitization of this portion of our natural capital is still missing. Since IPN, UNAM, and INECOL host the largest biological collections in Mexico, these institutions should lead, together with CONABIO, the development of a national policy on biological collections to achieve these objectives.

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Literature cited

Almendra, A. L., D. S. Rogers, and F. X. González-Cózatl. 2014. Molecular phylogenetics of the *Handleyomys chapmani* complex in Mesoamerica. Journal of Mammalogy 95:26–40.

Almendra, A. L., F. X. González-Cózatl, M. D. Engstrom, and D. S. Rogers. 2018. Evolutionary relationships and climatic niche evolution in the genus *Handleyomys* (Sigmodontinae: Oryzomyini). Molecular Phylogenetics and Evolution 128:12-25.

Álvarez, T., and J. Ramírez-Pulido. 1968. Descripción de una nueva especie de *Spermophilus* (Rodentia, Sciuridae) de Michoacán, México y estado taxonómico de *S. a. arceliae* (Villa R. 1942). Revista de la Sociedad Mexicana de Historia Natural 29:181-189.

Álvarez, T., and J. Arroyo-Cabrales. 1990. Cálculos de captura, densidad y ámbito hogareño de tres especies de roedores en un área de influencia humana en el sureste de Durango, México. Anales de la Escuela Nacional de Ciencias Biológicas 33:185-210.

Álvarez, T., and J. J. Hernández-Chávez. 1993. Taxonomía del metorito *Microtus mexicanus* en el centro de México con la descripción de una nueva subespecie. Pp. 137–156, *in* Avances en el estudio de los Mamíferos de México (Medellín, R. A., and G. Ceballos, eds.). Asociación Mexicana de Mastozoología, A. C., Ciudad de México, México.

Álvarez, T., S. T. Álvarez-Castañeda, and M. González-Escamilla. 1997. Localidades típicas de mamíferos terrestres en México. Centro de Investigaciones Biológicas del Noroeste y Escuela Nacional de Ciencias Biológicas, Ciudad de México, México.

Álvarez-Castañeda, S. T. 1993. Zoogeografía de los murciélagos de Chiapas. Anales de la Escuela Nacional de Ciencias Biológicas 38:139-159.

Álvarez-Castañeda, S. T., C. A Segura-Trujillo, and W. Z. Lidicker, Jr. 2019. Impact of the American Society of Mammalogists on the internationalization of mammalogy. Journal of Mammalogy 100:751-762

American Society of Mammalogists Systematics Collections Committee. 2004. Curatorial standards for systematic collections of mammals. Journal of Mammalogy 85:180-181.

Arcangeli, J., J. E. Light, and F. A. Cervantes. 2018. Molecular and morphological evidence of the diversification in the gray mouse opossum, *Tlacuatzin canescens* (Didelphimorphia), with description of a new species. Journal of Mammalogy 99:138–158.

Ávila-Valle, Z. A., A. Castro-Campillo, L. León-Paniagua, I. H. Salgado-Ugalde, A. G. Navarro-Sigüenza, B. E. Hernández-Baños, and J. Ramírez-Pulido. 2012. Geographic variation and molecular evidence of the blackish deer mouse complex (*Peromyscus furvus*, Rodentia: Muridae). Mammalian Biology 77:166-177.

Baird, A. B., J. K. Braun, M. A. Mares, J. C. Morales, J. C. Patton, C. Q. Tran, and J. W. Bickham. 2015. Molecular systematic revision of tree bats (Lasiurini): doubling the native mammals of the Hawaiian Islands. Journal of Mammalogy 96:1255–1274

Baker, R. J., L. C. Bradley, H. J. Garner, and R. D. Bradley. 2014. "Door to Drawer" costs of curation, installation, documentation, databasing, and long-term care of mammal voucher specimens in natural history collections. Occasional Papers, Museum of Texas Tech University 323:1-15.

Beaman, R. S., and N. Cellinese. 2012. Mass digitization of scientific collections: New opportunities to transform the use of biological specimens and underwrite biodiversity science. ZooKeys 209:7-17.

Bell, D. M., M. J. Hamilton, C. J. Edwards, L. E. Wiggins, R. Muñiz-Martínez, R. E. Strauss, R. D. Bradley, and R. J. Baker. 2001. Patterns of karyotypic megaevolution in *Reithrodontomys:* Evidence from a cytochrome-b phylogenetic hypothesis. Journal of Mammalogy 82:81-91.

Blagoderov, V., I. J. Kitching, L. Livermore, T. J. Simonsen, and V. S. Smith. 2012. No specimen left behind: industrial scale digitization of natural history collections. ZooKeys 209:133-146.

Borisenko, A. V., B. K. Lim, N. V. Ivanova, R. H. Hanner, and P. D. Hebert. 2008. DNA barcoding in surveys of small mammal communities: a field study in Suriname. Molecular Ecology Resources 8:471-479.

Botello, F., G. Monroy, P. Illoldi-Rangel, I. Trujillo-Bolio, and V. Sánchez-Cordero. 2007. Sistematización de imágenes obtenidas por fototrampeo: una propuesta de ficha. Revista Mexicana de Biodiversidad 78:207-210.

Bradley, R. D., L. K. Ammerman, R. J. Baker, L. C. Bradley, J. A. Cook, R. C. Dowler, C. Jones, D. J. Schmidly, F. B. Stangl, Jr., R. A. Van Den Bussche, and B. Würsiget. 2014. Revised checklist of North American mammals north of Mexico, 2014. Occasional Papers, Museum of Texas Tech University 327:1-27.

Briones-Salas, M., M. A. Peralta-Pérez, and M. García-Luis. 2013. Acoustic characterization of new species of bats for the state of Oaxaca, México. Therya 4:15–32.

Briones-Salas M., M. Cortes-Marcial, and M. C. Lavariega. 2015. Diversidad y distribución geográfica de los mamíferos terrestres del estado de Oaxaca, México. Revista Mexicana de Biodiversidad 86:685-710.

Carraway, L. N. 2007. Shrews (Eulypotyphla: Soricidae) of Mexico. Monographs of the Western North American Naturalist 3:1-92.

Cervantes, F. A., J. Vargas-Cuenca, and Y. Hortelano-Moncada. 2016. An overview of the Mammal Collection of Instituto de Biología, Universidad Nacional Autónoma de México. Journal of the Natural Sciences Collections Association 4:4-11.

Choate, J. R., and H. H. Genoways. 1975. Collections of recent mammals in North America. Journal of Mammalogy 56:452-502.

Comisión Nacional para el Conocimiento y uso de la Biodiversidad (CONABIO). 2019. Estadísticas de colecciones. http://www.biodiversidad.gob.mx/fichas-conabio-war/graficas.html. Accessed on March 20, 2019.

Consejo Nacional de Ciencia y Tecnología. 2020. Criterios SNI (Sistema Nacional de Investigadores). https://www.conacyt.gob.mx/index.php/sni/convocatorias-conacyt/convocatorias-sistema-nacional-de-investigadores-sni/marco-legal-sni/criterios-sni. Accessed on July 7, 2020.

Cook, J. A., and J. E. Light. 2019. The emerging role of mammal collections in 21st century mammalogy. Journal of Mammalogy 100:733–750.

Danks, H. V. 1991. Museum collections: fundamental values and modern problems. Collection Forum 7:95-111.

Distributed System of Scientific Collections (DiSSCo). 2019. What is DISSCo?. https://dissco.eu/. Accessed on February 26, 2019.

Dunnum, J. L., B. S. McLean, and R. C. Dowler. 2018. Mammal collections of the Western Hemisphere: a survey and directory of collections. Journal of Mammalogy 99:1307-1322.

Fontaine, B., A. Perrard, and P. Bouchet. 2012. 21 years of shelf life between discovery and description of new species. Current Biology 22:R943-R944.

Geist, V. 1992. Endangered species and the law. Nature 357:274-276.

Genoways, H. H. 1973. Systematics and evolutionary relationships of spiny pocket mice, genus *Liomys*. Special Publications, Museum of Texas Tech University 5:1-368.

Genoways, H. H., J. R. Choate, E. F. Pembleton, I. F. Greenbaum, and J. W. Bickham. 1976. Systematists, other users, and uses of North American collections of recent mammals. Museology, Texas Tech University 3:1-87.

González-Ruíz, N., J. Ramírez Pulido, and J. Arroyo-Cabrales. 2011. A new species of mastiff bat (Chiroptera: Molossidae: *Molossus*) from México. Mammalian Biology 76:461–469.

Greenbaum, I. F., R. L. Honeycutt, and S. E. Chirhart. 2019. Taxonomy and phylogenetics of the *Peromyscus maniculatus* species group. Pp. 559-575, *in* From field to laboratory: a memorial volume in honor of Robert J. Baker (Bradley, R. D., H. H. Genoways, D. J. Schmidly, and L. C. Bradley, eds.). Special Publications, Museum of Texas Tech University 71:xi+1–911.

Guevara-Chumacero, L. M., R. López-Wilchis, F. F. Pedroche, J. Juste, C. Ibáñez, and I. D. Barriga-Sosa. 2010. Molecular phylogeography of *Pteronotus davyi* (Chiroptera: Mormoopidae) in Mexico. Journal of Mammalogy 91:220-232.

Guzmán, G. 1994. Las colecciones de hongos en México y su problemática en la biodiversidad del país. Boletín de la Sociedad Botánica de México 55:35-37.

Hafner, D. J., B. R. Riddle, and S. T. Álvarez-Castañeda. 2001. Evolutionary relationships of white-footed mice (*Peromyscus*) on Islands in the Sea of Cortéz, México. Journal of Mammalogy 82:775-790.

Hafner, M. S., W. L. Gannon, J. Salazar-Bravo, and S. T. Álvarez-Castañeda. 1997. Mammal Collections in the Western Hemisphere. A Survey and Directory of existing Collections. American Society of Mammalogists. Lawrence, U.S.A.

Hanson, J. D., J. L. Indorf, V. J. Swier, and R. D. Bradley. 2010. Molecular divergence within the *Oryzomys palustris* complex: evidence for multiple species. Journal of Mammalogy 91:336–347.

Illoldi-Rangel, P., V. Sánchez-Cordero, and T. Peterson. 2004. Predicting distributions of Mexican mammals using ecological niche modeling. Journal of Mammalogy 85:658–662.

Integrated Digitized Biocollections (iDigBio). 2019. Project scope. https://www.idigbio.org/about/project-scope. Accessed on February 26, 2019.

Kemp, C. 2015. The endangered dead. Nature 518:292-294.

Lavoie, C. 2013. Biological collections in an ever-changing world: Herbaria as tools for biogeographic and environmental studies. Perspectives in plant Ecology, Evolution, and Systematics 15:68-76.

Léon-Tapia, M. Á., J. A. Fernández, Y. Rico, F. A. Cervantes, and A. Espinosa de los Monteros. 2020. A new mouse of the *Peromyscus maniculatus* species complex (Cricetidae) from the highlands of central Mexico. Journal of Mammalogy 101:1117-1132.

Lister, A. M., S. J. Brooks, P. B. Fenberg, A. G. Glover, K. E. James, K. G. Johnson, E. Michel, B. Okamura, M. Spencer, J. R. Stewart, J. A. Todd, E. Valsami-Jones, and J. Young. 2011. Natural history collections as sources of long-term datasets. Trends in Ecology and Evolution 26:153-154.

López-González, C., S. J. Presley, A. Lozano, R. D. Stevens, and C. L. Higgins. 2015. Ecological biogeography of Mexican bats: the relative contributions of habitat heterogeneity, beta diversity, and environmental gradients to species richness and composition patterns. Ecography 37:1-12.

López-González, C., D. F. García-Mendoza, J. C. López-Vidal, and C. Elizalde-Arellano. 2019. Multiple lines of evidence reveal a composite of species in the plateau mouse, *Peromyscus melanophrys* (Rodentia, Cricetidae). Journal of Mammalogy 100:1583–1598.

Lorenzo, C., E. Espinoza, M. Briones, and F. A. Cervantes (eds.). 2006. Colecciones Mastozoológicas de México. Instituto de Biología, UNAM and Asociación Mexicana de Mastozoología A. C., Mexico City, Mexico.

Lorenzo C., S. T. Álvarez-Castañeda, E. Arellano, J. Arroyo-Cabrales, J. Bolaños, M. Briones-Salas, F. A. Cervantes, J. Chablè-Santos, L. Corral, M. Cortés, P. Cortés-Calva, M. De La Paz-Cuevas, C. Elizalde-Arellano, E. Escobedo-Cabrera, E. Espinoza, E. Estrella, J. P. Gallo-Reynoso, D. F. García-Mendoza, H. A. Garza-Torres, C. A. González, F. X. González-Cózatl, R. M. González-Monroy, N. González-Ruíz, D. Guzmán, A. F. Guzmán, S. F. Hernández-Betancourt, Y. Hortelano-Moncada, L. I. Iñiguez, A. Jiménez-Guzmán, Y. N. Kantum, L. León-Paniagua, C. López-González, J. H. López-Soto, J. C. López-Vidal, N. Martin, J. Martínez-Vázquez, M. A. Mejenes-López, B. Morales-Vela, R. Muñiz-Martínez, J. A. Niño-Ramírez, A. Núñez-Garduño, C. Pozo, J. Ramírez-Pulido, O. G. Retana, I. Ruan, C. I. Selem, J. Vargas, and M. A. Zúñiga-Ramos. 2012. Los mamíferos de México en las colecciones científicas de Norteamérica. Therya 3:239-262.

McLean, B. S., K. C. Bell, J. L. Dunnum, B. Abrahamson, J. P. Colella, E. R. Deardorff, J. A. Weber, A. K. Jones, F. Salazar-Miralles, and J. A. Cook. 2016. Natural history collections-based research: progress, promise, and best practices. Journal of Mammalogy 97:287-297.

MacLean, H. J., M. E. Nielsen, J. G. Kingsolver, and L. B. Buckley. 2018. Using museum specimens to track morphological shifts through climate change. Philosophical Transactions of the Royal Society B 374:20170404, doi.org/10.1098/rstb.2017.0404.

Mantilla-Meluk, H. 2014. Defining Species and Species Boundaries in *Uroderma* (Chiroptera: Phyllostomidae) with a Description of a New Species. Occasional Papers, Museum of Texas Tech University 325:1-25.

Meineke, E. K., T. J. Davies, B. H. Daru, and C. C. Davis. 2018. Biological collections for understanding biodiversity in the Anthropocene. Philosophical Transactions of the Royal Society B 374:20170386, doi.org/10.1098/rstb.2017.0386.

Muñiz-Martínez, R. 1997. Relación roedor-vegetación en el sureste del estado de Durango, México. Pp. 223–237, *in* Homenaje al profesor Ticul Álvarez (Arroyo-Cabrales, J. and O. J. Polaco, eds.). Instituto Nacional de Antropología e Historia, Ciudad de México, México.

Neiswenter, S. A., D. J. Hafner, J. E. Light, G. D. Cepeda, K. C. Kinzer, L. F. Alexander, and B. R. Riddle. 2019. Phylogeography and taxonomic revision of Nelson's pocket mouse (*Chaetodipus nelsoni*). Journal of Mammalogy 100:1847–1864.

Ordóñez-Garza, N., C. W. Thompson, M. K. Unkefer, C. W. Edwards, J. G. Owen, and Robert D. Bradley. 2014. Systematics of the *Neotoma mexicana* species group (Mammalia: Rodentia: Cricetidae) in Mesoamerica: new molecular evidence on the status and relationships of *N. ferruginea* Tomes, 1862. Proceedings of the Biological Society of Washington 127:518-532.

Patton, J. L, and L. H. Emmons. 2015. Family Dasyproctidae Bonaparte, 1838. Pp. 733-762, *in* Mammals of South America Volume 2. (Patton, J. L., U. F: J. Pardiñas, and G. D'Elía, eds.). University of Chicago Press, Chicago, U.S.A.

Pavan, A. C., and G. Marroig. 2016. Integrating multiple evidences in taxonomy: species diversity and phylogeny of mustached bats (Mormoopidae: *Pteronotus*). Molecular Phylogenetics and Evolution 103:184-198.

Phillips, C. D., J. L. Dunnum, R. C. Dowler, L. C. Bradley, H. J. Garner, K. A MacDonald, B. K. Lim, M. A. Revelez, M. L. Campbell, H. L. Lutz, N. Ordóñez-Garza, J. A. Cook, R. D. Bradley, and Systematic Collections Committee of the American Society of Mammalogists. 2019. Curatorial guidelines and standards of the American Society of Mammalogists for collections of genetic resources. Journal of Mammalogy 100:1690–1694.

Porter, C. A., N. E. Beasley, N. Ordóñez-Garza, L. L. Lindsey, D. S. Rogers, N. Lewis-Rogers, J. W. Sites, Jr., and R. D. Bradley. 2017. A new species of big-eared climbing rat, genus *Ototylomys* (Cricetidae: Tylomyinae), from Chiapas, Mexico. Journal of Mammalogy 98:1310–1329.

Prieto-Bosch, M., and V. Sánchez-Cordero. 1993. Sistemas de Información geográfica, un caso de estudio: Veracruz. Pp. 455–464, *in* Avances en el estudio de los Mamíferos de México (Medellín, R. A., and G. Ceballos, eds.). Asociación Mexicana de Mastozoología, A. C. Mexico City, México.

Pyke, G. H., and P. R. Ehrlich. 2010. Biological collections and ecological/environmental research: a review, some observations, and a look to the future. Biological Reviews 85:247-266.

Ramírez-Pulido, J., and N. González-Ruíz. 2006. 3. Las colecciones de mamíferos de México, origen y destino. Pp. 73-110, *in* Colecciones Mastozoológicas de México (Lorenzo, C., E. Espinosa, M. A. Briones, and F. A. Cervantes, eds.). Asociación Mexicana de Mastozoología e Instituto de Biología, UNAM. Mexico City, México.

Ramírez-Pulido, J., N. González-Ruiz, A. L. Gardner, and J. Arroyo-Cabrales. 2014. List of recent land mammals of Mexico, 2014. Special Publications, Museum of Texas Tech University 63:1-69.

Redding, D. W., L. M. Moses, A. A. Cunningham, J. Wood, and K. E. Jones. 2016. Environmental-mechanistic modelling of the impact of global change on human zoonotic disease emergence: a case study of Lassa fever. Methods in Ecology and Evolution 7:646-655.

Ríos-Muñoz, C. A., J. Arroyo-Cabrales, and L. S. León-Paniagua. 2014. Historia de la Mastozoología en México: de dónde venimos y a dónde vamos. Pp. 293-314, *in* Historia de la Mastozoología en Latinoamérica, las Guayanas y el Caribe (Ortega, J., J. L. Martínez, and D. G. Tirira, eds.). Editorial Murciélago Blanco. Quito, Ecuador.

Rivera-León, V., Y. Hortelano-Moncada, and F. A. Cervantes. 2018. Health level of a mammal collection. Revista Mexicana de Biodiversidad 89:402-411.

Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). 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, 14 november 2019.

Schindel, D. E., and J. A. Cook. 2018. The next generation of natural history collections. PLoS Biology 16:e2006125.

Shaffer, H. B., R. N. Fisher, and C. Davidson. 1998. The role of natural history collections in documenting species declines. Trends in Ecology and Evolution 13:27-30.

Shetler, S. G. 1969. The herbarium: past, present, and future. Papers Presented at a Symposium on Natural History Collections: Past-Present-Future. Proceedings of the Biological Society of Washington 82:687-758.

Sosa-Escalante, J. E. 2018. Acoustic reference library of Mexican insectivorous bats: Phase I. Therya 9:197-199.

Sunderland, M. E. 2013. Computerizing natural history collections. Endeavor 37:150-161.

Thiers, B. M. 2018. The World's Herbaria 2017: a summary report based on data from *Index Herbariorum*. http://sweetgum.nybg.org/science/docs/The_Worlds_Herbaria_2017_5_Jan_2018.pdf. Accessed on August 31, 2018.

Uribe, F. 1997. The evaluation of natural history collections. Pp. 158-163, *in* The value and valuation of Natural Science collections (Nudds J. R., and C. W. Pettitt, eds.). The Geological Society Publishing House. Bath, United Kingdom.

Vázquez-Domínguez, E., and D. J. Hafner (eds.). 2006. Genética y mamíferos mexicanos: presente y futuro. New Mexico Museum of Natural History and Science, Vol 32. Albuquerque, U.S.A.

Winker, K. 2004. Natural History Museums in a postbiodiversity era. BioScience 54:455–459.

Yates, T. L., W. R. Barber, and D. M. Armstrong. 1987. Survey of North American collections of Recent mammals. Journal of Mammalogy Supplement 68:1-76.

Zamora-Gutierrez, V., J. Ortega, R. Avila-Flores, P. A. Aguilar-Rodríguez, M. Alarcón-Montano, L. G. Ávila-Torresagatón, J. Ayala-Berdón, B. Bolívar-Cimé, M. Briones-Salas, M. Chan-Noh, M. Chávez-Cauich, C. Chávez, P. Cortés-Calva, J. Cruzado, J. C. Cuevas, M. Del Real-Monroy, C. Elizalde-Arellano, M. García-Luis, R. García-Morales, J. A. Guerrero, A. A. Guevara-Carrizales, E. G. Gutiérrez, L. A. Hernández-Mijangos, M. P. Ibarra-López, L. I. Iñiguez-Dávalos, R. León-Madrazo, C. López-González,| M. C. López-Téllez, J. C. López-Vidal, S. Martínez-Balvanera, F. Montiel-Reyes, R. Murrieta-Galindo, C. L. Orozco-Lugo, J. M. Pech-Canché, L. Pérez-Pérez, M. M. Ramírez-Martínez,| A. Rizo-Aguilar, E. Robredo-Esquivelzeta, A. Z. Rodas-Martínez, M. A. Rojo-Cruz, C. I. Selem-Salas, E. Uribe-Bencomo, J. A. Vargas-Contreras, M. C. MacSwiney G. 2020. The Sonozotz project: Assembling an echolocation call library for bats in a megadiverse country. Ecology and Evolution DOI: 10.1002/ece3.6245.

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Appendix 1

Wild mammals of continental Mexico represented in the IPN collections. The classification follows Ramírez-Pulido *et al*. (2014) with some modifications as indicated in the far-right column. Taxa are listed up to subfamily; subspecies are not included. NOM: conservation status as per the official Mexican standard NOM-059-SEMARNAT-2010 (SEMARNAT 2019): Pr = Species subject to special protection, A = threatened, P = endangered, EM = endemic to Mexico.

	ENCB	**CRD**	**OAXMA**	**EM**	**NOM**	**Remarks**
Orden Didelphimorphia						
Familia Didelphidae						
Didelphis marsupialis	X	X	X			
Didelphis virginiana	X	X	X			
Marmosa mexicana	X		X			
Philander oposum	X	X	X			
Tlacuatzin canescens	X			X		May include *T. gaumeri* Arcangeli *et al.* (2018)
Tlacuatzin balsasensis	tipo		X	X		Arcangeli *et al.* (2018)
Tlacuatzin sinaloae		X		X		Arcangeli *et al.* (2018)
Orden Cingulata						
Familia Dasypodidae						
Dasypus novemcinctus	X	X	X			
Orden Pilosa						
Familia Myrmecophagidae						
Tamandua mexicana	X		X		P	
Familia Cyclopedidae						
Cyclopes didactylus	X				P	
Orden Soricomorpha						
Familia Soricidae						
Cryptotis goldmani	X			X	Pr	
Cryptotis mayensis	X				Pr	
Cryptotis mexicanus	X	X	X	X		Carraway (2007)
Cryptotis parvus	X				Pr	
Notiosorex crawfordi	X	X			A	
Notiosorex evotis	X			X	A	
Sorex emarginatus	X	X		X		Carraway (2007)
Sorex ixtlanensis			X	X		
Sorex mediopua		X		X		Carraway (2007)
Sorex oreopolus	X			X		
Sorex salvini			X		A	
Sorex saussurei	X			X		
Sorex sclateri	X			X	A	
Sorex ventralis	X			X		
Sorex veraepacis	X		X	X	A	
Sorex veraecrucis		X		X	Pr	= *S. salvini veraecrucis*
Orden Chiroptera						
Familia Emballonuridae						
Balantiopteryx io	X	X				
Balantiopteryx plicata	X	X	***			

Peropteryx kappleri	X				Pr	
Peropteryx macrotis	X		***			
Rhynchonycteris naso	X				Pr	
Saccopteryx bilineata	X	X				
Familia Molossidae						
Cynomops mexicanus	X	X	X***	X	Pr	
Eumops auripendulus	X					
Eumops ferox	X		X			
Eumops nanus	X	X			Pr	
Eumops perotis	X	X				
Eumops underwoodi	X					
Molossus alvarezi	tipo			X		
Molossus molossus	X	X	X***			
Molossus rufus	X	X	X***			
Molossus sinaloe	X		***			
Nyctinomops aurispinosus		*				
Nyctinomops femorosaccus	X	X				
Nyctinomops laticaudatus	X	X	***			
Nyctinomops macrotis	X	X	X			
Promops centrales	X		X			
Tadarida brasiliensis	X	X	X			
Familia Natalidae						
Natalus lanatus		X		X		
Natalus mexicanus	X	X	X			
Familia Mormoopidae						
Mormoops megalophylla	X	X	X***			
Pteronotus fulvus	X	X	X***			Pavan and Marroig (2016)
Pteronotus gymnonotus	X				A	Pavan and Marroig (2016)
Pteronotus mexicanus	X	X	X***	X		Pavan and Marroig (2016), may include *P. mesoamericanus*
Pteronotus psilotis	X	X	X***			Pavan and Marroig (2016)
Familia Noctilionidae						
Noctilio albiventris	X				Pr	
Noctilio leporinus	X	X	X***			
Familia Phyllostomidae						
Subfamilia Carolliinae						
Carollia perspicillata	X	X	X			
Carollia sowelli	X	X	X			
Carollia subrufa	X		X			
Subfamilia Desmodontinae						
Desmodus rotundus	X	X	X			
Diphylla ecaudata	X		X			
Subfamilia Glossophaginae						
Anoura geoffroyi	X	X	X			
Choeroniscus godmani	X		X			
Choeronycteris mexicana	X	X	X		A	
Glossophaga commissarisi	X	X	X			
Glossophaga leachii	X	X	X			
Glossophaga morenoi	X	X	X	X		
Glossophaga soricina	X	X	X			
Hylonycteris underwoodi	X		X			
Leptonycteris nivalis	X	X	X		A	
Leptonycteris yerbabuenae	X	X	X		Pr	
Lichonycteris obscura	X					
Musonycteris harrisoni	X			X	P	
Subfamilia Glyphonycterinae						
Glyphonycteris sylvestris	X	X				
Subfamilia Lonchorhininae						
Lonchorhina aurita	X				A	
Subfamilia Macrotinae						
Macrotus californicus	X	X				
Macrotus waterhousii	X		X			
Subfamilia Micronycterinae						
Lampronycteris brachyotis	X	X			A	
Micronycteris microtis	X	*	X			
Micronycteris schmidtorum	X				A	
Subfamilia Phyllostominae						
Chrotopterus auritus	X				A	
Lophostoma evotis	X	X			A	
Mimon cozumelae	X				A	
Phyllostomus discolor	X		X			
Trachops cirrhosus	X		X		A	
Vampyrum spectrum	X				P	
Subfamilia Stenodermatinae						
Artibeus hirsutus	X	X		X		
Artibeus jamaicensis	X	X	X			
Artibeus lituratus	X	X	X			
Centurio senex	X	X	X			
Dermanura azteca	X	X	X			
Dermanura phaeotis	X	X	X			
Dermanura tolteca	X	X	X			
Dermanura watsoni	X				Pr	
Enchisthenes hartii	X		X		Pr	
Chiroderma salvini	X	X	X			
Chiroderma villosum	X					
Platyrrhinus helleri	X					
Sturnira hondurensis	X	X	X			
Sturnira parvidens	X	X	X			
Uroderma davisi	X	X	X			Mantilla-Meluk (2014)
Vampyressa thyone	X		X			
Vampyrodes major	X		X			
Familia Vespertilionidae						
Subfamilia Antrozoinae						
Antrozous pallidus	X	X				
Bauerus dubiaquercus	X	*				
Subfamilia Myotinae						
Myotis albescens	X				Pr	
Myotis auriculus	X	X				
Myotis californicus	X	X	X***			
Myotis carteri	X	X			Pr	= *M. nigricans carteri*
Myotis elegans	X	X				
Myotis fortidens	X	X	X***	X		
Myotis keaysi	X		X***			

Myotis nigricans	X		X			
Myotis melanorhinus		X				
Myotis occultus	X					
Myotis thysanodes	X	X	X			
Myotis velifer	X	X	X***			
Myotis vivesi	X			X	P	
Myotis volans	X	X				
Myotis yumanensis	X	X				
Subfamilia Vespertilioninae						
Corynorhinus mexicanus	X	X		X		
Corynorhinus townsendii	X	X	X***			
Eptesicus furinalis	X	X	X			
Eptesicus fuscus	X	X	X***			
Euderma maculatum	X				Pr	
Idionycteris phyllotis	X	X	X***			
Lasiurus frantzi	X	X	X			Baird *et al.* (2015)
Lasiurus borealis	X	X				Baird *et al.* (2015)
Aeorestes cinereus	X	X	X***			Baird *et al.* (2015)
Dasypterus intermedius	X	X	X***			Baird *et al.* (2015)
Dasypterus xanthinus	X	X	X***			May include *D. ega* (Baird *et al.* 2015)
Nycticeius humeralis	X					
Parastrellus hesperus	X	X				
Perimyotis subflavus	X					
Rhogessa aeneus	X			X		
Rhogessa alleni	X		X	X		
Rhogessa gracilis	X		X	X		
Rhogeessa mira	X			X	Pr	
Rhogeessa parvula	X	X	X	X		
Rhogeessa tumida	X	X	***			
Orden Primates						
Familia Atelidae						
Ateles geoffroyi	X				P	
Alouatta villosa	X				P	
Orden Lagomorpha						
Familia Leporidae						
Lepus californicus	X	X				
Lepus callotis	X	X		X		
Lepus flavigularis			X	X	P	
Sylvilagus audubonii	X	X				
Sylvilagus brasiliensis	X					
Sylvilagus cunicularius	X		X	X		
Sylvilagus floridanus	X	X	X			
Romerolagus diazi	X			X	P	
Orden Rodentia						
Familia Sciuridae						
Subfamilia Pteromyinae						
Glaucomys volans	X				A	
Subfamilia Sciurinae						
Ammospermophilus leucurus	X					
Callospermophilus madrensis		X		X	Pr	
Cynomys mexicanus	X			X	P	
Ictydomis mexicanus	X			X		
Ictydomys parvidens		X				
Neotamias bulleri	X	X		X		
Neotamias dorsalis		X				
Neotamias durangae	X	X		X		
Notocitellus adocetus	X			X		
Notocitellus annulatus	X			X		
Otospermophilus variegatus	X	X	X			
Sciurus aberti		X			Pr	
Sciurus alleni	X			X		
Sciurus aureogaster	X		X			
Sciurus colliaei	X			X		
Sciurus deppei	X		X			
Sciurus nayaritensis	X	X		X		
Sciurus oculatus	X			X	Pr	
Sciurus yucatanensis	X	X				
Xerospermophilus perotensis	X				A	= *X. spilosoma perotensis*
Xerospermophilus spilosoma	X	X				
Familia Geomyidae						
Subfamilia Geomyinae						
Cratogeomys castanops	X	X				
Cratogeomys fumosus	X			X	A	
Cratogeomys goldmani	X			X		
Cratogeomys merriami	X			X		
Orthogeomys grandis	X		X			
Orthogeomys hispidus	X					
Pappogeomys bulleri	X			X	Pr	
Thomomys sheldoni	X	X		X		
Thomomys umbrinus	X	X		X		
Familia Heteromyidae						
Subfamilia Dipodomyinae						
Dipodomys deserti	X					
Dipodomys merriami	X	X				
Dipodomys nelsoni	X	X		X		
Dipodomys ordii	X	X				
Dipodomys phillipsii	X	X	X	X	A	
Dipodomys spectabilis	X	X				
Subfamilia Heteromyinae						
Heteromys desmarestianus	X		X			
Heteromys gaumeri	X	X		X		
Heteromys irroratus	X	X	X			
Heteromys pictus	X	X	X			
Heteromys salvini	X					
Heteromys spectabilis	X			X	Pr	
Subfamilia Perognathinae						
Chaetodipus arenarius	X			X		
Chaetodipus artus		X		X		
Chaetodipus baileyi	X					
Chaetodipus californicus	X					
Chaetodipus eremicus	X	X				
Chaetodipus fallax	X					

Chaetodipus goldmani		X		X		
Chaetodipus hispidus	X	X				
Chaetodipus lineatus	X			X		
Chaetodipus intermedius	X	X				
Chaetodipus nelsoni	X	X		X		May include *C. durangae* and *C. collis* (Neiswenter *et al.* 2019)
Chaetodipus penicillatus	X					
Chaetodipus pernix		X		X		
Chaetodipus spinatus	X					
Perognathus flavus	X	X				
Perognathus longimembris	X					
Familia Cuniculidae						
Cuniculus paca	X		X			
Familia Dasyproctidae						Patton and Emmons (2015)
Dasyprocta mexicana			**			
Dasyprocta punctata	X					
Familia Erethizontidae						
Coendou mexicanus	X		X		A	
Familia Cricetidae						
Subfamilia Arvicolinae						
Microtus mexicanus	X	X	X			
Microtus oaxacensis			X	X	A	
Microtus quasiater	X			X	Pr	
Microtus umbrosus			X	X	Pr	
Subfamilia Neotominae						
Baiomys musculus	X	X	X			
Baiomys taylori	X	X				
Habromys chinanteco			X	X		
Habromys ixtlani			X	X		
Habromys lepturus			X	X		
Habromys lophurus	X					
Hodomys alleni	X	X		X		
Nelsonia goldmani	X			X	Pr	
Nelsonia neotomodon	X	X		X	Pr	
Neotoma albigula	X					
Neotoma goldmani	X	X		X		
Neotoma lepida	X					
Neotoma leucodon	X	X		X		
Neotoma mexicana	X	X	X			May include *N. picta* and *N. ferruginea* (Ordoñez-Garza *et al.* 2014)
Neotoma micropus	X	X				
Neotoma palatina	X			X		
Neotomodon alstoni	X	X		X		
Megadontomys cryophilus			X	X	A	
Megadontomys nelsoni			X	X	A	
Megadontomys thomasi	X			X	Pr	
Onychomys arenicola	X	X				
Onychomys leucogaster	X	X				
Onychomys torridus	X					
Osgoodomys banderanus	X			X		
Peromyscus aztecus	X	X	X			
Peromyscus beatae	X	X		X		
Peromyscus boylii	X	X	X			
Peromyscus bullatus	X			X	Pr	
Peromyscus carletoni		X		X		
Peromyscus californicus	X					
Peromyscus difficilis	X	X	X	X		
Peromyscus eremicus	X	X				
Peromyscus eva	X	X		X		
Peromyscus furvus	X	X	X	X		May include *P. latirostris* (Ávila-Valle *et al.* 2012)
Peromyscus gratus	X	X	X	X		
Peromyscus guatemalensis	X					
Peromyscus gymnotis	X					
Peromyscus hooperi	X	X		X		
Peromyscus leucopus	X	X	X			
Peromyscus levipes	X	X	X	X		
Peromyscus labecula	X	X	X			May include *P. gambelii* (Greenbaum *et al.* 2019) and *P. purepechus* (León-Tapia et al. 2020)
Peromyscus leucurus	X			X		López-González *et al.* (2019)
Peromyscus megalops	X		X	X		
Peromyscus melanocarpus	X		X	X		
Peromyscus melanophrys	X		X	X		López-González *et al.* (2019)
Peromyscus micropus	X	X		X		López-González *et al.* (2019)
Peromyscus melanotis	X	X				Occurs in SW U.S.A., Bradley *et al.* (2014)
Peromyscus melanurus	X		X	X		
Peromyscus merriami	X	X				
Peromyscus mexicanus	X		X			
Peromyscus nasutus		X				
Peromyscus ochraventer	X			X		
Peromyscus pectoralis	X	X				
Peromyscus perfulvus	X			X		
Peromyscus polius	X	X		X		
Peromyscus schmidlyi	X	X		X		
Peromyscus sejugis	X			X	A	
Peromyscus simulus	X	X		X		
Peromyscus spicilegus	X	X		X		
Peromyscus yucatanicus	X	X		X		
Peromyscus zamorae	X	X		X		López-González *et al.* (2019)
Peromyscus zarhynchus	X			X	Pr	
Reithrodontomys chrysopsis	X	X		X		
Reithrodontomys fulvescens	X	X	X			
Reithrodontomys gracilis	X				A	
Reithrodontomys hirsutus	X	X		X		
Reithrodontomys megalotis	X	X	X			
Reithrodontomys mexicanus	X	X	X			
Reithrodontomys microdon	X	X	X		A	
Reitrodontomys sumichrasti	X	X	X			
Reithrodontomys zacatecae	X	X		X		
Subfamilia Sigmodontinae						
Nyctomys sumichrasti	X		X			

Oligoryzomys fulvescens	X	X	X			
Handleyomys alfaroi	X	X	X			Almendra *et al.* (2018)
Handleyomys chapmani	X		X	X	Pr	Almendra *et al.* (2018). May include *H. guerrerensis* (Almendra *et al.* 2014, 2018)
Oryzomys couesi	X		X		A	May include *O. fulgens* (Hanson *et al.* 2010)
Oryzomys fulgens		X	X		A	*sensu* Ramírez-Pulido *et al.* (2014), *O. mexicanus sensu* Hanson *et al.* (2010)
Handleyomys melanotis	X		X	X		Almendra *et al.* (2018)
Handleyomys rostratus	X	X		X		Almendra *et al.* (2018)
Handleyomys saturatior	X					Almendra *et al.* (2018)
Rheomys mexicanus			X	X	Pr	
Sigmodon alleni	X	X		X		
Sigmodon arizonae	X	X				
Sigmodon fulviventer	X	X				
Sigmodon hispidus	X	X				
Sigmodon leucotis	X	X		X		
Sigmodon mascotensis	X	X		X		
Sigmodon ochrognathus		X				
Sigmodon toltecus		X		X		
Otonyctomys hatti		X			A	
Ototylomys phyllotis	X	X				May include *O. chiapensis* (Porter *et al.* 2017)
Tylomys nudicaudus			X			
Tylomys tumbalensis	X			X	Pr	
Orden Carnivora						
Familia Felidae						
Herpailurus yagouaroundi	X		**		A	
Leopardus pardalis	X		**		P	
Leopardus wiedii	X	X	X		P	
Lynx rufus	X	X	X			
Puma concolor	X	X	**			
Panthera onca	X	X	**		P	
Familia Canidae						
Canis latrans	X	X	X			
Canis lupus	X		X		P	
Urocyon cinereoargenteus	X	X	X			
Vulpes macrotis	X				A	
Familia Mephitidae						
Conepatus leuconotus	X	X	**			
Conepatus semistriatus	X		**		Pr	
Mephitis macroura	X	X	X			
Mephitis mephitis	X	X				
Spilogale angustifrons	X		X			
Spilogale gracilis	X	X				
Spilogale pygmaea			X		A	
Familia Mustelidae						
Eira barbara	X		**		P	
Galictis vittata	X		X		A	
Lontra longicaudis	X		**		A	
Mustela frenata	X	X	X			
Taxidea taxus	X	X	X		A	
Familia Procyonidae						
Bassariscus astutus	X	X	X			
Bassariscus sumichrasti	X		**		Pr	
Potos flavus	X	X	X		Pr	
Nasua narica	X	X	X			
Procyon lotor	X	X	X			
Orden Artiodactyla						
Familia Tayassuidae						
Dicotyles angulatus		X	X			
Dicotyles crassus	X		X			
Tayassu pecari	X		**		P	
Familia Antilocapridae						
Antilocapra americana	X				P	
Familia Cervidae						
Mazama pandora	X					
Mazama temama			X			
Odocoileus hemionus	X	X				
Odocoileus virginianus	X	X	X			
Familia Bovidae						
Ovis canadensis	X				Pr	
Orden Perissodactyla						
Familia Tapiridae						
Subfamilia Tapirinae						
Tapirella bairdii	X		X		P	
TOTAL	313	192	157	116	88	

* Species represented only as tissue samples, ** Species represented as photographs or footprint casts, *** Species with echolocation recordings.

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Comparison of big cat food habits in the Amazon piedmont forest in two Bolivian protected areas

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The feeding habits of jaguar (*Panthera onca*) and puma (*Puma concolor*) were studied in the lower Tuichi, Hondo (PNANMI Madidi) and Quiquibey (RB Pilón Lajas) river valleys. A total of 122 large felid scats were collected and identified by an experienced local guide, with identifications later confirmed by morphometry. Of this total, 54 were also identified using associated tracks. For jaguar, prey species richness was 25 species considering all scats and 20 species only considering those scats associated with tracks. For puma, 28 and 22 prey species were obtained, respectively. The results indicate that there is low trophic niche overlap between both cats (0.46 and 0.44 respectively). The breadth of the trophic niche indicates that the jaguar is more specialized in its diet (0.28 and 0.42 respectively), mainly due to the high consumption of the white-lipped peccary (*Tayassu pecari*), which contributes significantly to jaguar prey biomass. The puma has a more generalist tendency (0.56 and 0.58 respectively), with the spotted paca (*Cuniculus paca*) the most frequently consumed prey and also contributing the most biomass. Despite the fact that both felines share most of their prey, jaguar and puma differ in the frequency of prey consumption.

Se estudiaron los hábitos alimentarios de jaguar (*Panthera onca*) y puma (*Puma concolor*) en áreas cercanas a los ríos Tuichi, Hondo (PNANMI Madidi) y Quiquibey (RB Pilón Lajas). Se recolectaron un total de 122 excretas con la ayuda de un guía de campo experimentado en identificación de heces de ambos felinos, mismas que fueron confirmadas mediante morfometría. De este total, 54 fueron identificadas además mediante huellas asociadas, observando que los resultados muestran similitud con ambos análisis. Se obtuvo una riqueza de especies para jaguar de 25 presas con el total de heces y 20 presas con el total de heces asociadas a huellas. Para puma se obtuvo 28 y 22 presas respectivamente en ambos análisis. Los resultados indican que hay poco solapamiento de nicho trófico entre ambos felinos (0.46 y 0.44 respectivamente), pues los resultados de amplitud de nicho trófico indican que el jaguar es más especialista en su dieta (0.28 y 0.42 respectivamente), principalmente por el elevado consumo de *Tayassu pecari*, el cual aportó más a la biomasa consumida por este felino. En tanto el puma se muestra más generalista (0.56 y 0.58 respectivamente), donde la presa consumida con mayor frecuencia y la que aportó más a su biomasa fue *Cuniculus paca*. A pesar de que ambos felinos comparten la mayor parte de sus presas, el jaguar consume unas con más frecuencia y el puma otras.

Keywords: biomass; diet; PNANMI Madidi; RBTCO Pilón Lajas; trophic niche.

Introduction

The jaguar (*Panthera onca*) and the puma (*Puma concolor*) are the largest felids in the Americas, and are sympatric in much of the Neotropics (Sunquist and Sunquist 1989; Farrell *et al.* 2000). Both are strict carnivores (Kitchener 1991), and knowledge of their diet help inform conservation strategies (Farrell *et al.* 2000), since food plays a fundamental role in evolutionary behavior of predators (Ramalho and Magnusson 2009). In addition, prey availability is one of the main factors that determines the presence, behavior and ecology of predators in the ecosystems where they live, and predator-prey relationships can influence community dynamics, maintaining the balance of an ecosystem (Sunquist and Sunquist 1989; Aranda 2000; Ramalho and Magnusson 2009; Chumacero and Sainoz 2010).

Studies on the diet of both species in South America show the capture of larger prey by jaguar, and medium to small prey by puma (Schaller and Crawchaw 1980; Emmons 1987; Maxit 2001; Crawshaw and Quigley 2002; Leite *et al.* 2002; Scognamillo *et al.* 2003; Caselli de Azevedo 2008). However, studies in Central America and North America report a greater consumption of larger prey by puma, especially deer species (Nuñez *et al.* 2000; Novack *et al.* 2005; Hernández 2008; Gómez 2010).

In this study we report on jaguar and puma food habits in the Madidi National Park and Natural Area of Integrated Management Area (PNANMI Madidi) and the Pilón Lajas Biosphere Reserve (RBTCO Pilón Lajas) in northwestern Bolivia, including trophic niche breadth, relative biomass consumed from the prey by each felid, and trophic niche overlap between the species. This study aims to improve our understanding of the diet of both cats at this study area and contribute towards actions for their protection and broader conservation strategies for the protected areas. It also seeks to obtain a greater understanding of variations in diet of both cats within their distribution, as compared to similar works at different study sites.

Methods

Study Area. The study was conducted in the Amazon piedmont forest along the beaches and in the riverine forests of the Tuichi, Hondo and Quiquibey rivers, tributaries of the Beni River (De la Quintana 2005). The first two rivers are found in the Franz Tamayo Province of the La Paz Department within PNANMI Madidi (De la Quintana 2005), and the third river is within the Ballivián Province of the Beni Department within RBTCO Pilón Lajas (Figure 1). The average elevation is around 251 masl, annual rainfall oscillates between 1,300 to 2,444 mm, and temperature averages 24.9 ºC (Navarro and Maldonado 2002).

Forests at the study site are a mixture of riverine *varzea* and terre firme primary forests on the alluvial fans between 300 and 500 m, both with tall emergent trees (Identidad Madidi and SERNAP 2019). The vegetation is characterized by a great diversity of palm trees, with the most representative genera *Acrocomia* sp., *Allagoptera* sp., *Astrocaryum* sp., *Attalea* sp., *Bactris* sp., *Chamaedorea* sp., *Desmoncus* sp., *Geonoma* sp. *Socratea* sp., *Syagrus* sp., *Scheelea* sp., *Jessenia* sp. (Wallace *et al.* 2003; Paniagua–Zambrana 2005), as well as families of vascular plants such as Pteridaceae, Sapindaceae, Lauraceae, Bignonaceae, Polypoidaceae, Melastomataceae, Rubiaceae, and Fabaceae (Jorgensen *et al.* 2005).

According to the field survey conducted by the scientific expedition Identidad Madidi in 2016, more than 428 species of vertebrates have been registered along the Hondo river, including 26 medium and large-sized mammals (Identidad Madidi and SERNAP 2019). The mammalian fauna of the area includes the following potential prey species for jaguar (*Panthera onca*) and puma (*Puma concolor*): tayra (*Eira barbara*), brown-nosed coati (*Nasua nasua*), crab-eating raccoon (*Procyon cancrivorus*), lowland tapir (*Tapirus terrestris*), white-lipped peccary (*Tayassu pecari*), collared peccary (*Pecari tajacu*), red brocket deer (*Mazama americana*), capybara (*Hydrochoerus hydrochaeris*), agoutis (*Dasyprocta* spp.), paca (*Cuniculus paca*), southern tamandua (*Tamandua tetradactyla*), black-eared opossum (*Didelphis marsupialis*) and forest rabbit (*Sylvilagus brasiliensis*) (Ríos-Uzeda *et al.* 2001; Gómez *et al.* 2005; Identidad Madidi and SERNAP 2019).

Fieldwork. Fieldwork was carried out between July and October 2008, with 62 days of sampling effort at sites near the Tuichi, Hondo and Quiquibey rivers, intensively searching beaches, streams, old river stretches and riparian forests which are the habitats with the highest probability of finding jaguar and puma scats. Searches were also carried out along 3 to 5 km transects within terra firme forest. The collected scats were identified using morphometry and local guide experience. All encountered scats were measured for diameter and length, to compare with previous descriptions (Aranda 2000; Chame 2003). In some cases, scats were identified with scat morphometry, as well as associated tracks,

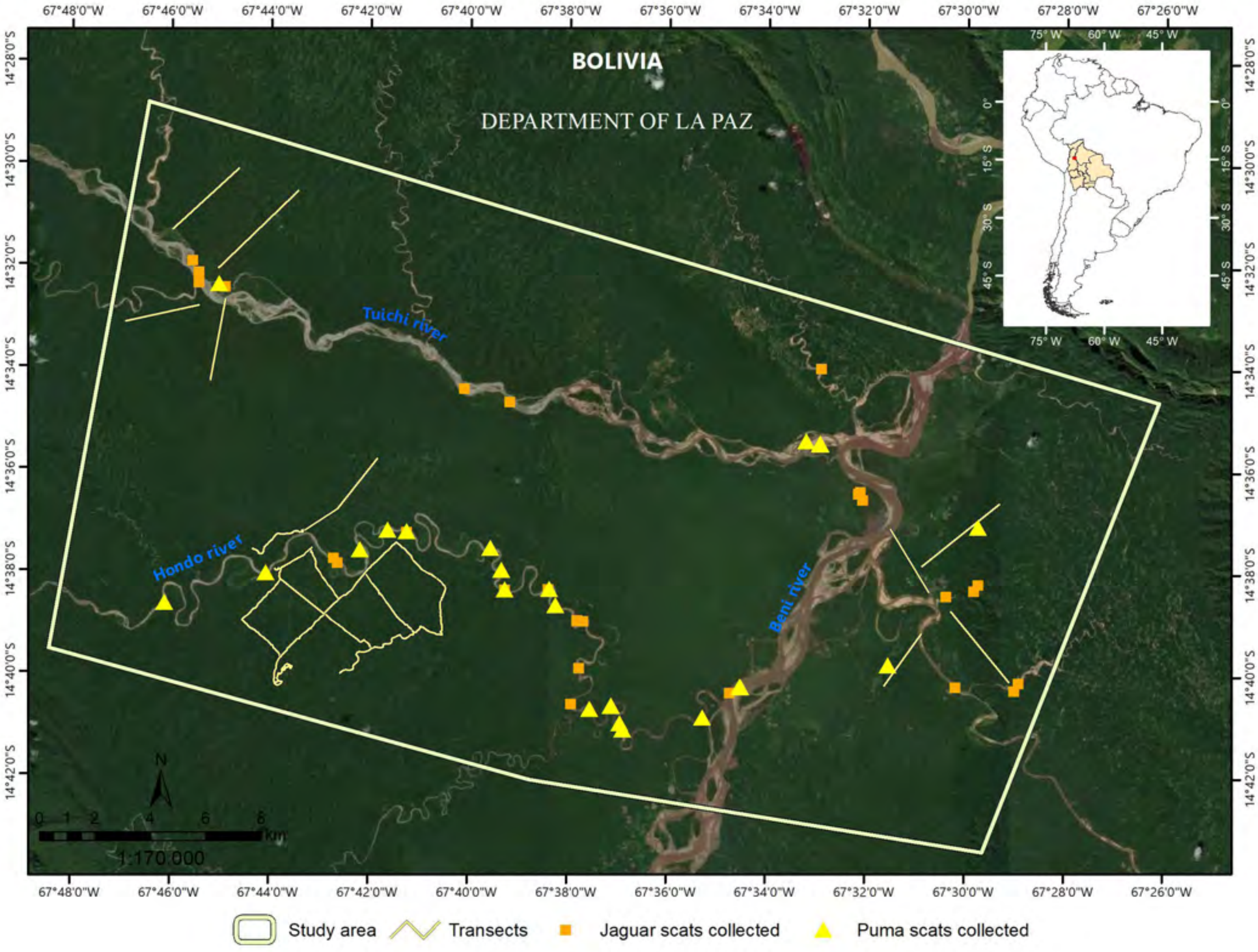

Figure 1. Study area for jaguar and puma diet in the PNANMI Madidi and RBTCO Pilón Lajas, Bolivia.

using track descriptions from De Angelo *et al.* (2008). More than 85 % of collected scats were fresh, and even old scats retained their morphological characteristics, allowing measurements for their identification. Each scat was collected in paper envelopes labeled with the date, site, coordinates, habitat and species. All samples were placed in the shade until sent to La Paz city for subsequent laboratory analysis.

Laboratory work. The scat samples were dried at room temperature before separating food items. For mammals, species identification was performed using teeth morphometry, and mainly hair descriptions, analyzing: A) *Morphological characteristics*, considering the size, color and thickness of the hair, and comparing with the Wildlife Conservation Society catalog of Bolivian mammal hairs (Viscarra *et al.* 2010). B) *Cuticle patterns*, the hairs were cleaned and stamped in a thin layer of transparent nail polish onto a slide, then dried and removed with a tweezer, observed under a microscope, considering the pattern, shape and size of the cuticles (Short 1978; Chehébar and Martin 1989; Fernández and Rossi 1998; Vásquez *et al.* 2000; Quadros and Monteiro-Filho 2006; Zafarina and Panneerchelvan 2009). C) *Core patterns*, following the methodology proposed by Fernández and Rossi (1998) and Tavera (2006), with modifications and adaptations standardizing the methodology to be applied to all types of hair thickness. The hairs were dipped in a mixture of 50 % sulfuric acid (98 %) and 50 % alcohol (96 %) for 48 hours, until the cuticles were shed. Subsequently, the hairs were washed with water. Finally, they were moistened with a drop of water and placed on a slide for identification, taking note of the shape, diameter and width of the marrow.

Data Analysis. The Frequency of Occurrence (FO %) and the Relative Biomass Consumption (RBC of the prey were calculated; Ackerman *et al.* 1984). The Relative Biomass Consumption (RBC %) of each prey was calculated for each cats' diet according to the live weight of the prey species, representing the importance of a prey type in the diet in proportion to its contribution (Farrell *et al.* 2000). Food items whose species identity was unknown were removed from the RBC % analyses due to undetermined weight.

An analysis by prey size category was carried out: large prey (> 15 kg), medium prey (from 1 to 15 kg) and small prey (< 1 kg), following the classifications of Iriarte *et al.* (1990), Taber *et al.* (1997), Scognamillo *et al.* (2003) and Paviolo (2010). The trophic niche overlap between both cats was calculated using the Pianka Index (Pianka 1973), and the trophic niche width for each feline using the Levins Index (Levins 1968). We performed these analyses using the entire scat sample for each large felid, as well as using only those scats associated with clearly identifiable tracks.

To determine whether jaguar and puma prey consumption was influenced by prey abundance, a linear regression analysis was performed between the relative abundance of prey species (events/100 camera trap night) at the study site during the same year (Ayala and Viscarra 2009) and the FO % of the prey consumed in this study for total scats collected, and scats associated with tracks, evaluating the significance of ANOVA ($F \leq 0.05$) and Pearson R^2.

For this analysis, the original data were previously transformed to natural logarithms, evaluating the normality of the data with Shapiro-Wilk ($P \leq 0.05$). The FO % and prey abundance variables for the total of collected scats reported in the zone for jaguar (abundance: $W = 0.9327$, $P = 0.3327$; FO %: $W = 0.8897$, $P = 0.08$) and puma (abundance: $W = 0.9398$, $P = 0.4944$; FO %: $W = 0.9546$, $P = 0.7054$) were normal. The FO % and prey abundance variables for scats associated with tracks for puma (abundance: $W = 0.9398$, $P = 0.4949$; FO %: $W = 0.9518$, $P = 0.6633$) were also normal. For jaguar scats associated with tracks the prey abundance variable ($W = 0.9327$ $P = 0.3327$) was normal, but for FO % (FO %: $W = 0.8241$ $P = 0.0134$) was not normal. These analyses were performed with the JMP Program version 7.0.1. (SAS Institute Inc. 2007).

Results

A total of 122 large felid scats were collected: 66 jaguar and 56 puma scats. Of the collected scats 68 were identified using morphometry and local guide experience, and 54 were identified with morphometry and associated tracks. The totality of collected jaguar scats included items from 25 species (Table 1), with a higher consumption of larger prey (55 %), mainly artiodactyls, and especially the white-lipped peccary (*Tayassu pecari*; 28 %). Using only scats associated with tracks, we detected 20 prey species, again with *T. pecari* the most frequently consumed prey (27 %). For puma (Table 1), items from 28 species were identified, with a higher consumption (56 %) of medium-sized prey, such as rodents, especially the paca (*Cuniculus paca*; 14 %). Using only scats associated with tracks, we detected 22 prey species, again with *C. paca* still the most frequently consumed prey (16 %).

Considering all collected scats, six main prey species were reported for jaguar (*T. pecari*, *Pecari tajacu*, *Dasyprocta* spp., *Mazama americana, Cuniculus paca* and *Nasua nasua*), representing 61 % of their total diet (Table 1). For puma, seven main prey were reported (*C. paca*, Didelphidae, Echimyidae, *P. tajacu*, *N. nasua*, *Sylvilagus brasiliensis* and *Didelphis marsupialis*), representing 54 % of their diet (Table 1). For scats associated with tracks, four main species were reported for jaguar (*T. pecari*, *P. tajacu*, *Dasyprocta* spp. and *N. nasua*), representing 51 % of their diet. For puma, and five main species for pumas (*C. paca*, *Didelphis marsupialis*, Didelphidae, *N. nasua* and Echimyidae), representing 51 % of their diet.

The RBC % analysis, found that the two peccaries, *T. pecari* (37 %) and *P. tajacu* (22 %), contributed the most to the biomass of jaguar diet. Although *Tapirus terrestris* did not have a high FO %, it was the second most important biomass contribution towards jaguar diet (Table 2). Using only those scats associated with tracks this analysis indicated that *T. pecari* (33 %), *T. terrestris* (16 %) and *P. tajacu*

(11 %) were the three most important prey species for jaguar. For puma, *C. paca* (18 %) and *P. tajacu* (11 %) were the prey that contributed the most biomass to diet (Table 2), and using only those scats associated with tracks *C. paca* (20 %), *D. marsupialis* (11 %) and *P. tajacu* (10 %) were the most important prey.

Regarding the breadth of the trophic niche, a more specialized trend was observed for jaguar (0.28), and a more general trend for puma (0.56). There was a relatively low trophic niche overlap between both cats (0.46). Using only scats associated with tracks, the breadth of the trophic niche for jaguar is 0.42 and for puma is 0.58, underlining a more specialized diet for jaguar as compared to puma. The trophic niche overlap was similar using only scats associated with tracks (0.44).

Table 1. Prey species richness for jaguar and puma in PNANMI Madidi and RBTCO Pilón Lajas, Bolivia.

	All scats				**Scats with tracks**			
	Jaguar		**Puma**		**Jaguar**		**Puma**	
Prey	**Nº**	**FO%**	**Nº**	**FO%**	**Nº**	**FO %**	**Nº**	**FO %**
Large prey								
Tayassu pecari	31	28	4	2	13	27	3	4
Pecari tajacu	20	18	11	7	5	10	5	6
Mazama americana	5	5	5	3	2	4	2	3
Tapirus terrestris	2	2			2	4		
Hydrochoerus hydrochaeris	2	2			1	2		
Myrmecophaga tridactyla	1	1			1	2		
Caiman yacare			1	1				
Medium-sized prey								
Dasyprocta spp.	6	5	5	3	4	8	1	1
Cuniculus paca	5	5	24	14	1	2	13	16
Nasua nasua	4	4	10	6	3	6	6	8
Ateles chamek	2	2	3	2	2	4	1	1
Sylvilagus brasiliensis	2	2	10	6	1	2	5	6
Didelphis marsupialis	2	2	10	6	1	2	8	10
Procyon cancrivorus	1	1	8	5	1	2	3	4
Eira barbara	1	1	4	2	1	2	3	4
Potos flavus	1	1	1	1			1	1
Echimyidae	1	1	12	7	1	2	6	8
Alouatta sara			1	1				
Tamandua tetradactyla			1	1			1	1
Bradypus variegatus			1	1			1	1
Galictis vittata			3	2				
Small prey								
Sciurus spadiceus	3	3	7	4	2	4	3	4
Ameiva sp.	1	1	3	2	1	2	1	1
Unidetified prey								
Plants	9	8	5	3	3	6	4	5
Unidentified rodents	3	3	5	3	1	2	2	3
Unidentified birds	3	3	8	5	2	4	2	3
Unidentified mammals	2	2	5	3				
Unidentified reptiles	2	2	2	1				
Didelphidae	1	1	14	8			7	9
Colubridae	1	1	2	1				
Hoplocercidae			1	1			1	1
Total occurrences	111		166		48		79	
Total items	25		28		20		22	
Total scats	66		56		28		26	

Nº = Number of occasions in which a prey was registered in jaguar and puma scats; FO% = Percentage of the Frequency of Occurrence of each consumed prey.

The FO % of total scats collected and the relative abundance of prey (Ayala and Viscarra 2009) for jaguar and puma are presented in Tables 3 and 4, respectively. Considering all collected scats, the linear regressions for FO % and prey abundance, showed significant positive relationships for both jaguar ($r^2 = 43$ %; F = 0.0104) and puma ($r^2 = 44$ %; F = 0.0179; Figure 2). For puma, the linear regression between FO % of scats associated with tracks and prey abundance was not significant ($r^2 = 25$ %; F = 0.0963).

Discussion

In this study, jaguar and puma foraging behavior responded to prey availability, with the most abundant prey at our study site contributing most to their diets. We recorded that

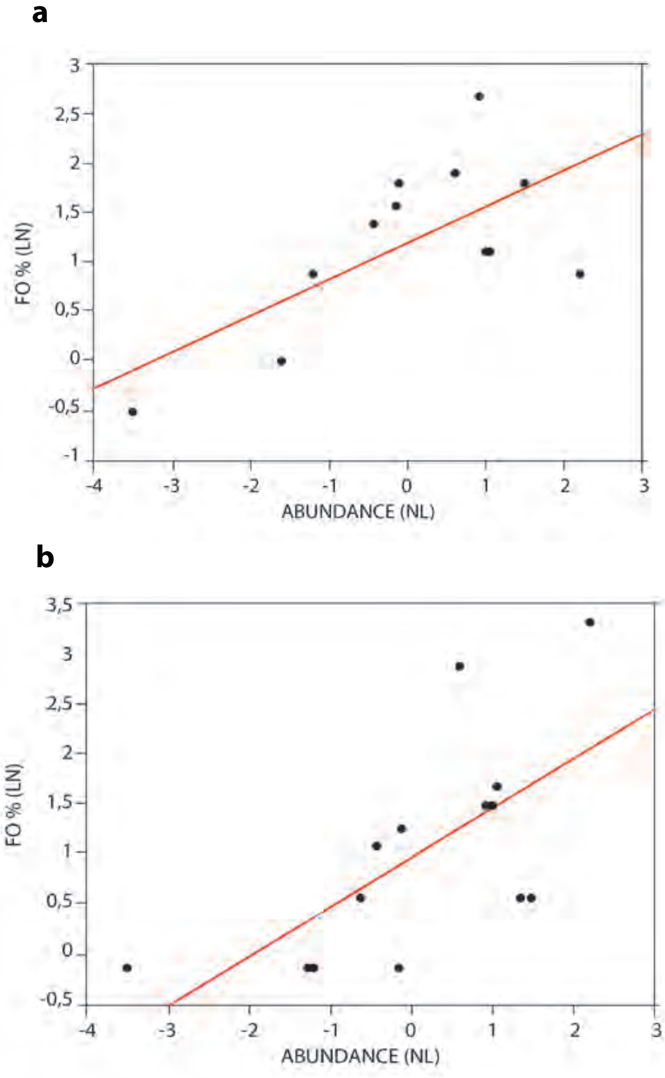

Figure 2. A) Linear regression between Natural Logarithm of FO % and Natural Logarithm of jaguar prey abundance ($r^2 = 43$ %; F = 0.0104). B) Linear regression between FO % and puma prey abundance ($r^2 = 44$ %; F = 0.0179).

Table 2. Prey contribution towards the biomass of jaguar and puma diet in PNANMI Madidi and RBTCO Pilón Lajas, Bolivia.

		All scats				**Scats with tracks**			
		Jaguar		**Puma**		**Jaguar**		**Puma**	
Species	**Weight (kg)**	**Nº**	**RBC %**	**Nº**	**RBC %**	**Nº**	**RBC %**	**Nº**	**RBC %**
Tayassu pecari	35	31	37	4	5	13	33	3	7
Pecari tajacu	26	20	22	11	11	5	11	5	10
Tapirus terrestris	239	2	8			2	16		
Mazama americana	36	5	6	5	6	2	5	2	5
Dasyprocta spp.	5	6	5	5	4	4	7	1	2
Cuniculus paca	5	5	4	24	18	1	2	13	20
Nasua nasua	5	4	3	10	8	3	5	6	9
Hydrochoerus hydrochaeris	50	2	3			1	3		
Sciurus spadiceus	1	3	2	7	5	2	3	3	4
Ateles chamek	8	2	2	3	2	2	4	1	2
Sylvilagus brasiliensis	4	2	2	10	8	1	2	5	7
Didelphis marsupialis	1	2	2	10	7	1	2	8	11
Myrmecophaga tridactyla	22	1	1			1	2		
Procyon cancrivorus	5	1	1	8	6	1	2	3	5
Eira barbara	5	1	1	4	3	1	2	3	5
Potos flavus	3	1	1	1	1			1	1
Echimydae	2	1	1	12	9	1	2	6	9
Ameiva sp.	1	1	1	3	2	1	2	1	1
Caiman yacare	34			1	1				
Alouatta sara	7			1	1				
Tamandua tetradactyla	6			1	1			1	2
Bradypus variegatus	4			1	1			1	1
Galictis vittata	2			3	2				
Total occurrences		90		124		42		63	
Total items		18		23		17		17	
Total scats		66		56		20		22	

Nº = Number of occasions in which a prey was registered in jaguar and puma scats; RBC% = Percentage of Relative Consumed Biomass that each prey contributes to jaguar and puma diet.

jaguars preyed mostly on large to medium sized prey, which mirrors many previous studies (Table 4). From an energy perspective it is preferable to hunt larger prey (Aranda 2002), and previous studies have suggested that peccary hunting by jaguars may be the result of preferential adaptive predation towards these ungulates, since, as these animals are dangerous and difficult to hunt, physical skills are required to kill them quickly (Schaller *et al.* 1984; Aranda 2002).

The collared peccary (*Pecari tajacu*) was the second most important prey in the jaguar diet at our study site. Many studies have reported this species as the most consumed by this feline, with only one study reporting the white-lipped peccary (*Tayassu pecari*) as the prey with the highest FO % and BRC % (Table 4), as in our study. Collared peccaries form small groups and are relatively quiet, while white-lipped peccaries form large and noisy herds that may attract the jaguar's attention (Garla *et al.* 2001). However, Polisar et al. (2003)1 suggest that jaguars consume both peccary species according to their availability. Estimated white-lipped peccary relative abundance at our study site was high at the time of the dietary study, with 9.1 independent events/100 camera trap nights (Ayala and Viscarra 2009), reflected in a population density of 10.74 individuals/100 km^2, with an average of 3.9 white-lipped peccary herds encountered every 10 km of transect (Romero-Valenzuela 2008). Thus, jaguars were probably responding to a high availability of this tropical ungulate.

Contrary to these results, studies in Central America have shown that medium-sized prey contribute more to jaguar diet (Table 4), again apparently responding to prey abundance, with jaguars smaller than in South America (Hernández 2008).

Across their range, pumas consume significantly more rodents than jaguars (Nuñez *et al.* 2000) and specialize on medium to small prey (Table 4). Our results also showed a preference for medium to small prey, especially rodents,

Table 3. Jaguar (*Panthera onca*) and puma (*Puma concolor*) prey relative abundance (events/100 camera trap night) and Percentage of the Frequency of Occurrence of each consumed prey (FO %).

		Jaguar		**Puma**	
Prey	**Abundance within the study area**	**FO % (All scats)**	**FO % (Scats with footprints)**	**FO % (All scats)**	**FO % (Scats with footprints)**
Tayassu pecari	9.10	28	27	2	4
Pecari tajacu	1.84	18	10	7	6
Dasyprocta spp.	2.86	5	8	3	1
Mazama americana	2.75	5	4	3	3
Cuniculus paca	2,51	5	2	14	16
Nasua nasua	0.90	4	6	6	8
Sciurus spadiceus	0.66	3	4	4	4
Tapirus terrestris	3.87	2	4		
Hydrochoerus hydrochaeris	0.53	2	2		
Sylvilagus brasiliensis	14.79	2	2	6	6
Didelphis marsupialis	4.42	2	2	6	10
Myrmecophaga tridactyla	0.28	1	2		
Procyon cancrivorus	0.87	1	2	5	4
Eira barbara	0.30	1	2	2	4
Potos flavus	0.03	1		1	1
Tamandua tetradactyla				1	1

with the paca (*Cuniculus paca*) the most consumed species in the puma's diet in both FO % and BRC %. Although this species is reported as part of the puma diet in several studies (Emmons 1987; Novack *et al*. 2005; Caselli de Azevedo 2008; Hernández 2008), none record it as the main species in the puma diet. Rather, agoutis (*Dasyprocta* spp.) are reported as more frequently consumed by puma in previous studies (Table 4). At our study

Table 4. Jaguar (*Panthera onca*) and puma (*Puma concolor*) diet reported in this study compared to previous studies across the jaguar and puma range.

		Jaguar				**Puma**			
Studies	**Prey size preferences**	**Mostly consumed prey**	**FO %**	**Prey with greate contibution to RBC**	**RBC %**	**Mostly consumed prey**	**FO %**	**Prey with greate contibution to RBC**	**RBC %**
Data form this study (all scats) (Bolivia)	large to medium	*Tayassu pecari*	28	*Tayassu pecari*	37	Cuniculus paca	14	*Cuniculus paca*	18
Data form this study (scats with footprints; Bolivia)	large to medium	*Tayassu pecari*	27	*Tayassu pecari*	33	Cuniculus paca	16	*Cuniculus paca*	20
Emmons 1987 (Perú)	large to medium	tortoise/turtles	33			Dasyprocta variegata	33		
Chinchilla 1997 (wet season; Costa Rica)	large to medium	*Iguana iguana*	37	*Tayassu pecari*	57	Proechimys semispinosus	29	*Ateles geoffroyi*	32
Chinchilla 1997 (dry season; Costa Rica)	large to medium	*Choleopus hoffmani*	21	*Tayassu pecari*	55	Mazama americana	25	*Mazama americana*	66
Taber et *al*. 1997 (Paraguay)	large to medium	*Mazama gouazoubira*	23	*Mazama gouazoubira*	37	Mazama gouazoubira	12	*Mazama gouazoubira*	24
Nuñez 2000 (México)	large to medium	Odocoileus virginianus	52	*Odocoileus virginianus*	54	Odocoileus virginianus	55	*Odocoileus virginianus*	66
Garla 2001 (Brasil)	large to medium	*Tayassu pecari*	21	*Tayassu pecari*	26				
Polisar *et al*. 2003 (Venezuela)	large to medium	*Pecari tajacu*	26			H. hydrochaeris /O. virginianus	20 /10		
Scognamillo 2003 (Venezuela)	large to medium	*Pecari tajacu*	26	*Pecari tajacu*	27	*Pecari tajacu* (juvenil)	12	*Hydrochoerus hydrochaeris*	21
Novack *et al*. 2005 (Guatemala)	medium	*Dasypus novemcinctus*	47	*Dasypus novemcinctus*	32	Dasyprocta punctata	26	*Dasyprocta punctata*	16
Weckel *et al*. 2006 (Belize)	large to medium	*Dasypus novemcinctus*	33						
Caselli and Murray 2007 (Brasil)	large to medium	*Hydrochoerus hydrochaeris*	14	*Hydrochoerus hydrochaeris*	24				
Caselli de Azevedo 2008 (Brasil)	large to medium	*Pecari tajacu*	27	*Pecari tajacu*	32	Dasyprocta azarae	21	*Mazama* sp.	20
Hernández 2008 (México)	medium	*Pecari tajacu*	54	*Pecari tajacu*	27	Venados	38	Venados	45
Gómez 2010 (México)	large to medium	*Dasypus novemcinctus*	38	*Dasypus novemcinctus*	32	Dasypus novemcinctus	55	*Dasypus novemcinctus*	49
Rosas 2003 (México)	large to medium					Ovis canadensis	40	*Ovis canadensis*	47
De la Torre and De la Riva 2009 (México)	large to medium					*Odocoileus virginianus*	42	*Odocoileus virginianus*	36

site, *Cuniculus* and *Dasyprocta* showed a similar relative abundance (Table 3). Pacas are significantly larger than agoutis (Emmons and Feer 1999). Puma activity patterns within our study area are cathemeral, with intermittent activities during the day and at night, but the majority of camera trap photographic records at night (Gómez *et al.* 2005; Ayala *et al*. in press). This suggests another possible selection factor, because *Cuniculus* is nocturnal, whilst *Dasyprocta* is diurnal (Avila-Nájera *et al.* 2016; Ayala and Viscarra 2009; Briones-Salas *et al.* 2015).

Nevertheless, other studies reported that larger prey, especially deer, contribute a higher percentage of puma diet in terms of BRC %, even in areas where they are sympatric with the jaguar (Table 4). Comparative studies of puma diet across the range revealed wide variation in the frequency of occurrence of deer from 37.3 in México, 6.4 in Brazil, to zero in Perú (MacBride 1976; Emmons 1987; Iriarte *et al.* 1990; Crawshaw and Quigley 2002). Thus, the puma maybe opportunistic (Hernández 2008) and as deer abundance decreases, they change their diet to more abundant prey types (Ackerman *et al.* 1986; Iriarte *et al.* 1990; Aranda 2000; Pacheco *et al.* 2004). A predictive model for puma diet indicates the ability to change their habits and hunt small prey when they are very abundant (Ackerman *et al.* 1986).

Within our study area, the only species of deer is the red brocket (*Mazama americana*), found at a similar relative abundance to the paca (Ayala and Viscarra 2009; Table 3), but is consumed considerably less. Some authors attribute the consumption of medium sized prey by pumas to their sympatry with jaguars, since in areas where the two felines do not coexist, pumas hunt more large prey, especially deer (Iriarte *et al.* 1990; Laundré and Hernández 2002; Rosas *et al.* 2003; De la Torre and De la Riva 2009).

At our study site, there was a low trophic niche overlap between the two cats: although they share most prey species, the jaguar consumes more large prey and the puma more medium-sized prey. Other studies have reported greater (0.84) and lower (0.33) trophic niche overlaps between the two cats (Table 5). Taber *et al.* (1997) indicate that despite the similarity in diet of jaguar and puma, in general, there is no evidence of competition, and Nuñez *et al.* (2000) conclude that, as in our study, both felines consume similar prey, but in different proportions.

We found that the jaguar is more selective in its diet and the puma more generalist. Similar results were observed in South America (Table 5), but a different pattern is observed in Central America (Table 5), where pumas tend to be more selective in their prey and jaguars more generalist. In conclusion, prey selection for jaguar and puma largely depends on prey abundance. However, it may also be related to activity patterns, since jaguars are more active during the day (Ayala *et al*. in press), as are both peccaries species that are their main prey. Jaguars typically taking larger prey than pumas, suggesting adaptive predation according to the body shape of both cats.

Table 5. Overlap and trophic niche width of jaguar and puma reported in this study and other studies in the Neotropics

Studies	Trophic niche overlap	Niche width for jaguar	Niche width for puma
Data form this study (all scats; Bolivia)	0.46	0.28	0.56
Data form this study (scats with footprints; Bolivia)	0.44	0.42	0.58
Taber *et al.* 1997 (Paraguay)	0.65		
Nuñez 2000 (México)	0.84	0.50	0.38
Scognamillo 2003 (Venezuela)	0.70	0.32	0.85
Novack *et al.* 2005 (Guatemala)	0.33		
Caselli de Azevedo 2008 (Brasil)	0.56	0.35	0.47
Hernández 2008 (México)	0.51	0.80	0.69
Gómez 2010 (México)	0.83	0.64	0.13

Finally, our study occurred within neighboring protected areas and indigenous territories, and the information herein significantly improves our knowledge about the ecology of these apex predators that are conservation objectives for the national parks. Subsistence hunting by Indigenous People is permitted within the Natural Area of Integrated Management portion of the Madidi protected area and the Pilón Lajas Biosphere Reserve, with both peccaries and the paca popular targets, coinciding with the most consumed prey for both cats (CSF 2011; CIPTA and WCS 2017). Our information stresses the importance of monitoring and managing subsistence hunting into the future, so the availability of prey for both cats and the indigenous communities is not affected. Protected areas aim to conserve biodiversity, including predator-prey dynamics as an indicator of a healthy ecosystem. Therefore, continued monitoring of the population dynamics of jaguars, pumas and their prey, will help evaluate ecosystem health. This will facilitate decision-making to ensure the conservation and protection of the natural heritage of the country.

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Literature Cited

Ackerman, B. B., F. G. Lindzey, and T. P. Hemker. 1984. Cougar food habits in southern Utah. The Journal of Wildlife Management 48:147-155.

Ackerman, B. B., F. G. Lindzey, and T. P. Hemker. 1986. Predictive energetics model for cougars. Pp. 333–352, *in* Cats of the world: biology, conservation, and management (Miller, S. D., and D. D. Everet, eds.). National Wildlife Federation. Washington, U.S.A.

Aranda, M. 2000. Huellas y Otros Rastros de los Mamíferos Grandes y Medianos de México. Instituto de Ecología A. C. Xalapa, Veracruz.

Aranda, M. 2002. Importancia de los pecaríes para la conservación del jaguar en México. Pp. 101–105, *in* El Jaguar en el Nuevo Milenio (Medellín R., C. Equihua, C. Chetkiewicz, P. Crawshaw, A. Rabinowitz, K. Redford, J. Robinson, E. Sanderson, and A. Taber, eds.). Fondo de Cultura Economica. Ciudad de México, México.

Aranda, M., and V. Sánchez-Cordero. 1996. Prey spectra of jaguar (*Panthera onca*) and puma (*Puma concolor*) in tropical forests of Mexico. Studies on Neotropical Fauna and Environment 31:65-67.

Ávila-Nájera, D. M., C. Chávez, M. A. Lazcano-Barrero, G. D Mendoza, and S. Perez-Elizalde. 2016. Traslape en patrones de actividad entre grandes felinos y sus principales presas en el norte de Quintana Roo, México. Therya 7:439-448.

Ayala, G., and M. Viscarra. 2009. Estimando la densidad de jaguar y la abundancia relativa de mamíferos medianos y grandes en el Parque Nacional Madidi y la Reserva Pilón Lajas, Bolivia. Unpublished report. Wildlife Conservation Society. La Paz, Bolivia.

Briones-Salas, M., I. Lira-Torres, R. Carrera-Treviño, and G. Sánchez-Rojas. 2015. Relative abundance and activity patterns of wild felids in Chimalapas rainforest, Oaxaca, Mexico. Therya 7:123-134.

Caselli de Azevedo, F. C. 2008. Food habits and livestock depredation of sympatric jaguars and pumas in the Iguacu National Park area, south Brazil. Biotropica 40:494-500.

Caselli de Azevedo, F. C., and D. L. Murray. 2007. Spatial organization and food habits of jaguars (*Panthera onca*) in a floodplain forest. Biological Conservation 137:391-402.

Chame, M. 2003. Terrestrial mammal scats: a morphometric summary and description. Memórias do Instituto Oswaldo Cruz 98:71-94.

Chehébar, C., and S. Martín. 1989. Guía para el reconocimiento microscópico de los pelos de los mamíferos de la Patagonia. Doñana Acta Vertebrata 16:247-291.

Chinchilla, F. A. 1997. La dieta del jaguar (*Panthera onca*), el puma (*Felis concolor*) y el manigordo (*Felis pardalis*) (Carnivora: Felidae) en el Parque Nacional Corcovado, Costa Rica. Revista de Biología Tropical 45:1223-1229.

Chumacero, L. M. G., and A. Sainoz. 2010. Mamíferos–depredadores ¿controlan las densidades poblacionales de los mamıferos–presa? Universidad Autónoma Metropolitana-Iztapalapa. Departamento de Biología, División de CBS. ContactoS 76:5-9.

Crawshaw, P. G., and H. B. Quigley. 2002. Hábitos alimentarios del jaguar y el puma en el Pantanal, Brasil, con implicaciones para su manejo y conservación. Pp. 223–235, *in* El Jaguar en el Nuevo Milenio (Medellín R., C. Equihua, C. Chetkiewicz, P. Crawshaw, A. Rabinowitz, K. Redford, J. Robinson, E. Sanderson, and A. Taber, eds.). Fondo de Cultura Economica. Ciudad de México, México.

Consejo Indígena del Pueblo Indígena Tacana and Wildlife Conservation Society (CIPTA and WCS). 2017. Caza de subsistencia en el territorio indígena Tacana. La Paz, Bolivia.

Conservation Strategy Fund (CSF). 2011. Reglas para la cacería en comunidades indígenas de la Reserva de la Biósfera y Tierra Comunitaria de Origen Pilón Lajas: Un análisis desde la economía experimental. La Paz, Bolivia.

De Angelo, C., A. Paviolo, Y. Di Blanco, and M. Di Bitetti. 2017. Guía para la Identificación de Huellas de Mamíferos de Misiones y otras Áreas del Subtrópico de Argentina. Ediciones del Subtrópico, Fundación ProYungas. Yungas, Argentina.

De la Quintana, D. 2005. Diversidad florística y estructura de una parcela permanente en un bosque amazónico preandino del sector del Río Hondo, Área Natural de Manejo Integrado Madidi (La Paz, Bolivia). Ecología en Bolivia 40:418-442.

De la Torre, J. A., and G. De la Riva. 2009. Food habits of pumas (*Puma concolor*) in a semiarid region of central Mexico. Mastozoología Neotropical 16:211-216.

Emmons, L. H. 1987. Comparative feeding ecology of felids in a neotropical rainforest. Behavioral Ecology and Sociobiology 20:271-283.

Emmons, L. H., and F. Feer. 1999. Mamíferos de los Bosques Húmedos de América Tropical. Fundación Amigos de la Naturaleza. Santa Cruz de la Sierra, Bolivia.

Farrell, L. E., J. Roman, and M. E. Sunquist. 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. Molecular Ecology 9:1583-1590.

Fernández, G. J., and S. M. Rossi. 1998. Medullar type and cuticular scale patterns of hairs of rodents and small marsupials from the Monte Scrubland (San Luis Province, Argentina). Mastozoología Neotropical 5:109-116.

Garla, R. C., E. Z. Setz, and N. Gobbi. 2001. Jaguar (*Panthera onca*) food habits in Atlantic rain forest of southeastern Brazil. Biotropica 33:691-696.

Gau, R. J., R. Mulders, T. Lamb, and L. Gunn. 2001. Cougars (*Puma concolor*) in the Northwest Territories and Wood Buffalo National Park. Arctic 54:185-187.

Gómez, H., R. B. Wallace, G. Ayala, and R. Tejada. 2005. Dry season activity periods of some Amazonian mammals. Studies on Neotropical Fauna and Environment 40:91-95.

Gómez, Y. 2010. Nicho Trófico de Jaguar y Puma en la Reserva Natural Sierra Nanchititla, México. Masters Thesis. Universidad Autónoma de Baja California. Mexicalli. Baja California, México.

Hernández, C. G. E. 2008. Dieta, uso de hábitat y patrones de actividad del puma (*Puma concolor*) y el jaguar (*Panthera onca*) en la Selva Maya, Centroamérica. Revista Mexicana de Mastozoología 12:113-130.

Identidad Madidi and SERNAP. 2019. Informe de la Expedición Científica Identidad Madidi 2016. Relevamientos de Biodiversidad en el Parque Nacional y Área Natural de Manejo Integrado Madidi. La Paz, Bolivia.

Iriarte, J. A., W. L. Franklin, W. E. Johnson, and K. H. Redford. 1990. Biogeographic variation of food habits and body size of the America puma. Oecologia 85:185–190.

Jorgensen, P. M., M. J. Macía, A. Fuentes, S. G. Beck, M. Kessler, N. Paniagua, R. Seidel, C. Maldonado, A. Araujo–Murakami, L. Cayola, T. Consiglio, T. J. Killeen, W. H. Cabrera, F. Bascopé, D. De la Quintana, T. Miranda, F. Canqui, and V. Cardona–Peña. 2005. Lista anotada de las plantas vasculares registradas en la región de Madidi. Ecología en Bolivia 40:70-169.

Kitchener, A. 1991. The Natural History of the Wild Cats. Comstock Pub. Associates. New York, U.S.A.

Laundré, J. W., and L. Hernández. 2003. Winter hunting habitat of pumas (*Puma concolor*) in northwestern Utah and southern Idaho, USA. Wildlife Biology 9:123-129.

Leite R., R. Boulhosa, F. Galvao, and L. Cullen. 2002. Conservación del jaguar en las áreas protegidas del bosque Atlántico de la costa del Brasil. Pp 25–41, *in* El Jaguar en el Nuevo Milenio (Medellín R., C. Equihua, C. Chetkiewicz, P. Crawshaw, A. Rabinowitz, K. Redford, J. Robinson, E. Sanderson, and A. Taber eds.). S.L. Fondo de Cultura Economica. Fondo de Cultura Economica. Ciudad de México, México.

Levins, R. 1968. Evolution in Changing Environments. Princeton Univ. Press, Princeton. New Jersey, U.S.A.

Maxit, I. E. 2001. Prey Use by Sympatric Puma and Jaguar in the Venezuelan Llanos. Masters Thesis, Universidad de Florida, U.S.A.

McBride, R. T. 1976. The Status and Ecology of the Mountain Lion *Felis concolor stanleyana*, of the Texas-Mexico Border. Doctoral Thesis, Sul Ross State University, Alpine, U.S.A.

Navarro, G., and M. Maldonado. 2002. Geografía Ecológica de Bolivia: Vegetación y Ambientes Acuáticos. Centro de Ecología Simón I. Patiño. Cochabamba, Bolivia.

Novack, A. J., M. B. Main, M. E. Sunquist, and R. F. Labisky. 2005. Foraging ecology of jaguar (*Panthera onca*) and puma (*Puma concolor*) in hunted and non-hunted sites within the Maya Biosphere Reserve, Guatemala. Journal of Zoology 267:167-178.

Núñez, R., B. Miller, and F. Lindzey. 2000. Food habits of jaguars and pumas in Jalisco, Mexico. Journal of Zoology 252:373-379.

Pacheco, L. F., A. Lucero, and M. Villca. 2004. Dieta del puma (*Puma concolor*) en el Parque Nacional Sajama, Bolivia y su conflicto con la ganadería. Ecología en Bolivia 39:75–83.

Paniagua-Zambrana, N. Y. 2005. Diversidad, densidad, distribución y uso de las palmas en la región del Madidi, noreste del departamento de La Paz (Bolivia). Ecología en Bolivia 40:265-280.

Paviolo, A. J. 2010. Densidad de yaguareté (*Panthera onca*) en la selva paranaense: su relación con la disponibilidad de presas, presión de caza y coexistencia con el puma (*Puma concolor*). Mastozoología Neotropical 17:397-398.

Pianka, E. R. 1973. The structure of lizard communities. Annual Review of Ecology and Systematics 4:53-74.

Polisar, J., I. Maxit, D. Scognamillo, L. Farrell, M. E. Sunquist, and J. F. Eisenberg. 2003. Jaguars, pumas, their prey base, and cattle ranching: ecological interpretations of a management problem. Biological Conservation 109:297-310.

Quadros, J., and E. L. D. A. Monteiro-Filho. 2006. Revisão conceitual, padrões microestruturais e proposta nomenclatória para os pêlos-guarda de mamíferos brasileiros. Revista Brasileira de Zoologia 23:279-292.

Ramalho, E. E., and W. E. Magnusson. 2009. Uso do habitat por onça-pintada (*Panthera onca*) no entorno de lagos de várzea, Reserva de Desenvolvimento Sustentável Mamirauá, AM, Brasil. Scientific Magazine UAKARI 4:33-39.

Ríos-Uzeda, B., R. B. Wallace, H. Aranibar, and C. Veitch. 2001. Evaluación de mamíferos medianos y grandes en el bosque semideciduo del alto Tuichi (PN y ANMI Madidi, Depto. La Paz). Ecología en Bolivia 36:31-38.

Romero–Valenzuela, D. 2008. Estimación de la Densidad Poblacional del Chancho de Labios Blancos (*Tayassu pecari*) en el Valle del Río Hondo, Parque Nacional y Área Natural de Manejo Integrado (Bolivia). Tesis de Licenciatura Universidad Mayor de San Andrés. La Paz, Bolivia.

Rosas, O. C., R. Valdez, L. C. Bender, and D. Daniel. 2003. Food habits of pumas in northwestern Sonora, Mexico. Wildlife Society Bulletin 31:528-535.

SAS Institute Inc. 2007. JMP user's guide. arXiv:1004.3173.

Schaller, G. B., and P. G. Crawshaw Jr. 1980. Movement patterns of jaguar. Biotropica 12:161-168.

Schaller, G. B., H. B. Quigley, and P. G. Crawshaw. 1984. Biological investigations in the Pantanal, Mato Grosso, Brazil. National Geographic Society Research Reports 17:777-792.

Scognamillo, D., I. E. Maxit, M. Sunquist, and J. Polisar. 2003. Coexistence of jaguar (*Panthera onca*) and puma (*Puma concolor*) in a mosaic landscape in the Venezuelan llanos. Journal of Zoology 259:269-279.

Seymour, K. L. 1989. *Panthera onca*. Mammalian Species 340:1-9.

Short, H. L. 1978. Analysis of cuticular scales on hairs using the scanning electron microscope. Journal of Mammalogy 59:261-268.

Sunquist, M. E., and F. C. Sunquist. 1989. Ecological constraints on predation by large felids. Pp. 283–301 *in* Carnivore Behaviour, Ecology and Evolution (Gittleman, J. L., ed.). Springer Science. Ithaca, U.S.A.

Taber, A. B., A. J. Novaro, N. Neris, and F. H. Colman. 1997. The food habits of sympatric jaguar and puma in the Paraguayan Chaco. Biotropica 29:204–213.

Tavera, G. 2006. Patrones Cuniculares y Medulares de Pelos de Cinco Especies de Roedores Altoandinos del Departamento de Cochabamba. Tesis de Licenciatura, Carrera de Biología, Universidad Mayor de San Simón. Cochabamba, Bolivia.

Vázquez, D. E., P. G. Perovic, and A. A. de Olsen. 2000. Patrones cuticulares y medulares de pelos de mamíferos del noroeste argentino (Carnivora y Artiodactyla). Mastozoología Neotropical. 7:131-147.

Viscarra, M., C. Flores–Turdera, and G. Ayala. 2010. Catálogo de Pelos Guardia de Mamíferos Medianos y Grandes. Wildlife Conservation Society. La Paz, Bolivia

Wallace, R. B., H. Gomez, G. Ayala, and F. Espinoza. 2003. Camera trapping for jaguar (*Panthera onca*) in the Tuichi Valley, Bolivia. Mastozoología Neotropical 10:133-139.

Weckel, M., W. Giuliano, and S. Silver. 2006. Cockscomb revisited: Jaguar diet in the Cockscomb Basin Wildlife Sanctuary, Belize. Biotropica 38:687-690.

Zafarina, Z., and S. Panneerchelvam. 2009. Analysis of hair samples using microscopical and molecular techniques to ascertain claims of rare animal species. The Malaysian Journal of Medical Sciences 16:35.

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A checklist of the helminth parasites of invasive murid rodents in Mexico

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El presente trabajo provee un listado helmintológico actualizado de los roedores múridos invasores (*Mus musculus*, *Rattus norvegicus*, *R. rattus* y *Rattus* sp.) en México, que incluye 35 taxa de helmintos (3 tremátodos, 7 céstodos, 2 acantocéfalos y 23 nematodos). Los registros helmintológicos hasta mayo de 2020, provienen de 18 estudios procedentes de siete estados del país. Se incluye información de los registros acerca de los hábitats, estadios de desarrollo, localidades de registro, hospedadores, colecciones helmintológicas donde se encuentran depositados los especímenes, prevalencias y referencias bibliográficas, cuando estuvieron disponibles. Finalmente, se adiciona un nuevo registro de localidad para el nematodo *Hassalstrongylus musculi* en Yucatán.

The present work provides an updated checklist of helminth species infecting invasive murid rodents (*Mus musculus*, *Rattus norvegicus*, *R. rattus*, and *Rattus* sp.) in Mexico, including 35 helminth taxa (3 trematodes, 7 cestodes, 2 acanthocephalans, and 23 nematodes). The helminthological records were compiled from 18 studies conducted in seven Mexican states up to May 2020. Information on habitats, life stages, geographical locations, hosts, helminthological collections, prevalences, and bibliographic references are included, when available. Finally, a new locality record of the nematode *Hassalstrongylus musculi* in Yucatan is provided.

Keywords: Helminthological record; *Mus musculus*; *Rattus norvegicus*; *Rattus rattus*.

Introducción

Los roedores representan aproximadamente el 39 % de los mamíferos del mundo (Burgin *et al.* 2018). A pesar de este porcentaje, pocas especies son consideradas como invasoras en hábitats naturales, urbanos y rurales (Stenseth *et al.* 2003). El ratón doméstico *Mus musculus*, la rata café o noruega *Rattus norvegicus* y la rata negra o de los tejados *Rattus rattus* (Rodentia: Muridae), son consideradas las especies invasoras más importantes a nivel mundial. Estos roedores han causado extinciones de plantas, invertebrados y pequeños mamíferos en numerosas islas del mundo (Leitschuh *et al.* 2018), y pérdidas económicas a nivel mundial por las toneladas de cereales que consumen o degradan anualmente (Singleton *et al.* 2010), así como mortalidades y morbilidades en los seres humanos causadas por patógenos zoonóticos como los helmintos (*e. g.*, *Angiostrongylus cantonensis*, *Hymenolepis diminuta*) que éstos hospedan (Meerburg *et al.* 2009).

En México, los estudios helmintológicos en pequeños roedores iniciaron en la década de 1930 (Ochoterena y Caballero y Caballero 1932; Chitwood 1938; Caballero y Caballero 1939). En roedores invasores los primeros registros helmintológicos fueron publicados por Caballero y Caballero (1939) quien registró tres especies de céstodos (*Hydatigera taeniaeformis*, *H. diminuta* y *Rodentolepis nana*) y un nematodo (*Heterakis spumosa*) en *R. norvegicus* en la Ciudad de México. Recientemente, García-Prieto *et al.* (2012) compilaron el listado de helmintos de los mamíferos silvestres de México, incluyendo aquellos reportados en roedores invasores. En este estudio se listaron 10 especies de helmintos en *M. musculus* y *R. rattus* y 12 especies en *R. norvegicus*. Sin embargo, este trabajo carece de los registros más recientes y posteriores al año 2012, especialmente aquellos realizados en el sureste de México, donde los estudios en roedores múridos invasores se han incrementado debido a su papel como reservorios de helmintos zoonóticos (*e. g.*, Panti-May *et al.* 2015, 2020a).

El objetivo del presente trabajo es actualizar el listado de helmintos parásitos de roedores múridos invasores en México, compilando los trabajos más recientes y añadiendo un nuevo registro no publicado para el estado de Yucatán.

Materiales y métodos

Se realizó una búsqueda electrónica de literatura sobre helmintos parásitos de roedores múridos invasores en México. Para ello se consultaron tres bases de datos electrónicas (Google Scholar, PubMed y Web of Science) utilizando las palabras clave "helminto", "céstodo", "nematodo", "tremátodo", "acantocéfalo", "parásito", "*Mus musculus*", "*Rattus rattus*", "*Rattus norvegicus*", "roedor invasor", "roedor comensal", "roedor sinantrópico" y "México" en español e inglés. La búsqueda incluyó trabajos desde el año 2012 hasta mayo de 2020. La revisión incluyó estudios con infecciones naturales en poblaciones sinantrópicas o comensales de roedores invasores; no se incluyen infecciones experimentales o naturales en animales de líneas o cepas usadas en laboratorios o centros comerciales de mascotas. Los registros de los trabajos identificados en la búsqueda electrónica fueron añadidos al listado helmintológico publicado por García-Prieto *et al.* (2012). Las referencias bibliográficas de este último trabajo fueron consultadas para confirmar la información en la fuente original.

Los registros helmintológicos fueron agrupados por filo, y ordenados alfabéticamente a nivel familia, género y especie. Para cada especie de helminto se presenta información sobre el hábitat, estado de desarrollo, localidades de registro, hospedadores, colecciones helmintológicas donde están depositados los ejemplares, prevalencias y referencias bibliográficas, siempre y cuando estén disponibles. El registro referenciado en el presente estudio proviene de un muestreo realizado en un rancho ganadero en Sotuta, Yucatán (20° 39′ 14.8″ N, -89° 02′ 28.8″ O, 21 msnm), en marzo de 2019. Todos los procedimientos de captura y manejo de los animales fueron aprobados por el Comité de Bioética (No. aprobación CB-CCBA I-2018-001) del Campus de Ciencias Biológicas y Agropecuarias de la Universidad Autónoma de Yucatán. La recolección de los roedores contó con el permiso de colecta científica (No. permiso SGPA/DGVS/05995/19) expedido a S. F. Hernández-Betancourt por la Secretaría de Medio Ambiente y Recursos Naturales de México.

Las abreviaciones para las colecciones helmintológicas son las siguientes: CNHE, Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, México; HWML, Harold W. Manter Laboratory of Parasitology, University of Nebraska-Lincoln, Nebraska, Estados Unidos de América; USNM, Smithsonian National Museum of Natural History (ahora alberga la U.S. National Parasite Collection), Washington, D.C., Estados Unidos de América; MLP-He, Museo de La Plata-Colección Helmintológica, La Plata, Argentina; CHE-UAEH, Colección de Helmintos, Universidad Autónoma del Estado de Hidalgo, Hidalgo, México.

Resultados

La revisión de literatura incluye 111 registros helmintológicos en roedores múridos invasores obtenidos de 18 estudios, incluyendo el reporte realizado en el presente trabajo. Los registros se incluyen en un capítulo de libro, cuatro tesis y 13 artículos científicos realizados desde 1939 hasta mayo de 2020 (Caballero y Caballero 1939; Cerecero 1943a, b; Gutiérrez-González 1980; García-Prieto 1986; Hierro-Huerta 1992; Tay Zavala *et al.* 1999; Pulido-Flores *et al.* 2005; Falcón-Ordaz *et al.* 2010; Rodríguez-Vivas *et al.* 2011; García-Prieto *et al.* 2012; Falcón-Ordaz *et al.* 2015; Panti-May *et al.* 2015; Cigarroa-Toledo *et al.* 2017; Panti-May *et al.* 2017, 2018; Medina-Pinto *et al.* 2019). De estos registros, 48 fueron reportados hasta el año 2012 y 63 entre 2013 y 2020. De los 18 estudios, 11 se realizaron hasta el año 2012 (Caballero y Caballero 1939; Cerecero 1943a, b; Gutiérrez-González 1980; García-Prieto 1986; Hierro-Huerta 1992; Tay Zavala *et al.* 1999; Pulido-Flores *et al.* 2005; Falcón-Ordaz *et al.* 2010; Rodríguez-Vivas *et al.* 2011; García-Prieto *et al.* 2012) y siete entre los años 2013 y 2020 (Falcón-Ordaz *et al.* 2015; Panti-May *et al.* 2015; Cigarroa-Toledo *et al.* 2017; Panti-May *et al.* 2017, 2018; Medina-Pinto *et al.* 2019; presente estudio). Los helmintos fueron registrados en siete de los 32 estados de México (Chiapas, Ciudad de México, Hidalgo, Michoacán, Nuevo León, Tabasco y Yucatán). Los estados con mayor número de taxa de helmintos fueron Tabasco (13 taxa) y Yucatán (12 taxa), mientras que aquellos con mayor número de estudios fueron Yucatán (seis estudios) y Ciudad de México (cinco estudios; Figura 1).

Se identificaron un total de 35 taxa de helmintos en roedores múridos invasores en México. El número de taxa por especie de roedor fue 19 en *M. musculus*, 16 en *R. norvegicus*, 18 en *R. rattus* y dos en *Rattus* sp. Sin embargo, éstos presentan una asimetría en el número de registros y diversidad entre los estados (Figura 1). Los helmintos reportados incluyeron tremátodos (Diplostomidae y Echinostomatidae), céstodos (Anoplocephalidae, Hymenolepididae y Taeniidae), acantocéfalos (Moniliformidae y Oligacanthorhynchidae) y nematodos (Ascarididae, Gongylonematidae, Heligmonellidae, Heligmosomidae, Heterakidae, Heteroxynematidae, Oxyuridae, Spirocercidae, Strongyloididae, Trichinelloidea y Trichuridae).

Lista parásito–hospedador:

Platyhelminthes Gegenbaur, 1859
Trematoda Rudolphi, 1808
Diplostomidae Poirier, 1886
Fibricola caballeroi Cerecero, 1943

Intestino delgado (adultos). Ciudad de México: Zoológico de Chapultepec, *R. norvegicus*, CNHE (802, 1372), no se reportan datos de prevalencia (Cerecero 1943a).

Echinostomatidae Looss, 1899
Echinostoma revolutum (Frölich, 1802)

Intestino delgado (adulto). Ciudad de México: Zacatenco, *Rattus* sp., no se reportan datos de material en colección y de prevalencia (Cerecero 1943b).

Euparyphium ochoterenai Cerecero, 1943

Intestino delgado (adultos). Ciudad de México: Bosque de Chapultepec, *R. norvegicus*, CNHE (800, 1359), no se reportan datos de prevalencia (Cerecero 1943a).

Cestoda Rudolphi, 1808
Anoplocephalidae Cholodkovsky, 1902
Monoecocestus sigmodontis (Chandler y Suttles, 1922)

Intestino (adultos). Nuevo León: Casas Blancas, el Brasil y la Perla, Apodaca, *M. musculus* y *R. rattus*, no se reportan datos de material en colección y de prevalencia (Gutiérrez-González 1980).

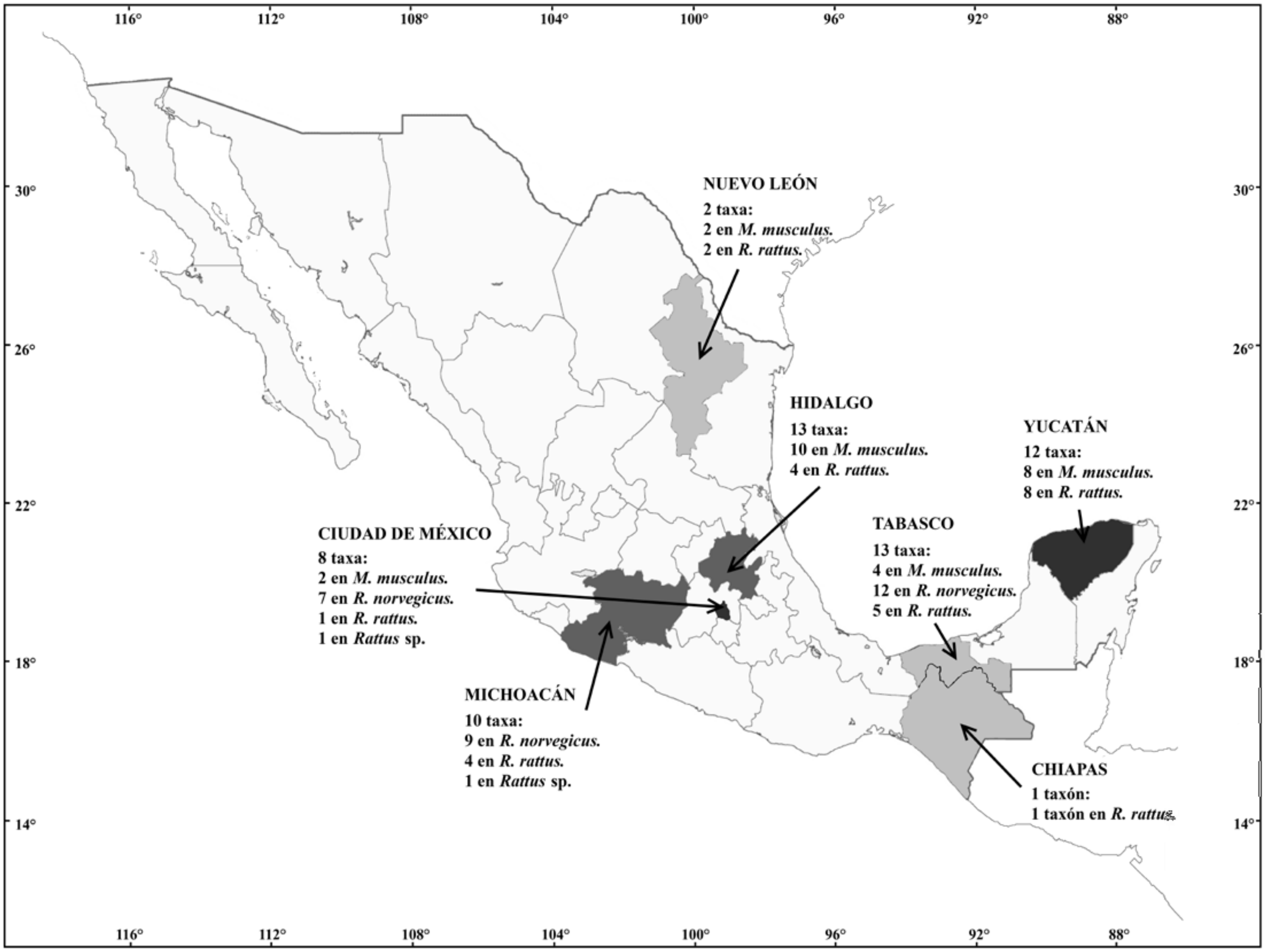

Figura 1. Número de estudios y taxa de helmintos reportados en roedores múridos invasores en siete estados de México. Gris claro, estados con un estudio; gris oscuro, estados con tres estudios; negro, estados con cinco o seis estudios.

Hymenolepididae Ariola, 1899

Hymenolepididae gen. sp. Intestino (adultos).

Chiapas: Pueblo Nuevo, *R. rattus*, HWML (31364), no se reportan datos de prevalencia (García-Prieto *et al.* 2012).

Hymenolepis diminuta (Rudolphi, 1819)

Intestino delgado (adultos), heces (huevos). Ciudad de México: no se reporta la localidad, *R. norvegicus*, CNHE (451), no se reportan datos de prevalencia (Caballero y Caballero 1939); Bosque de Chapultepec y rastro de la Ciudad de México, *R. norvegicus*, CNHE (453, 454), no se reportan datos de prevalencia (Cerecero 1943b). Michoacán: Ciudad de Morelia, *R. norvegicus*, CNHE (1316), no se reportan datos de prevalencia (Hierro-Huerta 1992); Ciudad de Morelia, *R. norvegicus* y *R. rattus*, no se reportan datos de material en colección y de prevalencia (Tay Zavala *et al.* 1999). Tabasco: Ciudad de Villahermosa, *R. norvegicus*, no se reportan datos de material en colección, 13 % (3/23; Cigarroa-Toledo *et al.* 2017). Yucatán: San José Tecoh y Plan de Ayala Sur, Ciudad de Mérida, *R. rattus*, no se reportan datos de material en colección, 14.3 % (12/84; Panti-May *et al.* 2017); comunidad de Opichén, Opichén, *M. musculus* y *R. rattus*, no se reportan datos de material en colección, 1.9 % (1/52) y 23.1 % (3/13; Panti-May *et al.* 2017); comunidad de Xkalakdzonot, Chankom, *R. rattus*, MLP-He (7423) y CNHE (10699), 15.2 % (18/118; Panti-May *et al.* 2018).

Rodentolepis microstoma (Dujardin, 1845)

Ductos biliares (adultos). Yucatán: Comunidad de Paraíso, Maxcanú, *M. musculus*, MLP-He (7424) y CNHE (10700), 4.4 % (7/159; Panti-May *et al.* 2018).

Rodentolepis nana (von Siebold, 1852)

Intestino delgado (adultos), heces (huevos). Ciudad de México: No se reporta la localidad, *R. norvegicus*, CNHE (379, 398-400), no se reportan datos de prevalencia (Caballero y Caballero 1939); Bosque de Chapultepec y rastro de la Ciudad de México, *R. norvegicus*, CNHE (450), no se reportan datos de prevalencia (Cerecero 1943b); no se reporta la localidad, *M. musculus*, CNHE (381), no se reportan datos de prevalencia (García-Prieto 1986). Hidalgo: Meztitlán y San Cristóbal, Reserva de la Biosfera Barranca de Metztitlán, *M. musculus*, CHE-UAEH (F00006), 33.3 % (7/21; Pulido-Flores *et al.* 2005). Michoacán: Ciudad de Morelia, *R. norvegicus*, CNHE (1321), no se reportan datos de prevalencia (Hierro-Huerta 1992); Ciudad de Morelia, *R. rattus*, no se reportan datos de material en colección y de prevalencia (Tay Zavala *et al.* 1999). Tabasco: Ciudad de Villahermosa, *R. norvegicus* y *R. rattus*, no se reportan datos de material en colección, 30.4 % (7/23) y 9.1 % (1/11; Cigarroa-Toledo *et al.* 2017).

Taeniidae Ludwing, 1886

Hydatigera taeniaeformis (Batsch, 1786)

Hígado (larvas). Ciudad de México: No se reporta la localidad, *R. norvegicus*, CNHE (460, 464, 471, 472), no se reportan datos de prevalencia (Caballero y Caballero 1939); no se reporta la localidad, *M. musculus*, CNHE (685), no se reportan datos de prevalencia (García-Prieto *et al.* 2012). Hidalgo: San Cristóbal, Reserva de la Biosfera Barranca de Metztitlán, *R. rattus*, CHE-UAEH (P00027), 50 % (2/4; Pulido-Flores *et al.* 2005). Michoacán: Ciudad de Morelia, *R. norvegicus*, CNHE (1320), no se reportan datos de prevalencia (Hierro-Huerta 1992). Yucatán: Comunidad de Molas, Mérida, *M. musculus* y *R. rattus*, no se reportan datos de material en colección, 9 % (29/322) y 3.5 % (2/57; Rodríguez-Vivas *et al.* 2011); comunidad de Molas, Mérida, *M. musculus* y *R. rattus*, MLP-He (6684), 9.7 % (15/154) y 4.3 % (2/46; Panti-May *et al.* 2015); San José Tecoh y Plan de Ayala Sur, Ciudad de Mérida, *M. musculus* y *R. rattus*, no se reportan datos de material en colección, 5 % (6/121) y 14.3 % (12/84; Panti-May *et al.* 2017); comunidad de Opichén, Opichén, *M. musculus* y *R. rattus*, no se reportan datos de material en colección, 17.3 % (9/52) y 15.4 % (2/13; Panti-May *et al.* 2017); comunidad de Xkalakdzonot, Chankom, *M. musculus* y *R. rattus*, CNHE (10701), 1.3 % (1/74) y 28.8 % (34/118; Panti-May *et al.* 2018); comunidad de Paraíso, Maxcanú, *M. musculus*, CNHE (10702), 6.9 % (11/159; Panti-May *et al.* 2018); comunidad de Cenotillo, Cenotillo, *R. rattus*, no se reportan datos de material en colección, 18.5 % (12/65; Medina-Pinto *et al.* 2019).

Taenia sp.

Dermis, hígado (larvas). Hidalgo: Huasca, *M. musculus*, no se reportan datos de material en colección y de prevalencia (Falcón-Ordaz *et al.* 2015).

Acanthocephala (Rudolphi, 1808)

Moniliformidae Van Cleave, 1924

Moniliformis moniliformis (Bremser, 1811)

Intestino delgado (adultos), heces (huevos). Michoacán: Ciudad de Morelia, *R. norvegicus*, CNHE (617), no se reportan datos de prevalencia (Hierro-Huerta 1992); Ciudad de Morelia, *R. norvegicus* y *R. rattus*, no se reportan datos de material en colección y de prevalencia (Tay Zavala *et al.* 1999). Tabasco: Ciudad de Villahermosa, *R. norvegicus*, no se reportan dados de material en colección, 8.7 % (2/23; Cigarroa-Toledo *et al.* 2017).

Oligacanthorhynchidae Southwell y MacFie, 1925

Oligacanthorhynchidae gen. sp.

Intestino delgado (adultos). Yucatán: Comunidad de Xkalakdzonot, Chankom, *R. rattus*, MLP-He (7426), 0.8 % (1/118; Panti-May *et al.* 2018).

Nematoda Rudolphi, 1808

Ascarididae Baird, 1853

Toxocara sp.

Heces (huevos). Tabasco: Ciudad de Villahermosa, *R. norvegicus*, no se reportan datos de material en colección, 4.3 % (1/23; Cigarroa-Toledo *et al.* 2017).

Gongylonematidae (Hall, 1916)

Gongylonema neoplasticum (Fibiger y Ditlevsen, 1914).

Estómago (adultos). Michoacán: Ciudad de Morelia, *R. norvegicus*, CNHE (2392), no se reportan datos de prevalencia (Hierro-Huerta 1992). Yucatán: Comunidad de Xkalakdzonot, Chankom, *M. musculus* y *R. rattus*, MLP-He (7438, 7441) y CNHE (10710), 6.7 % (5/75) y 11.9 % (14/118; Panti-May *et al.* 2018).

Gongylonema sp.

Estómago (adultos). Hidalgo: Meztitlán, Reserva de la Biosfera Barranca de Metztitlán, *M. musculus*, CHE-UAEH (F00002), 33.3 % (1/3; Pulido-Flores *et al.* 2005).

Heligmonellidae (Skrjabin y Schikhobalova, 1952)

Hassalstrongylus aduncus (Chandler, 1932).

Intestino delgado (adultos). Yucatán: Comunidad de Xkalakdzonot, *R. rattus*, MLP-He (7431) y CNHE (10713), 1.7 % (2/118; Panti-May *et al.* 2018).

Hassalstrongylus musculi (Dickmans, 1935).

Intestino delgado (adultos). Yucatán: Comunidad de Paraíso, Maxcanú, *M. musculus*, MLP-He (7432) y CNHE (10711), 17 % (27/159; Panti-May *et al.* 2018); Rancho Kuncheil, Sotuta, *M. musculus*, CNHE (11050), 100 % (5/5; presente estudio).

Nippostrongylus brasiliensis (Travassos, 1914).

Intestino delgado (adultos), heces (huevos). Hidalgo: San Cristóbal, Reserva de la Biosfera Barranca de Metztitlán, *R. rattus*, CHE-UARH (F00007) y CNHE (5320), 100 % (4/4; Pulido-Flores *et al.* 2005). Michoacán: Ciudad de Morelia, *R. norvegicus*, CNHE (2391), no se reportan datos de prevalencia (Hierro-Huerta 1992). Tabasco: Ciudad de Villahermosa, *M. musculus*, *R. norvegicus* y *R. rattus*, no se reportan datos de material en colección, 6.2 % (1/16), 34.8 % (8/23) y 18.2 % (2/11; Cigarroa-Toledo *et al.* 2017). Yucatán: Comunidad de Molas, Mérida, *M. musculus* y *R. rattus*, MLP-He (6687), 81.2 % (125/154) y 43.5 % (20/46; Panti-May *et al.* 2015); San José Tecoh y Plan de Ayala Sur, Ciudad de Mérida, *M. musculus* y *R. rattus*, no se reportan datos de material en colección, 28.1 % (34/121) y 4.8 % (4/84; Panti-May *et al.* 2017); comunidad de Opichén, Opichén, *M. musculus* y *R. rattus*, no se reportan datos de material en colección, 21.1 % (11/52) y 7.7 % (1/13; Panti-May *et al.* 2017); comunidad de Paraíso, Maxcanú, *R. rattus*, MLP-He (7433) y CNHE (10712), 14.3 % (1/7; Panti-May *et al.* 2018).

Heligmosomidae (Travassos, 1914)

Heligmosomoides polygyrus (Dujardin, 1845).

Heces (huevos). Tabasco: Ciudad de Villahermosa, *R. norvegicus* y *R. rattus*, no se reportan datos de material en colección, 56.5 % (13/23) y 36.4 % (4/11; Cigarroa-Toledo *et al.* 2017).

Longistriata sp.

Intestino (adultos). Nuevo León: Casas Blancas, el Brasil y la Perla, Apodaca, *M. musculus* y *R. rattus*, no se reportan datos de material en colección y de prevalencia (Gutiérrez-González 1980).

Heterakidae Ralliet y Henry, 1914

Heterakis spumosa Schneider, 1866.

Intestino grueso (adultos), estómago (adultos), heces (huevos). Ciudad de México: no se reporta la localidad, *R. norvegicus*, CNHE (2003), no se reportan datos de prevalencia (Caballero y Caballero 1939); Bosque de Chapultepec, *R. norvegicus*, CNHE (2001, 2002), no se reportan datos de prevalencia (García-Prieto *et al.* 2012). Michoacán: Ciudad de Morelia, *R. norvegicus*, CNHE (2393), no se reportan datos de prevalencia (Hierro-Huerta 1992). Tabasco: Ciudad de Villahermosa, *R. norvegicus*, no se reportan datos de material en colección, 13 % (3/23; Cigarroa-Toledo *et al.* 2017).

Heteroxynematidae Skrjabin y Shikhobalova, 1948

Aspiculuris huascaensis Falcón-Ordáz, Monks y Pulido-Flores, 2010.

Ciego (adultos). Hidalgo: Cerro del Tezontle y San Juan Hueyapan, Huasca de Ocampo, *M. musculus*, CNHE (6395-7) y HWML (64565), 13.8 % (4/29; Falcón-Ordaz *et al.* 2010).

Aspiculuris cf. *lahorica* Akhtar, 1955.

Ciego (adultos). Hidalgo: Meztitlán, Reserva de la Biosfera Barranca de Metztitlán, *M. musculus*, CHE-UAEH (F00001), 33.3 % (1/3; Pulido-Flores *et al.* 2005).

Aspiculuris sp.

Heces (huevos). Yucatán: San José Tecoh y Plan de Ayala Sur, Ciudad de Mérida, *R. rattus*, no se reportan datos de material en colección, 8.3 % (7/84; Panti-May *et al.* 2017).

Oxyuridae Cobbold, 1864

Syphacia muris (Yamaguti, 1935).

Intestino grueso (adultos), heces (huevos). Hidalgo: San Cristóbal, Reserva de la Biosfera Barranca de Metztitlán, *R. rattus*, CHE-UAEH (F00008) y CNHE (5319), 50 % (2/4; Pulido-Flores *et al.* 2005). Tabasco: Ciudad de Villahermosa, *R. rattus*, no se reportan datos de material en colección, 27.3 % (3/11; Cigarroa-Toledo *et al.* 2017). Yucatán: Comunidad de Molas, Mérida, *R. rattus*, MLP-He (6686), 17.4 % (8/46; Panti-May *et al.* 2015); San José Tecoh y Plan de Ayala Sur, Ciudad de Mérida, *R. rattus*, no se reportan datos de material en colección, 17.9 % (15/84; Panti-May *et al.* 2017); comunidad de Opichén, Opichén, *R. rattus*, no se reportan datos de material en colección, 15.4 % (2/13; Panti-May *et al.* 2017); comunidad de Xkalakdzonot, Chankom, *R. rattus*, MLP-He (7435) y CNHE (10706), 65.3 % (77/118; Panti-May *et al.* 2018).

Syphacia obvelata (Rudoplhi, 1802).

Intestino grueso (adultos), heces (huevos). Hidalgo: San Cristóbal y Metztitlán, Reserva de la Biosfera Barranca de Metztitlán, *M. musculus*, CHE-UAEH (F00003) y CNHE (5318), 28.6 % (6/21; Pulido-Flores *et al.* 2005). Tabasco: Ciudad de Villahermosa, *M. musculus* y *R. norvegicus*, no se reportan datos de material en colección, 6.2 % (1/16) y 4.3 % (1/23; Cigarroa-Toledo *et al.* 2017). Yucatán: Comunidad de Opichén, Opichén, *M. musculus*, no se reportan datos de material en colección, 9.6 % (5/52; Panti-May *et al.* 2017); comunidad de Paraíso, Maxcanú, *M. musculus*, MLP-He (7434) y CNHE (10708), 3.8 % (6/159; Panti-May *et al.* 2018).

Syphacia sp. Ciego (adultos).

Hidalgo: Cerro del Tezontle y San Juan Hueyapan, Huasca de Ocampo, *M. musculus* y *R. rattus*, no se reportan datos de material en colección y de prevalencia (Falcón-Ordaz *et al.* 2015).

Spirocercidae (Chitwood y Wehr, 1933)

Mastophorus muris (Gmelin, 1790).

Estómago (adultos), heces (huevos). Michoacán: Uruapan, *Rattus* sp., USNM (1345984), no se reportan datos de prevalencia (García-Prieto *et al.* 2012). Tabasco: Ciudad de Villahermosa, *R. norvegicus*, no se reportan datos de material en colección, 17.4 % (4/23; Cigarroa-Toledo *et al.* 2017).

Strongyloididae Chitwood y McIntosh, 1934

Strongyloides ratti Sandground, 1925.

Heces (huevos). Tabasco: Ciudad de Villahermosa, *M.*

musculus, *R. norvegicus* y *R. rattus*, no se reportan datos de material en colección, 12.5 % (2/16), 65.2 % (15/23) y 45.5 % (5/11; Cigarroa-Toledo *et al.* 2017).

Trichosomoides crassicauda (Bellingham, 1840).

Heces (huevos). Tabasco: Ciudad de Villahermosa, *R. norvegicus*, no se reportan datos de material en colección, 8.7 % (2/23; Cigarroa-Toledo *et al.* 2017).

Trichuridae (Ransom, 1911)

Capillaria sp.

Vejiga urinaria (adultos). Michoacán: Ciudad de Morelia, *R. norvegicus*, CNHE (2394), no se reportan datos de prevalencia (Hierro-Huerta 1992).

Trichinella spiralis (Owen, 1835).

Músculo (larvas). Michoacán: Ciudad de Morelia, *R. norvegicus* y *R. rattus*, no se reportan datos de material en colección y de prevalencia (Tay Zavala *et al.* 1999).

Trichinella sp. Músculo (larvas).

Hidalgo: San Cristóbal, Reserva de la Biosfera Barranca de Metztitlán, *M. musculus*, CHE-UAEH (F00004), 5.5 % (1/18; Pulido-Flores *et al.* 2005).

Trichuris muris (Schrank, 1788).

Intestino, ciego (adultos), heces (huevos). Ciudad de México: Bosque de Chapultepec, *R. norvegicus*, CNHE (2399), no se reportan datos de prevalencia (Cerecero 1943b); no se reporta la localidad, *R. rattus*, CNHE (2247), no se reportan datos de prevalencia (García-Prieto *et al.* 2012). Hidalgo: San Cristóbal y Meztitlán, Reserva de la Biosfera Barranca de Metztitlán, *M. musculus*, CNHE (5316) y CHE-UAEH (F00005), 9.5 % (2/21; Pulido-Flores *et al.* 2005). Tabasco: Ciudad de Villahermosa, *M. musculus* y *R. norvegicus*, no se reportan datos de material en colección, 6.3 % (1/16) y 13 % (3/23; Cigarroa-Toledo *et al.* 2017). Yucatán: Comunidad de Molas, Mérida, *M. musculus*, MLP-He (6685), 11.7 % (18/154; Panti-May *et al.* 2015); San José Tecoh y Plan de Ayala Sur, Ciudad de Mérida, *M. musculus*, no se reportan datos de material en colección, 13.2 % (16/121; Panti-May *et al.* 2017); comunidad de Opichén, Opichén, *M. musculus*, no se reportan datos de material en colección, 32.7 % (17/52; Panti-May *et al.* 2017); comunidad de Paraíso, Maxcanú, *M. musculus*, MLP-He (7427) y CNHE (10703), 15.1 % (24/159; Panti-May *et al.* 2018).

Trichuris sp.

Ciego (adultos). Hidalgo: Cerro del Tezontle y San Juan Hueyapan, Huasca de Ocampo, *M. musculus*, no se reportan datos de material en colección y de prevalencia (Falcón-Ordaz *et al.* 2015).

Discusión

Los roedores múridos invasores están presentes a lo largo del territorio nacional en áreas rurales, urbanas e incluso en áreas naturales como islas. Los estudios helmintológicos en estos roedores han sido escasos en comparación, por ejemplo, con aquellos realizados con roedores cricétidos o heterómidos (ver listados helmintológicos de García-Prieto *et al.* 2012; Preissar y Falcón-Ordaz 2019). La presente revisión actualiza el número de helmintos registrados en roedores múridos a 35 taxa identificados en siete estados de México, incluyendo tremátodos, céstodos, acantocéfalos y nematodos. De éstos, 12 fueron reportados posteriormente a la publicación del listado helmintológico de García-Prieto *et al.* (2012). Para *M. musculus*, *R. norvegicus* y *R. rattus*, el número de taxa de helmintos se actualizó a 19, 16 y 18, respectivamente. Anteriormente, García-Prieto *et al.* (2012) reportaron 10 taxa para *Mus* y 12 para *Rattus*. A pesar de esto, es probable que el número de especies de helmintos que estos roedores albergan esté subestimado debido al escaso número de estudios realizados en estos hospedadores, el número de especímenes y especies de hospedadores examinados, así como el número de taxa que no han sido identificados a nivel especie.

La mayoría de las especies de helmintos registradas en los roedores múridos en México son específicas de estos roedores, y por lo general ocasionan infecciones leves no patogénicas y no causan signos clínicos en los animales (Taffs 1976; Baker 2007). Sin embargo, algunas especies de helmintos pueden ser introducidas con sus hospedadores invasores y afectar negativamente la salud de hospedadores no naturales como los roedores silvestres. *Hydatigera taeniaeformis*, un parásito del gato doméstico (hospedador definitivo) y roedores múridos (hospedadores intermediarios), ha sido mencionado como el causante de muertes masivas en *Ondrata zibethicus* en Republica Checa (Dvorakova y Prokopic 1984). En México, esta especie ha sido reportada en los roedores silvestres *Sigmodon hispidus* en Nuevo León (Gutiérrez-González 1980), *S. toltecus* en Yucatán (Panti-May *et al.* 2018) y en *Sciurus yucatanensis* en Yucatán (Panti-May *et al.* 2019) usualmente con intensidades de infección mayores (intensidad media 19.7, rango 1-50, Panti-May *et al.* 2018, 2019) a las reportadas en roedores múridos (intensidad media 1.1-1.5, rango 1-4; Rodríguez-Vivas *et al.* 2011; Panti-May *et al.* 2015; 2018; Medina-Pinto *et al.* 2019), debido probablemente a una respuesta inmune más fuerte de los roedores múridos hacia este cestodo (Rodríguez-Vivas *et al.* 2011). También, se ha reportado la introducción de *T. muris* con su hospedador *R. rattus* en localidades donde viven poblaciones de *Peromyscus maniculatus*, en las Islas del Mar de Cortés (Smith y Carpenter 2006), aunque sin estudios del impacto en la salud de los roedores silvestres.

El listado helmintológico de roedores múridos invasores en México mostró una mayor diversidad de nematodos en comparación con los tremátodos, céstodos o acantocéfalos. Esto puede estar relacionado con el ciclo de vida directo de la mayoría de las especies de nematodos registradas, con pocas excepciones como *G. neoplasticum* y *T. spiralis* que tiene un ciclo de vida indirecto. En parásitos con ciclo de vida directo, la infección y distribución de los parásitos está determinada por factores micro-ambientales favorables (*e. g.*, temperatura, humedad) para los estadios infectantes, así como la distribución y abundancia de los hospedadores (Arneberg 2001; Wilson *et al.* 2002). La mayor diversidad de nematodos puede estar relacionada con las características estables de los asentamientos humanos que favorecen la transmisión de los nematodos en las poblaciones roedores múridos que generalmente son abundantes. En contraste, las especies de céstodos (con excepción de *R. nana*) y acantocéfalos reportadas requieren de un hospedador intermediario (*e. g.*, escarabajos, cucarachas, pulgas, e incluso roedores) para completar su ciclo de vida, y los tremátodos requieren de dos hospedadores intermediarios (*e. g.*, moluscos y anfibios). Considerando que factores como la abundancia de los hospedadores intermediarios y hábitos alimenticios de los hospedadores definitivos afectan la transmisión de los helmintos con ciclo de vida indirecto (Krasnov *et al.* 2006), es posible que en las áreas muestreadas estos factores presenten variaciones marcadas que promuevan una baja transmisión para algunas especies de tremátodos, céstodos o acantocéfalos. Sin embargo, es necesario estudiar los múltiples factores que determinan los patrones de infección de los helmintos que parasitan roedores múridos en México.

Aunque el listado de helmintos que parasitan roedores múridos invasores en México incluye más de 30 taxa, la mayoría de éstos presentan pocos registros. Considerando el número de especies de hospedadores infectadas y la distribución en el país, siete especies de helmintos podrían considerase como las más frecuentes, incluyendo céstodos (*H. taeniaeformis*, *H. diminuta* y *R. nana*) y nematodos (*N. brasiliensis*, *S. obvelata*, *S. muris* y *T. muris*). Estas especies han sido reportadas también como frecuentes en estos roedores en distintas regiones del mundo (Oldham 1931; McKenna 1997; Gliga *et al.* 2020).

Los registros helmintológicos mostraron una asimetría en el número de registros y diversidad de helmintos entre las tres especies de roedores de los siete estados (ver Figura 1). Por ejemplo, en la única especie, *R. rattus*, con registros en los siete estados, el número de especies de helmintos varió de uno a siete considerando los diferentes estudios, y de uno a ocho considerando los estados. Estas variaciones pueden deberse a múltiples factores como el número de estudios y hospedadores examinados, el periodo de colecta, así como la abundancia de los hospedadores (intermediarios y definitivos) y características micro-ambientales presentes en las áreas de colecta (selvas, ranchos, comunidades rurales, ciudades).

Uno de los motivos que ha favorecido el incremento de los estudios parasitológicos en los roedores múridos invasores en los últimos años, es la investigación sobre patógenos zoonóticos (*e. g.*, helmintos) presentes en sus poblaciones sinantrópicas. En México, los estudios helmintológicos han reportado en los roedores algunas especies de tremátodos (*E. revolutum*), céstodos (*H. diminuta*, *R. nana*, *R. microstoma*, *H. taeniaeformis*), acantocéfalos (*M. moniliformis*) y nematodos (*T. spiralis*) que pueden transmitirse directa o indirectamente a los humanos (Macnish *et al.* 2003; Larrieu *et al.* 2004; Salehabadi *et al.* 2008; Sohn *et al.* 2011; Panti-May et al. 2020a; b). Entre estos resaltan los hymenolepididos *R. nana* e *H. diminuta* que causan la hymenolepiasis humana, principalmente en niños de áreas con carencias socioeconómicas. El primero es el céstodo más común en humanos con una prevalencia mundial estimada de 4 % (Bogitsh *et al.* 2012), mientras que el segundo cestodo tiene más de 500 registros en humanos entre 2000-2018 (Panti-May *et al.* 2020b). La relevancia de estos hospedadores en asentamientos humanos como ciudades o comunidades rurales se debe a sus poblaciones abundantes y el contacto frecuente con los habitantes, lo que puede favorecer la transmisión de patógenos zoonóticos (Panti-May *et al.* 2012).

Basados en el listado de helmintos registrados en *M. musculus*, *R. norvegicus* y *R. rattus*, es evidente que la diversidad de especies de helmintos en roedores múridos invasores en México aún está subestimada. En menos del 25 % de los estados de México se han estudiado los helmintos de estos roedores, y además, el número de hospedadores y localidades muestreados en cada estudio han sido heterogéneos. Es necesario realizar más estudios para incrementar el conocimiento de los helmintos que albergan los roedores múridos invasores, así como su distribución en el país, en especial considerando la presencia de especies zoonóticas.

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Literatura citada

Arneberg, P. 2001. An ecological law and its macroecological consequences as revealed by studies of relationships between host densities and parasite prevalence. Ecography 24:352-358.

Baker, D. G. 2007. Parasites of rats and mice. Pp. 303-397, *in* Flynn's parasites of laboratory animals (Baker, D. G., ed.). Blackwell Publishing. Iowa, U.S.A.

Bogitsh, B. J., C. E. Carter, y T. N. Oeltmann. 2012. Human parasitology. Academic Press. New York, U.S.A.

Burgin, C. J., J. P. Colella, P. L. Kahn, y N. S. Upham. 2018. How many species of mammals are there? Journal of Mammalogy 99:1-14.

Caballero y Caballero, E. 1939. Algunos endoparásitos de *Rattus rattus norvegicus* y de *Rattus norvegicus albinus*, del Laboratorio de Investigaciones Médicas del Hospital General de la Ciudad de México. Anales del Instituto de Biología 10:285-291.

Cerecero, M. C. 1943a. Algunos helmintos de las ratas domésticas y silvestres de México con descripción de dos nuevas especies. Lic. Tesis, Facultad de Ciencias, Universidad Nacional Autónoma de México, México.

Cerecero, M. C. 1943b. Algunos trematodos de las ratas domésticas de la Ciudad de México. Anales del Instituto de Biología 14:507-526.

Chitwood, B. G. 1938. Some nematodes from the caves of Yucatan. Publications of Carnegie Institute of Washington 491:51-66.

Cigarroa-Toledo, N., Y. Santos-Martinez, C. V. Zaragoza-Vera, M. M. Garcia-Rodriguez, C. M. Baak-Baak, C. Machain-Williams, J. E. Garcia-Rejon, J. A. Panti-May, y O. M. Torres-Chable. 2017. Occurrence of gastrointestinal helminths in commensal rodents from Tabasco, Mexico. Helminthologia 54:170-173.

Dvotakova, L., y J. Prokopic. 1984. *Hydatigera taeniaeformis* (Batsch, 1786) as the cause of mass deaths of muskrats. Folia Parasitologica 31:127-131.

Falcón-Ordaz, J., S. Monks, G. Pulido-Flores, L. García-Prieto, y G. Lira-Guerrero. 2015. Riqueza de helmintos parásitos de vertebrados silvestres del estado de Hidalgo, México. Pp. 20–37, *in* Estudios de Biodiversidad: Volumen I (Pulido-Flores, G. , S. Monks y M. López-Herrera, eds.). Zea Books. Lincoln, U.S.A.

Falcón-Ordaz, J., G. Pulido-Flores, y S. Monks. 2010. New species of *Aspiculuris* (Nematoda: Heteroxynematidae), parasite of *Mus musculus* (Rodentia: Muridae), from Hidalgo, Mexico. Revista Mexicana de Biodiversidad 81:669-676.

García-Prieto, L. 1986. Estudio taxonómico de algunos céstodos de vertebrados de México. Lic. Tesis, Facultad de Ciencias, Universidad Nacional Autónoma de México, México.

García-Prieto, L., J. Falcón-Ordaz, y C. Guzmán-Cornejo. 2012. Helminth parasites of wild Mexican mammals: list of species, hosts and geographical distribution. Zootaxa 92:1-92.

Gliga, D. S., B. Pisanu, C. Walzer, y A. Desvars-Larrive. 2020. Helminths of urban rats in developed countries: a systematic review to identify research gaps. Parasitology Research 119:2383-2397.

Gutiérrez-González, J. 1980. Algunos helmintos parásitos en ratas silvestres de Apodaca, Nuevo León, México. Lic. Tesis, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, México.

Hierro-Huerta, P. 1992. Helmintofauna de la rata de alcantarilla *Rattus norvegicus* Erxleben 1777, de la Ciudad de Morelia, Michoacán, México. Lic. Tesis, Escuela de Biología, Universidad Michoacana de San Nicolás de Hidalgo, México.

Krasnov, B. R., R. Poulin, y S. Morand. 2006. Patterns of macroparasite diversity in small mammals. Pp. 197–231, *in* Micromammals and macroparasites: From evolutionary ecology to management (Morand, S., B. R. Krasnov y R. Poulin, eds.). Springer. Tokyo, Japan.

Larrieu, E., V. Molina, S. Albarracín, S. Mancini, R. Bigatti, L. Ledesma, C. Chiosso, S. Krivokapich, E. Herero, y E. Guarnera. 2004. Porcine and rodent infection with *Trichinella*, in the Sierra Grande area of Rio Negro province, Argentina. Annals of Tropical Medicine and Parasitology 98:725-731.

Leitschuh, C. M., D. Kanavy, G. A. Backus, R. X. Valdez, M., Serr, E. A. Pitts, D. Threadgill, y J. Godwin. 2018. Developing gene drive technologies to eradicate invasive rodents from islands. Journal of Responsible Innovation 5:S121-S138.

Macnish, M. G., U. M. Ryan, J. M. Behnke, y R. C. A. Thompson. 2003. Detection of the rodent tapeworm *Rodentolepis* (=*Hymenolepis*) *microstoma* in humans. A new zoonosis? International Journal for Parasitology 33:1079-1085.

Mckenna, P. B. 1997. Checklist of helminth parasites of terrestrial mammals in New Zealand. New Zealand Journal of Zoology 24:277-290.

Medina-Pinto, R. A., M. A. Torres-Castro, R. A. Medina-Pinto, M. E. Bolio-González, y R. I. Rodríguez-Vivas. 2019. Natural *Cysticercus fasciolaris* infection in rodents from a rural area in Yucatan, Mexico. Veterinaria México 6:1–10.

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

Ochoterena, I., y E. Caballero y Caballero. 1932. Filaria parásita de las ratas de campo *Micropleura sigmodoni* spec., nov. Anales del Instituto de Biología 3:123-125.

Oldham, J. N. 1931. The helminth parasites of common rats. Journal of Helminthology 9:49-90.

Panti-May, J. A., M. C. Digiani, E. E. Palomo-Arjona, Y. M. Gurubel-Gonzalez, G. T. Navone, C. Machain-Williams, S. F. Hernández-Betancourt, y M. R. Robles. 2018. A checklist of the helminth parasites of sympatric rodents from two Mayan villages in Yucatán, México. Zootaxa 4403:495-512.

Panti-May, J. A., E. E. Palomo-Arjona, Y. M. Gurubel-Gonzalez, M. A. Torres-Castro, M. V. Vidal-Martínez, C. Machain-Williams, S. F. Hernández-Betancourt, y M. R. Robles. 2017. New host, geographical records, and factors affecting the prevalence of helminths infection from synanthropic rodents in Yucatán, Mexico. Helminthologia 54:231-239.

Panti-May, J. A., A. Servían, W. Ferreri, M. L. Zonta, D. I. Hernández-Mena, S. F. Hernández-Betancourt, M. R. Robles, y C. Machain-Williams. 2020a. Morphological and molecular identification of hymenolepidid cestodes in children and synanthropic rodents from rural Mexico. Parasitology International 75:102042.

Panti-May, J. A., S. F. Hernández-Betancourt, y L. García-Prieto. 2019. *Hydatigera taeniaeformis* (Cestoda: Taeniidae) in the Yucatán squirrel *Sciurus yucatanensis* (Rodentia: Sciuridae), Mexico. Therya 10:179-182.

Panti-May, J. A., S. F. Hernández-Betancourt, R. I. Rodríguez-Vivas, y M. R. Robles. 2015. Infection levels of intestinal helminths in two commensal rodent species from rural households in Yucatan, Mexico. Journal of Helminthology 89:42-48.

Panti-May, J. A., S. Hernández-Betancourt, H. Ruíz-Piña, y S. Medina-Peralta. 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., R. I. Rodríguez-Vivas, L. García-Prieto, A. Servián, y F. Costa. 2020b. Worldwide overview of human infections with *Hymenolepis diminuta*. Parasitology Research 119:1997-2004.

Preisser, W. C., y J. Falcón-Ordaz. 2019. A checklist of the parasitic helminths of cricetid and heteromyid rodents in Mexico. Therya 10:329-341.

Pulido-Flores, G., S. Moreno-Flores, y S. Monks. 2005. Helminths of rodents (Rodentia: Muridae) from Metztitlán, San Cristobal, and Rancho Santa Elena, Hidalgo, Mexico. Comparative Parasitology 72:186-192.

Rodríguez-Vivas, R. I., J. A. Panti-May, J. Parada-López, S. F. Hernández-Betancourt, y H. Ruiz-Piña. 2011. The occurrence of the larval cestode *Cysticercus fasciolaris* in rodent populations from the Cuxtal ecological reserve, Yucatan, Mexico. Journal of Helminthology 85:458-461.

Salehabadi, A., G. Mowlavi, y S. M. Sadjjadi. 2008. Human infection with *Moniliformis moniliformis* (Bremser 1811) (Travassos 1915) in Iran: another case report after three decades. Vector-Borne and Zoonotic Diseases 8:101-104.

Singleton, G. R., S. Belmain, P. R. Brown, K. Aplin, y N. M. Htwe. 2010. Impacts of rodent outbreaks on food security in Asia. Wildlife Research 37:355-359.

Smith, K. F., K. F. Smith, S. M. Carpenter, y S. M. Carpenter. 2006. Potential spread of introduced black rat (*Rattus rattus*) parasites to endemic deer mice (*Peromyscus maniculatus*) on the California Channel Islands. Diversity and Distributions:742-748.

Sohn, W. M., J. Y. Chai, T. S. Yong, K S. Eom, C. H. Yoon, M. Sinuon, D. Socheat, y S. H. Lee. 2011. *Echinostoma revolutum* infection in children, Pursat Province, Cambodia. Emerging Infectious Diseases 17:117-119.

Stenseth, N. C., H. Leirs, A. Skonhoft, S. A. Davis, R. P. Pech, H. P. Andreassen, G. R. Singleton, M. Lima, R. M. Machangu, R. H. Makundi, Z. Zhang, P. B. Brown, D. Shi, y X. Wan. 2003. Mice and rats: the dynamics and bio- economics of agricultural rodents pests. Frontiers in Ecology and the Environment 1:367-375.

Taffs, L. F. 1976. Pinworm infections in laboratory rodents: a review. Laboratory Animals 10:1-13.

Tay Zavala, J., T. Alvarez, M. Gutiérrez Quiroz, J. Sánchez Vega, y A. Fenández Presas. 1999. Zoonosis por helmintos en mamíferos de Morelia, Michoacán, República Mexicana. Revista de la Facultad de Medicina 42:64-65.

Wilson, K., O.N. Bjornstad, A.P. Dobson, S. Merler, G. Poglayen, S.E. Randolph, y A. Skorping. 2002. Heterogeneities in macroparasite infections: patterns and processes. Pp. 6–44, *in* The Ecology of Wildlife Diseases (Hudson, P. J., A. Rizzoli, B. T. Grenfell, H. Heesterbeek y A. P. Dobson, eds.). Oxford University Press. New York, U.S.A.

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Neotropical otter diet variation between a lentic and a lotic systems

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Neotropical otters have been scarcely studied at the northern edge of their distribution in Mexico. The species has nocturnal-twilight habits, and their principal food is fish and crustaceans. The aim of this work was to know the foraging habits of Neotropical otters (*Lontra longicaudis*) in two monitoring sites one lentic and the other one lotic systems of the Río San Lorenzo, Sinaloa México. From February 2009 to January 2010, we surveyed the area during the four seasons by walking 15 km of the river edge of Río San Lorenzo, and 15 km at the El Comedero reservoir. We collected 318 scats, 155 in the reservoir and 163 in the river. Fish were the most abundant prey item in both places, with a frequency of occurrence of 98.1 % in the river and 100 % in the reservoir. The percentage of occurrence varied largely in the other prey items in both places. Seven taxonomic groups compose their foraging habits in the river (relative frequency in parentheses): fish (64.3 %), insects (9.2 %), mollusks (6.8 %), birds (6.0 %), mammals (5.6 %), crustaceans (5.2 %) and fruits (2.8 %). Five taxonomic groups in the reservoir compose it: fish (65.1 %), birds (22.7 %), mammals (9.2 %), fruits (2.5 %) and insects (0.4 %). The most important fish in the diet belonged to the genus *Oreochromis*, which includes an introduced species. We conclude that the Neotropical otter is an opportunistic carnivore that preys mainly on fish, probably due to their high availability, but they also forage in other taxa that are available at Río San Lorenzo basin.

Las nutrias neotropicales han sido poco estudiadas en el norte de su distribución en México. Esta especie tiene hábitos nocturno-crepusculares, y su principal alimento consiste en peces y crustáceos. El objetivo de este trabajo fue conocer los hábitos alimentarios de la nutria neotropical (*Lontra longicaudis*) en dos sitios uno léntico y otro lótico del Río San Lorenzo, Sinaloa México. De febrero de 2009 a enero de 2010, se hicieron recorridos estacionales a pie, 15 km en el río San Lorenzo y 15 km en el embalse El Comedero. Se colectaron 318 heces, 155 en el embalse y 163 en el río. En ambos lugares los peces fueron la presa más abundante, con una frecuencia de ocurrencia de 98.1 % en el río y un 100 % en el embalse; para los grupos restantes los porcentajes variaron entre grupos y entre sitios. La alimentación se compuso de siete grupos taxonómicos en el río (entre paréntesis se presenta la frecuencia relativa): peces (64.3 %), insectos (9.2 %), moluscos (6.8 %), aves (6.0 %), mamíferos (5.6 %), crustáceos (5.2 %) y frutos (2.8 %); y de cinco grupos taxonómicos en el embalse: peces (65.1 %), aves (22.7 %), mamíferos (9.2 %), frutos (2.5%) e insectos (0.4%). Entre los peces, las presas más importantes correspondieron al género *Oreochromis*, entre las cuales existe una especie introducida. La nutria neotropical es un carnívoro oportunista, cuyo alimento principal lo constituyen los peces, probablemente debido a su alta disponibilidad y, en menor medida, se alimenta de otros taxones también disponibles en la cuenca del Río San Lorenzo.

Keywords: Foraging habits; *Lontra longicaudis;* Neotropical otters; Cosalá; Sinaloa.

Introduction

Energy intake through feeding is essential for metabolic processes like growth and individual physiological maintenance (Saint 2006). Poor nutrition leads to malnutrition, limiting growth in body size (Yom-Tov *et al.* 2006) and impacting individual performance.

In carnivores, feeding habits are drivers of social organization, habitat use, and reproductive rates, especially when food availability is seasonal (Braña *et al.* 1987). If food is abundant, the predator can become selective (Young *et al.* 2008), hunting for those preys that provide the highest amount of energy with the lowest searching effort (Stephens and Krebs 1986). By contrast, if food is scarce, prey diversity increases because predators feed on any prey available (Tinker *et al.* 2008).

There are different methods for studying the feeding habits of carnivores, including the analysis of scats, direct observations, collection of prey remains in feeders (this option yields limited results by not reflecting the whole range of prey), and stomach content analysis (highly invasive). Of these, scat analysis is the method most commonly used for being non-invasive, relatively easy to analyze, and posing no risk on endangered or threatened species (Marucco *et al.* 2008). Besides, scat analysis yields information on habitat use, hunting area, trophic niche, home range, behavior, parasitic fauna, heavy metal concentration, and genetic diversity, among other aspects (Chame 2003; Trinca *et al.* 2007; Josef *et al.* 2008; Weber *et al.* 2009; Ramos-Rosas *et al.* 2013; Guerrero-Flores *et al.* 2015; Hernández *et al.* 2018).

Otters are top predators in aquatic ecosystems (Smiroldo *et al.* 2009; Prigioni *et al.* 2006). *Lontra longicaudis*, one of the four species in the genus, is distributed from northern Mexico to southern Argentina (Gallo-Reynoso 1989; Eisenberg and Redford 1999; Perini *et al.* 2009; Kasper *et al.* 2008). The diet of the Neotropical otter have been studied extensively in recent years (Perez-Claros and Palmqvist 2008; Monroy-Vilchis and Mundo 2009; Gallo-Reynoso 1997; Gallo-Reynoso *et al.* 2008; Rangel-Aguilar and Gallo-Reynoso 2013), reporting the local availability of its potential prey, as well as the morphological, behavioral, and physiological adaptations allowing otters to locate, capture, and ingest biomass from a wide variety of species (Kok and Nel 2004). In general, these studies indicate that its main diet consists of fish and crustaceans (Gallo-Reynoso 1989; 1997; Macías-Sánchez 1999; Casariego-Madorell *et al.* 2006); however, this opportunistic predator occasionally feeds on reptiles, amphibians, insects, birds, and small mammals such as mice (Gallo-Reynoso 19899; Gallo-Reynoso *et al.* 2008), and even on insects (Rangel-Aguilar and Gallo-Reynoso 2013). Because of its relative selectivity, the Neotropical otter is considered a bioindicator of well-conserved aquatic ecosystems (Lodé 1993).

There are no specific studies on this species in Sinaloa. Its presence has been reported in Ahome and Escuinapa, El Verde estuary (Gallo-Reynoso and Navarro-Serment, personal observation 2002) and El Fuerte River and its dams (Gallo-Reynoso, unpublished data), as well as in Durango rivers flowing to Sinaloa, such as the San Diego river (Cruz *et al.* 2017) and Mezquital and San Pedro rivers (Servin *et al.* 2003; Charre-Medellín *et al.* 2011). Studies to define its current distribution and conservation status in Sinaloa are needed because this area is close to its northern distribution limit. This paper aims to determine the diet composition of the Neotropical otter at two locations in the State of Sinaloa, namely El Comedero dam a lentic system and the San Lorenzo River a lotic system, during one year to explore any variability in the otter's diet associated with the climatic seasons. This study will provide data on the distribution of the Neotropical otter and the characteristics of aquatic ecosystems in Sinaloa, which could be used in management plans for the conservation of the species and the maintenance of river water quality.

Materials and Methods

Study Area. The work was carried out on the San Lorenzo river that drains its waters into the José López Portillo dam (locally known as El Comedero), a reservoir shared between the States of Durango and Sinaloa. The San Lorenzo river basin is located in the Sierra Madre Occidental, running

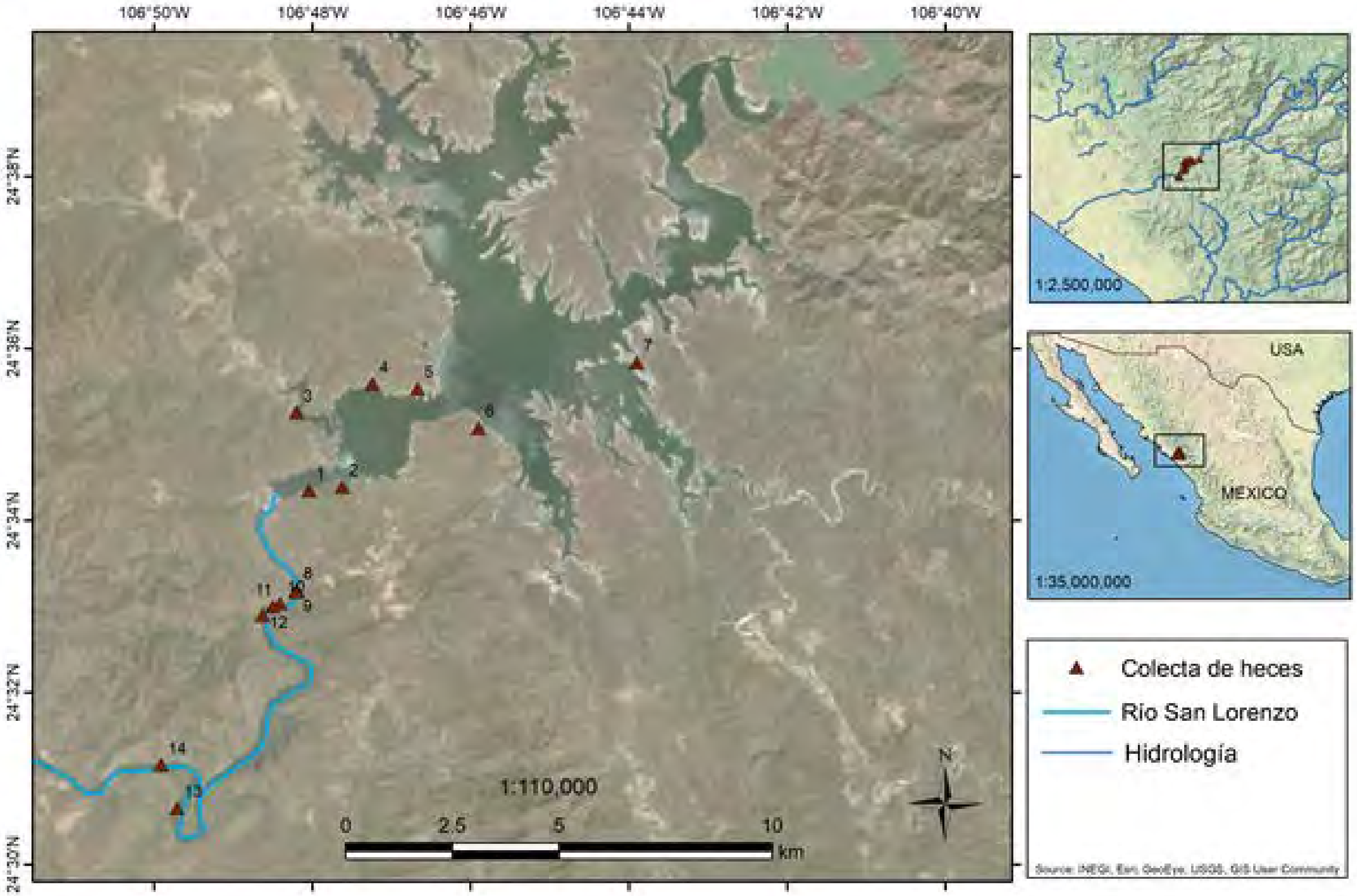

Figure 1. El Comedero dam (above) and San Lorenzo river (below - left) as the sites where scats of Neotropical otter were collected. Municipality of Cosalá, Sinaloa (map drafted by Gloria Ponce García).

across parts of Sinaloa and Durango and catching runoff waters from summer rains. Both study sites are located in the municipality of Cosalá, Sinaloa. The dam a lotic system, is located at an altitude of 258 meters above sea level, centered geographically at 24° 35′ 03.8″ N and -106° 45′ 53.4″ W; the study site on the San Lorenzo River a lotic system is located downstream at 168 meters above sea level, with a geographic center located at the coordinates 24° 33′ 07.4″ N and -106° 48′ 12.8″ W (Figure 1).

The dominant vegetation on the riverbanks corresponds to low deciduous forest (classification of Miranda and Hernández 1963) or deciduous tropical forest (classification of Rzedowski 1978). Its most representative species include mauto (*Lysiloma divaricata*), amapa (*Tabebuia palmeri*), palo blanco (*Ipomoea arboressen*), pochote (*Ceiba acuminata*), papelillo (*Bursera grandifolia*), Brazilwood (*Haematoxyllum brasiletto),* mora (*Chlorophora tinctoria*) and yellow rose (*Cochlopermum vitium*). Common animal species include carnivores such as coyote (*Canis latrans*), coatí (*Nasua narica*), raccoon (*Procyon lotor*), gray fox (*Urocyon cinereoargenteus*), *capixtle* (*Bassariscus astutus*), bobcat (*Lynx rufus*), cougar (*Puma concolor*), jaguar (*Panthera onca*), ocelot (*Leopardus pardalis*), margay (*Leopardus wiedii*), jaguarundi (*Puma yaguarundi*), and skunks (*Conepatus leuconotus*, *Mephitis macroura,* and *Spilogale* sp.). Ungulates include the white-tail deer (*Odocoileus virginianus*) and the necklace peccari (*Pecari tajacu*). Chachalaca (*Ortalis poliocephala*) and other birds are common to this type of vegetation. Riverbank vegetation includes *Ficus* and *Salix* as the most representative genera, as well as vines. Riverbank vegetation comprises some a strip of land stretching 15 to 20 m from the riverbank, with deciduous tropical forests growing farther inland.

Sample Collection. Seasonal sampling was carried out at each study site to represent the different climatic seasons and explore any seasonal variations in the diet of otters. We selected one month as representative of each climatic season (spring, summer, autumn, and winter). Sampling was carried out by walking along the riverbank and collecting any otter scats found that could be quantified as a single unit, i.e., complete scats that could be determined as otter scats with certainty. Otter scats were identified based on their dark color with a slight musk odor when fresh, or whitish when dry, and visibly composed of fish scales, insect legs, prawn or shrimp heads, fragments of crab exoskeletons, fruit seeds, pastures, organic matter, etc. Individual scat samples were stored in self-sealing plastic bags labeled with the geographic location of the sample as recorded with a manual geopositioner (GPS). In the dam, the sites for scat collection were marked considering a separation of approximately 3 km between them.

Sample Analysis. Scat samples were transferred to the Zoology Laboratory at *Universidad Autónoma de Sinaloa* (Culiacan Campus) for analysis. Once in the lab, samples were immersed in soapy water for 48 hours, then rinsed and screened through a 1 mm mesh to remove digested organic matter and other soluble materials. The hard remains of preys contained in scats were sorted using dissecting needles and forceps; these were dried in an oven at 60 °C for 48 hours and stored in labeled bottles or self-sealable bags until analysis. For species other than fish (i.e., invertebrates, birds, mammals, reptiles, amphibians, etc.), the remains were observed under a stereomicroscope for identification aided with guides and catalogs (García and Ceballos 1994). In the case of fish, scales and vertebrae found in scats were compared (based on morphological traits) with the respective parts of fish collected from the sampling sites as reference material. Fish were collected by local fishers using seine nets (locally called *chinchorro*), hooks, fishing rods, and by freediving with rudimentary harpoons.

For each prey species identified, the frequency of occurrence (*FO*) was estimated as: $FO = FE_i/N$, where FE_i is the total number of samples showing species *i* and *N* is the total number of samples. The percentage of occurrence (*PO*) of each prey category was estimated according to Maher and Brady (1986) as modified by Gallo-Reynoso and García-Aguilar (2008): $PO = FCi \times 100/\sum F$, where FC_i is the total frequency of the prey *i* and *F* is frequency.

The differences in the otter's diet (number and identity of prey species) between the two water bodies studied were explored with the Bray-Curtis Dissimilarity Index (Bray and Curtis 1957). This index is commonly used to quantify the difference in the number of species between two sites. In this case, it was used to determine the magnitude of the difference in the otter's diet (number of elements in the diet) in both water bodies. The Bray-Curtis dissimilarity figure ranges between 0 and 1, where 0 (zero) indicates total similarity of elements in the diet in both sites, while 1 (one) indicates that the diet is completely dissimilar (i.e., it shares no common elements) in the two sites. The values obtained are usually multiplied by 100 to report them as percent dissimilarity. The Bray-Curtis dissimilarity index is calculated as: $BC_{ij}= 1 - 2C_{ij} / S_i+S_j$, where *i* and *j* are the two sites, *Si* is the total number of species at site *I*, and *Sj* is the number of species at site *j*. *Cij* is the sum of the lowest counts for each species found in both sites.

Finally, the Schoener index (Schoener 1974) was used to determine the diet overlap between the dam and San Lorenzo river (using the data in Table 1) to explore the potential effect of reservoir waters on the overall composition of the diet. According to the Schoener index, a value of 0 indicates no overlap, values close to 1 suggest marked overlap, and 1 indicates complete overlap. Overlap values above 0.6 are considered biologically relevant. C_{xy} estimates the overlap in the diet between the dam (*xi*) and the river (*yi*). *P* is the proportion of total resources in the diet in the dam (*xi*) and the river (*yi*). $C_{xy} = 1 – 0.5\ (\sum|p_{xi} – p_{yi}|)$.

Results

Frequency of Occurrence. A total of 318 scat samples were collected, from which food items belonged to seven taxonomic groups: mammals, birds, fish, crustaceans, insects, mollusks, and fruits. Of these groups, only five were found in the dam, where no mollusks and crustaceans were observed. In both sites, fish were the dominant food item in the otter's diet, with a frequency of occurrence of 98.1 % in the river and 100 % in the dam. The percentage of occurrence of the remaining food types varies considerably; for example, insects were the second most frequent group (14.1 %) in the river, whereas they made a mere 0.6 % in the dam. The groups not detected in dam scats were mollusks and crustaceans, which attained frequencies of 10.4 % and 7.9 %, respectively, in the river. Seeds accounted for 3.8 % of food items in the dam and 4.2 % in the river (Table 1).

Dietary Dissimilarity. According to the Bray-Curtis index, the dissimilarity in the otter's diet between the water bodies studied was not significant, with 0.205 (*i. e.*, a difference of 20.5%), suggesting that the feeding habits of otters were not markedly different in the two water bodies.

Diet Overlap. The Schoener overlap index showed a non-significant difference between the two water bodies, with 0.85, indicating that the otter's diet is highly overlapped in both water bodies. However, there are some differences regarding some elements of the diet (refer to the following section).

Identification of Prey Species. Most of the prey species identified were fish. In both the dam and the river, *mojarra* or *tilapia* (*Oreochromis* sp.) was the fish species showing the highest percentage of occurrence, with 90.8 % in the river and 98.1 % in the dam. This was followed by the carp (*Cyprinus carpio*, 46 % and 56.8 %) and, in smaller percentages, largemouth bass (*Micropterus salmoides*), catfish (*Ictalurus* sp.), and charal (*Dorostoma smithi*; Table 1).

In the river, the group that ranked second in importance after fish were insects. Of these, the family Tettigoniidae (Orthoptera) had a percentage of occurrence of 9.2 %, followed by unidentified coleopterans with 6.7 %, and the family Gryllidae (Orthoptera) with 2.5 %. In the dam, the most abundant group after fish was birds (which were not identified), with 34 %; a significant difference was observed versus the river, where birds represented only 9.2 %.

As for mammals, we recorded field mouse remains, representing 8.2% in the river and 14.2% in the dam. The river prawn *Macrobrachium americanum* and the river snail (unidentified Gasteropoda) were absent in scat samples from the dam but were found in river samples with frequencies of 8.0 % and 9.2 %, respectively. Seeds of guamúchil, *P. dulce*, were observed at lower frequencies, 4.3% in the river and 0% in the dam; to note, 10% of the seeds found in otter scats could not be identified.

Table 1. Number of occurrences (NO), frequency of occurrence (FO), and percentage of occurrence (PO) of the supra-specific taxonomic groups and species in the otter diet, from 318 scat samples collected in the San Lorenzo river and El Comedero dam, municipality of San Juan Cosalá, Sinaloa.

	San Lorenzo River			**El Comedero dam**		
Species	**NO**	**FO**	**PO**	**NO**	**FO**	**PO**
FISH	160	64.3	98.2	155	65.1	100
Tilapia *(Oreochromis sp)*	148		90.8	152		98.1
Bass (*Micropterus salmoides)*	35		21.5	46		29.7
Catfish *(Ictalurus sp)*	27		16.6	37		23.9
Carp (*Cyprinus carpio)*	75		46.0	88		56.8
Charal (*Dorostoma smithi*)	0		0	15		9.7
INSECTS	23	9.2	14.1	1	0.4	0.6
Grasshopper: Tetigonidae	15		9.2	1		0.6
Coleoptera: Unidentified	11		6.7	0		0
Crickets: Grillidae	4		2.5	0		0
MOLLUSKS	17	6.8	10.4	0	0	0
River snail: Unidentified	15		9.2	0		0
Bivalve: Unidentified	2		1.2	0		0
BIRDS	15	6.1	9.2	54	22.7	34.8
Unidentified birds	15		9.2	54		34.8
MAMMALS	14	5.6	8.6	22	9.2	14.2
Unidentified mice	14		8.6	22		14.2
CRUSTACEANS	13	5.2	8.0	0	0	0
River prawn: *Macrobachium americanum*	13		8.0	0		0
PLANTS (FRUITS)	17	2.8	10.4	6	2.6	3.9
Guamuchil: *Pithecellobium dulce*	7		4.3	0		0
Unidentified	10		6.1	6		3.9
Total SAMPLES	163			155		

Seasonal Variation. The months selected were those with no minor differences in the number of samples collected. Accordingly, we analyzed 66 samples from the dam and 65 from the river (Tables 2, 3).

Regarding the proportion of groups and species, a marked difference was found both between seasons and between the two sites (Tables 2, 3). In the dam, fish showed no variations, as they were recorded in the same proportion of the diet in the four seasons, although with fluctuations in the proportion of species. *Oreochromis* sp. remained stable at 50 % in spring and summer, increasing to 92 % in autumn and then decreasing to 63 % in winter. Fish and birds were identified in all seasons, with significant variations between them. Insects and plants were found in otter scats only in the spring, with 5 % each.

Discussion

Prey Abundance. This work supports the hypothesis that the otter is a predominantly ichthyophagous species (Gallo-Reynoso 1989, 1997; Macías-Sánchez 1999; Perini *et al.* 2009), as fish were the most abundant food item in the diet of otters, both in the river and in the reservoir. The predominance of fish in otter scats could be either a direct indicator of its dietary preferences or a reflection of the local abundance of their different types of prey (Gallo-Reynoso 1989 and 1997; Rangel-Aguilar and Gallo-Reynoso 2013). Birds, mammals, and insects are also groups of greater importance than crustaceans. These differences may reflect the relative availability of each of these groups due to the type of ecosystem they inhabit. In addition, we found two species of plants whose fruits were consumed by otters.

Similar results supporting the predominance of ichthyophagous feeding habits have been recorded for *Lontra longicaudis*, as fish represented 93 % in southern Mexico (Gallo-Reynoso 1989) and Costa Rica (Pardini 1998), and 50 % to 85 % in southern Brazil (Quadros and Monteiro-Filho 2001). This was also the case for *Lutra lutra*, with fish representing 88 % of the diet in Scotland (Durbin 1997), and *Lutra perspicillata*, with 92 % in India (Anoop and Hussain 2005).

Several works on the feeding habits of the otter report crustaceans, particularly river prawn (*M. americanum*), as an important component in the diet (Gallo-Reynoso 1989 and 1997; Pardini 1998; Casariego-Madorell *et al.* 2006). Crustaceans were the second-most important group, even being the main food type consumed by otters in some smaller rivers (Gallo-Reynoso 1989 and 1997).

On the other hand, insects are an important component of the diet of *L. longicaudis*, only second to fish in the river. Three taxa, Coleoptera, Orthoptera, and Grilleidae, of which only coleopterans (Gallo-Reynoso 1997) and crickets (Rangel-Aguilar and Gallo-Reynoso 2013) had been reported, were identified in this category.

This feeding behavior of the otter is consistent with the optimal foraging theory (Stephens and Krebs 1986). This theory predicts that a predator will feed preferentially on the most abundant organism because of the ease of capture involving minimum energy expenditure; however, the same outcome is predicted when the most energy-profitable prey is also the most abundant one. Thus, the otter could be either behaving according to optimal foraging or forage opportunistically on the most abundant prey, similar to the coyote (*Canis latrans*; Boutin and Cluff 1989). The optimal foraging theory (Stephens and Krebs 1986) also predicts that an individual predator will become selective towards the prey providing the highest energy profit per unit of energy spent in the capture (Charnov 1976; Pyke *et al.* 1977; Stephens and Krebs 1986). This is what we would expect if species that are more energy-profitable become available. In the present study, the bass would be the most energy-profitable prey because of its larger size relative to

Table 2. Seasonal variations in the diet of the Neotropical otter in El Comedero dam, Cosalá, Sinaloa. Findings from 66 samples analyzed, representing a single collection per month and climatic season. Data shown are the percentage of occurrence, with the frequency of occurrence (FO) in parentheses.

Group and species occurrences	Spring	Summer	Fall	Winter
	(*n* = 20)	(*n* = 10)	(*n* = 14)	(*n* = 22)
Fish (FO)	*n* = 140 (7)	*n* = 190 (19)	*n* = 143 (10.2)	*n* = 113 (5.1)
Oreochromis sp. (FO)	35.7	26.3	92.8	63.6
Ictalurus sp. (FO)	21.4	10.6	28.5	18.1
Micropterus salmoides (FO)	25	36.8	7.1	13.6
Cyprinus carpio (FO)	17.9	26.3	14.2	18.1
Birds (FO)	*n* = 35 (1.8)	*n* = 50 (5)	*n* = 43 (3.1)	*n* = 32 (0.7)
Unidentified (FO)	35	50	42.8	31.8
Mammals (FO)	*n* =15 (0.8)			
Mice (FO)	15			
Insects (FO)	*n* = 5 (0.3)			
Grillidae (FO)	5			
Plants (fruits) (FO)	*n* = 5 (0.3)			
Unidentified (FO)	5			

tilapia; indeed, this species predominates in otter scats in summer, in both the river and the dam.

Identification of Prey Species. Tilapia, *Oreochromis* sp., which is an introduced fish, is the most important prey for the Neotropical otter, attaining the highest frequency in scats, besides clearly being the most available species, with various growth stages observed. This species has been reported in other works, albeit not as the most abundant species (Gallo-Reynoso *et al.* 2008; Rangel-Aguilar and Gallo-Reynoso 2013). The largemouth bass (*M. salmoides*) and other salmonids (Crait and Ben-David 2006), as well as cyprinids, have been reported as the groups of fish most consumed by several species of otters (Britton and Shepherd 2005; Crait and Ben-David 2006). In contrast, the charal (*D. smithi*) had not been previously recorded as a prey of *L. longicaudis.*

As for plants (fruits), those consumed by otters include Myrtaceas, Sapotaceae, and Rubiaceae (Quadros and Monteiro-Filho 2001), as well as remains of cultivated plants such as papaya (Gallo-Reynoso 1989; Kasper *et al.* 2008). However, there are no previous records of guamúchil (*P. dulce*), which was found with a frequency of 4.1 % in scats collected in the river, along with other unidentified seeds. Two of the invertebrate species found — a gastropod and a bivalve — could not be identified for lack of published studies on them, although both have been reported previously by Rangel-Aguilar and Gallo-Reynoso (2013).

There are records of field mice as prey of otters (Gallo-Reynoso 1997; Pardini 1998), but the species found in the present study could not be identified because it was represented by long bone fragments only.

Seasonal Variation. Seasonal variations in the diet composition of the Neotropical otter have been reported (Gori *et al.* 2003; Kasper *et al.* 2008), a finding that was confirmed in this study. In summer alone, the composition of the diet in the river varied in terms of the proportion of fish species. In all other stations in both sites, this parameter showed a stable behavior. This is likely because fish are highly available, with different growth stages observed; an alternative explanation is that the otter specifically forages on them because the energy and time employed in capturing them is compensated by the amount of energy provided by these organisms (Pyke *et al.* 1977; Hernández *et al.* 2002).

In the dam, the main prey was *Oreochromis* sp. This species was observed in high frequencies in otter scats throughout the year, being constant in spring-summer and increasing in frequency in autumn-winter, which is when the largemouth bass, *M. salmoides* (a top predator in the fish food chain), shows lower frequencies of occurrence. The shortage of bass in the diet was compensated by an

Table 3. Seasonal variations in the feeding habits of the Neotropical otter in El Comedero dam, Cosalá, Sinaloa. Findings from 65 samples analyzed, representing a single collection per month and climatic season. Data shown are the percentage of occurrence, with the frequency of occurrence (FO) in parentheses.

Group and species occurrences	Spring	Summer	Fall	Winter
	(n= 20)	(n= 21)	(n= 12)	(n= 12)
Fish (FO)	*n* = 110 (5.5)	*n* = 153 (7.3)	*n* = 84 (7)	*n* = 117 (9.8)
Oreochromis sp. (FO)	55	48	59	59
Ictalurus sp. (FO)	5		25	17
Micropterus salmoides (FO)	30	76		41
Cyprinus carpio (FO)	20	29		
Dorostoma smithi (FO)				
Birds (FO)	*n* = 20 (1)			*n* = 8 (0.7)
Unidentified (FO)	20			8.3
Mammals (FO)	5		8.3	
Mice (FO)	5		8.3	
Insects (FO)	*n* = 30 (1.5)	*n* = 5 (0.2)	*n* = 17 (1.4)	
Coleoptera (FO)	30	4.7		
Tetigonidae (FO)			16.6	
Grillidae (FO)				
Mollusks (FO)	*n* = 35 (1.8)	*n* = 5 (0.2)	*n* = 17 (1.4)	*n* = 17 (1.4)
Gasteropod (FO)	35		8.3	16.6
Bivalve (FO)		4.7	8.3	
Crustaceans (FO)		*n* = 33 (1.6)		
Macrobrachium americanum (FO)		33		
Plants (fruits) (FO)	*n* = 15 (0.8)	*n* = 14 (0.7)	*n* = 8 (0.6)	
Pithecellobium dulce (FO)		14.2		
Unidentified (FO)	15		8.3	

increased consumption of *Oreochromis* sp. In autumn, the frequency of tilapia in otter scats increased to 92.8 % and charal (*D. smithi*) was absent.

In the river, the tilapia remains as a nearly constant prey, with no noticeable changes in frequency; it showed a slight increase in autumn when bass and carp were completely absent as prey. The bass occurred in spring-summer, being more abundant in summer — the season when catfish *Ictalurus* sp were not recorded. No charal was observed in the river.

In the river, insects made up an important part of the diet of otters; however, these were insignificant in the dam, with only traces of insects found in a single sample of otter scats. This suggests that otters are capable of adapting to altered environments such as dams, and although there is no presence of insects in their diet, they can modify their diet by predating on species that are in greater availability.

The absence of mollusks and crustaceans in scat samples collected in the dam may be due to their absence in this site. Other scenarios are that these invertebrates are present, but otters do not feed on them due to the high abundance of fish, or these invertebrates are hard to capture by otters because they probably occur in deep habitats that are hard to access since the dam is deeper than the river.

Birds were twice as frequent in otter scats in the dam relative to the river, similar to the findings reported by Gallo-Reynoso *et al.* (2008). This suggests a higher diversity and availability of aquatic birds in the dam or that birds use the greater area of the dam than the river.

In this study, the diet of the Neotropical otter included seven taxonomic groups in the San Lorenzo river and five in El Comedero dam, with fish showing the highest percentage of occurrence in both sites. These results are consistent with works conducted for this same species in southern and northern Mexico (Gallo-Reynoso 1989, 1997; Macías-Sánchez 1999; Rangel-Aguilar and Gallo-Reynoso 2013) and in other countries (Pardini 1998; Quadros and Monteiro-Filho 2001; Perini *et al.* 2009).

Finally, a biologically significant difference was found with the Schoener index (Schoener 1974) as to the species that make up the diet of the Neotropical otter; this is expected because the diversity of prey in the lentic system (dam) is much lower than in the lotic system (river).

The Neotropical otter shares the riparian habitat with the gray fox (*Urocyon cinereoargenteus*), the coatí (*Nasua narica*), and the raccoon (*Procyon lotor*), and resembles the latter in the consumption of crustaceans and insects. Most otter latrines also showed mingled scats from gray fox, coati, and raccoon. This may be explained by the musk odor in otter scats that attract these other carnivorous species to mark the territory or display a communication behavior similar to that of otters (Gallo-Reynoso *et al.* 2016).

This work underlines the importance of the Neotropical otter as a bioindicator of the presence of other species in water bodies, including mollusks, crustaceans, insects, and fish on which they feed. At the same time, otters act as umbrella species. As top predators in water bodies of northwestern Mexico, these indicate the presence of a certain diversity of species in these environments, therefore suggesting that they are suitable for use as an indicator of the good conservation status of these ecosystems.

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Literature cited

Anoop, K. R., and S. A. Hussain. 2005. Food and feeding habits of smooth-coated otters (*Lutra perspicillata*) and their significance to the fish population of Kerala, India. Journal of Zoology 266:15-23.

Azevedo, F. C. C., V. Lester, W. Gorsuch, A. S. Lariviere, and J. W. D. L. Murray. 2005. Dietary breadth and overlap among five sympatric prairie carnivores. Zoology 269:127-135.

Boutin, S., and H. D. Cluff. 1989. Coyote prey choice; optimal or opportunistic foraging? A comment. Wildlife Management 53:663-666.

Braña, F., J. Naves, and G. Palomero. 1987. Hábitos alimenticios y configuración de la dieta del oso pardo en la cordillera cantábrica. Coloquio Internacional sobre el oso pardo 1:14-15.

Bray, J. R., and J. T. Curtis. 1957. An ordination of upland forest communities of southern Wisconsin. Ecological Monographs 27:325-349.

Britton, J. R., and J. S. Shephered. 2005. Presence of carp, *Cyprinus carpio*, in the diet of the otter, *Lutra lutra*. Fisheries Management and Ecology 12:221-223.

Casariego-Madorell, M. A., R. List, and G. Ceballos. 2006. Aspectos básicos sobre la ecología de la nutria de río (*Lontra longicaudis annectens*) para la costa de Oaxaca. Revista Mexicana de Mastozoología 10:71-74.

Crait, J. R., and M. Ben-David. 2006. River otters in Yellowstone lake depend on a declining cutthroat trout population. Journal of Mammalogy 87:485-494.

Cruz García, F., A. J. Contreras Balderas, J. A. García Salas, and J. P. Gallo-Reynoso. 2017. Dieta de la nutria neotropical (*Lontra longicaudis annectens*) en Pueblo Nuevo, Durango, México. Revista Mexicana de Biodiversidad 88:701-709.

Charre-Medellín, J. F., C. López-González, A. Lozano, and A. F. Guzmán. 2011. Conocimiento actual sobre la nutria neotropical (*Lontra longicaudis annectens*) en el estado de Durango. Revista Mexicana de Biodiversidad 82:1343-1347.

Chame, M. 2003. Terrestrial Mammal Feces: a Morphometric Summary and Description. Memórias do Instituto Oswaldo Cruz 98:71-94.

Charnov, E. L. 1976. Optimal foraging: attack strategy of a mantid. The American Naturalist 110:141-151.

Durbin, L. S. 1997. Composition of salmonid species in the estimated diet of otters (*Lutra lutra*) and in electrofishing catches. Journal of Zoology 243:821-825.

Eisenberg, J. F., and K. H. Redford. 1999. Mammals of the Neotropics: The Central Neotropics. The University of Chicago Press. U.S.A.

Foster-Turley, P., S. Macdonald, and C. Mason. 1990. Otters: An Action Plan for their Conservation. IUCN - Otter Specialist Group. Gland Switzerland.

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. Tesis de Maestría. Facultad de Ciencias, UNAM. 236 p. Disponible en: jpgallo@ciad.mx

Gallo-Reynoso, J. P. 1997. Situación y distribución de las nutrias de México, con énfasis en *Lontra longicaudis annectens* MAJOR, 1897. Revista Mexicana de Mastozoología 2:10-32.

Gallo-Reynoso, J. P., N. N. Ramos-Rosas, and O. Rangel-Aguilar. 2008. Aquatic bird predation by Neotropical river otter (*Lontra longicaudis annectens*), at Río Yaqui, Sonora, México. Depredación de aves acuáticas por la nutria neotropical (*Lontra longicaudis annectens*), en el río Yaqui, Sonora, México. Revista Mexicana de Biodiversidad 79:275-279.

Gallo-Reynoso, J. P., and M. C. García-Aguilar. 2008. Análisis preliminar de la presencia de perros ferales en la Isla Cedros, Baja California. Revista Mexicana de Mastozoología 12:130-140.

Gallo-Reynoso, J. P., S. Macías-Sánchez, L. Armenta-Méndez, I. Barba-Acuña, V. A. Nuñez-Ramos, A. Loya-Jaquez, G. Ponce-García, and A. Gardea-Béjar. 2016. Letrinas de nutrias y cámaras trampa ¿qué nos dicen? XIII Congreso Nacional de Mastozoología, Tuxtla Gutiérrez, Chiapas. 24 a 28 de Octubre. Disponible en: jpgallo@ciad.mx

García, A., and G. Ceballos. 1994. Guía de campo de los reptiles y anfibios de la costa de Jalisco, México. Fundación Ecológica de Cuixmala, A.C. e Instituto de Biología, UNAM. Ciudad de México.

Glen, A. S., and C. R. Dickman. 2008. Niche overlap between marsupial and eutherian carnivores: does competition threaten the endangered spotted-tailed quoll? Applied Ecology 45:700-707.

Gori, M., G. M. Carpaneto, and P. Ottino. 2003. Spatial distribution and diet of the Neotropical otter *Lontra longicaudis* in the Ibera Lake (northern Argentina). Acta Theriologica 48:495-504.

Guerrero-Flores, J. J., J. P. Gallo-Reynoso, and R. Biek. 2015. Mitochondrial DNA diversity, genetic structure, and demographic history of the Neotropical Otter (*Lontra longicaudis*) in Mexico. (JMAMM2014048.R2) Published on line in the Journal of Mammalogy 96:1162-1173.

Hardin, G. 1960. The Competitive Exclusion Principle. Science 131:1292-1297.

Hernández, L., R. R. Parmenter, J. W. Dewitt, D. C. Lightfoot, and J. W. Laundre. 2002. Coyote diets in the Chihuahuan Desert, more evidence for optimal foraging. Arid Environments 51:613-624.

Hernández-Romero, P. C., C. Gutiérrez-Rodríguez, C. Valdespino, and D. A. Prieto-Torres. 2018. The Role of Geographical and Ecological Factors on Population Divergence of the Neotropical otter *Lontra longicaudis* (Carnivora, Mustelidae). Evolutionary Biology 45:37-55.

Josef, C. F., L. R. Adriano, E. J. De Frañasa, G. G. Arantes De Carvalho, and J. R. Ferreira. 2008. Determination of Hg and diet identification in otter (*Lontra longicaudis*) feces. Environmental Pollution 152:592-596.

Kasper, C. B., V. A. G. Bastazini, J. Salvi, and H. C. Z. Grillo. 2008. Trophic ecology and the use of shelters and latrines by the Neotropical otter (*Lontra longicaudis*) in the Taquari Valley, Southern Brazil. Iheringia, Série Zoologia 98:469-474.

Kok, O. B., and J. A. J. Nel. 2004. Convergence and divergence in prey of sympatric canids and felids: opportunism or phylogenetic constraint? Biological Journal of the Linnean Society 83:527-538.

Lodé, T. T. 1993. The decline of otter *Lutra lutra* populations in the region of the Pays de Loire, western France. Biological Conservation 65:9-13.

Macías-Sánchez, S., and M. Aranda. 1999. Análisis de la alimentación de la nutria *Lontra longicaudis* (Mammalia: Carnivora) en un sector del Río Los Pescados, Veracruz, México. Acta Zoológica Mexicana (nueva serie) 76:49-57.

Maher, D. S., and J. R., Brady. 1986. Food habits of bobcat in Florida. Journal of Mammalogy 67:133-138.

Marucco, F., D. H. Pletscher, and L. Boitani. 2008. Accuracy of scat sampling for carnivore analysis: wolves in the Alps as a case study. Mammalogy 89:665-673.

Miranda, F., and E. Hernández. 1963. Los tipos de vegetación de México y su clasificación. Boletín de la Sociedad Botánica de México 28:29-179.

Mitchell, B. D., and P. B. Banks. 2005. Do wild dogs exclude foxes? Evidence for competition from dietary and spatial overlaps. Austral Ecology 30:581-591.

Monroy-Vilchis, O., and V. Mundo. 2009. Food niche of the neotropical river otter (*Lontra longicaudis*) in a modified environment, Temascaltepec, México. Revista Mexicana de Biodiversidad 80:801-806.

Nowak, R. M. 1991. Walker's mammals of the world. London.

Pardini, R. 1998. Feeding ecology of the neotropical river otter *Lontra longicaudis* in an Atlantic Forest stream, south-eastern Brazil. Journal of Zoology 245:385-391.

Perez-Claros, J. A., and P. Palmqvist. 2008. How many potential prey species account for the bulk of the diet of mammalian predators? Implications for stable isotope paleodietary analyses. Zoology 275:9-17.

Perini, A. A., E. M. Vieira, and U. H. Schulzb. 2009. Evaluation of methods used for diet analysis of the Neotropical otter *Lontra longicaudis* (Carnivora, Mustelidae) based on spraints. Mammal Biology 74:230-235.

Prigioni, C., A. Balestrieri, L. Remonti, A. Gargaro, and G. Priore. 2006. Diet of the Eurasian otter (*Lutra lutra*) in relation to freshwater habitats and alien fish species in southern Italy. Ethology Ecology and Evolution 18:307-320.

Pyke, G. H., H. R. Pulliam, and E, L, Charnov. 1977. Optimal foraging: a selective review of theory and tests. The Quarterly Review of Biology 52:137-154.

Quadros, J., and E. L. A. Monteiro-Filho. 2001. Diet of the Neotropical otter, *Lontra longicaudis*, in an Atlantic forest area, Santa Catarina State, southern Brazil. Studies on Neotropical Fauna and Environment 36:15-21.

Ramos-Rosas, N., C. Valdespino, J. García Hernández, J. P. Gallo-Reynoso, and E. Olguín. 2012. Heavy metals in the habitat and throughout the food chain of the Neotropical otter, *Lontra longicaudis*, in protected Mexican wetlands. Environmental Monitoring and Assessment 184:1-11.

Rangel-Aguilar, O., and J. P. Gallo-Reynoso. 2013. Hábitos alimentarios de la nutria neotropical (*Lontra longicaudis annectens*) en el Río Bavispe-Yaqui, Sonora, México. Feeding habits of the Neotropical otter (*Lontra longicaudis annectens*) in the Bavispe-Yaqui River, Sonora, Mexico. Therya 4:297-309.

Roth, J. D., D. L. Murray, and T. D. Steury. 2008. Spatial dynamics of sympatric canids: Modeling the impact of coyotes on red wolf recovery. Ecological modelling 214: 391-403.

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

Saint, A. D. H. 2006. Hábitos alimentarios del puma (*Puma concolor*) en la sierra de Nanchititla. Tesis de Licenciatura. Universidad Autónoma del Estado de México.

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

Servín, J. E., N. Chacón, N. Alonso-Pérez, and C. Huxley. 2003. New records of mammals from Durango, Mexico. The Southwestern Naturalist 48:312-313.

Smiroldo, G., A. Balestrieri, L. Remonti, and C. Prigioni. 2009. Seasonal and habitat-related variation of otter *Lutra lutra* diet in a Mediterranean river catchment (Italy). Folia Zoologica 58:87-97.

Stephens, D. W., and J. R. Krebs. 1986. Foraging Theory. Princeton University Press. New Jersey. U.S.A.

Tinker, M. T., G. Bentall, and J. A. Estes. 2008. Food limitation leads to behavioral diversification and dietary specialization in sea otters. Proceedings of the National Academy of Sciences 105:560–565.

Trinca, C. S., H. F. Waldemarin, and E. Eizirik. 2007. Genetic diversity of the Neotropical otter (*Lontra longicaudis* Olfers, 1818) in Southern and Southeastern Brazil. Brazilian Journal of Biology 67:813-818.

Weber, L. I., C. G. Hildebrand, A. Ferreira, G. Pedarassi, J. A. Levy, and E. P. Colares. 2009. Microsatellite genotyping from faeces of *Lontra longicaudis* from southern Brazil. Iheringia Serie Zoologia 99:5-11.

Yom-Tov, Y., T. M. Heggberget, Ø. Wiig, and S. Yom-Tov. 2006. Body size changes among otters, *Lutra lutra*, in Norway: the possible effects of food availability and global warming. Oecologia 150:155-160.

Young, J. K., S. N. Glasscock, and J. A. Shivik. 2008. Does spatial structure persist despite resource and population changes? Effects of experimental manipulation on coyotes. Mammalogy 89:1094-1104.

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Postnatal growth and development of *Natalus mexicanus* (Chiroptera: Natalidae)

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Natalus mexicanus is a bat species distributed from northern México to Central America. It inhabits various types of tropical vegetation, roosting mainly in caves with high relative humidity and temperature, and feeding mostly on arachnids. This study investigated postnatal growth and flight development of populations under natural conditions inhabiting "Los Laguitos" cave, Chiapas, southern México. Forty-four females and 50 males were monitored from birth to 55 days of age; at five-day intervals, we measured body mass, forearm length, cartilaginous epiphyseal gap of the fourth metacarpal-phalangeal joint, and development of four characters. We used statistical analyses and growth models to quantify the changes in morphometric parameters. Neonates are altricial; the ears began to unfold since day one, while the eyes opened at day 25. Greyish hair appeared between 25 to 35 days. Forearm length and body mass increased linearly over 35 days, then the growth rate decreased. The cartilaginous epiphyseal gap increased in size until day 25 and then started to close. The logistic equation yielded the best fit for forearm length ($K = 0.07$) and body mass ($K = 0.10$). Sustained flight was first achieved at 35 days of age. *N. mexicanus* neonates are altricial and relatively small compared with other bats. Eye-opening and fur development took place at a slower rate than in most species of insectivorous bats. In the Chiapas population, eye-opening, fur development, and volancy occurred more slowly relative to the population inhabiting Álamos, Sonora. These differences are probably consequences of local variations. The morphometric postnatal growth pattern of *N. mexicanus* was like that of other insectivorous bats, *i. e.*, linear growth rate before the onset of flight and slowing down thereafter. As in other studies, the logistic model best fitted the growth pattern of body mass and forearm length, but growth coefficients were lower versus other tropical bats. The cartilaginous epiphyseal gap of the fourth digit closes at an intermediate age relative to other insectivorous bats. Sustained flight was attained when the relative body mass and forearm length of individuals approached adult body dimensions, as in other bat species.

Natalus mexicanus se distribuye desde México hasta Centroamérica. Este murciélago se refugia principalmente en cuevas con elevada humedad relativa y temperatura. Habita diferentes tipos de vegetación tropical y se alimenta sustancialmente de arácnidos. Debido a que es una especie con amplia distribución y que no existe información sobre el crecimiento postnatal y desarrollo para las poblaciones del sur de México, en el presente trabajo se realiza un estudio bajo condiciones naturales en la cueva "Los Laguitos" Chiapas, México. Se midió el desarrollo de algunos caracteres y los cambios de la masa corporal, longitud del antebrazo, epífisis cartilaginosa metacarpo-falange del cuarto dedo cada cinco días, desde el día del nacimiento hasta los 55 días de edad, en 44 hembras y 50 machos. Para analizar los datos se emplearon una prueba de T de Student y tres modelos de crecimiento. Los neonatos son altricios, las orejas empezaron a separarse desde el primer día, mientras que los ojos se abrieron hasta los 25 días. El pelaje grisáceo apareció entre los 25 a 35 días. La longitud del antebrazo y masa corporal crecieron linealmente hasta los 35 días, después el incremento disminuyó. La ecuación logística ofreció el mejor ajuste para los datos de longitud del antebrazo ($K = 0.07$) y masa corporal ($K = 0.10$). La epífisis cartilaginosa aumentó hasta el día 25 y después empezó a cerrarse. El vuelo sostenido inició a los 35 días de edad. En comparación con otros microquirópteros, los neonatos de *N. mexicanus* son relativamente pequeños y altricios. La apertura de los ojos y desarrollo del pelaje fueron más lentos en comparación con la mayoría de las especies insectívoras. En la población de Chiapas, la apertura de los ojos, desarrollo del pelaje y vuelo fueron más lentos en comparación con los de Álamos, Sonora. Estas diferencias se consideran resultado de variaciones locales. El patrón de crecimiento resultó similar a otros murciélagos insectívoros, fue lineal previo al inicio del vuelo, posteriormente la tasa de incremento disminuyó. Como en otros estudios, el modelo logístico fue el que mejor describió el patrón de crecimiento de la masa corporal y longitud del antebrazo, pero los coeficientes de crecimiento fueron menores con respecto a otros murciélagos tropicales. La epífisis cartilaginosa metacarpo-falange del cuarto dedo, se cierra a edad intermedia en comparación con otras especies insectívoras. El vuelo sostenido inició cuando los juveniles son casi del tamaño del adulto, como en la mayoría de las especies estudiadas.

Keywords: flight development; life strategies; "Los Laguitos" cave; neonatal size; neonates; tropical bats.

Introduction

Postnatal development and growth are life-history traits related to reproductive success, age at onset of adulthood, and longevity (Barclay and Harder 2003). Organisms with a faster development that reach a larger size in a shorter time have advantages over organisms with a slower growth rate (Kunz and Hood 2000; Dmitriew 2011). In bats, development and growth rate are affected by inheritance, maternal health, shelter conditions, and available food resources, among others (Kunz and Hood 2000). Bats undergo accelerated growth and development, lasting about one month in most Yangochiroptera (Kunz and Hood 2000). Likewise, weaning occurs at one month of age, although it is delayed to up to nine months in vampires (Kunz and Hood 2000; Delpietro and Russo 2002). Most studies on postnatal growth in bats focus on species with temperate distribution, rather than on tropical species (Kunz and Hood 2000; Richardson and Kunz 2000; Lin *et al.* 2010; Chaverri and Vonhoff 2011). For this reason, it is valuable to estimate the parameters related to postnatal development and growth in tropical and subtropical bat species, to understand the evolution of reproductive strategies of these organisms in different environments.

Natalus mexicanus is a bat species ranges from México to Panama and in Pacific and Caribbean islands; its habitat comprises from xeric shrubland to tropical rainforest, from sea level to 2,300 masl (Tejedor 2011, 2019). This species occupies mainly caves as daytime shelters and has also been found in mines and tree holes with high temperature and relative humidity (RH), from 22 to 26 °C and 74 to 99 %, respectively (Torres-Flores and López-Wilchis 2010; Tejedor 2011). Its diet includes a variety of arthropods, mainly arachnids (Torres-Flores and López-Wilchis 2019). Reproductive data from a population of Sonora indicate that the species is monoestrous, mating in summer and giving birth to a single offspring (Mitchell 1965). Since the species is widespread in México and thrives in different environments, some characteristics of its life history are considered to vary, as there is no reproductive information available for populations inhabiting southern México. The present study investigated the growth and flight development in *N. mexicanus* based on a population inhabiting "Los Laguitos"cave, Tuxtla Gutiérrez, Chiapas.

Materials and Methods

Study Area. The "Los Laguitos" cave is located 4 km to the NW of Tuxtla Gutiérrez (16° 46' N, -93° 8' W; altitude 781 masl), Chiapas, México. The cave has a horizontal structure consisting of three galleries with a single entrance. Inside the cave, the mean annual temperature is 32.3 °C and RH is 95.4 %; these values are higher than outdoor conditions (Martínez-Coronel *et al.* 2010). The local climate is warm subhumid, with a mean annual temperature of 24.7 °C (Cardoso 1979); the rainy season spans from May to October, with a well-marked summer drought or canicula in July and August and a dry season the rest of the year. The local vegetation is a tropical deciduous forest (Miranda 1998).

Growth and Development. To describe and quantify the growth and development of *Natalus mexicanus*, numbered plastic rings were attached to specimens at birth. The rings measured 4 mm in diameter by 4 mm width, with a mean weight of 0.04 g. The ring was attached around the right forearm in females and on the left forearm in males. Newborn specimens were determined based on the presence of fresh placenta (Kunz 1973), in addition to having a naked body, pink skin, folded ears, auditory meatus, closed eyes, and for being unable to move away from the perch site (Martínez-Coronel *et al.* 2014).

Individuals were recaptured at five-day intervals until their flight capacity made it impossible to locate and catch them. The age categories included were as follows: 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 days post-birth, corresponding to the chronological age. After day 55, the capture of individuals was no longer possible due to the high number of individuals in the cave. Each specimen was individually manipulated next to its perch; on each occasion, the site, recapture date, ring number, and sex were recorded.

The separation of the ears, opening of the eyes, and emergence of the fur are continuous phenomena; however, for descriptive purposes, these characteristics were classified into discrete categories, defined as follows. As regards ears, three categories were recognized: Folded ears - ears attached to the sides of the head, with the auditory meatus closed. Partially upright ears - auditory meatus open, ears separated from the head, but with the tip bent. Upright ears - ears completely upright, as in adults. For the opening of the eyes, we defined three categories: Closed eyes - eyes completely covered by the eyelids, which are fused; separation line not visible. Partially opened eyes - eyes do not open completely, because the eyelids remain fused at the lateral commissures. Opened eyes - eyes completely visible, as the eyelids are fully separated. Regarding pigmentation and fur, five categories were used: I) pink skin, body completely naked; vibrissae absent, with only red spots observed in sites where vibrissae will emerge. II) Light-pink body, off-white membranes; vibrissae present; hairs smaller than 2 mm in genitals and toes. III) Grayish dorsum, with darker crown, interscapular region, patagium, forearm, and legs; shiny patagium and pinkish abdomen. IV) Pigmented abdomen; body covered with short grayish hairs measuring less than 3 mm between legs and hips. V) Fur darker, with brown hair on the dorsum and whitish hair in the abdomen; fur longer on legs and dorsum (> 3 mm) than on the abdomen.

Also, the forearm length and the length of the epiphyseal gap of the fourth metacarpal-phalangeal joint of the right wing were measured to the nearest 0.05 mm with a Mitutoyo digital caliper (Kunz and Anthony 1982); body mass was measured to the nearest 0.1 g with a spring balance. Temperature and RH were also recorded in each visit using a Taylor psychrometer. The readings were taken at 21:00 and 2:30 h at the center of the roost and at 1.20 m height.

Growth was quantified through the changes in forearm length, length of the cartilaginous epiphyseal gap of the fourth metacarpal-phalangeal joint of the right-wing, and body mass (Kunz and Anthony 1982). Flight development was described using the three categories proposed by Stern *et al.* (1997), namely: non-volant, individuals who hang from walls and are unable to fly, remaining still in the perching site or moving only through walls; semi-volant, individuals who attempt to fly but fail in staying in the air, colliding with walls and failing to perch; volant, individuals capable of flying in a straight line, gaining height and succeeding to perch.

Statistical analysis. The basic statistics, *i. e.,* mean, and standard deviation, were calculated for all variables. Differences between sexes in individuals of the same age were evaluated with a Student's-*t* test. This method was used for being robust to deviations from normality, as the sample sizes analyzed were small for the older age classes.

To determine the growth rate of each variable, the linear portion of each empirical curve was considered separately, and a linear regression was applied to estimate the slope. To obtain the growth model with the best fit for the relationship between age and forearm length, as well as between age and body mass, we used the growth equations of Gompertz, Von Bertalanffy and the logistic model (Zullinger *et al.* 1984). In the case of the length of the epiphyseal cartilage of the fourth metacarpal-phalangeal joint, different polynomial models were used (Brown and Rothery 1994). All statistical analyses were performed in NCSS (2020). A value of ≤ 0.05 was used as a statistical significance criterion.

Results

We studied a total of 94 neonates (44 females and 50 males) of *N. mexicanus* specimens inhabiting "Los Laguitos" cave. All the individuals studied belonged to single-pup litters, as confirmed through the examination of pregnant females collected in previous months and at the beginning of the study. The parturition period lasted 25 days, from 25 May to 19 June 2009. During the day, pups slept hanging from their mothers, while at night, when mothers went out to forage, pups were transported to communal sites or nurseries, with mothers returning several times to breastfeed their pups along night. The mean temperature in the nursery was 32.8 ± 0.55 °C; this fluctuated over the first 30 days and tended to stabilize thereafter. RH was 96.8 ± 2.71 %, increasing from the beginning of the study to 45 days, tending to decrease thereafter.

Neonate condition. All neonates had umbilical cord and placenta; the abdominal skin was translucent, with visible viscera; ears folded and attached to the head; auditory meatus and eyes closed; pink body and skin completely devoid of hair. Red spots were observed in sites where vibrissae were to emerge. The patagium and uropatagium were whitish; the skull lacked the anterior fontanel but showed the posterior fontanel between the occipital and parietals; fat in shoulders and supra-scapular area (Figure 1a). Neonates emitted audible sounds and remained still at the site where they were left by their mothers, moving their heads upon hearing any sound; they attempted to suck milk when an object was brought closer.

Development of ears, fur, and eye opening. The ears began to separate from day one, and by day five of age, all recaptured specimens already had the auditory meatus open and the ears separated with the tip slightly bent. At day 10 of age, most individuals already had their ears fully upright (Table 1). The eyes remained closed over the first 15 days post-birth; in some 20-day individuals, the eyelids began to separate, disclosing the eyes in mid portion, as the lateral commissures remained attached. The last specimens with semi-closed eyes were 35 days old, although in other 25-day-old specimens the eyelids were already separated, and the eyes were fully open (Table 1).

Table 1. Detachment of the ears and opening of the eyes in *Natalus mexicanus* inhabiting "Los Laguitos" cave, Chiapas, Mexico. For each age category, the number of individuals in each development stage of each character is given.

Age/ days	Ear detachment			Eye opening		
	Folded	Semi-erect	Erect	Closed	Semi-open	Open
1	93	1		94		
5		33		33		
10		3	20	23		
15			24	24		
20			11	8	3	
25			14	11	2	1
30			11	6	4	1
35			12	1	4	7
40			12			12
45			3			3
50			4			4
55			3			3

The growth of the fur was slowly; individuals remained pink and naked during the first five days post-birth (Figure 1a). Between 10 and 15 days of age, vibrissae had already grown, and hairs less than two mm long were observed in the genital area and toes (Table 2). At postnatal day 15, some individuals had the crown and dorsum darker than the rest of the body (Figure 1b), a condition that occurred up to day 35 in some individuals. However, in other 30-day-old individuals, the body was already covered with a grayish fur (< 2 mm), with the abdomen paler than the dorsum (Figure 1c). Also, in some 35-day-old individuals, the dorsum was covered with a dark brown fur more than three mm in length, being shorter and lighter-colored in the abdomen (Figure 1d).

Neonates had a forearm length of 10.68 ± 0.54 mm in females and 10.72 ± 0.73 mm in males, representing

Table 2. Development of the fur in *Natalus mexicanus* inhabiting "Los Laguitos" cave, Chiapas, Mexico. For each age category, the number of individuals in each development stage is given. I) pink skin, body completely naked, with no vibrissae. II) light-pink body, white membranes; vibrissae have emerged, hair on genitals and toes. III) grayish dorsum, with darker crown, interscapular region, patagium, forearm, and legs; bright patagium and pink abdomen. IV) pigmented abdomen; body covered with gray and short hair. V) all skin darker, with brown hair on the dorsum and whitish hair in the abdomen.

Age/days	Category				
	I	II	III	IV	V
1	94				
5	33				
10	5	18			
15		21	3		
20		8	3		
25		8	6		
30		3	7	1	
35			2	6	4
40				3	9
45				1	2
50				1	3
55					3

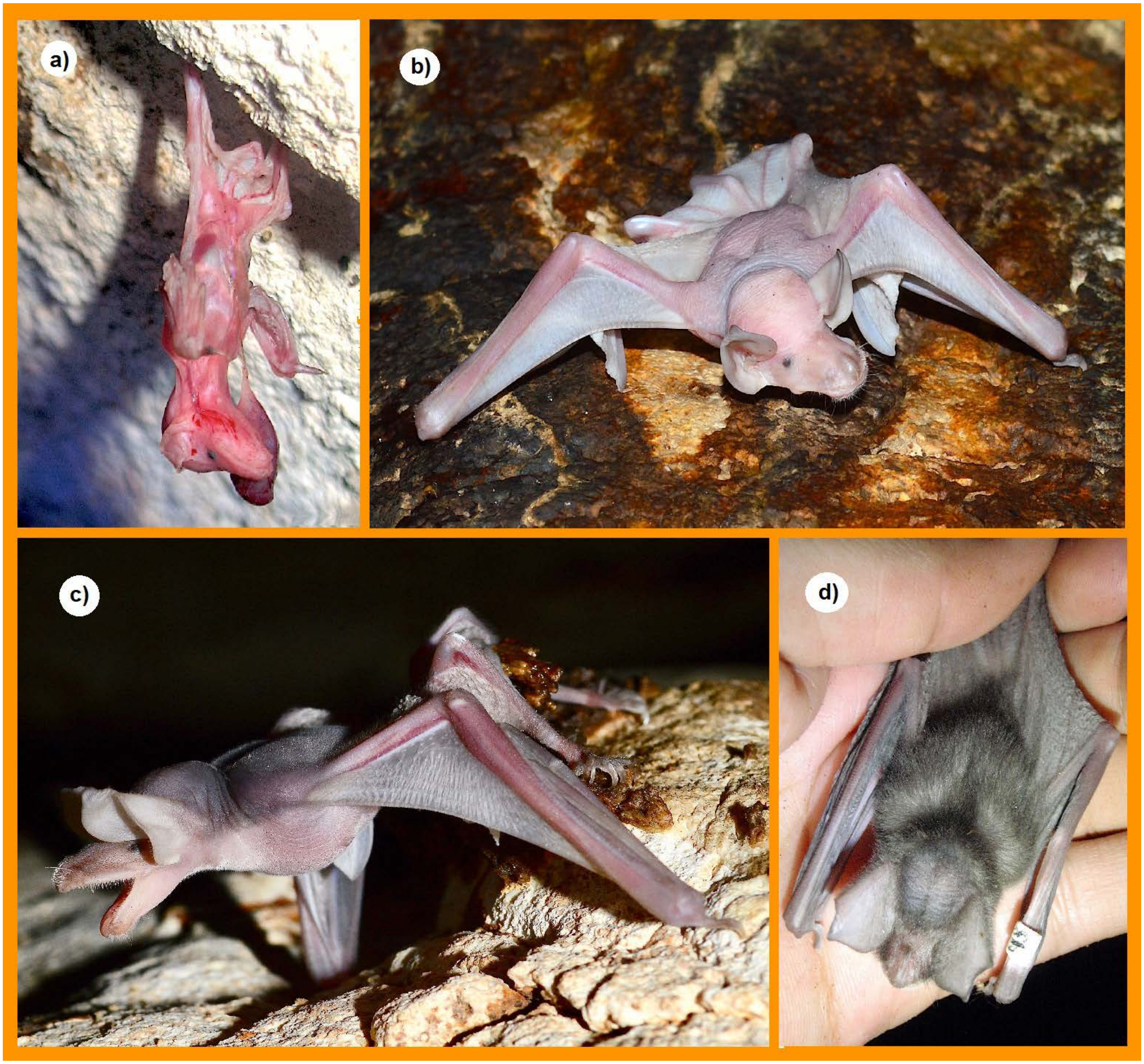

Figure 1. Specimens of *Natalus mexicanus* of different ages observed in "Los Laguitos" cave, Chiapas, Mexico, in 2009. a) Neonate with placenta, closed eyes, folded ears, pink, and naked body. b) 15-day-old individual with closed eyes; pigmentation appears in the dorsum and crown. c) 30-day individual with open eyes and covered with fine grayish fur; and d) 40-day individual covered with dark fur.

27.15 % and 27.26 %, respectively, of postpartum females. Body mass was 0.93 ± 0.21 g in females and 1.03 ± 0.22 g in males, representing 17.27 % and 19.20 % of postpartum females. The epiphyseal gap measured 2.3 ± 0.25 mm in females and 2.4 ± 0.27 mm in males.

Secondary sexual dimorphism. Of the three morphometric variables analyzed, only neonate body mass showed significant differences between sexes ($T_{93} = 2.37$, $p = 0.01$), while sexes were statistically similar across the rest of the age categories. As regards forearm length and epiphyseal cartilage length, no differences between sexes were significant in any age category ($P > 0.05$); thus, data for both sexes were pooled together for subsequent analyses to increase the sample size.

Growth. Forearm length and body mass increased continuously over 55 days post-birth (Table 3). In the first 35 days, these variables increased linearly at a rate of 0.72 mm/day in length of and 0.07 g/day in body mass. After day 35, the growth rate of both variables decreased to 0.19 mm/day and 0.05 g/day, respectively (Figure 2a, b). The length of the epiphyseal gap increased over the first 25 days post-birth at 0.098 mm/day, and then closed at 0.075 mm/day (Table 3, Figure 2b).

Growth models. Of the three models used to describe the forearm and body mass growth patterns, the logistic model yielded the best fit; therefore, the only equations shown below correspond to this model: forearm = $42.44 / (1 + 3.29^{-0.07\ (age)})$, $R^2 = 0.96$; mass = $5.67 / (1 + 4.63^{0.10\ (age)})$,

R^2 = 0.91. For its part, the growth pattern of the length of the epiphyseal gap fitted a second-order polynomial model (epiphyseal gap length = $-0.0032x^2 + 0.1678x + 2.2084$, R^2 = 0.80; Figure 2a, b), which predicts the complete closure or calcification of the cartilaginous epiphysis between 63 and 64 days of age.

Flight development. At birth, 100 % of neonates were classified as non-volant. The first individual classified as a semi-volant was 20 days old, and the first to achieve sustained flight was 35 days old. At 50 and 55 days of age, all recaptured specimens were volant (Table 4). The number of specimens recaptured after day 1 post-birth decreased with time, because after marking, some pups were moved from the original perch site by the mother. Besides, as pups developed, they began moving through the walls and mingling with the rest of the population; finally, when they started flying, they flew away from the nursery as soon as they noticed our presence, so recapturing them became impossible.

Discussion

Natalus mexicanus uses daytime shelters with temperatures ranging from 25.0 to 36.6 °C and RH above 74 % (Mitchell 1965; Torres-Flores and López-Wilchis 2010). These conditions occur all year round in "Los Laguitos", maintaining a mean temperature of 32.3 °C and RH of 95.4 % (Martínez-Coronel *et al.* 2010) and were above average during the study period. The conditions inside the cave were favorable for the growth and development of pups because they are born naked and lose moisture easily (Mitchell 1965; Kunz and Hood 2000; Ruczynski 2006; Pretzlaff *et al.* 2010).

In "Los Laguitos" cave, the period of births of *N. mexicanus* lasted 25 days, from late May to late June; in Alamos, Sonora, it spans from late July to early August (Mitchell 1965), one month after it ended in the Chiapas population. These results differ from the findings by Tejedor (2019), who reported similar patterns in northern and southern populations. However, for a species with a wide geographic distribution such as *N. mexicanus*, with populations that do not migrate latitudinally, differences are likely to exist because of local adaptations to different conditions (Kunz and Hood 2000; Barclay and Harder 2003). Geographic differences in birth season, growth, and postnatal development have also been reported for *Leptonycteris yerbabuenae* and *Miniopterus schreibersii* (Gould 1975; Richardson 1977; Martínez-Coronel *et al.* 2014), species with wide distributions.

Table 3. Postnatal growth of *Natalus mexicanus* inhabiting "Los Laguitos" cave, Chiapas, Mexico. For each age category, changes in body mass (grams), forearm length and length of the cartilaginous epiphyseal gap of the fourth metacarpal-phalangeal joint (millimeters) of the right wing are shown. For each measurement, the sample size (*n*) and mean ± standard deviation are shown.

Age/days	n	Weight	Forearm	Epiphysis
1	94	0.98 ± 0.02	10.71 ± 0.64	2.36 ± 0.02
5	33	1.63 ± 0.06	12.70 ± 0.15	2.96 ± 0.08
10	23	2.43 ± 0.13	16.22 ± 1.67	3.53 ± 0.08
15	24	2.85 ± 0.08	20.05 ± 2.02	4.07 ± 0.08
20	11	3.69 ± 0.19	24.60 ± 2.73	4.10 ± 0.17
25	14	4.20 ± 0.21	28.74 ± 3.05	4.44 ± 0.10
30	11	4.55 ± 0.19	31.22 ± 3.58	4.40 ± 0.13
35	12	5.08 ± 0.17	34.41 ± 1.23	4.05 ± 0.16
40	12	5.45 ± 0.20	36.90 ± 1.28	3.52 ± 0.12
45	3	5.40 ± 0.40	38.00 ± 0.42	3.60 ± 0.20
50	4	5.97 ± 0.25	38.30 ± 2.67	2.72 ± 0.30
55	3	6.16 ± 0.28	39.20 ± 0.30	1.93 ± 0.23

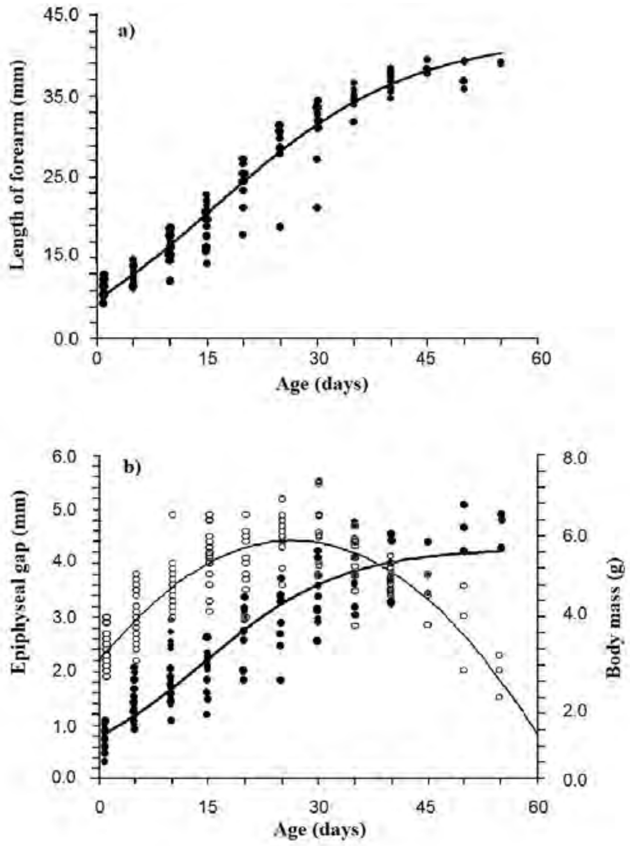

Figure 2. Growth trends of three morphometric variables evaluated in *Natalus mexicanus* from "Los Laguitos", Chiapas, Mexico, in 2009. a) forearm length. b) length of the cartilaginous ephiphyseal gap of the metacarpal-phalangeal joint of the fourth finger and body mass. The length of forearm and epiphysis gap curves correspond to those generated by the logistic equation model, while the one for body mass corresponds to a polynomial model.

Bats give birth to altricial pups, although there are differences in the degree of development between species (Kunz and Hood 2000). Examples of neonates that are born with the body covered with hair and open eyes and auditory meatus include *Rosettus leschenaulti* and *R. amplexicaudatus* (Elangovan *et al.* 2002; Giannini *et al.* 2006), *Artibeus* sp., *Carollia perspicillata*, *Desmodus rotundus*, *Diphylla ecaudata*, *Glossophaga* sp., and *Uroderma bilobatum* (Kleiman and Davis 1979; Kunz and Hood 2000; Delpietro and Russo 2002; Cretekos *et al.* 2005; Reyes-Amaya and Jerez 2013; Kohles *et al.* 2018), *Myotis albescens* and *Eumops patagonicus* (Rodríguez *et al.* 2018). Most of these species thrive in tropical areas, use outdoor shelters, and neonates are carried around by their mothers during foraging from day one post-birth (Kleiman and Davis 1979; Hernández-Mijangos *et al.* 2009). However, other species such *as Hipposideros speoris*, *Leptonycteris* *yerbabuenae*, *Myotis emarginatus, Rhinolophus mehelyi*, and *Tadarida brasiliensis* (McCracken and Gustin 1991; Sharifi 2004; Martínez-Coronel *et al.* 2014; Doss *et al.* 2018; Eghbali and Sharifi 2018), give birth to completely naked pups that remain with the mother during the day, presumably because of their poor thermoregulation (Studier and O'Farrell 1972). Pups are left in nurseries during the night and are recovered by mothers upon returning from foraging to be breastfed (Martínez-Coronel *et al.* 2014). These data contrast with studies on a population of *N. mexicanus* in Sonora and on *Pipistrellus pipistrellus* (Mitchell 1965; Hughes *et al.* 1995). These studies reported that pups remained separate from adults during the day and were only recovered at night to be breastfed; the authors provided no explanation for this behavior.

In the population of *N. mexicanus* inhabiting "Los Laguitos", Chiapas, pups are born with eyes closed and remain so for the first 15 days post-birth; eyes are completely open by day 25. On the other hand, in a population of the same species living in Alamos, Sonora (Mitchell 1965), eyes were fully open by day 17. The results of this study contrast with findings for some species of insectivorous bats, such as *Myotis capaccinii*, *M. emarginatus*, *Pipistrellus mimus, Rhinolophus mehelyi,* and *Vespertilio sinensis,* in which eyes open between four and eight days of age (Isaac and Marimuthu 1996; Jin *et al.* 2012 a, b; Eghbali and Sharifi 2018; Mehdizadeh *et al.* 2018), whereas in *Hipposideros pomona* eyes open completely until day 13 post-birth (Lin *et al.* 2011). Eye opening in the population *of N. mexicanus* studied occurs later, possibly because pups are born in a less advanced development stage compared to the other species. Likewise, the development of the fur of *N. mexicanus* in "Los Laguitos" follows a pattern like that of the eyes. The body remains apparently naked during the first 15 days post-birth, with long hairs evident only in the genital area and rostrum. A short, grayish fur is visible until 30 days of age, while a long, dark fur appears between 35 and 40 days old. In contrast, Mitchell (1965) reported that in the population of Sonora, the body of pups was already covered with a short fur by day 17 post-birth. In *Thyroptera tricolor* and *V. sinensis*, a grayish fur appears at 21 and 16 days of age, respectively (Chaverri and Vonhoff 2011; Jin *et al.* 2012 b), and in *M. emarginatus* between 25 to 30 days (Eghbali and Sharifi 2018). These data indicate that *N. mexicanus* is a species in which eye opening and fur development take longer.

Bats give birth to relatively large neonates compared to terrestrial mammals of the same size. This reduces the risk of dying from sudden changes in environmental temperature, relative humidity, and predation, as bat pups require less investment in parental care before reaching independence (Racey and Entwistle 2000). In *N. mexicanus*, the body mass of neonates is 18.23 % relative to adults, a value that is below the average of 23.0 ± 8.0 % reported for Chiroptera (Barclay and Harder 2003). In other bat species, neonate body mass is equivalent to 20.7 % of adult biomass in *Phyllostomus hastatus* (Stern and Kunz 1998), 25 % in *Tadarida brasiliensis* (Kunz and Robson 1995); 26 % in *T. tricolor* (Chaverri and Vonhoff 2011), 28 % in *Carollia perspicillata* (Kleiman and Davis 1979) and *M. lucifugus* (O´Farrell and Studier 1973), 39 % in *H. pomona,* 40 % in *M. capaccini* (Mehdizadeh *et al.* 2018), and 56 % in *Artibeus watsoni* (Chaverri and Kunz 2006).

Table 4. Flight development in *Natalus mexicanus* inhabiting "Los Laguitos" cave, Chiapas, Mexico. For each age category, the number of individuals in each flight development stage is given.

Age (days)	**Category**		
	Non-volant	**Semi-volant**	**Volant**
1	94		
5	33		
10	23		
15	24		
20	10	1	
25	9	5	
30	5	6	
35	1	8	3
40		3	9
45		1	2
50			4
55			3

The secondary sexual dimorphism in bats is variable; in most species, females tend to be larger than males (Ralls 1977; Hurtado *et al.* 2015; Ruelas 2019). In the case of small-sized species, when females are larger than males, this is a consequence of their reproductive role. During nursing, females take care of pups from conception to weaning; therefore, they must store energy during the first weeks of gestation, since by the end of pregnancy the amount of food consumed decreases because of the space occupied by the fetus (De Camargo and Oliveira 2013; Barclay and Harder 2003). Besides, carrying the fetus or pup during foraging implies a greater winged load; consequently, wings tend to be larger to improve flight performance (De Camargo and Oliveira 2013; Hurtado *et al.* 2015; Stevens and Platt 2015). When males are larger than females, these differences are explained as related to sexual competition (Ralls 1977). In *N. mexicanus* living in "Los Laguitos" cave, of the three morphometric variables analyzed, we only found significant differences in neonate body mass, with males being significantly larger than females. However, no evidence of secondary sexual dimorphism was found in the other variables at any age categories. These results are consistent with those of Tejedor (2011), who found that in adults of *N. mexicanus* the sexes are similar in forearm length and body mass.

The pattern of forearm growth, body mass, and epiphyseal gap in *N. mexicanus* was like that of *Hipposideros cineraceus*, *M. capaccinii*, *T. brasiliensis*, and *T. tricolor* (Kunz and Robson 1995; Jin *et al.* 2010; Chaverri and Vonhoff 2011; Mehdizadeh *et al.* 2018). In the first two variables, growth was linear in the period prior to sustained flight, which started at 35 days of age, and the growth rate decreased thereafter. Similar results were observed in the population of Alamos, Sonora (Mitchell 1965). During flight, wing fingers are subjected to stress; thus, phalanges must be sufficiently resistant, and the wing membrane must have a minimum area, for flapping to produce the aerodynamic force necessary to sustain the body and move it through air (Hughes *et al.* 1995; Stern *et al.* 1997; Elangovan *et al.* 2002). Therefore, phalanges were expected to experience accelerated growth before the onset of sustained flight, while the cartilaginous areas of the epiphyses undergo increasing calcification to avoid dislocations. In *N. mexicanus*, the epiphyseal gap reached its maximum size at 25 days of age, closing in the following days before sustained flight was achieved, as in other species (Stern and Kunz 1998; Lin *et al.* 2010; Jin *et al.* 2012a, b; Mehdizadeh *et al.* 2018). The growth model obtained predicted that the epiphysis gap should close completely at day 64. However, this prediction could not be corroborated in the present study, since specimens could no longer be recaptured after 55 days post-birth because they mingled with the rest of the population, which included several thousands of individuals. The epiphyseal gap closure time was intermediate relative to other insectivorous species such as *Pipistrelus subflavus* (6.6 g), with closure at 45 days (Hoying and Kunz 1998); *R. mehelyi* (14 g), at 55 days (Sharifi 2004); and *R. hipposideros* (8 g), at 80 days (Reiter 2004).

As mentioned above, body mass increased in the period prior to sustained flight and no decrease was observed during the learning process, as reported for other bat species (Kunz 1973; Hughes *et al.* 1995; Hoying and Kunz 1998; Lin *et al.* 2010). Hoying and Kunz (1998) suggest that the decrease in body mass during this stage may be a consequence of the energy demand required by flight. However, Hughes *et al.* (1995) mention that it may be a strategy of the species, since when young bats start flying, their muscles still do not produce sufficient force and thus the wing load is greater; hence, reducing body mass facilitates learning to perform the necessary flight maneuvers. To note, these theories are not entirely satisfactory, as more than half of the species studied do not undergo a decrease in body mass during this stage; therefore, further research is needed (Hoying and Kunz 1998; Lin *et al.* 2010).

Of the three models used to describe the increase in body mass and forearm length in *N. mexicanus,* the logistic model best fitted the empirical data, as reported elsewhere (Hoying and Kunz 1998; Lin *et al.* 2010; Mehdizadeh *et al.* 2018). In *N. mexicanus,* the growth constants of body mass and forearm length were 0.10 and 0.07, respectively. These values indicate a lower growth rate relative to other tropical insectivorous species such as *H. cineraceus*, with weight and forearm values of 0.12 and 0.10, respectively, or *H. larvatus*, with 0.12 and 0.13, respectively (Jin *et al.* 2010; Lin *et al.* 2010). Also, Kunz and Hood (2000) and Lin *et al.* (2010) mention that although it is valid to compare growth rates between species, it should be noted that some variables affect growth rate, such as temperature, food availability, maternal size, and colony size, which cannot be controlled by the researcher, especially in field studies.

Regardless of the degree of development of the eyes and fur in neonates, bats are altricial before flight and depend entirely on the mother during the first postnatal days (Kunz and Hood 2000). In most species, sustained flight begins when pups attain a relative mass above 70 % and a forearm length of more than 90 % in relation to adults (Barclay 1994; Stern and Kunz 1998). There are exceptions, as the case of *L. yerbabuenae,* which attains sustained flight when body mass is 50 % and forearm length is 84 % relative to adults (Martínez-Coronel *et al.* 2014), while *R. leschenautti* attains it with 35 % body mass and 75 % forearm length (Elangovan *et al.* 2002). In the present study, we found that *N. mexicanus* displayed sustained flight at 35 days of age, when body mass was 86.38 % and forearm length was 95.35 % relative to adults. These values are above those recorded for other insectivorous species such as *M. capaccinii*, which achieves flight at day 28, with a weight of 82.60 % and forearm length of 90.31 % (Mehdizadeh *et al.* 2018); *P. mimus* at day 29, with a weight of about 80 % (Isaac and Marimuthu 1996); *H. larvatus* at day 24, with a weight of 61.4 % and forearm length of 92.4 % (Lin *et al.* 2010); *R. mehelyi* at day 28; with a weight of 92 % and forearm length of 87 % (Sharifi 2004). In contrast, in *T. tricolor* the onset of flight occurs up to day 60, with a weight of 77 % and forearm length of 95 % (Chaverri and Vonhoff 2011); and in *Artibeus watsoni* flight starts at day 35, when forearm length reaches 100 % and weight is 80 % relative to adults (Chaverri and Kunz 2006). Except for pteropodids, these results suggest that there is no trend regarding the age at onset of flight in the different species and families studied in temperate and tropical areas, nor between insectivorous and frugivorous bats (Kunz and Hood 2000; Lin *et al.* 2010).

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Literature cited

Barclay, R. M. R. 1994. Constraints on reproduction by flying vertebrates: energy and calcium. American Naturalist 144:1021-1031.

Barclay, R. M. R., and L. D., Harder. 2003. Life histories of bats: life in the slow lane. Pp. 209-253, *in* Bat ecology (Kunz, H., and M. B. Fenton, eds.). University of Chicago Press, Chicago.

Brown, D., and P. Rothery. 1994. Models in Biology: Mathematics, Statistics and Computing. Wiley. Inglaterra.

Cardoso, C. M. D. 1979. El clima de Chiapas y Tabasco. Universidad Autónoma de México. Ciudad de México, México.

Chaverri, G., and T. H. Kunz. 2006. Reproductive biology and postnatal development in the tent-making bat *Artibeus watsoni* (Chiroptera: Phyllostomidae). Journal of Zoology 270:650-656.

Chaverri, G., and M. J. Vonhof. 2011. Reproduction and growth in a Neotropical insectivorous bat. Acta Chiropterologica 13:147-155.

Cretekos, C. J., S. D. Weatherbee, C. H. Chen, N. K. Badwaik, L. Niswander, R. R. Behringer, and J. J. Rasweiler IV. 2005. Embryonic staging system for the short-tailed fruit bat, *Carollia perspicillata*, a model organism for the mammalian order chiroptera, based upon time pregnancies in captive-bred animals. Developmental Dynamics 233:721-738.

De Camargo, N. F., and H. F. M. de Oliveira. 2013. Sexual dimorphism in *Sturnira lilium* (Chiroptera, Phyllostomidae): can pregnancy and pup carrying be responsible for differences in wing shape? PLoS ONE 8:10.1371

Delpietro, V. H. A., and R. G. Russo. 2002. Observations of the common vampire bat (*Desmodus rotundus*) and the hairy-legged vampire bat (*Diphylla ecaudata*) in captivity. Mammalian Biology 67:65-78.

Dmitriew, C. M. 2011. The evolution of growth trajectories: what limits growth rate? Biological Reviews 86:97-116.

Doss, D. P. S., H. Raghuran, S. Muthuselvan, M. R. Sudhakaran, and S. S. Isaac. 2018. Postnatal growth and age estimation in a tropical insectivorous bat, *Hipposideros speoris*. International Journal of Zoology and Applied Biosciences 3:34-40.

Eghbali, H. y M. Sharifi. 2018. Postnatal growth, age estimation, and wing development in Geoffroy´s bat *Myotis emarginatus* (Chiroptera: Vespertilionidae). Mammal Study 43:153-165

Elangovan, V., H. Raghuram, E. Y. S. Priya, and G. Marimuthu. 2002. Postnatal growth, age estimation and development of foraging behaviour in the fulvous fruit bat *Rousettus leschenaulti*. Journal of Biosciences 27:695-702.

Giannini, N., A. Goswami, and M. R. Sánchez-Villagra. 2006. Development of integumentary structures in Rousettus amplexica*udatus* (Mammalia: Chiroptera: Pteropodidae) during late-embryonic and fetal stages, Journal of Mammalogy 87:993-1001.

Gould, E. 1975. Neonatal vocalizations in bats of eight genera. Journal of Mammalogy 56:15-29.

Hernández-Mijangos, L. A., A. Horváth, and R. Pérez Canales. 2009. Observations on female bats transporting non-volant juveniles during flight. Chiroptera Neotropical 15:472-476.

Hoying, K. M., and T. H. Kunz. 1998. Variation in size at birth and pos-natal growth in the insectivorous bat *Pipistrellus subflavus* (Chiroptera: Vespertilionidae). Journal of Zoology 245:15-27.

Hughes, P., J. M. V. Rayner. and G. Jones. 1995. Ontogeny of 'true' flight and other aspects of growth in the bat *Pipistrellus pipistrellus*. Journal of Zoology (London) 235:291-318.

Hurtado, N., R. D. Sepúlveda, and V. Pacheco. 2015. Sexual size dimorphism of a sensory structure in a monomorphic bat. Acta Chiropterologica 17:75-83.

Isaac, S. S., and S. S. Marimuthu. 1996. Postnatal growth and age estimation in the Indian pygmy bat *Pipistrellus mimus*. Journal of Mammalogy 77:199-204.

Jin, L., A. Lin, K. Sun, Y. Liu, and J. Feng. 2010. Postnatal growth and age estimation in the Ashy leaf-nosed bat, *Hipposideros cineraceus*. Acta Chiropterologica 12:155-160.

Jin, L., L. Bo., K. Sun, Y. Liu, J. P. Ho, and J. Feng. 2012a. Postnatal growth and age estimation in Marsahll´s horshoe bat, *Rinolophus marshalli*. Acta Chiropterologica 14:105-110.

Jin, L., J. Wang, Z. Zhang, K. Sun, J. S. Kanwal, and J. Feng. 2012b. Postnatal development of morphological and vocal features in Asian parti-colored bat, *Vespertilio sinensis*. Mammalian Biology 77:339-344.

Kleiman, D. G., and T. M. Davis. 1979. Ontogeny and maternal care. Pp. 387-402 in Biology of bats of the New World family Phyllostomidae. Part III (Baker, R. J., J. K. Jones, Jr., and D. C. Carter, eds.). Special Publications of the Museum, Texas Tech University 16:1-441.

Kohles, J.E., R. A. Page, D. K. N. Dechmann, and M. T. O'Mara. 2018. Rapid behavioral changes during early development in Peters' tent-making bat (*Uroderma bilobatum*). PLoS ONE 13: e0205351.

Kunz, T. H. 1973. Population studies of the cave bat (*Myotis velifer*): reproduction, growth and development. Occasional Papers of the Museum of Natural History, University of Kansas 15:1-43.

Kunz, T. H., and W. R. Hood. 2000. Parental care and postnatal growth in the Chiroptera. Pp. 415-468, *in* Reproductive biology of bats (Crichton, E. G., and P. H. Krutzsch, eds.). Academic Press.

Kunz, T. H., and E. L. P. Anthony. 1982. Age estimation and post-natal growth in the bat *Myotis lucifugus*. Journal of Mammalogy 63:23-32.

Kunz, T. H., and S. K. Robson. 1995. Postnatal growth and development in the mexican free-tailed bat (*Tadarida brasiliensis mexicana*): birth size, growth rates, and age estimation. Journal of Mammalogy 76:769-783.

Lin, A-Q., L.-R. Jin, Y. Liu, K.-P. Sun, and J. Feng. 2010. Postnatal growth and age estimation in horsefield`s leaf-nosed bat *Hipposideros larvatus*. Zoological Studies 49:789-796.

Lin, A.-Q., L.-R. Jin, L.-M. Shi, K.-P. Sun, S. W. Berquist, Y. Liu, and J. Feng. 2011. Postnatal development in Andersen´s leaf-nosed bat *Hipposideros pomona*: flight, wing shape, and wing bone lengths. Zoology 114:69-77.

Martínez-Coronel, M., E. García, C. Müdespacher-Zihel, and A. Torres. 2010. El microclima de la cueva de "Los Laguitos" y su relación con los murciélagos. Mundos Subterráneos 21:74-89.

Martínez-Coronel, M., F. A. Cervantes, and Y. Hortelano-Moncada. 2014. Crecimiento y desarrollo postnatal del vuelo en el murciélago *Leptonycteris yerbabuenae* en Chiapas, México. Therya 5:303-322.

McCracken, G. F., and M. K. Gustin. 1991. Nursing behavior in mexican free-tailed bats maternity colonies. Ethology 89:305-321.

Mehdizadeh, R. H., Eghbali, and M. Sharifi. 2018. Postnatal growth and vocalization development in the long-fingered bat, *Myotis capaccinii* (Chiroptera, Vespertilionidae). Zoological Studies 57: 1-13.

Miranda, F. 1998. La vegetación de Chiapas. Tercera edición. Consejo Estatal para la Cultura y las Artes de Chiapas, México.

Mitchell, G. C. 1965. A natural history study of the funnel-eared bat *Natalus stramineus*. Unpublished M.Sc. thesis, University of Arizona. hdl.handle.net/10150/318407

NCSS. 2020. The Number Cruncher Statistical System. Published by NCSS. Hintze, L. J. Kaysville, Utah.

O'Farrell, M. J., and E. H. Studier. 1973. Reproduction growth and development in *Myotis thysanodes* and *M. lucifugus* (Chiroptera: Vespertilionidae). Ecology 54:18-30.

Pretzlaff, I., G. Kerth, and K. H. Dausmann. 2010. Communally breeding bats use physiological and behavioural adjustments to optimise daily energy expediture. Naturwissenschaften 97:353-363.

Racey, P. A., and A. C. Entwistle. 2000. Life history and reproductive strategies of bats. Pp. 364-414 in Reproductive biology of bats (Crichton, E. G., and P. H. Krutzsch, eds.). Academic Press.

Ralls, K. 1977. Sexual dimorphism in mammals: Avian models and unanswered questions. The American Naturalist 111:917–938.

Reiter, G. 2004. The importance of woodland for *Rhinolophus hipposideros* (Chiroptera, Rhinolophidae) in Austria. Mammalia 68:403-410.

Reyes, Amaya, N., and A. Jerez. 2013. Postnatal cranial ontogeny of the common vampire bat *Desmodus rotundus* (Chiroptera: Phyllostomidae). Chiroptera Neotropical 19:1198-1211.

Richardson, C. S., and T. H. Kunz. 2000. Growth rates and age estimation in free-ranging bats: a comparison of longitudinal and cross-sectional sampling methods. Journal of Mammalogy 81:709-781.

Richardson, E. G. 1977. The biology and evolution of the reproductive cycle *of Miniopterus schreibersii* and *M. australis* (Chiroptera: Vespertilionidae). Journal of Zoology 183:353-375.

Rodríguez, F. E., M. T. Sandoval, B. B. Álvarez, and D. M. Lombardo. 2018. Comparative study of prenatal development between *Myotis albescens* (Chiroptera: Vespertilionidae) and *Eumops patagonicus* (Chiroptera: Molossidae): the chorionic vesicle and extraembryonic membranes considerations. The Anatomical Record 301:1527-1543.

Ruczynski, I. 2006. Influence of temperature on maternity roost selection by noctule bats (*Nyctalus noctula*) and Leisler's bats (*N. leisleri*) in Białowieza Primeval Forest, Poland. Canadian Journal of Zoology 84:900-907.

Ruelas, D. 2019. Variación ontogenética y dimorfismo sexual en *Platalina genovensium* (Chiroptera: Phyllostomidae). Revista Peruana de Biología 26: 201-210.

Sedgeley, J. A. 2001. Quality cavity microclimate as a factor influencing selection of maternity roosts by a tree-dwelling bat, *Chalinolobus turberculatus*, in New Zealand. Journal of Applied Ecology 38:425-438.

Sharifi, M. 2004. Postnatal Growth and Age Estimation in the Mehely's Horseshoe Bat (*Rhinolophus mehelyi*). Acta Chiropterologica 6:155-161.

Stern, A. A., and T. H. Kunz. 1998. Intraspecific variation in postnatal growth in the greater spear-nosed bat. Journal of Mammalogy 79:755-763.

Stern, A. A., T. H. Kunz, and S. S. Bahtt. 1997. Seasonal wing loading and the ontogeny of flight in *Phyllostomus hastatus* (Chiroptera: Phyllostomidae). Journal of Mammalogy 78:1199-1209.

Stevens, R. D., and R. N. Platt. 2015. Patterns of secondary sexual size dimorphism in New World *Myotis* and a test of Rensch's rule. Journal of Mammalogy 96:1128-1134.

Studier, E. H., and M. J. O'Farrell. 1972. Biology of *Myotis thysanodes* and *M. lucifugus* (Chiroptera: Vespertilionidae) I. Thermoregulation. Comparative Biochemistry and Physiology, Part A Physiology 41:567-595.

Tejedor, A. 2011. Systematics of funnel-eared bats (Chiroptera: Natalidae). Bulletin of the American Museum of Natural History 353:1-140.

Tejedor, A. 2019. Family Natalidae (Funnel-eared bats). Pp. 584-596, *in* Handbook of the Mammals of the World. Volume 9. Bats (Wilson, D. E., and R. A. Mittermeier, eds.). Lynx Edicions, Barcelona.

Torres-Flores, J.W., and R. López-Wilchis. 2010. Condiciones microclimáticas, hábitos de percha y especies asociadas a los refugios de *Natalus stramineus* en México. Acta Zoológica Mexicana (n. s.) 26:191-213.

Torres-Flores, J.W., and R. López-Wilchis. 2019. Trophic niche and diet of *Natalus mexicanus* (Chiroptera: Natalidae) in a tropical dry forest of western Mexico. Acta Chiropterologica 20:343-350.

Zullinger, E. M., R. E. Ricklefs, K. H. Redford, and G. M. Mace. 1984. Fitting sigmoidal equations to mammalian growth curves. Journal of Mammalogy 65:607-636.

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Biology and parasites of *Pteronotus gymnonotus* from the Caatinga shrublands of Ceará (Brazil)

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Mormoopid bats are distributed from southern United States of America to Brazil and comprise the genera *Mormoops* and *Pteronotus*. Although forms of *Mormoops* in Bahia, Brazil were described for the Quaternary, only some of the extant species of *Pteronotus* occur in this country, including *P. gymnonotus*. The species distribution ranges from southeastern México to northeastern Bolivia and central Brazil. This work presents information about food preference, reproduction, and the ectoparasitological fauna of *P. gymnonotus* in the state of Ceará. Fieldwork took place over ten consecutive days in the rain and dry seasons, in 2000, 2012, 2013, and 2019, on trails within the Serra das Almas Private Natural Heritage Reserve. A total of 14 *P. gymnonotus* specimens were caught in the three main phytophysiognomies present in the region. They were inspected for ecto and endoparasites, and fecal material was collected and analyzed under a light microscope. Of the total collected females, four were pregnant and one was lactating in January, during the rain season. No food residues or evidence of endoparasites were observed in the gastric content of the animals. Regarding the fecal samples, fragments of insects were found and identified as belonging to the orders Coleoptera and Lepidoptera. Seven individuals were infested with ectoparasites of the subfamily Acari and newly described associations with Diptera genera are presented. Due to the scarcity of data about these bats in the Caatinga biome, data reported here help explain the ecological interactions of *P. gymnonotus* in this semiarid area in Brazil.

Los murciélagos Mormopidos se distribuyen desde el sur de los Estados Unidos de América hasta Brasil y comprenden los géneros *Mormoops* y *Pteronotus*. Aunque las formas de *Mormoops* son conocidas para el Cuaternario de Bahía, Brasil, solamente algunas de las especies recientes de *Pteronotus* se encuentran en este país. La distribución de la especie comprende desde el sureste de México hasta el noroeste de Bolivia y centro de Brasil. Este estudio presenta información sobre la preferencia alimentaria, reproducción y fauna ectoparasitológica de *P. gymnonotus* en el Estado de Ceará. El trabajo de campo se llevó a cabo durante diez días consecutivos en las estaciones lluviosa y seca en los años 2000, 2012, 2013 y 2019 en senderos dentro de la Reserva Privada del Patrimonio Natural Serra das Almas. Se capturaron un total de 14 especímenes de *P. gymnonotus* en las tres principales fitofisionomías presentes en la región. Fueron inspeccionados en busca de ecto y endoparásitos y el material fecal se recogió y analizó con un microscopio óptico. Del total de hembras recolectadas en enero, cuatro estaban preñadas y una en lactancia, durante la época de lluvias. No se observaron residuos de alimentos ni evidencia de endoparásitos en el contenido gástrico de los animales. En cuanto a las muestras fecales, se encontraron fragmentos de insectos que se identificaron como pertenecientes a los órdenes Coleoptera y Lepidoptera. Siete individuos encontraron infestados por ectoparásitos pertenecientes a la subfamilia Acari y se presentan nuevas asociaciones con géneros del orden Diptera. Debido a la escasez de datos sobre estos murciélagos en el Bioma de Caatinga, los datos presentados aquí ayudan a comprender las interacciones ecológicas de *P. gymnonotus* en una zona semiárida de Brasil.

Keywords: diet; ectoparasite; geographic distribution; hemoparasite; reproduction.

Introduction

Of the 1,386 species of bats in the world (Burgin *et al.* 2018), the greatest biodiversity occurs in Brazil where there are 182 species (Nogueira *et al.* 2018; Quintela *et al.* 2020). The Mormoopidae was recognized as a family by Smith(1972); previously it was part of the family Phyllostomidae. This group of bats occurs from southern United States of America (U.S.A.) to Brazil and comprises the genera *Mormoops* and *Pteronotus*. Although forms of *Mormoops* in Bahia, Brazil were described for the Quaternary (Czaplewski and Cartelle 1998), only mormoopid extant species occur in this country: *Pteronotus alitonus*, *P. gymnonotus*, *P. personatus,* and *P. rubiginosus*.

Studies by Mares *et al.* (1981), Willig (1983) and Willig and Mares (1989) on the chiropterofauna of the Brazilian shrubland areas report the occurrence of *P. davyi* in the states of Ceará and Piauí. Later, Simmons (2005) and Patton and Gardner (2007) considered *P. gymnonotus* as a junior synonym of *P. davyi*, and thus these authors did not recognize the occurrence of this species in Brazil. The distribution of *P. gymnonotus* ranges from southeastern Mexico to northeastern Bolivia and central Brazil (Pavan and Tavares 2020). The first record of *P. gymnonotus* for the state of Ceará was published by Mares *et al.* (1981), who mistakenly identified specimens collected in the municipality of Crato as *P. davyi* (Patton and Gardner 2007). However, studies carried out in the Crato region, and the municipalities of Crateús and Aiuaba (Novaes 2012; Novaes and Laurindo 2014; Silva *et al.* 2015; Feijó and Rocha 2017), confirmed the occurrence of *P. gymnonotus* in this state.

Mormopids are found up to 3,000 m elevation and inhabit caves, rock crevices, open areas and dry deciduous and humid forests (Herd 1983; Patton and Gardner 2007). They are classified as aerial insectivorous bats (Simmons and Conway 2001; Ladle *et al.* 2012) and their diet consists mainly of insects of the order Coleoptera, as well as other hexapods, such as those belonging to the orders Lepidoptera, Diptera, Orthoptera, Hymenoptera, Hemiptera and Odonata (Rolfe and Kurta 2012).

Machado-Allison (1965) highlighted the high degree of specificity between *Cameronieta* species (Acari) and their respective hosts and advocated for the removal of Chilonycterine bats from the family Phyllostomidae. Subsequently, Smith (1972a) showed that ectoparasites of spinturnicid mites and flies found in mormopids are different from those recorded in phyllostomid bats.

The list of ectoparasites associated with *P. gymnonotus* includes the mite *Cameronieta* sp. (Almeida *et al.* 2016), Dipterans *Trichobius johnsonae* and *Nycterophilia fairchildi* (Figueiredo *et al.* 2015; Dick *et al.* 2016), and the tick *Ornithodoros marinkellei* (Luz *et al.* 2016). Regarding endoparasitic fauna, there are no records of helminths infesting these bats, only the presence of protozoan hemoparasites (Lima *et al.* 2015). Due to the scarcity of data on ecological associations of mormoopid bats in the Caatinga biome (northeastern Brazilian shrublands), this work provides information on food preference, reproduction, and evidences of new ectoparasites in association to *P. gymnonotus* in the state of Ceará.

Material and Methods

Fieldwork took place over ten consecutive days in the rain and dry seasons of 2000, 2012, 2013 and 2019, on trails within the Serra das Almas Private Natural Heritage Reserve (SANR; -5° 5' to -5° 15' S, -40° 50' to -41° 00' W) in the western part of Ceará State. The reserve is 5,845 ha (Figure 1) and was recognized in 2018 by the Latin American Network for the Conservation of Bats (RELCOM) as the first "Area of Importance for the Conservation of Bats" (AICOM - A-BR-002) in the Caatinga biome. The climate of the region is BSh (semi-arid climate) according to the Köppen classification system (Alvares *et al.* 2013), with a dry summer and an average annual temperature of 26.5 °C. Rain is highly irregular, which is typical for this Brazilian semiarid zone. The average rainfall is 870 mm/year, the rain season occurs from January to May, and the dry season occurs from June to December (INMET 2020). Three main phytophysiognomies of Caatinga vegetation, in different successional stages, are in the reserve: dry forest, carrasco scrub, and arboreal caatinga (Associação Caatinga 2012).

Bats were captured using eight mist nets extended from 18 to 24h, at elevations varying from 569 to 700 masl, in the three main phytophysiognomies in the region. Fecal samples were placed in plastic microtubes, ectoparasites were removed with fine-tipped tweezers and hematological slides were made according to Baptista and Esbérard (1997) to verify the occurrence of hemoparasites. For each specimen body measurements (total lenght, forearm, foot, ear, tibia, calcaneus and tail) were obtained using a digital caliper; weight and reproductive condition were also recorded.

Abdominal and thoracic cavities of necropsied specimens were examined to search for endoparasites. The fragments of insects found in the feces were mounted on permanent slides. Blood smears were prepared by using the "Panotic" rapid staining method (NewProv®) and observed using a Biofocus optical microscope (model Bio1600 BA-H; 100x and 400x). Dipterans were identified using a stereomicroscope (40x) and based on descriptions and keys by Wenzel *et al.* (1966) and Guerrero (1993; 1994; 1995). Mites were prepared using a clarification method following the Flechtmann (1990) technique, mounted on slides with a cover slip, and identified using the taxonomic key by Machado-Allison (1965). The bat specimens collected as vouchers are in the Adriano Lúcio Peracchi Collection (ALP-UFRRJ), currently housed at the Federal Rural University of Rio de Janeiro (UFRRJ / RJ), and the slides with the ectoparasites were deposited in the Acarological Collection at the Oswaldo Cruz Foundation - FIOCRUZ (Rio de Janeiro - RJ). The capture and collection of specimens was authorized by the Chico Mendes Institute for the Conservation of Biodiversity (ICMBio) of the Brazilian government (authorization number 32684-1).

Results

From a sampling effort (Straube and Bianconi 2002) of 103,313 m^2 h, a total of 14 *P. gymnonotus* specimens (12 females and two males) were caught between 620 and 700 masl elevation in the three phytophysiognomies in the region (Appendix 1). Of the total collected females (*n*= 12), four were pregnant and one was lactating (producing milk in the breast) in January, during the rain season. Two males and seven females were sexually inactive and were collected in both dry and rain seasons. Biometric data from these specimens are available in Table 1.

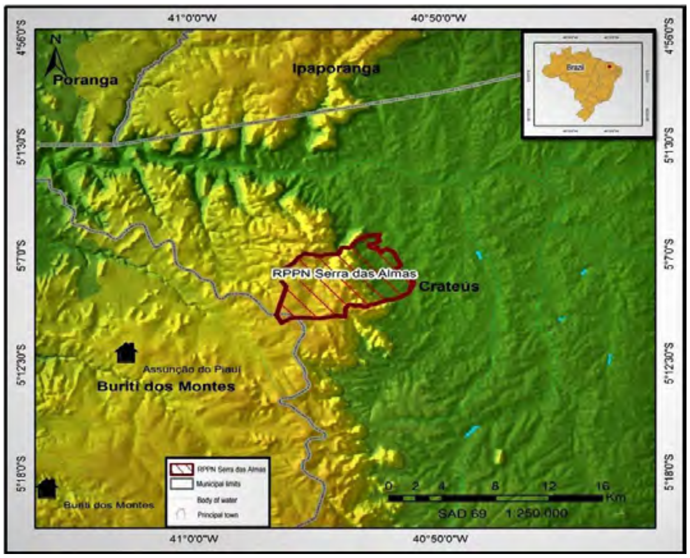

Figure 1. Location of Serra das Almas Private Natural Heritage Reserve in Crateús (Ceará, Brazil; Software ArcGis 10.1- Esri Maps; Prepared by: Sergio Carvalho Moreira, modified by Shirley Seixas Pereira da Silva).

Table 1. Mean biometric (in mm) data from *P. gymnonotus* specimens from Crateús (Ceará, Brazil).

	Pteronotus gymnonotus	
	Males (*n* = 2)	**Females (*n* = 12)**
Weight (g)	11.0 - 13.0	10.0 - 17.0
Total lenght	46.6 - 54.1	48.8 - 57.4
Forearm	48.7 - 51.0	48.4 - 51.2
Ear	12.8 - 17.6	11.9 - 18,7
Tibia	21.0 - 21.6	18.1 - 22.0
Foot	9.5 - 10.7	9.1 - 11.5
Tail	20.6 - 22.7	16.1 - 21.3

Of the *P. gymnonotus* specimens captured, it was possible to analyze the gastrointestinal content and fecal samples of three and 13 specimens, respectively. For three bats, both gastrointestinal and fecal samples were analyzed. No food residue or evidence of endoparasites was observed in the gastric content of the animals. Regarding the fecal samples, fragments of insects were found and identified as belonging to the orders Coleoptera and Lepidoptera (Figure 2). Some fragments were crushed, which made it impossible to identify them (38.2 %). The Coleoptera families consumed by bats in SANR were identified as Carabidae and Elateridae (Figure 3). Regarding the order Lepidoptera, the wing scales found were associated with the family Sphingidae (hawk moths: Figure 3).

When comparing diet between males and females of *P. gymnonotus* in our sample at SANR, both consumed Lepidoptera in dry and rain periods; only males consumed coleopterans in the rain season; the Coleoptera / Lepidoptera combination was consumed only by females in the rain season.

Seven individuals of *P. gymnonotus* were infested with ectoparasites. Specimens of the order Diptera and subfamily Acari were collected after checking the coat, patagium and uropatagium of the bats (Figure 3). Dipterans of Streblidae were identified as *Nycterophilia parnelli* (ALP 10194), *Megistopoda aranea* (ALP 10390), and *Trichobius johnsonae* (ALP 10386; ALP 10389; ALP 10422). Mite specimens were identified as *Cameronieta* sp. (Spinturnicidae).

Three bats were infested with both mites and dipterans, and one specimen had two Diptera species in its coat (Table 2). Regarding endoparasitic fauna, there were no records of infestation by helminths in the three bats inspected, and the analysis of the hematological slides did not show presence of hemoparasites.

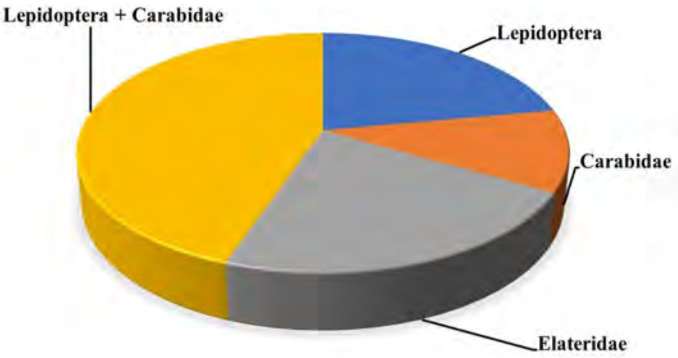

Figure 2. Food preferences of 13 *P. gymnonotus* specimens from Crateús (Ceará, Brazil).

Table 2. Ectoparasites found in association with *P. gymnonotus* from Crateús (Ceará, Brazil).

	Ectoparasites			
Bats	**Spinturnicidae**	**Streblidae**		
	***Cameronieta* sp.**	***Trichobius johnsonae***	***Nycterophilia parnelli***	***Megistopoda aranea***
ALP 10194			2♀	
ALP 10386	1♂	1♀		
ALP 10389		1♂ / 2♀		
ALP 10390				1♂ / 1♀
ALP 10422	1 pn / 3♂	2♂ / 3♀		
REG 188	1 pn		1♂	
REG 201	1♂			
REG 314		1♀		
REG 362		1♂ / 1♀	1♀	

pn = protonymph

Discussion

Pteronotus gymnonotus is an aerial insectivorous bat species that uses closed spaces as a foraging and feeding strategy, in the lower strata of the canopy and understory, and occurs in dry environments at elevations up to 1,000 masl (Norberg and Rayner 1987; Bredt *et al.* 1999; Denzinger and Schnitzler 2013; Novaes and Laurindo 2014; Pavan and Tavares 2020). In the Serra das Almas Private Natural Heritage Reserve, individuals were collected in the understory in all three phytophysiognomies in the area, at elevations ranging from 620 to 700 masl, confirming the pattern found for the species by previous studies.

Silva *et al.* (2015) publication on the biodiversity of bats in SANR included *P. gymnonotus*, but these authors did not record the new area of distribution of the species in Ceará State. The records of specimens near to the border of Piauí State extends its distribution to the north by 167 km in relation to the municipality of Aiuaba and 266 km in relation to the municipality of Crato, locations in Ceará where the occurrence of the species has already been registered.

Measurements taken from SANR *P. gymnonotus* specimens are in accordance with those previously published (Pavan and Tavares 2020). The reproductive pattern known for mormoopid bats is the seasonal monoestrous type, where pregnancy occurs from January to May and the offspring is born at the end of the rain season, giving birth to one young per pregnancy (Nowak 1994; Reis *et al.* 2006; Pavan and Tavares 2020). Feijó and Rocha (2017) registered pregnant females at the end of December in the municipality of Aiuaba (Ceará), which corresponds to the end of the dry season and the beginning of the rain season. The results presented here confirm this pattern; pregnant females were collected in January at the beginning of the rain season. However, the record of a lactating female in January differs from that reported by Nowak (1994), who recorded births in May at the end of the rain season.

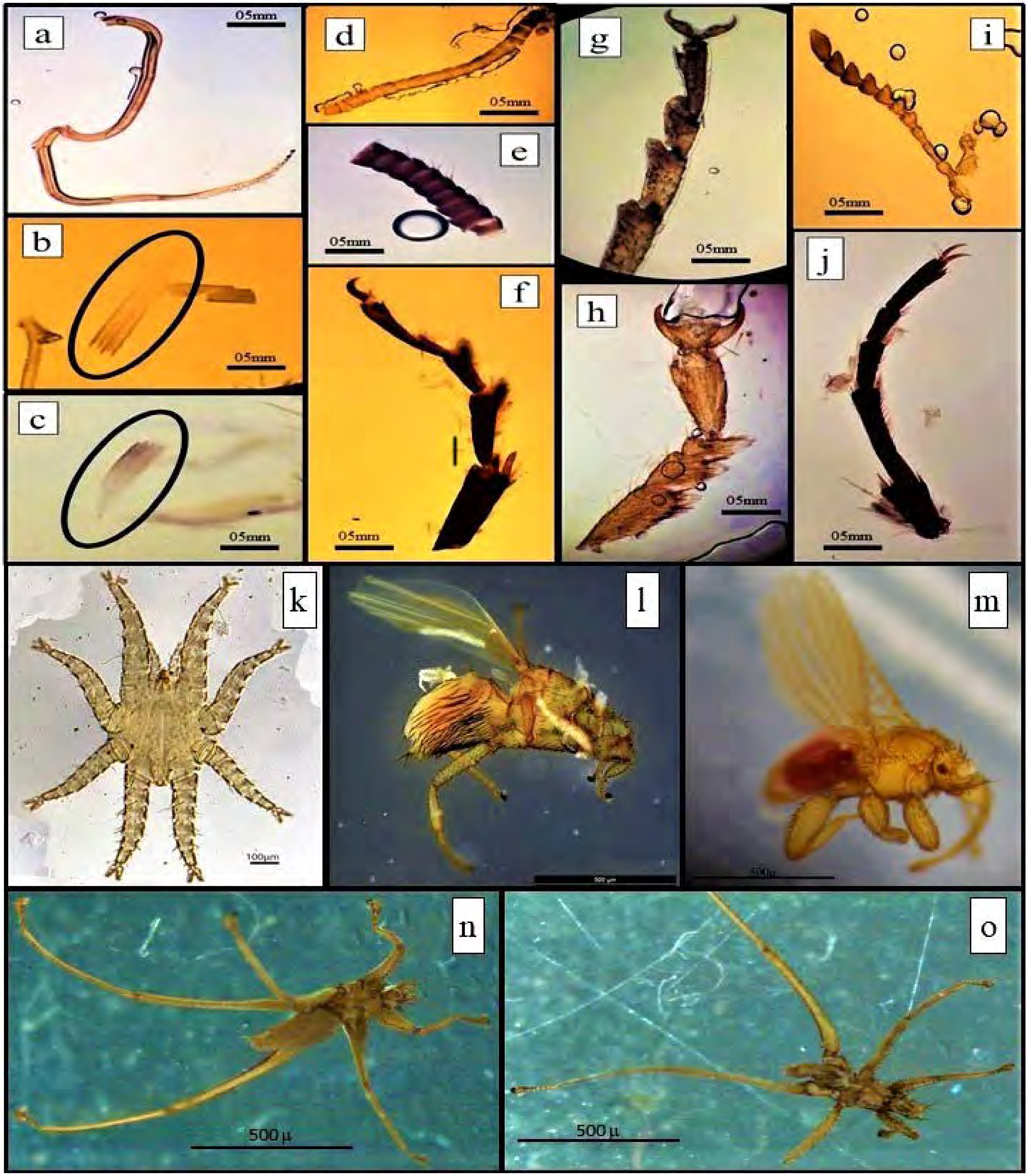

Figure 3. Material associated to *P. gymnonotus* from Crateús (Ceará, Brazil). Hexapoda fragments found in fecal samples: Lepidoptera proboscis (a), scales (b, c), antennae (d, e), and leg (f); Elateridae (Coleoptera) leg and antennae (g, i); undetermined Coleoptera leg (h); Carabidae (Coleoptera) leg (j). Ectoparasites: *Cameronieta* sp. (male; k); *Nycterophilia parnelli* (female; l); *Trichobius johnsonae* (male; m); *Megistopoda aranea* (male; n); *Megistopoda aranea* (female; o; Photos of fragments by Flávia Silva Severino and ectoparasites by Juliana Cardoso de Almeida).

With a strictly insectivorous diet, Mormopid bats food items include mainly coleopterans and lepidopterans, and smaller quantities of dipterans, orthopterans, homopterans, odonates and hymenopterans (Howell and Burch 1974; Whitaker and Findley 1980; Mancina 2005; Rolfe and Kurta 2012; Pavan and Tavares 2020). According to Rolfe and Kurta (2012), factors such as sex, age and reproductive condition could influence the feeding preference of insectivorous bats.

The consumption of Lepidoptera (15.4 %) and Coleoptera (15.4 %) in SANR reinforces the preference for these groups of Hexapoda. Some individuals consumed a combination of prey that included Coleoptera + Lepidoptera, which represented the highest percentage of fragments found (31 %). These results differ from those found by Whitaker and Findley (1980), who recorded a preferential intake of Coleoptera, followed by a much smaller amount of Hymenoptera and, lastly Lepidoptera; the Coleoptera family consumed was not mentioned by the authors. The difference in diet found in this study with that recorded by Whitaker and Findley (1980) may be related to the local entomofauna where the studies were carried out. Studies conducted by Molinari *et al.* (2000) in Venezuela indicated that *P. gymnonotus* consumes 35 families of insects from ten orders, of which Coleoptera is the most representative. Howell and Burch (1974) observed the ingestion of only lepidopterans by *P. gymnonotus* in Costa Rica. In the case of SANR food residues, only the consumption of moths from family Sphingidae was verified.

Parasitological studies on South America Mormopids bats were conducted by Guerrero (1985), Guerrero *et al.* (2003; 2006) and Travassos (1965). These authors identified microfilaria (Nematoda) in the systemic blood and intestine of *P. parnellii*. The necropsied individuals of *P. gymnonotus* from SANR did not display any endoparasites. Helminthic infestations for this species reported in the literature are unknown. There are only records of *Trypanosoma wauwau* hemoparasites in the states of Pará and Sergipe (Lima *et al.* 2015; Pavan and Tavares 2020).

Studies on *P. gymnonotus* ectoparasites collected in the Caatinga biome are scarce. The only records are cited by Almeida *et al.* (2016) and Luz *et al.* (2016). The former was conducted in SANR and lists *Cameronieta* sp. (Spinturnicidae) in association with this species in the area. Regarding Diptera, the results of the present study are significant considering that Frank *et al.* (2014) reported *Nycterophilia parnelli* in association with *P. davyi* and *P. parnellii* in Belize, Costa Rica and Venezuela; *Trichobius johnsonae* parasitizing *P. davyi* in Belize, Costa Rica and Venezuela; *P. gymnonotus* in Venezuela and Panama; *P. parnellii* in Belize; and *P. personatus* in Venezuela and Panama. That is, all of these fly species are new records for *P. gymononotus* in Brazil. The association of *Megistopoda aranea* with *P. gymnonotus* is a new evidence that makes this mormopid bat a potential new host for this Diptera species. Although a protocol to avoid contamination was implemented, further investigation is required.

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Literature cited

Almeida, J. C., D. Gettinger, and S. L. Gardner. 2016. Taxonomic review of the wingmite genus *Cameronieta* (Acari: Spinturnicidae) on Neotropical bats, with a new species from northeastern Brazil. Comparative Parasitology 83:212–220.

Alvares, C. A., J. L. Stape, P. C. Sentelhas, J. L. M. Gonçalves, and G. Sparovek. 2013. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22:711–728.

Associação Caatinga. 2012. Plano de Manejo da Reserva Natural Serra das Almas. 3ª Interação, Crateús.

Baptista, M., and C. E. L. Esbérard. 1997. Valores hematológicos de *Artibeus* sp. e *Desmodus rotundus* (Mammalia, Chiroptera). Revista Científica do Instituto de Pesquisa Gonzaga da Gama Filho 3:11–22.

Bredt, A., W. Uieda, and E. D. Magalhães. 1999. Morcegos cavernícolas da região do Distrito Federal, centro-oeste do Brasil (Mammalia, Chiroptera). Revista Brasileira de Zoologia 16:731–770.

Burgin, C. J., J. P. Colella, P. L. Kahn, and N. S. Upham. 2018. How many species of mammals are there? Journal of Mammalogy 99:1–14.

Czaplewski, N. J., and C. Cartelle. 1998. Pleistocene bats from cave deposits in Bahia, Brazil. Journal of Mammalogy 79:784–803.

Denzinger A., and H U. Schnitzler. 2013. Bat guilds, a concept to classify the highly diverse foraging and echolocation behaviors of microchiropteran bats. Frontier Physiology 4:1–15.

Dick, C. W., G. Graciolli, and R. Guerrero. 2016. Family Streblidae. Zootaxa 4122:784–802.

Feijó, A., and P. A. Rocha. 2017. Morcegos da Estação Ecológica Aiuaba, Ceará, nordeste do Brasil: uma unidade de proteção integral na Caatinga. Mastozoología Neotropical 24:333–346.

Figueiredo, D., G. Graciolli, and L. M. S. Aguiar. 2015. New records of bat flies (Diptera, Streblidae and Nycteribiidae) in Cerrado of central Brazil. Check List 11:1–5.

Flechtmann C.H.W. 1990. Ácaros de importância médica e veterinária. Nobel. São Paulo, Brasil.

Frank, R., J. Münster, J. Schulze, A. Liston, and S. Klimpel. 2014. Macroparasites of Microchiroptera: Bat Ectoparasites of Central and South America. Pp. 87-130, *in* Bats (Chiroptera) as Vectors of Diseases and Parasites - Facts and Myths (Sven, K., and H. Mehlhorn, eds.). Springer-Verlag. Berlin, Germany.

Guerrero, R. 1985. Parasitología. Pp. 35–91, *in* El Estudio de los Mamíferos en Venezuela. Evaluación y Perspectivas (Aguilera, M., ed.). Fondo Editorial Acta Científica Venezolana. Caracas, Venezuela.

Guerrero, R. 1993. Catálogo de los Streblidae (Diptera: Pupipara) parásitos de murciélagos (Mammalia: Chiroptera) del Nuevo Mundo. I. Clave para los géneros y Nycterophilinae. Acta Biológica Venezuelica 14:61–75.

Guerrero, R. 1994. Catálogo de los Streblidae (Diptera: Pupipara) parásitos de murciélagos (Mammalia:Chiroptera) del Nuevo Mundo. IV. Trichobiinae com alas desarrolladas. Boletim de Entomología Venezuela 9:161–192.

Guerrero, R. 1995. Catálogo de los Streblidae (Diptera: Pupipara) parásitos de murciélagos (Mammalia: Chiroptera) del Nuevo Mundo. III. Los grupos: *dugesii, dunni y phyllos-*

tomae del género *Trichobius* Gervais, 1844. Acta Biológica Venezuelica 15:1–27.

Guerrero, R., C. Martin, and O. Bain. 2003. *Litomosoides yutajensis* n. sp., first record of this filarial genus in a mormoopid bat. Parasite 10:219–225.

Guerrero, R., O. Bain, T. Attout, and C. Martin. 2006. The infective larva of *Litomosoides yutajensis* Guerrero *et al*., 2003 (Nematoda: Onchocercidae), a Wolbachia-free filaria from bat. Parasite 13:127–130.

Herd, R. M. 1983. *Pteronotus parnellii*. Mammalian Species 209:1–5.

Howell, D. J, and D. Burch. 1974. Food habits of some Costa Rican bats. Revista de Biologia Tropical 21:281–294.

Instituto Nacional de Meteorologia (INMET). Temperatura Média Compensada (°C). Available from: https://clima.inmet.gov.br/GraficosClimatologicos/DF/83377. Accessed on 15 May 2020.

Ladle, R. J., J. V. L. Firmino, A. C. M. Malhado, and A. Rodríguez-Durán. 2012. Unexplored diversity and conservation potential of neotropical hot caves. Conservation Biology 26:978–982.

Lima, L., O. Espinosa-Álvarez, C. M. Pinto, M. Cavazzana Jr., A. C. Pavan, J. C. Carranza, B. K. Lim, M. Campaner, C. S. A. Takata, E. P. Camargo, P. B. Hamilton, and M. M. G. Teixeira. 2015. New insights into the evolution of the *Trypanosoma cruzi* clade provided by a new trypanosome species tightly linked to Neotropical *Pteronotus* bats and related to an Australian lineage of trypanosomes. Parasites and Vectors 8:1–18.

Luz, H. R., S. Muñoz-Leal, J. C. Almeida, J. L. H. Faccini, and M. B. Labruna. 2016. Ticks parasitizing bats (Mammalia: Chiroptera) in the Caatinga Biome, Brazil. Brazilian Journal of Veterinary Parasitology 25:484–491.

Machado-Allison, C. E. 1965. Notas sobre Mesostigmata Neotropicales III. *Cameronieta thomasi*: nuevo género y nueva especie parasita de Chiroptera (Acarina, Spintumicidae). Acta Biológica Venezuelica 4:243–258.

Mancina, C. A. 2005. *Pteronotus macleayii*. Mammalian Species 778:1–3.

Mares M. A., M. R. Willig, K. E. Streilein, and T. E. Larcher Jr. 1981. The mammals of northeastern Brazil: a preliminary assessment. Annals of the Carnegie Museum of Natural History 50:81–137.

Molinari, J., A. Ascenção, and E. K. V. Kalko. 2000. Feeding ecology of the naked-backed bats, *Pteronotus gymnonotus* and *P. davyi*, in Venezuela. Pp. 130, *in* Abstracts of the Annual North American Symposium on Bat Research (NASBR), NASBR 30. Miami, U.S.A.

Nogueira M. R., I. P. Lima, G. S. T. Garbino, R. Moratelli, V. C. Tavares, R. Gregorin, and A. L. Peracchi. 2018. Updated checklist of Brazilian bats: versão 2018. 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 15 May 2020.

Norberg U. M., and J. M. Rayner. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society B 316:335–427.

Novaes, R. L. M. 2012. Morcegos e cavernas: história escondida de evolução, conservação e preconceito. Ciência Hoje 49:40–44.

Novaes, R. L. M., and R. S. Laurindo. 2014. Morcegos da Chapada do Araripe, Nordeste do Brasil. Papéis Avulsos de Zoologia 54:315–328.

Nowak, R. M. 1994. Bats of the World. Johns Hopkins University Press. Baltimore, U.S.A.

Patton, J. L., and A. L. Gardner. 2007. Family Mormoopidae. Pp. 376–384, *in* Mammals of South America. Volume 1: Marsupials, xenarthrans, shrews, and bats (Gardner, A. L., ed.). University of Chicago Press. Chicago, U.S.A.

Pavan, A. C., and V. C. Tavares. 2020. *Pteronotus gymnonotus* (Chiroptera: Mormoopidae). Mammalian Species 52:40–48.

Quintela, F. M., C. A. Da Rosa, and A. Feijó. 2020. Updated and annotated checklist of recent mammals from Brazil. Annals of the Brazilian Academy of Sciences 92:e20191004.

Reis, N. R., A. L. Peracchi, W. A. Pedro, and I. P. Lima. 2006. Mamíferos do Brasil. Imprensa da Universidade Estadual de Londrina. Londrina, Brazil.

Rolfe, A. K., and A. Kurta. 2012. Diet of Mormoopid bats on the Caribean Island of Puerto Rico. Acta Chiropterologica 14:369–377.

Simmons, N. B. 2005. Order Chiroptera. Pp. 312-529, *in* Mammal Species of the World: A Taxonomic and Geographic Reference. Vol 1. 3rd ed. (Wilson, D. E., and D. M. Reeder, eds). Johns Hopkins University Press. Baltimore, U.S.A.

Simmons, N. B., and T. M. Conway. 2001. Phylogenetic relationships of Mormoopid bats (Chiroptera: Mormoopidae) based on morphological data. Bulletin of the American Museum of Natural History 258:1– 97.

Smith, J. D. 1972. Chiropteran evolution. Pp. 49-69, *in* Biology of bats of the New Word Family Phyllostomatidae part I. (Baker, R. J., J. K. Jones Jr., and D. C. Carter, eds.). Special Publications of the Museum nº. 10, Texas Tech University Press, Lubbok, U.S.A.

Smith, J. D. 1972 a. Systematics of the chiropteran family Mormoopidae. Miscellaneous Publications, University of Kansas, Museum of Natural History 56:1–132.

Silva S. S. P., D. Dias, M. A. Martins, P. G. Guedes, J. C. Almeida, A. P. Cruz, N. M. Serra-Freire, J. S. Damascena, and A. L. Peracchi. 2015. Bats (Mammalia: Chiroptera) from the Caatinga scrublands of the Crateús region, Northeastern Brazil, with new records for the state of Ceará. Mastozoologia Neotropical 22:335–348

Straube, F. C., and G. V. Bianconi. 2002. Sobre a grandeza e a unidade utilizada para estimar esforço de captura com utilização de redes-de-neblina. Chiroptera Neotropical 8:150–152.

Travassos, L. 1965. Contribuição para o inventário crítico da zoologia no Brasil: Fauna helmintológica: considerações preliminares – cestódeos. Publicações Avulsas do Museu Nacional, Rio de Janeiro 48:1–84.

Wenzel, R. L., V. J. Tipton, and A. Kiewlicz. 1966. The streblid batflies of Panama (Diptera: Calyptera: Streblidae). Pp. 405–675, *in* Ectoparasites of Panama (Wenzel, R. L. and V. J. Tipton, eds.) Field Museum of Natural History. Chicago, U.S.A.

Willig, M. R. 1983. Composition microgeographic variation and sexual dimorphism in caatingas and cerrado bats communities from Northeastern Brazil. Bulletin of the Carnegie Museum of Natural History 23:1–131.

Willig, M. R., and M. A. Mares. 1989. Mammals of the Caatinga: an updated list and summary of recent research. Revista Brasileira de Biologia 49:361–367.

Whitaker Jr., J. O., and J. S. Findley. 1980. Foods eaten by some bats from Costa Rica and Panama. Journal of Mammalogy 61:540–544.

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Appendix 1

Examined material (Abbreviations: ALP - catalogue number for the Adriano Lúcio Peracchi Collection, UFRRJ; REG - specimen released): *Pteronotus gymnonotus* – ALP 10192, ALP 10194, ALP 10386, ALP 10389, ALP 10390, ALP 10422, REG 107, REG 138, REG 140, REG 141, REG 188, REG 201, REG 314, REG 362.

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Molecular data suggest that *Heteromys irroratus bulleri* should be recognized as a species-level taxon

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Recent collecting surveys in the Sierra de Manantlán Biosphere Reserve, Jalisco (México), uncovered the existence of a new population of the Mexican spiny pocket mouse, *Heteromys irroratus*. Because the subspecies *H. i. bulleri* and *H. i. jaliscensis* occur nearby, our objective was to properly determine the taxonomic identity of the novel sample. Also, we evaluate its distinctiveness, both in terms of genetic divergence and phylogenetic placement, relative to other members of *H. irroratus*. Preliminary, we compared external and cranial measurements of recently collected individuals to those reported for representatives of the subspecies of *H. irroratus* occurring close by. In addition, we examined levels of genetic differentiation and phylogenetic relationships, based on sequence data of the Cytochrome *b*, among this population and other members of *H. irroratus*, including an individual from Sierra de Juanacatlán, Jalisco, the type locality of *H. i. bulleri*. Phylogenetic relationships were estimated using Maximum Likelihood and Bayesian Inference methods and levels of genetic divergence were assessed employing Kimura-2-parameters genetic distances. Samples from Sierra de Manantlán and Sierra de Juanacatlán were grouped together in a well-supported haplogroup and genetic distances between them were lower than < 1.02 %. Our phylogenetic hypothesis shows that *H. i. bulleri* represents the sister group to all other samples of *H. irroratus* but that, genetically, is a very divergent lineage. Distances values between *H. i. bulleri* to any of the other groups were > 10.05 %. Comparison of external and cranial measurements showed that, in average, individuals from Sierra de Manantlán are larger than those of *H. i. jaliscensis*, but more similar in size to *H. i. bulleri*. Our results show that the new population of the Mexican spiny pocket mouse from Sierra de Manantlán belongs to *H. i. bulleri*. In addition, the phylogenetic separation and the high levels of genetic divergence of *H. i. bulleri*, relative to other members of *H. irroratus*, suggest that the first should be recognized as a species-level taxon. Considering the scarce collecting records of *H. i. bulleri* and the known area of occurrence, that is very restricted, this taxon may warrant special conservation status.

Colectas realizadas recientemente en la Reserva de la Biosfera Sierra de Manantlán, Jalisco (México), permitieron descubrir la existencia de una nueva población del ratón espinoso de abazones mexicano, *Heteromys irroratus*. Debido a que las subespecies *H. i. bulleri* y *H. i. jaliscensis* ocurren en áreas cercanas, nuestro objetivo fue determinar de forma apropiada la identidad taxonómica de la nueva muestra. Adicionalmente, evaluamos su posible distinción, en términos de su divergencia genética y posición filogenética, en relación con otros miembros de *H. irroratus*. Inicialmente comparamos medidas externas y craneales de los individuos colectados recientemente con las reportadas para representantes de las subespecies de *H. irroratus* que ocurren en zonas cercanas. Adicionalmente, examinamos niveles de diferenciación genética y las relaciones filogenéticas, basadas en secuencias del gen Citocromo *b*, entre esta población y representantes de otros miembros de *H. irroratus*, incluyendo un individuo de Sierra de Juanacatlán, Jalisco, la localidad tipo de *H. i. bulleri*. Las relaciones filogenéticas fueron estimadas con métodos de Máxima Verosimilitud e Inferencia Bayesiana y los niveles de divergencia genética fueron evaluados con distancias genéticas corregidas con el modelo de 2 parámetros de Kimura. Las muestras de Sierra de Manantlán y Sierra de Juanacatlán fueron agrupadas en un haplogrupo bien soportado y las distancias genéticas entre ellas fueron menores a < 1.02 %. Nuestra hipótesis filogenética indica que *H. i. bulleri* representa el grupo hermano de todas las otras muestras de *H. irroratus*, pero que, genéticamente, es un linaje muy divergente. Los valores de distancia entre *H. i. bulleri* y cualquiera de los otros grupos fueron > 10.05 %. La comparación de medidas externas y craneales mostró que, en promedio, los individuos de Sierra de Manantlán son más grandes que los de *H. i. jaliscensis* y más similares en tamaño a los de *H. i. bulleri*. Nuestros resultados muestran que la nueva población del ratón espinoso de abazones mexicano de Sierra de Manantlán pertenece a *H. i. bulleri*. Adicionalmente, la separación filogenética y los altos niveles de divergencia genética de *H. i. bulleri*, en relación con otros miembros de *H. irroratus*, sugieren que el primero debe ser reconocido como un taxón con la categoría de especie. Considerando los escasos registros de colecta reportados para *H. i. bulleri* y el área de distribución conocida, que es muy restringida, este grupo podría ser candidato a tener un estatus especial de conservación.

Keywords: Cytochrome *b*; genetic divergence; Mexican spiny pocket mouse; phylogeny; valid species.

Introduction

Heteromys irroratus, the Mexican spiny pocket mouse, is a species widely distributed in México and in a small portion of the United States. In México, it is found on the Mexican Plateau and in adjacent areas, and along the main mountain systems and coastal slopes (Genoways 1973; Dowler and Genoways 1978). Altitudinally, *H. irroratus* occurs from sea level, across the coasts of Tamaulipas and Veracruz to nearly 3,050 m in the highlands of the Sierra Madre de Sur, in México. It can be found mainly in xerophilous scrubland thorny forest, although it also occurs in coniferous and oak forests, and agricultural, and grazing lands (Dowler and Genoways 1978). Currently, seven subspecies are recognized (*H. irroratus alleni, H. i. bulleri, H. i. guerrerensis, H. i. irroratus, H. i. jaliscensis, H. i. texensis,* and *H. i. torridus*; Genoways 1973; Dowler and Genoways 1978). Of the seven, *H. i. bulleri* has the most restricted distribution.

Heteromys i. bulleri was described by Thomas (1893) from La Laguna, Sierra de Juanacatlán, Jalisco (México) and is only known from seven specimens collected in the mid-1960's; two from the type locality and five more from the vicinity of Soyatlán del Oro, Jalisco (Genoways 1973). These two localities are approximately 60 km from each other in the western portion of the state (Figure 1). Although this taxon was described almost 130 years ago, little is known about its natural history.

Recent surveys in the Sierra de Manantlán Biosphere Reserve, in the southwestern portion of Jalisco (México), uncovered the existence of a new population of *H. irroratus*. Although *H. i. jaliscensis* has been reported from localities not far from the Biosphere Reserve and its type locality is about 50 km distant from Sierra de Manantlán, specimens representing this population did not resemble *H. i. jaliscensis* morphologically. External and cranial measurements of individuals of the new population (Table 1) are larger than the ones reported for individuals of *H. i. jaliscensis* (including samples from its type locality; Genoways 1973). Therefore, considering that *H. i. bulleri* also occurs in Jalisco, although in a different area, our aim was to assess the subspecific assignment of the Manantlán Biosphere Reserve specimens. Due to the fact that there are only two specimens of *H. i. bulleri* from the type locality (holotype at the British Museum (Natural History), London, United Kingdom, and a topotype at the National Museum of Natural History, United States) and a direct comparison with them was not possible, field work was conducted at the type locality of *H. i. bulleri* to obtain samples to compare with specimens of the new population using Cytochrome *b* (Cyt *b*) sequences. In this study, we report the findings of the molecular identification of the novel samples and their distinctiveness, both in terms of genetic divergence and phylogenetic placement, compared to available sequence data for *H. irroratus*.

Material and methods

Eight specimens of the new population of *Heteromys irroratus* were collected at Las Joyas Scientific Station, located in the Sierra de Manantlán Biosphere Reserve, Municipality of Autlán, Jalisco, México (19° 35.443′ N, -104° 16.913′ W) at 1,957 masl. The area is characterized by pine-oak forest and cloud forest, but spiny pocket mice were captured near a field of *Zea diploperennis*, an endemic perennial species of teosinte or wild corn. Voucher specimens (preserved in alcohol) were deposited in the Colección Zoológica de Vertebrados, Centro Universitario de la Costa Sur, Universidad de Guadalajara (CVUDG: 1675, 1676, 1710, 1711, 1713, 1714, 1715, 1716). An additional individual of *H. irroratus* was collected at La Laguna, Sierra de Juanacatlán, Municipality of Mascota, Jalisco, México (20° 37.687′ N, -104° 43.752′ W), at 2,050 masl, which represents the type locality of the subspecies *H. i. bulleri*. This specimen was caught in secondary vegetation within a pine-oak forest next to a lake. The voucher specimen (preserved as skin and squeleton) is stored in the Colección de Mamíferos del Centro de Investigación en Biodiversidad y Conservación de la Universidad Autónoma del Estado de Morelos (CMC 3590). Sequences generated as part of this study were uploaded to GenBank and accession numbers are included in Appendix 1. Capture and handling methods followed the animal care and use guidelines of the American Society of Mammalogists (Sikes *et al.* 2016).

Three external and nine cranial measurements, described by Genoways (1973), were recorded for seven adult specimens of *H. irroratus* from Sierra de Manantlán (four females and two males) and one adult specimen from Sierra de Juanacatlán (one female; see Table 1). External measurements were obtained from collecting field catalogs, while cranial measurements were recorded by means of a digital caliper with accuracy of 0.1 mm. Discrimination of age categories that represent adult individuals was based on toothrow wear patterns described by Genoways (1973). Measurements were compared to data recorded by Genoways (1973) for individuals of *H. i. bulleri* and *H. i. jaliscensis* occuring near Sierra de Manantlán and Sierra de Juanacatlán. Because

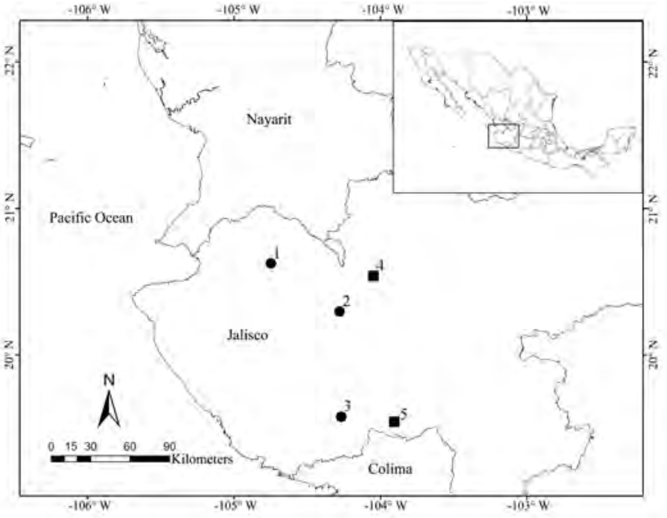

Figure 1. Map illustrating collecting records of *Heteromys irroratus bulleri* (dots) and *H. i. jaliscensis* (squares). 1) La Laguna, Sierra de Juanacatlán (*H. i. bulleri* type locality). 2) Soyatlán del Oro (Genoways 1973). 3) Sierra de Manantlán Biosphere Reserve (current study). 4) Vicinity of Ameca (molecular data from specimens collected at this locality are included in current study as *H. i. jaliscensis*). 5) Las Canoas (*H. i. jaliscensis* type locality).

Genoways (1973) documented that female specimens of *H. i. jaliscensis* are smaller than males, comparison of measurements among specimens of *H. irroratus* from Sierra de Manantlán and Sierra de Juanacatlán and *H. i. jaliscensis* were performed by sex. Box plots of external and cranial measurements were generated using STATISTICA v.8.0 (StatSoft 2007).

Total genomic DNA from the newly collected specimens was extracted from liver tissue preserved in 95 % ethanol following the procedure described by Fetzner (1999). Four microliters of DNA were electrophoresed on 1.75 to 2.0 % agarose gels stained with SYBR Green to visualize quality of genomic DNA. MVZ05 and MVZ14 primers (Smith and Patton 1993; Arellano *et al.* 2005) were used to amplify the Cyt *b* gene (1140 bp). PCR master mix contained: 1.0 µl of template DNA (approximate concentration estimated on a 2 % agarose gel), 1 µl of deoxynucleosidetriphosphates (10 mM), 5 µl of 10x Taq buffer containing $MgCl_2$, 1 µl of each primer (100 mM concentration), 40.7 µl of distilled water, and 0.3 µl of Taq polymerase (5 U/µl; Promega Corp., Madison, Wisconsin) for a 50 µl final volume. Standard amplification conditions consisted of 2 to 4 min at 94 °C for initial denaturation (1 cycle); then, 1 min at 94 °C for denaturation, 1 min at 45 °C for annealing, and 1 min at 72 °C for extension (35 cycles); lastly, 5 min at 72 °C for final extension (1 cycle). Four microliters of PCR-amplified product were assayed by electrophoresis on a 2 % agarose gel. PCR products were purified with a Gene-Clean PCR purification kit (Bio 101, La Jolla, California). Sequencing reactions of purified PCR products were done with the Perkin–Elmer ABI PRISM dye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, California). Excess dye terminator was removed using a Sephadex 50G solution (3 g/50 ml H_2O). Light- and heavy-strand sequences were determined with an ABI 3100 automated sequencer (Applied Biosystems) housed in the DNA Sequencing Center at Brigham Young University. Final sequences were edited using BioEdit v.7.0.8.0 (Hall 1999). GenBank sequences accession numbers are MT709150, 709151, 709152, 709153, 709154, 709155, 709156, T709157, and (see Appendix 1 for correspondence of accession numbers and voucher collecting information).

New sequences were added to a matrix together with 33 Cyt *b* sequences of *H. irroratus* available from GenBank and representing the following subspecies: *H. i. alleni, H. i. guerrerensis, H. i. irroratus, H. i. jaliscensis, H. i. torridus*, and *H. i. texensis* (first described by Rogers and Vance 2005; Appendix 1). Also, sequences of *H. pictus* accessible from GenBank were downloaded and used as the outgroup in phylogenetic analyses (Appendix 1). Our final matrix included 786 bp of Cyt *b*.

Sequence alignment was done with BioEdit v.7.0.8.0 (Hall 1999). Hypotheses concerning phylogenetic relationships among sequences were estimated using Maximum Likelihood (ML) and Bayesian Inference (BI) employing IQ-TREE v.1.6.10 (Kalyaanamoorthy *et al.* 2017; Nguyen *et al.* 2015) and MrBayes v.3.2.2 (Ronquist *et al.* 2012) on XSEDE, respectively. The model of DNA evolution most appropriate for our data was selected using jModelTest2 on XSEDE (Darriba *et al.* 2012) under the Bayesian information criterion (BIC). To assess if data needed to be partitioned by codon position, we employed ModelFinder (Kalyaanamoorthy *et al.* 2017). The transition model 2 with invariable sites and rate heterogeneity (TIM_2+I+G4; Tamura and Nei 1993) was determined as the best-fit model of nucleotide substitution (πA = 0.288, πC = 0.248, πG = 0.142, and πT = 0.323; rAC/AT = 1.000/1.000, rCG/GT = 1.000/1.000; I = 0.475; α = 0.224), and the data were not partitioned. For the ML analysis, tree searches were performed employing IQ-TREE default search parameters. Branch support was assessed through ultrafast bootstrap (UB) with 1,000 replicates (Hoang *et al.* 2018). For the BI analyses, runs were specified with four of MCMC chains and 50,000,000 generations, with a sample frequency of 1,000 generations. This resulted in 50,000 samples from the posterior probability (PP) distribution. Burn-in value was set to 10,000. This resulted in 50,000 samples and the burn-in value was set to 10,000. The posterior probability (pP) was computed for individual branches by constructing a majority-rule consensus with the trees not discarded as burn-in. Once the main phylogenetic haplogroups were identified, uncorrected (p-distances) and Kimura-2-parameters (K2P; Kimura 1980) genetic distances were calculated employing Mega v.7.0.3 (Kumar *et al.* 2016). The former genetic measurement is provided for further comparison to other mammal taxa. The latter genetic distance was compared between selected sequences of *H. irroratus* since it has been widely used to compare levels of DNA sequence divergence in mammals under the Genetic Species Concept framework (Bradley and Baker 2001; Baker and Bradley 2006).

Results

External and cranial measurements among specimens of the new population of *H. irroratus* from Sierra de Manantlán and individuals of *H. i. jaliscensis* showed overlap in the ranges displayed by most variables for both females and males (Table 1; Figures 2, 3). On average, the four adult females of *H. irroratus* from Sierra de Manantlán are larger than the females of *H. i. jaliscensis*. This is particularly evident for three external measurements (total length, length of tail, length of hind foot; Figures 2a-c) and five cranial variables (greatest length of skull, mastoid breadth, length of nasals, length of maxillary toothrow, depth of braincase; Table 1; Figures 2d-j). The two adult males from Sierra de Manantlán showed larger mean values for three cranial measurements (mastoid breadth, length of nasals, and depth of braincase) compared to males of *H. i. jaliscensis* (Table 1; Figures 3f-j). The only adult female specimen of *H. irroratus* from Sierra de Juanacatlán, also displayed larger values for all three external and seven cranial measurements compared to average values depicted by females of *H. i. jaliscensis* (Table 1; Figures 2a-j). Mean lengths of the adult females of *H. irroratus* from Sierra de Manantlán and Sierra de Juanacatlán were more similar to the mean measurements of specimens of *H. i. bulleri* reported by

Table 1. External and cranial measurements (in mm) for specimens of *H. i. jaliscensis* and *H. i. bulleri*. When more than one individual was available, the range is shown for each variable. Mean values are in parentheses. Sierra de Manantlán (SM), Sierra de Jaunacatlán (SJ).

External and cranial measurements	Genoways (1973)			This study		
	H. i. jaliscensis		*H. i. bulleri**	*H. i. bulleri* SM		*H. i. bulleri* SJ
	Females (*n* = 51)	*Males* (*n* = 38)	*Females* (*n* = 3)	*Females* (*n* = 4)	*Males* (*n* = 2)	*Female* (*n* = 1)
Total length	207.0-251.0 (226.9)	212.0-272.0 (238.3)	245.0-250.0 (247.4)**	226.0-255.0 (245.5)	228.0-249.0 (238.0)	249.0
Length of tail	101.0-131.0 (114.1)	103.0-140.0 (118.7)	127.0-128.0 (127.4)**	113.0-134.0 (125.0)	125.0	128.0
Length of hind foot	25.0-30.0 (27.3)	26.5-32.0 (28.9)	27.0-32.0 (29.3)**	26.0-32.0 (29.2)	25.9-30.0 (27.8)	33.0
Greatest length of skull	30.1-33.2 (31.6)	30.4-35.1 (32.6)	33.5-34.1 (33.8)	31.8-33.5 (32.7)	33.7	32.0
Interorbital constriction	7.2-8.6 (7.8)	7.2-9.0 (8.0)	8.2-8.7 (8.5)	7.6-8.1 (8.0)	7.7-7.9 (7.8)	8.7
Mastoid breadth	13.8-14.7 (14.2)	13.8-15.9 (14.8)	14.8-15.5 (15.1)	14.4-15.4 (14.8)	14.8-15.3 (15.0)	14.6
Length of nasals	11.0-13.5 (12.1)	11.4-13.4 (12.3)	13.2-14.1 (13.6)	13.2-14.6 (14.1)	14.4-14.6 (14.5)	13.9
Length of rostrum	12.9-14.9 (13.8)	12.2-15.7 (13.7)	15.3** (15.3)	12.8-14.4 (13.9)	14.1-14.5 (14.3)	13.6
Length of maxillary toothrow	4.4-5.4 (4.8)	4.8-5.8 (5.3)	5.9-6.0 (5.9)	5.5-5.9 (5.6)	5.2-5.3 (5.2)	5.3
Depth of braincase	8.2-9.1(8.6)	8.2-9.3 (8.7)	9.4-10.0 (9.7)**	9.2-9.8 (9.5)	9.4-9.7 (9.5)	9.4
Interparietal width	7.3-9.5 (8.3)	7.9-9.8 (8.7)	5.4-8.0 (6.7)	7.4-7.8 (7.8)	7.4	8.6
Interparietal length	2.5-4.4 (3.2)	3.0-4.4 (3.6)	3.2-3.9 (3.6)	3.7-4.2 (3.9)	3.9	4.1

*Samples included two adult females from La Laguna, Sierra de Juanacatlán (type locality) and one adult female from Soyatlán del Oro.

**Data preceded by two asterisks were recorded for only two specimens.

Genoways (1973), particularly two external variables (total length and length of tail; Table 1; Figures 2a, 2b) and one cranial measurement (length of nasals; Table 1; Figure 2g).

Phylogenies derived from ML (Figure 4) and BI (not shown) were highly congruent. Sequences from Sierra de Manantlán grouped together with high nodal support (UB = 91 and PP = 0.99). In turn, the sequence from Sierra de Juanacatlán (CMC 3590) joined those sequences from Sierra de Manantlán forming a strongly supported haplogroup (UB = 100 and PP = 1.0; Figure 4). The *H. i. bulleri* haplogroup was positioned as a divergent sister group to the other sequences of *H. irroratus*. The remaining sequences of *H. irroratus* formed a monophyletic group with strong nodal support values (84 / 0.99). This clade contained representatives of the other six subspecies of *H. irroratus* (*alleni, guerrerensis, irroratus, jaliscensis, texensis,* and *torridus*). Sequences of each subspecies formed separate haplogroups, with the exception of *H. i. alleni*. Sequences representing this subspecies split into two independent lineages, *H. i. alleni* (1) and *H. i. alleni* (2). Although two divergent lineages were also found within *H. i. texensis,* they are sister to each other. In summary, we identified a total of eight haplogroups within *H. irroratus*.

Pairwise K2P genetic distances (Kimura 1980) between localities 1 and 3 of *H. i. bulleri* (Figure 1) ranged between 0.63 % and 1.02 % with a mean of 0.84 % (Table 2). In contrast, the mean K2P genetic distance between individuals of *H. i. bulleri* and the other haplogroups ranged from 10.05 % (*H. i. jaliscensis*) to 11.94 % (*H. i. irroratus*). Among all haplogroups of *H. irroratus,* except *H. i. bulleri*, K2P distances ranged between 1.59 % (*H. i. guerrerensis-H. i. irroratus*) and 8.34 % (*H. i. jaliscensis-H. i. alleni* (2); Table 2).

Table 2. Pairwise Kimura-2-parameter distances (below the diagonal) and p-distances (above the diagonal), between samples of *Heteromys irroratus* based on 786 bp of the Cytochrome *b* gene.

Taxon	*H. i. irroratus*	*H. i. texensis*	*H. i. alleni (1)*	*H. i. jaliscensis*	*H. i. alleni (2)*	*H. i. guerrerensis*	*H. i. torridus*	*H. i. bulleri*
H. i. irroratus		0.0694	0.0635	0.0705	0.0734	0.0157	0.0564	0.1079
H. i. texensis	0.0742		0.0407	0.0505	0.0744	0.0637	0.0405	0.1016
H. i. alleni (1)	0.0675	0.0423		0.0364	0.0657	0.0619	0.0233	0.0930
H. i. jaliscensis	0.0751	0.0527	0.0375		0.0776	0.0636	0.0449	0.0927
H. i. alleni (2)	0.0789	0.0800	0.0701	0.0834		0.0725	0.0623	0.1049
H. i. guerrerensis	0.0159	0.0678	0.0659	0.0674	0.0780		0.0534	0.1025
H. i. torridus	0.0595	0.0421	0.0238	0.0467	0.0662	0.0563		0.0973
H. i. bulleri	0.1194	0.1116	0.1013	0.1005	0.1160	0.1129	0.1064	

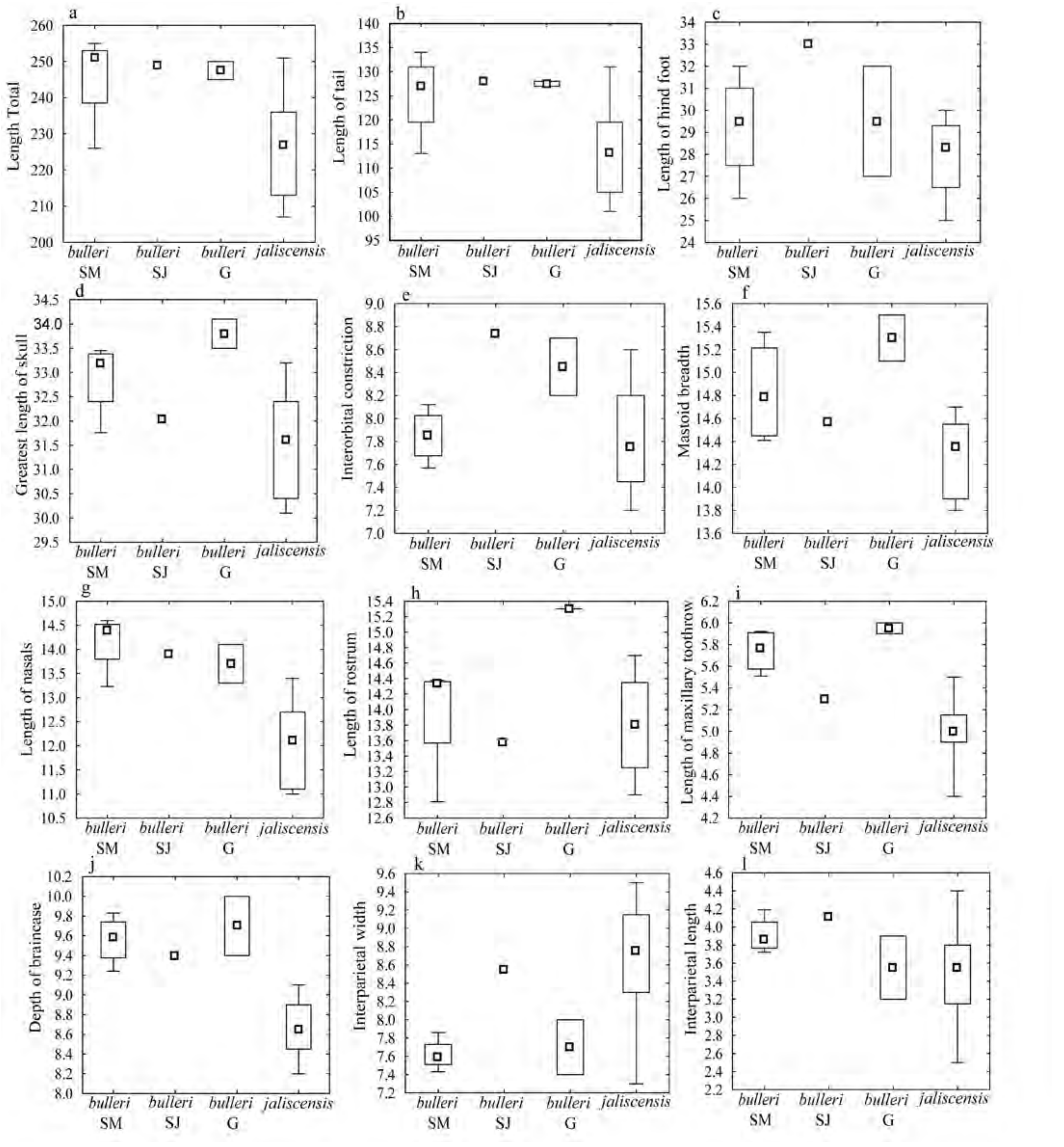

Figure 2. Box plots of female measurements of individuals of *H. i. bulleri* from Sierra de Manantlán (*bulleri* SM), *H. i. bulleri* from Sierra de Juanacatlán (*bulleri* SJ), *H. i. bulleri* from Genoways (1973; *bulleri* G), and *H. i. jaliscensis* from Genoways (1973; *jaliscensis*). Median (small squares), 25% to 75% confidence interval (rectangles), and minimum and maximum values (whiskers).

Discussion

This study focuses on the molecular taxonomic identification of individuals of a new populations of *H. irroratus* from Sierra de Manantlán Biosphere Reserve, a locality in southern Jalisco (México), and its phylogenetic placement within this group. Our initial identification assessment involved the comparison of external and cranial measurements to *H. i. jaliscensis* and *H. i. bulleri*, two subspecies of this taxon occurring not far from this new collecting locality. Yet, ranges of measurements between adult females from Sierra de Manantlán and those of *H. i. jaliscensis* overlap, the former are on average larger than the latter (Table 1, Figures 2, 3). Although differences in length between males from Sierra de Manantlán and *H. i. jaliscensis* were not evident for most variables, specimens from Sierra de Manantlán displayed larger mastoid breadth, length of nasals, length of rostrum,

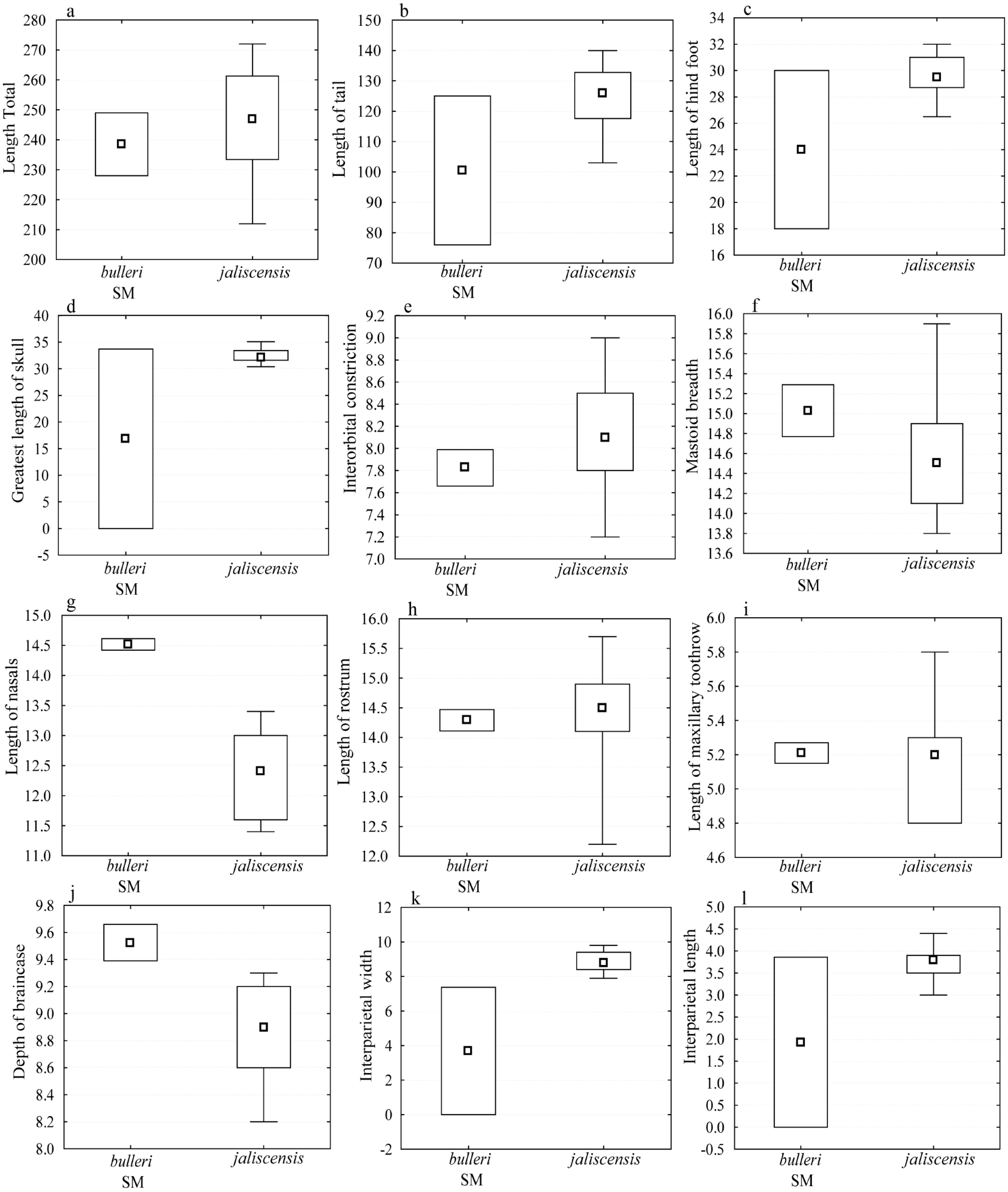

Figure 3. Box plots of male measurements of individuals of *H. i. bulleri* from Sierra de Manantlán (*bulleri* SM) and *H. i. jaliscensis* from Genoways (1973; *jaliscensis*). Median (small squares), 25% to 75% confidence interval (rectangles), and minimum and maximum values (whiskers).

and depth of braincase. On the other hand, measurements of females from Sierra de Manantlán were similar to those of *H. i. bulleri* reported by Genoways (1973). These results, principally the data recorded for females, preliminary suggest that specimens of the new population of *H. irroratus* from Sierra de Manantlán were not representatives of *H. i. jaliscensis*, but rather, represented the subspecies *H. i. bulleri*. In addition, the female specimen collected at the type locality of *H. i. bulleri* (La Laguna, Sierra de Juanacatlán), exhibited measurements that better agree with those reported for *H. i. bulleri* than for *H. i. jaliscensis* (Genoways, 1973 Genoways 1973), suggesting that this individual also belongs to the former taxon. This is in agreement with the findings reported by Genoways (1973) who concluded that *H. i. bulleri* can be discriminated from *H. i. jaliscensis* (and other subspecies) by its larger external and cranial size.

Although, it was not possible to make a comparison with type specimens of *H. i. bulleri*, color patterns (fresh pelage) of the individual collected at the type locality (upperparts gray mixed with black and ochraceous hairs; sides with a pale ochraceous line; underparts white; feet white; ears dusky with a white edge; tail bicolor, brown above and white below) match those described for *H. i. bulleri* (Thomas 1893; Goldman 1911), supporting the assignment of this specimen to this taxon. Although *Heteromys pictus* could also occur at the type locality of *H. i. bulleri*, it can be distinguished from *H. irroratus* by the presence of six plantar tubercles on each hind foot (compared to five plantar tubercles displayed by *H. irroratus*; Genoways 1973) and overall smaller size. The fact that all the specimens of *Heteromys* from Sierra de Manantlán and Sierra de Juanacatlán collected for our study possess five plantar tubercles on each hind foot, supports the hypothesis they are representatives of *H. irroratus*.

When comparing Cyt *b* sequence data between the sample from Sierra de Juanacatlán (type locality of *H. i. bulleri*) to any of the individuals from Sierra de Manantlán, the genetic distance values were < 1.02 % (mean 0.84 %), lower than the upper bound (1.8 %) of the intrasubspecific range reported by Bradley and Baker (2001) for Cyt *b* in rodents. This suggests a close affinity between individuals from the Sierras de Juanacatlán and Manantlán and supports that they represent the same subspecies. This hypothesis is also supported by the fact that all these sequences formed a single haplogroup with strong nodal support. Since the sample from Sierra de Juanacatlán represents the type locality of *H. i. bulleri*, we propose that specimens from the Sierra de Manantlán also represent *H. i. bulleri*. Certainly, confirmation of this findings would be desirable by analyzing additional data such as nuclear markers and morphological features.

Although we did not have molecular data for *H. i. jaliscensis* from its type locality (Las Canoas, Jalisco) to confirm that specimens from Sierra de Manantlán do not belong to that subspecies, we included representatives of *H. i. jaliscensis* from Ameca, Jalisco. Samples of *H. irroratus* from these two localities (Las Canoas and Ameca), were analyzed morphologically by Genoways (1973) and regarded as the same subspecies. The levels of genetic differentiation between *H. i. jaliscensis* and specimens from Sierra de Manantlán were comparatively high (10.05 %; Table 2), supporting the hypothesis that they represent distinct subspecies.

Our phylogenetic hypothesis places *H. i. bulleri* as the sister group to all other forms of *H. irroratus* (Figure 4). The reciprocal monophyly of these two clades was well supported (*H. i. bulleri* = 100/1.0; other *H. irroratus* = 84/0.98). Likewise, the high degree of genetic divergence among sequences of these two clades was documented with K2P;

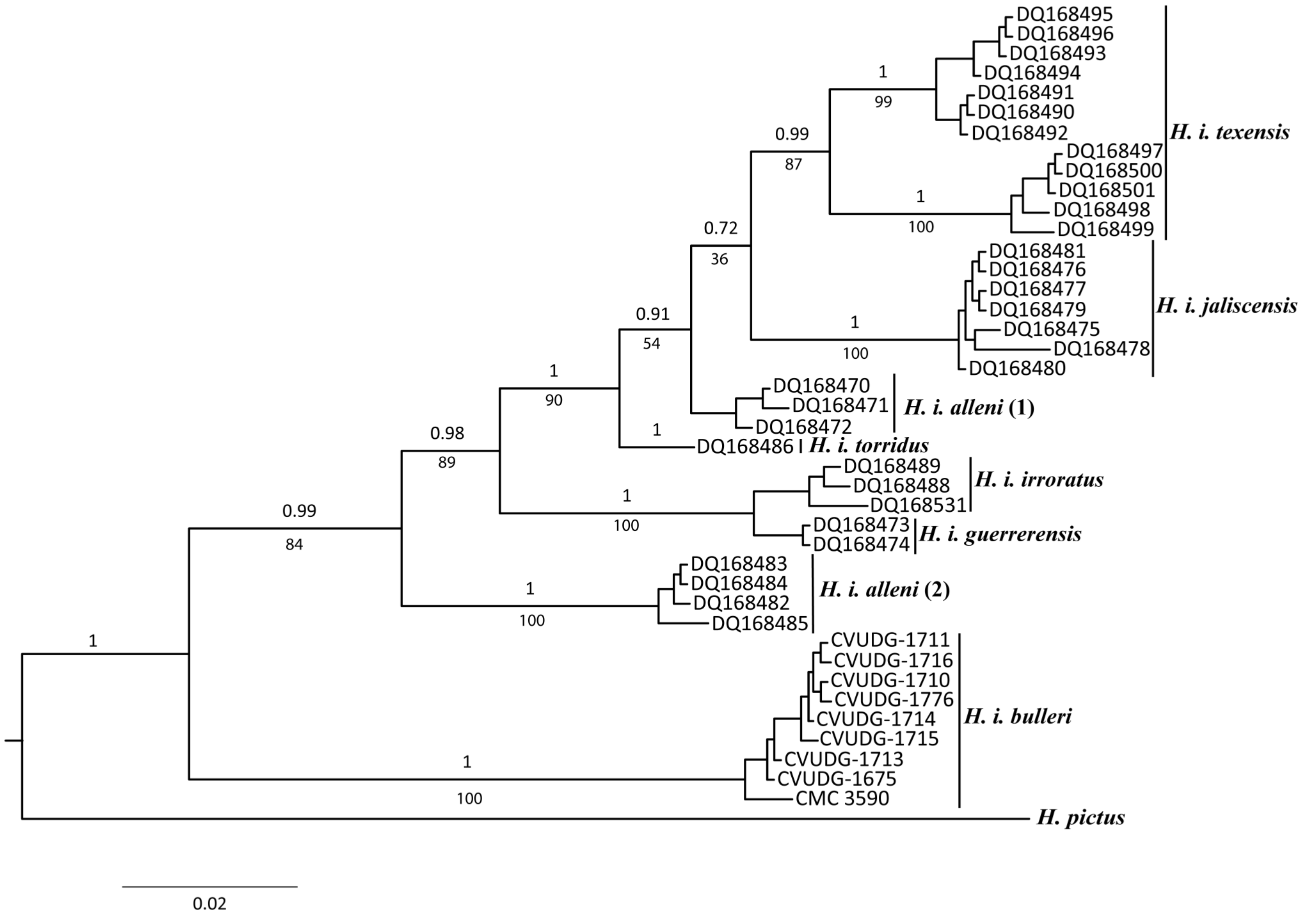

Figure 4. Maximum likelihood phylogenetic tree of *Heteromys irroratus* sequences based on 786 bp of the Cytochrome *b* gene (lnL = -3357.567). Posterior probability estimates are shown above branches and bootstrap values are below branches.

in any pairwise comparison between these two groups, genetic distances exceeded 10 %. This level of Cyt *b* genetic differentiation can be indicative of distinct species (Bradley and Baker 2001; Baker and Bradley 2006).

Although *H. i. bulleri* is currently recognized as a subspecies (Genoways 1973), it was originally described as a species by Thomas (1893:330) because it had a "skull strong and stoutly built, differing mainly from that of *H. alleni* in its much greater size, and from that of *H. irroratus* by its differently shaped interparietal". The specific status of *H. i. bulleri* was supported by Goldman (1911:62) who wrote: "In external appearance it resembles *alleni* and *jaliscensis*, but the cranial characters, especially the decidedly smaller size and more triangular shape of the interparietal, distinguish it from either". Genoways (1973), based on a morphological analysis of all known forms of the Mexican spiny pocket mouse, found that samples of *H. i. bulleri* possessed deeper braincases, compared to other groups of *H. irroratus*. *Heteromys i. bulleri* also was separated from all other samples of *H. irroratus* in a Principal Component Analysis. In addition, a UPGMA phenogram showed that, at least for the sample from Soyatlán del Oro, is quite distinct from the rest of samples representing the Mexican spiny pocket mouse. Finally, Genoways (1973:106) observed that "the small size of the interparietal is rather unique" for *bulleri*, although he pointed out that this feature was shared with a population of *H. i. alleni* from Michoacán. However, he concluded that, due to the high variation of the shape and size of the interparietal bone in *H. irroratus* and other species in the genus, specific distinction of *bulleri* should not be based on that single character, and therefore, he relegated it to the subspecific level.

Although our molecular data represent a portion of a single mitochondrial gene, the level of genetic differentiation documented by this marker for *H. i. bulleri* relative to other samples of *H. irroratus*, suggest that it represents a distinct species-level taxon. However, we believe that additional data (both molecular and morphological) are necessary to further test the specific status of this taxon.

The fact that *H. i. bulleri* should be considered a distinct species supports previous views suggesting that *H. irroratus* is a species complex containing some species-level lineages (Rogers and Vance 2005). It has been demonstrated that individuals of *H. irroratus* from near Pátzcuaro, Michoacán, México (represented by our *H. i. alleni* (2) haplogroup; Figure 2) were genetically distinct and can be considered as a candidate species. Also, it was concluded that populations of *H. irroratus* from Guerrero and Oaxaca are genetically differentiated and may represent a second candidate species (Rogers and Vance 2005).

Since its description in 1893, only 16 specimens of *H. i. bulleri* have been collected: two reported at the time of its description, five more collected between 1956 and 1966, and nine more reported in this study were trapped between 2014 and 2020. The scarce number of voucher specimens collected in almost 130 years suggests that either the trapping efforts have been insufficient to properly sample it or this taxon is uncommon. The Sierra de Manantlán is a new locality for *H. i. bulleri* and represents a range extension of about 125 km southeast from the type locality and 80 km south from Soyatlán del Oro. Given that *H. i. bulleri* is distinctive molecularly and morphologically and coupled with its restricted distribution, it is crucial to pay attention to conservation issues surrounding this taxon.

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Literature cited

Arellano, E., F. X. González-Cozátl, and D. S. Rogers. 2005. Molecular systematics of the Middle American harvest mice *Reithrodontomys* (Muridae), estimated from mitochondrial cytochrome b gene sequences. Molecular Phylogenetics and Evolution 37:529-540.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy 87:643-662.

Bradley, R. D., and R. J. Baker. 2001. A test of the genetic species concept: Cytochrome-b sequences and mammals. Journal of Mammalogy 82:960-973.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

Dowler, R. C., and H. H. Genoways. 1978. *Liomys irroratus*. Mammalian Species 82:1-6.

Fetzner, J. W., Jr. 1999. Extracting high quality DNA from shed reptile skins: a simplified method. Biotechniques 26:1052-1054.

Genoways, H. H. 1973. Systematics and evolutionary relationships of spiny pocket mice, genus *Liomys*. Special Publications The Museum Texas Tech University 5:1-368.

Goldman, E. A. 1911. Revision of the spiny pocket mice (genera *Heteromys* and *Liomys*). North American Fauna 34:1-70.

Hall, T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Hoang, D. T., O. Chernomor, A. von Haeseler, B. Q. Minh, and L. S. Vinh. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35:518-522.

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermiin. 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14:587-589.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.

Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.

Nguyen, L. T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32:268-274.

Rogers, D. S., and V. L. Vance. 2005. Phylogenetic relationships among spiny pocket mice (*Liomys*: Family Heteromyidae): Analysis of cytochrome *b* based on multiple heuristic approaches. *Journal of Mammalogy* 86:1085-1094.

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. Systematic Biology 61:539-542.

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

Smith, M., and J. Patton. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of the Linnaean Society 50:149-177.

StatSoft, Inc. 2007. STATISTICA (data analysis software system). version 8.0. www.statsoft.com.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.

Thomas, O. 1893. Description of two new "pocket-mice" of the genus *Heteromys*. The Annals and Magazine of Natural History 11:329-332.

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Appendix 1

Specimens of Mexican *Heteromys* used in the molecular analysis listed by subspecies, state, collecting locality (specific locality, elevation, and geographic coordinates), museum voucher (acronym and number), and GenBank accession number (DQ series first reported by Rogers and Vance 2005). Museum abbreviations are as follows: Brigham Young University (BYU); Colección de Mamíferos at Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos (CMC); Carnegie Museum of Natural History (CM); Biodiversity Research and Teaching Collection, Texas A&M University (TCWC); Colección Zoológica de Vertebrados, Centro Universitario de la Costa Sur, Universidad de Guadalajara (CVUDG).

Taxon (Haplogroup)	State	Collecting locality	Museum Voucher	GenBank Accession No.
H. i. alleni (1)	Durango	25 km E, 22 km S Vicente Guerrero, Municipio Vicente Guerrero, 1,950 m (23.713, -104.000)	BYU 15765	DQ 168470
			BYU 15766	DQ 168471
			BYU 15767	DQ 168472
H. i. alleni (2)	Michoacán	10 km S (by road) Pátzcuaro, 2,200 m (19.470, -101.609)	BYU 16051	DQ 168482
			BYU 16052	DQ 168483
			BYU 16053	DQ 168484
			BYU 16055	DQ 168485
H. i. bulleri	Jalisco	Estación Científica las Joyas, Reserva de la Biósfera Sierra de Manantlán, 1,957 m (19.586, -104.2743)	CVUDG 1675	MT 709150
			CVUDG 1713	MT 709151
			CVUDG 1711	MT 709152
			CVUDG 1710	MT 709153
			CVUDG 1714	MT 709154
			CVUDG 1715	MT 709155
			CVUDG 1716	MT 709156
			CVUDG 1676	MT 709157
H. i. bulleri	Jalisco	La Laguna, Sierra de Juanacatlán, 2,050 m (20.499, -103.145)	CMC 3590	MT 709149
H. i. guerrerensis	Guerrero	6.1 km SW (by road) Omiltemi, 2,490 m (17.549, -99.721)	BYU 20646	DQ 168473
			CMC 399	DQ 168474
H. i. irroratus	Oaxaca	El Polvorín, 5.3 km turn off Lachao Viejo (by road), 1,735 m (16.453, -97.002)	CMC 408	DQ 168488
			CMC 410	DQ 168489
H. i. irroratus	Oaxaca	18.5 km S (by road) Sola de Vega, 2,175 m (16.199, -97.134)	BYU 20652	DQ 168487
			CMC 419	DQ 168531
H. i. jaliscensis	Jalisco	24 km W (by road) Ameca, 1,470 m (20.522, -104.076)	BYU 16044	DQ 168481
			BYU 16045	DQ 168475
			BYU 16046	DQ 168476
			BYU 16047	DQ 168477
			BYU 16048	DQ 168478
			BYU 16049	DQ 168479
			BYU 16050	DQ 168480
H. i. texensis	San Luis Potosí	Rancho Plan de la Laja, Xilitlilla, 6 km W Xilitla, Municipio Xilitla, 785 m (21.355, -99.031)	BYU 15265	DQ 168493
			BYU 15266	DQ 168494
			BYU 15267	DQ 168495
			BYU 15268	DQ 168496
H. i. texensis	Puebla	3.5 miles SW Xicotepec de Juárez (20.279, -97.964)	TCWC 41724	DQ 168490
			CM 70450	DQ 168491
			CM 70451	DQ 168492
H. i. texensis	Tamaulipas	2.2 miles N Soto la Marina (23.814, -98.189)	TCWC 42044	DQ 168497
			TCWC 42045	DQ 168498
			TCWC 42046	DQ 168499
			TCWC 42047	DQ 168500
			TCWC 42048	DQ 168501
H. i. torridus	Morelos	Cuernavaca, 2,210 m (18.986, -99.236)	CMC 404	DQ 168486
H. pictus	Oaxaca	El Polvorín, 5.3 km turn off Lachao Viejo (by road), 1,735 m (16.453, -97.002)	BYU 20656	DQ 168534

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Factors influencing the activity patterns of two deer species and their response to predators in two protected areas in Indonesia

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Facing change of environmental conditions, the activity rhythm of animals may habituate. Remote cameras were used to quantify Bawean deer and red muntjac activity patterns and to examined differences by season, sex and lunar cycle to respond predator presence, in Bawean Island and Ujung Kulon National Park, Indonesia. Photographs of Bawean deer (*n* = 118) were taken during March to November 2014 and for red muntjac (*n* = 4,142) were taken during January 2013 to July 2014. Data were analyzed by using Generalized Additive Models (GAMs) to test the relationship between activity patterns and the lunar cycle, further, the pattern of daily activity overlap between deer and their predators calculated by kernel density estimation. The number of captures by camera trapping were less for both sexes of both deer in wet season. Male and female Bawean deer were active throughout the day and night during all seasons, with several activity peaks during the 24h period, while male and female red muntjac show diurnal activity levels with higher peaks 1h after sunrise until 1h before sunset. There was no significant difference in activities between males and females for both deer. The amount of nocturnal activity corresponding to differences in nocturnal luminosity for Bawean deer varied, but not for the red muntjac. Free-roaming dog and Bawean deer presented opposite peak activities, while dhole as (the predator of the red muntjac) and red muntjac were both cathemeral. The differences between the two similar-sized species could be closely related to reduction of predation risk and increasing foraging success.

Ante el cambio de las condiciones ambientales, la actividad de los animales puede habituarse. Se utilizaron cámaras remotas para cuantificar los patrones de actividad del ciervo de Bawean y el muntjac rojo y para examinar las diferencias por estación, sexo y ciclo lunar como respuesta a la presencia de depredadores, en la isla de Bawean y el Parque Nacional Ujung Kulon, Indonesia. Se tomaron fotografías de ciervos de Bawean (*n* = 118) durante marzo a noviembre de 2014 y de muntjac rojo (*n* = 4,142) durante enero de 2013 y julio de 2014. Los datos se analizaron mediante el uso de modelos aditivos generalizados (GAM) para probar la relación entre los patrones de actividad y el ciclo lunar, además, el patrón de superposición de la actividad diaria entre los ciervos y sus depredadores calculado mediante la estimación de la densidad del kernel. El número de capturas por cámara trampa fue menor para ambos sexos de ambos ciervos en la temporada de lluvias. Los ciervos bawean machos y hembras estuvieron activos durante el día y la noche durante todas las estaciones, con varios picos de actividad durante el período de 24 horas, mientras que los muntjac rojo macho y hembras muestran niveles de actividad diurna con picos más altos 1 h después del amanecer hasta 1 h antes del atardecer. No hubo diferencias significativas en las actividades entre machos y hembras para ambos ciervos. La cantidad de actividad nocturna correspondiente a las diferencias en la luminosidad nocturna para el ciervo de Bawean varió, pero no para el muntjac rojo. Los perros ferales y el ciervo de Bawean presentaron actividades pico opuestas, mientras que el dhole (el depredador del muntjac rojo) y el muntjac rojo eran ambos catemeraless. Las diferencias entre las dos especies de tamaño similar podrían estar estrechamente relacionadas con la reducción del riesgo de depredación y el aumento del éxito de la búsqueda de alimentos.

Keywords: Activity; carnivore; Cervidae; lunar cycle; seasonal; tropical rainforest.

Introduction

Throughout the day, wild animals show changes in their behavior and activity (Pipia *et al.* 2008; Ikeda *et al.* 2015; Porfirio *et al.* 2016; Rahman *et al.* 2018). The patterns of the temporal activity changes indicate important dimensions in the ecological niche and has been known to be directly affected individual fitness (Kronfeld-Schor and Dayan 2003). Questions about how species are able to survive, adapt and persist in their environment can be answered by understanding the key factors that influence the timing of activity (Buchholz 2007; Krop-Benesch *et al.* 2012). Many factors may affect the activity of animals, including predation (Harmsen *et al.* 2011; Cozzi *et al.* 2012), habitat fragmentation (Norris *et al.* 2010), sex and reproductive status (Kolbe and Squires 2007), human related disturbance (Ikeda *et al.* 2015), as well as environmental factors such as seasonal changes (McLellan and McLellan 2015), and lunar luminosity (Michalski and Norris 2011; Pratas-Santiago *et al.* 2017).

As for seasonal changes, there were predictable variations in activity pattern related to the physiological state of the animal, including the reproductive stage, and the environment condition, notably the availability of food resources and climatic conditions (Scheibe *et al.* 2001). There has been growing evidence that environmental conditions, mainly season, changes activity level of ungulates, and affects spatial behavior as well (Bowyer and Kie 2009; Bourgoin *et al.* 2011). High ambient temperatures due to seasonal changes, alone or in combination with low wind speed, have been found to lead to heat stress in ungulates inhabiting both temperate and polar environments (Cain III 2006). However, study on how the season influence the activity level on tropical ungulates is still lacking, while understanding that influences is a key to understanding how species survive. The dry season is known to cause phenological changes that lead to natural scarcity of the diversity and availability of food resources for herbivores (Rahman *et al.* 2017; Contreras-Moreno *et al.* 2019). The reduction in leaves and stems during the dry season, which is the main diet of white-tailed deer in tropical lowland of Mexico, is a factor that motivates the deer to travel long distances to find food, meet metabolic needs, and increase the fulfillment of energy needs during the breeding season, especially in female deer so that they can sustain the next generation. Furthermore, changing light conditions when certain phases of the moon is also known to cause changes in behavior, especially for nocturnal animals. In most cases, predation risk or prey availability are the two main forces explaining changes in the behavior. The moon phase can affect animals differently, whether they are predators, prey, or both. Lunar phobia and lunar philia, the latter for nocturnal species, are two patterns explaining variations in activity level of many species (Michalski and Norris 2011).

Visually orienting nocturnal predators may benefits from bright moonlight because their prey is easier to detect, known as the visual acuity hypothesis (Traill *et al.* 2016). This, in turn, would cause prey to adopt a more cryptic behavior through reduction in activity during moonlight. This assertion is supported by observations of white-tailed deer (*Odocoileus virginianus*) in North Carolina (USA), which individual vigilance was increased during diurnal and moonlit nocturnal hours, presumably to avoid visually hunting coyotes, bobcats or human (Lashley *et al.* 2014). In line with this, red brocket deer (*Mazama americana*) in Central Amazon, whose records evenly distributed through the moon cycle, was found to avoid the brighter times of the night regardless of the moon phase. Red brocket deer shifted the temporal distribution of their activities under different moon phases when predators were present (Pratas-Santiago *et al.* 2017). This pattern of behavior can also be found in Baird's tapir (*Tapirus bairdii*), which was found to be more active on dark nights in Southern México (Sánchez-Pinzón *et al.* 2020). Conversely, studies on ungulate that lived in tropical ecosystem such as the mountain tapir (*Tapirus pinchaque*) in Central Andes (Columbia) showed that night time activity was higher during full moon than during quarter and new moons (Lizcano and Cavalier 2000) or it may not affect the activities of zebra and wildebeest in Kruger National Park, where there was no evidence for increased foraging activity over the full moon for both species (Traill *et al.* 2016).

Previous studies on activity pattern of Bawean deer (*Axis kuhlii*; Blouch and Atmosoedirdjo 1987) have shown that the species was solitary and nocturnal, active intermittently through the night. The red muntjac (*Muntiacus muntjak*) was known to be mostly diurnal (Kawanishi and Sunquist 2004) and in several areas were classified as cathemeral (*i. e.*, sporadic and random intervals of activity during the day or night; van Schaik and Griffiths 1996). Both deer are similar in size, elusive, and poorly known. Bawean deer and red muntjac are good examples of medium-sized ungulates, cryptic, secretive, and uncommon species in Southeast Asian tropical rainforests. Like most tropical ungulates worldwide, little has been published on their ecology and their role in the tropical rainforest ecosystem.

Through data analysis from a 9- and 19-month camera trapping in Bawean Island Nature Reserve and Wildlife Sanctuary (for Bawean deer) and Ujung Kulon National Park (for red muntjac), respectively, we ascertained how season, sex and lunar illumination along with their predators affect Bawean deer and red muntjac activity. This study aimed to test the hypothesis on seasonal, sex, and lunar influence along with their predators on activity patterns on the two tropical deer species, the Bawean deer and the red muntjac in the tropical rainforest in Southeast Asia. The hypothesis that specifically tested was that both deer species will minimize their activity during the most luminous season and moon phase as a response to the presence of predators or human disturbance.

Material and methods

Study areas. The research was conducted in Bawean Island Nature Reserve and Wildlife Sanctuary (hereafter refer to as Bawean Island) and Ujung Kulon National Park (hereafter refer to as Ujung Kulon; Figure 1). Bawean deer was studied in Bawean Island, an isolated 200 km^2 island in Java Sea (-5° 40', -5° 50' S; 112° 3', 112° 36' E). The study area in this island comprised of a nature reserve (725 ha) and a wildlife sanctuary (3,832 ha) having a steep topography of terrain slopes more than 60° and a wide altitudinal gradient (-11 to 630 masl).

Based on Schmidt and Ferguson (1951) classification, Bawean Island climate is categorized as type C with temperature varies between 22 and 32 °C (Semiadi 2004), the annual rainfall is 2,298 to 2,531 mm on the southern coast. Rainfall is mostly high during the northwest monsoon, which normally lasting from the end of October until April.

The main vegetation type on Bawean Island is tropical rainforest which can be divided into four major forest types: primary forest, secondary forest, teak (*Tectona grandis*) forest, and shrubs. The Bawean Island protects a small patch of tropical rainforest (approximately 23 % of the island area), including teak forest (60 %), a habitat type which is globally endangered due to deforestation and climate change. The remaining natural forests are confined to the steep sides and top of the higher hills and mountains, often occurring as habitat islands surrounded by teak.

Moreover, the Bawean Island serves as one of the last strongholds in the country for endemic medium-sized ungulates, including the Bawean deer and Bawean warty pig (*Sus blouchi*). Therefore, although the island is tiny, the Bawean Island plays a key role in conservation of medium-

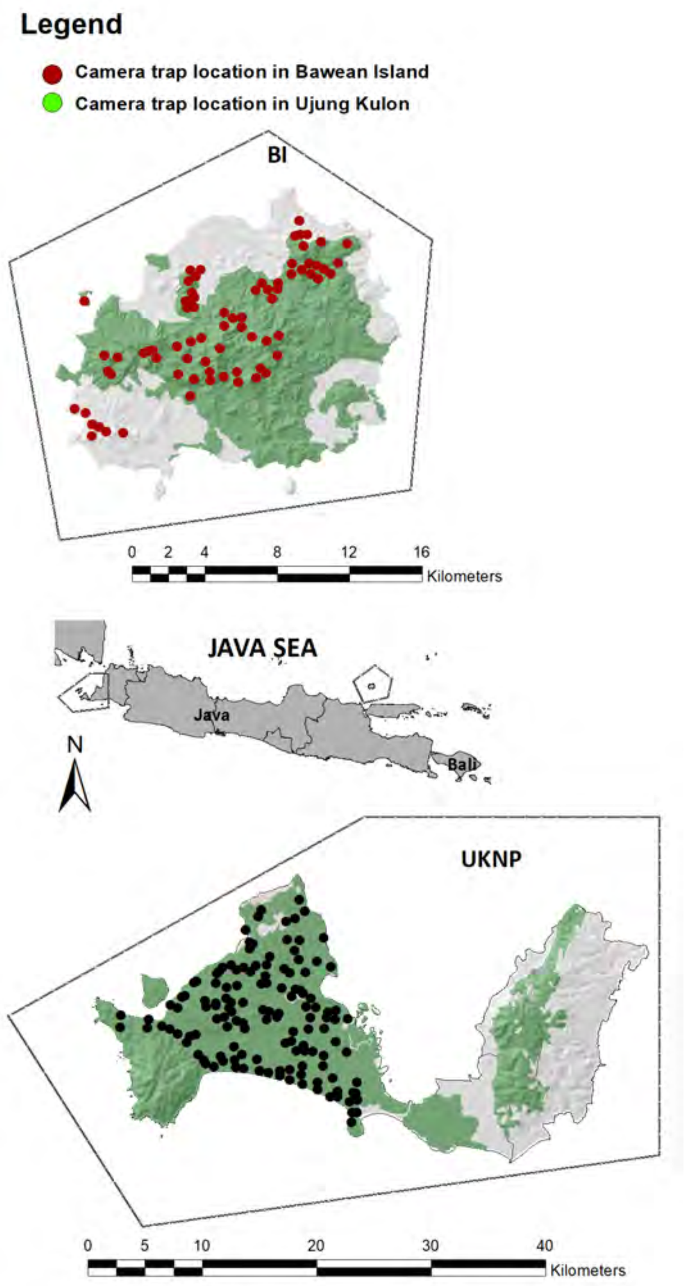

Figure 1. Location of the two study areas (BI: Bawean Island; Ujung Kulon: Ujung Kulon National Park) in Java, Indonesia where we studied the activity patterns of Bawean deer and red muntjac, respectively. Red dots location in Bawean Island and black dots location in Ujung Kulon.

sized mammals in Indonesia. Unfortunately, land cover has been changing and continue to occur until now. The need of space for settlements and agricultural areas due to human population growth increasingly urges the existence of protected areas as the only safe habitat for Bawean deer. In addition, other pressures due to hunting activities and the presence of free-roaming dogs and invasive agricultural weeds (*e. g.*, *Ageratum conyzoides*, *Chromolaena odorata*) are believed to cause a decline in Bawean deer habitat and population (Semiadi 2004).

Red muntjac was studied in Ujung Kulon, a peninsular national park at the extreme southwestern tip of Java Island, Indonesia (-6 °38', -6° 52' S; 105°30', 105°41 'E). The Park encompasses an area of 105.694,46 ha, of which 44,337 ha are marine areas (Ministry of Environment and Forestry 2014). Ujung Kulon climate is categorized as type A (Hommel 1987) with a temperatures range of 25 to 30 °C and relative humidity ranges between 65 and 100 % (Blower and van der Zon 1977; Hommel 1987). Under the tropical maritime condition, the mean annual rainfall is approximately 3,250 mm. The heaviest rainfall occurs between October and April during the northwest monsoon. A noticeably drier period occurs between May and September with approximately 100 mm rainfall per month during the southeast monsoon. The Park has varied topography (with terrain slopes steeper than 15°) and a wide altitudinal gradient (0 to 620 m).

The vegetation in the Park is a tropical rainforest, which has suffered several anthropogenic and natural disturbances in the past and the present. It is mainly secondary growth, following the destructive tsunami of 1883 caused by the eruption of the Krakatau supervolcano and landslide in the Sunda Strait following a massive eruption of the Anak Krakatau volcano in December 2018. The main habitat types are primary forest, secondary forest, mangrove-swamp and beach forest. The *Arenga* palms, which grow on thick volcanic ash, may be dominant as a result of long-past volcanic disturbance. As a result, the natural vegetation cover, primary lowland rainforest, now occupies only 50 % of the total area, and is largely confined to the Mount Payung and Mount Honje massifs.

The Ujung Kulon constitutes one of the last strongholds for endemic large ungulates such as the Javan rhino (*Rhinoceros sondaicus sondaicus*) and banteng (*Bos javanicus*), as well as common carnivores such as Javan leopard (*Panthera pardus melas*) and dholes (*Cuon alpinus*). On the contrary to Bawean Island, human access, and the presence of forest edges in this Park is lower.

Gridding methodology. Camera trapping was used as the main method to study both deer species. The use of camera traps to study ecological attributes and behavioral aspects of deer has become popular and extensive lately (Caravaggi *et al.* 2017). Field surveys were carried out for nine months (March to November 2014) in Bawean Island and 19 months (January 2013 to July 2014) in Ujung Kulon. The sampling periods corresponded to two consecutive season cycles (wet and dry season). The distribution of season was based on the monsoon in Indonesia, *i. e.*, wet season in November with transition in April and dry season in May with transition in October (Tjasyono 2008). Sampling effort during the survey was 5,500 trap-days in Bawean Island and 62,316 trap-days in Ujung Kulon.

In both study areas, camera traps were positioned according to the methodology of Karanth and Nichols (1998). The cameras were positioned in grids, in a way to cover the whole study area by applying a buffer equivalent to half of the mean maximum distance moved (MMDM). This means that any individual in the study area had a probability greater than zero to be photographed by at least one camera. Because the goal was obtaining as many photographs as possible in each grid, when a camera did not capture any object (zero presence) for some time, camera location was changed in the same grid. Based on the availability of camera traps and representation of major forest types, Bawean Island was divided into 20 trap stations of 2-km^2, while Ujung Kulon was divided into 329 trap stations of 1-km^2.

Camera traps with heat-in-motion detectors were used to continuously (24h) record the activity of the deer species and their main predator. Date and time of all photos were set automatically. Twenty units of Bushnell Trophy Cam HD Max analog cameras were deployed in Bawean Island. Each camera was mounted on a tree, positioned 30 to 50 cm above the forest floor to record small to large animals. In Ujung Kulon, 108 units Bushnell Trophy Cam 119467 and Bushnell Trophy Cam 119405 analog cameras were deployed. Cameras were positioned at 170 cm above the ground with an angle 10 to 20 degree toward to the ground, following the standard design of camera trapping by the on-going Rhino Monitoring Unit Team to survey the Javan rhino.

Cameras were checked every 28 to 30 days to replace battery and the memory card, and to check condition of the camera trap in case of malfunction to avoid data loss. Each photograph of the wild animal was identified to species, and if the quality of the photograph did not allow absolute identification, the photograph was excluded from the dataset. Sequential frames of the same species were counted as one photographic event, and unless individual identification was possible, any subsequent photograph of the same species taken within half hour of the first was not considered a new photographic event.

The study by Meek *et al.* (2016) conveys that there were differences in the level of detection produced by trap cameras that were placed at different heights (0.9 m and 3 m) and different orientations (placing the camera vertically or horizontally), where cameras placed 3 m high and those facing downwards reduced the detection rate of all species compared to those at 0.9 m. Although there were differences of placement of camera trapping in both of study areas, it seemed that it did not affect the capture probability. The height of camera traps differences in this study (0.5 m and 1.7 m with an angle of 10 to 20° lead to the ground), were still within the height limit which gave good detection results (see the number of species detection at each height).

Camera-trapping captures showed the presence of all small-to large-sized mammal species that were known to be existed in both study areas. For analysis purposes, findings of wild animals were separated in pair of deer species and their predators (*i. e.*, Bawean deer with free-roaming dogs in Bawean Island; red muntjac with Javan leopard, and red muntjac with dholes in Ujung Kulon).

Each individual deer captured by camera trap was distinguished by sex based on the sexual dimorphism, presence or absence of antler, and the appearance of the genital organ (Figure 2). In most deer, males tend to be larger than females and possess antlers. Fawns were characterized by always being close to the mother. Individuals who did not show clear sex characteristics were categorized as unsex.

Figure 2. Photos of A) male and B) female Bawean deer in Bawean Island. C) male and D) female red muntjac in Ujung Kulon.

Data analysis

Temporal patterns of activity. Every camera location for each species site was used as an individual sample unit, furnishing hour/date and sex for each single event recorded. The activity level or capture frequency was defined as the proportion of photographic events per hour. The total photographic events occurring during each hour of a-24h day was considered as a proxy to the activity patterns of both species (Figure 3). Sequential frames of the same species were counted as one photographic event. Instances where an individual or group with same sex or composition respectively were captured by the same camera more than once within half hour were discarded from analyses. The number of Bawean deer and red muntjac events per hour of the day were grouped and tested the null hypothesis that both deer capture was uniform throughout the day, applying the Rayleigh test (Batschelet 1981; Zar 2010). The program Oriana V4.05 (Kovach Computing Services 2012) was used to apply this test. To complete this analysis, the Bawean deer and red muntjac events were grouped in three time periods: diurnal, nocturnal, and crepuscular (dawn and dusk). Diurnal (1h after sunrise to 1h before sunset), nocturnal (1h after sunset to 1h before sunrise), dawn (from 1h before to 1h after sunrise), and dusk (from 1h before to 1h after sunset; Theuerkauf *et al.* 2003).

Differences in the capture frequency between sexes and times of the day were evaluated using Chi-square tests. Two-factor analysis of variance were used to test differences in captures among daily time periods and seasons. For the seasonal analysis, data were pooled into 3h periods, to obtain a larger and more uniform number of activity-fixed in each period. Tukey's honestly significant difference test was used to evaluate variation differences in the frequency of events for each period, when necessary. Results were considered significant if alpha < 0.05.

Moon phase and activity. Moon phase was enumerated for each calendar day of the sampling period using the software Quickphase Pro 3.3.4 (BlueMarmot.com). The effect of moonlight on capture frequency was obtained by assigning one of the four moon phases to each day. Following Batschelet (1981), circular statistical analyses for temporal data that follow a cycle was used.

To include the moon phase as a covariate in subsequent analysis, a lunar light index (LMw) was created according to the following calculation. For a given week, the number of nights corresponding to the four lunar phases was calculated and converted to a lighting coefficient according this quantification: new moon = 0, first and last quarter = 4, full moon = 10 (Lang *et al.* 2006; Manfredi *et al.* 2011). The coefficients values were added to obtain a LMw for each week. For example, a week of which all the nights were in full moon received an index of 70, while for a new moon week, the value was 0.

In analyzing the data sets according the variables of interest, particularly the lunar light, a Generalized Additive Models (GAMs as implemented in the R package mgcv) that was applied as a rough examination of the relationship between activity patterns and lunar cycle suggested a non-linear pattern. Indeed, deviance explained was better with additive rather linear models (37 % vs 10 % for additive and linear calculations respectively). GAMs are similar to generalized linear models except that a component of each predictor is a sum of smooth, nonlinear functions of the numerical predictor variables in the model. Additive modeling would be well suited to analyze non-linear response of species to multiple explanatory variables, situations encountered frequently for ecological data. Accordingly, temporal changes of rate of capture per week was modeled as a function of date (week number), habitat, season, elevation and an index evaluating the amount of moonlight received each week.

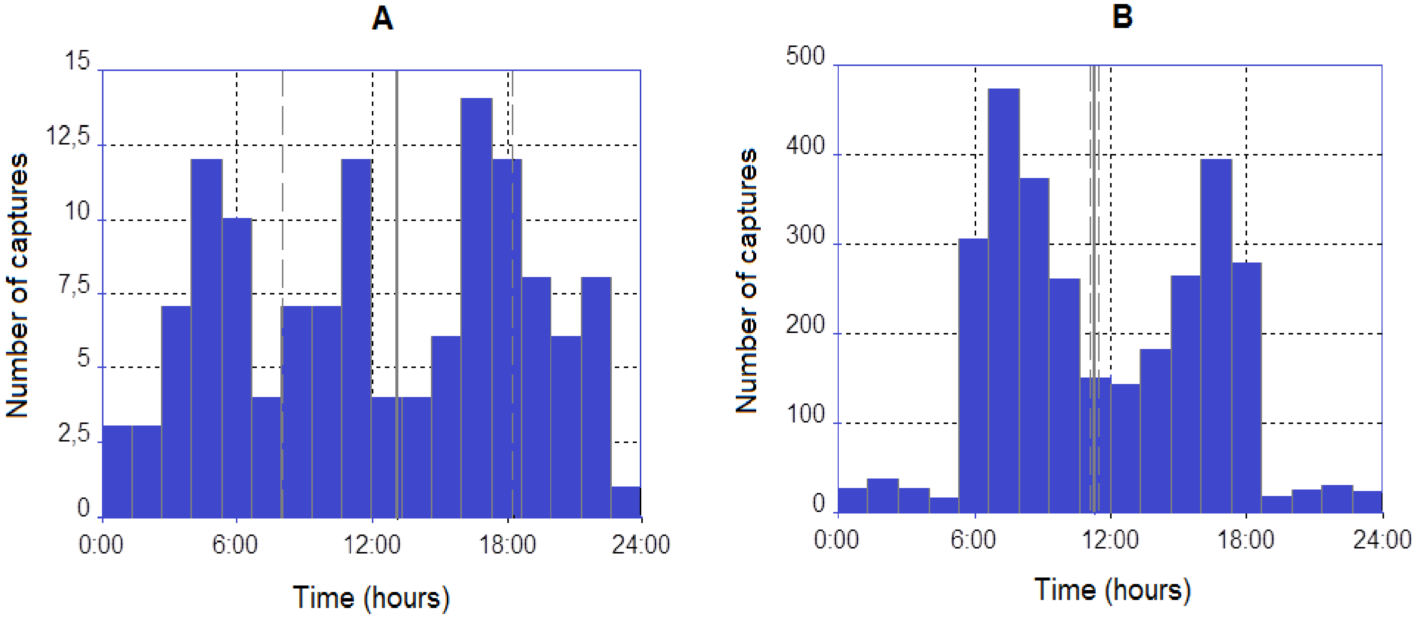

Figure 3. Circular histogram illustrating the distribution of deer activity by camera traps throughout the day. Black bars indicate levels of activity of A) Bawean deer, and B) red muntjac. Radius and indicate location of average hour of activity and its 95 % confidence interval, respectively. *n*: total number of captures.

The sampling records at each camera trap station was divided into seven-day segments according to the week number. Accordingly, a weekly capture rate was estimated for each camera trap inside its operating period and constituted the modeled variable. Covariates used as predictor were the week number, habitat type, season simplified to two (wet and dry), elevation and a weekly index of lunar illumination (Appendix 1). Furthermore, the importance of covariates for determining the number of photographs/camera-week for each species based on Δ AICc were also measured accordingly (Appendix 2). GAMs analyses were run using R version 3.3.3. (R Core Team 2017) and coded using the "gam" function in the mgcv package (Wood 2006).

Temporal interactions between prey-predator. The activity patterns of ungulates were believed to be strongly related to their predators. The daily activity overlap patterns between a prey and their main predator were quantified with a two-step procedure that resulted in a coefficient of overlap, with the values ranging from 0 (no overlap in activity pattern) to 1 (identical activity pattern; Meredith and Ridout 2014). For the first step, each capture frequency along the day was estimated separately either non-parametrically, using kernel density estimation or by fitting a distribution from the flexible class of non-negative trigonometric sum distributions (Fernández-Durán 2004). For the second step, a measure of overlap between the two estimated distributions was calculated. Ridout and Linkie (2009) reviewed several alternative measures of overlap between two probability distributions, favouring the coefficient of overlapping, Δ (Weitzman 1970). In this research, Δ_1 and Δ_4 analysis were applied respectively (Ridout and Linkie 2009; Meredith and Ridout 2014) because of the mixture of small sample size (for Bawean deer-free roaming dog) and large sample size (for red muntjac-Javan leopard and red muntjac-dholes). The overlap package, developed by Meredith and Ridout (2014), was used to fit kernel density functions, estimate the coefficient of overlap, and calculate bootstrap estimates of the confidence intervals. The Mardia-Watson-Wheeler test (MWW test) was used to compare the distribution of detections across the dual cycle for all sampling pairs to determine whether two activity patterns significantly differ (Batschelet 1981). However, due to the limited number of records for Bawean deer-free roaming dog, the observed temporal interactions for both species surely need to be treated with caution.

Results

Temporal pattern of activity. For the Bawean deer in Bawean Island, a total of 5,406 photographs were obtained, both in wet and dry season. Of those photographs, the number of photographs qualified to be used for analysis were 118 photographs of Bawean deer (wet: 18 vs. dry: 99 independent photographs) and 86 photographs of free-roaming dog (wet: 33 vs. dry: 50 independent photographs).

Bawean deer capture frequency did not varied significantly with time of day (F = 0.197, d. f. = 2, $P > 0.05$) and nor among seasons (F = 0.644, d. f. =1, $P > 0.05$; Figure 4A). There was no interaction between time of day and season in all activity levels (diurnal F = 1.179, d. f. = 2, $P > 0.05$; nocturnal F = 0.095, d. f. = 2, $P > 0.05$; crepuscular F = 0.047, d. f. = 2, $P > 0.05$). The null hypothesis that Bawean deer was distributed uniformly through the day was accepted (Rayleigh test; Z = 2.22; $P > 0.05$). This indicates that Bawean deer were active throughout most of the day. Although males of Bawean deer seemed to show slightly different pattern throughout the day than females especially during June and August, statistical tests showed that there were no significant differences in the activity patterns of both sexes ($\chi^2 = 19.72$, d. f. = 23, $P > 0.05$; Figure 4B).

For red muntjac in Ujung Kulon, a total of 86,432 photographs were exposed, with 4,363 photographs of red muntjac recorded in both seasons (wet: 1,639 independent photographs vs, dry: 1,986 independent photographs). Moreover, the potential predators for red muntjac were also recorded: 699 independent photographs of Javan leopard (229 and 470 in wet and dry season, respectively) and 351 independent photographs of dholes (95 and 256 in wet and dry season, respectively; Table 1).

Red muntjac showed a significant variation of activity level with time of day (F= 344.92, d. f. =2, $P > 0.01$) and also between seasons (F = 13.33, d. f. =1, $P > 0.01$; Figure 4C). There was an interaction between time of day and season in crepuscular activity levels (F = 54.48, d. f. =2, $P > 0.001$), but not for diurnal and nocturnal activity levels (F= 0.317, d. f. =2, $P > 0.05$ for diurnal; F = 0.991, d. f. =2, $P > 0.05$ for nocturnal). Tukey post-hoc comparisons indicated that crepuscular (M = 4.745) was significantly greater than diurnal (M = 1.445) and nocturnal (M = 2.112) activity levels. Based on the Rayleigh test, the null hypothesis that red muntjac activity was distributed uniformly throughout the day was rejected (Z = 756.85; $P > 0.01$). Although male and female red muntjac showed diurnal activity levels with higher peaks during more or less one hour after sunrise until one hour before sunset, there was no significant differences in activity patterns ($\chi^2 = 31$, d. f. =23, $P > 0.05$). Most activities occurred during August and September for both sexes (Figure 4D).

Moon phase and activity. Results of GAMs showed that the relationship between deer activity and LMw gave very similar pattern between Bawean deer and red muntjac during the wet season with estimates of 1.716 (p-value = 0.236) and 8.875 (p-value = 0.000) respectively (Table 2A, B;

Table 1. Total number of Bawean deer (*Axis kuhlii*) and red muntjac (*Muntiacus Muntjak*) records from March to November 2014 in Bawean Island Nature Reserve and Wildlife Sanctuary and from January 2013 to July 2014 in Ujung Kulon National Park, Indonesia.

Species	Trap-days	Number of photographs				Sex ratio[a]
		Males	Females	Fawn	Unsexed	
Bawean deer	5,500	78	46	20	11	1.70:1
Red muntjac	62,316	2290	1694	84	116	1.35:1

[a] Adult sex ratio (M/F); calculated only from known sex.

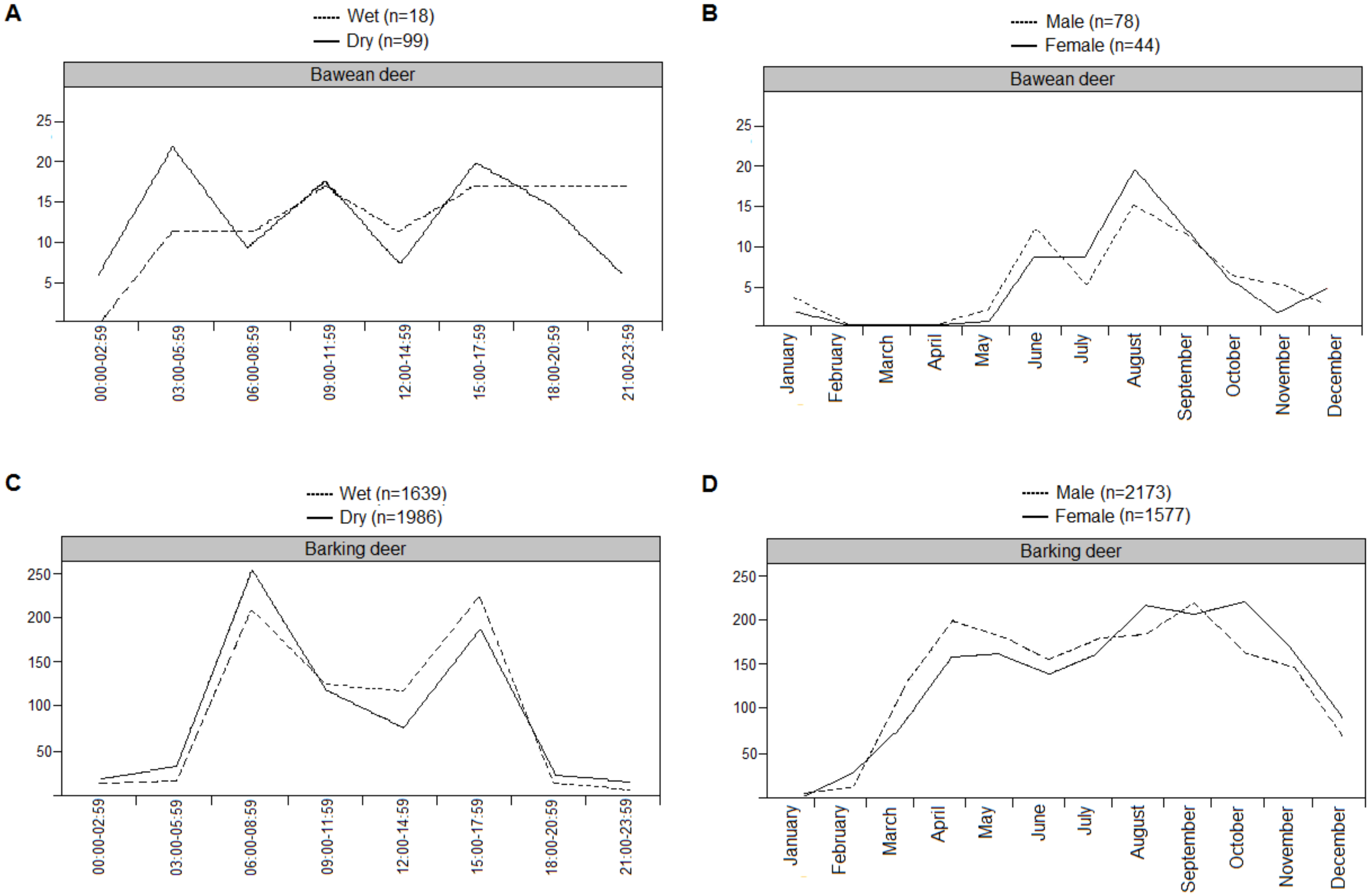

Figure 4. Pattern of seasonal activity of A) Bawean deer and B) red muntjac and intersexual monthly activity of C) Bawean deer and D) red muntjac, respectively, in Bawean Island Nature Reserve and Wildlife Sanctuary and Ujung Kulon National Park. *n*: total number of captures.

Figure 5). These patterns were in agreement with the differences that appeared between the activity rhythms estimated by the proportion of captures per time slot. While red muntjac was active in a bimodal mode, primarily during the daytime, the Bawean deer had a larger temporal distribution over the day and night periods.

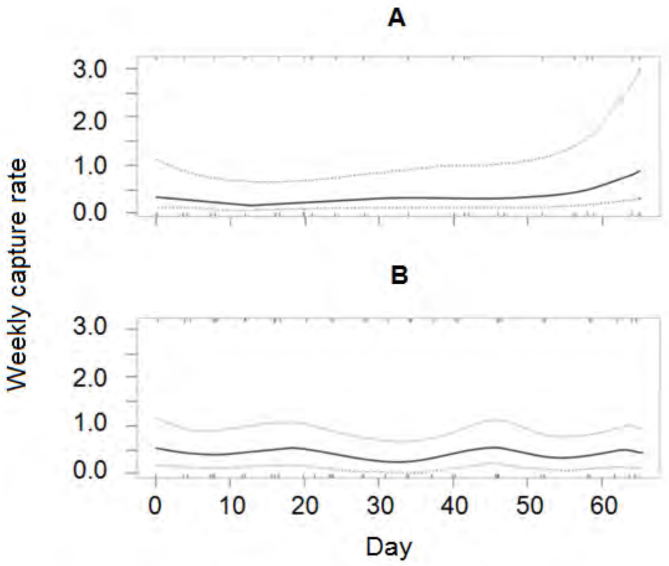

Figure 5. Estimated capture rate (per week) by idCam as a function of GAM covariate MPw (weekly index of lunar light), a) Bawean deer, b) red muntjac. Dashed line indicates the 95 % confidence interval.

Accordingly, the relationship with lunar light of both species unveiled very distinctive patterns. The red muntjac activity revealed as diurnal was not influenced by lunar light, conversely to the Bawean deer that tend to increase their activity with lunar light intensity.

Temporal interactions between prey-predator. The results of the MWW test revealed that patterns of daily activities were significantly different in all pairs of species (*i. e.*, Bawean deer-free-roaming dogs, red muntjac-Javan leopard, red muntjac-dholes; Table 3, Figure 6). The values of temporal overlap coefficients were < 0.72 for all pairs of animals, which means that there was either low or intermediate overlap in their activity patterns. The most similar pairs was between the red muntjac and dholes, which were cathemeral species. Free-roaming dog and Bawean deer showed different peak activities. The free-roaming dog was most active in early morning and late evening, whereas the peak activity of Bawean deer was in mid-day.

Discussion

Several authors have previously analyzed the activity pattern of Bawean deer and red muntjac. Blouch and Atmosoedirdjo (1978, 1987) noted that Bawean deer was primarily nocturnal, emerging from dense cover just after dark (around 6pm) and being active intermittently throughout the night. Peak of activity occur approxi-

Table 2. Result of the Generalized Additive Models (GAMs) relating the number of photographs/camera-week of Bawean deer to habitat type (Hab), index of lunar light received by week (LMw) and season (Seas).

A (n = 190)

Parametric coefficients	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	0.2109	-3.809	-0.8031	0.00014***
SeasWet	-2.7177	1.9125	-1.421	0.15531
Smooth terms	Edf	Ref.df	Chi.sq	p-value
s(LMw:SeasDry)	5.095	6.13	34.525	6.45e-06 ***
s(LMw:SeasWet)	1.716	2.109	3.217	0.236
s(IdCatr)#	7.259	12.000	37.133	8.23e-08 ***

R-sq.(adj) = 0.283 Deviance explained = 34.7 %; UBRE = 0.23925 Scale est. = 1

B (*n* = 6835)

Parametric coefficients	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	-2.0101	0.2564	-7.841	4.48^{-15}***
HabPr_f	1.5620	0.4349	3.592	3.29^{-4}***
HabSe_f	1.0790	0.2601	3.5920	3.33^{-5}***
HabSwMgr	0.4487	0.5469	0.8200	0.4119
Smooth terms	Edf	Ref.df	Chi.sq	p-value
s(LMw:SeasDry)	8.710	8.974	116.66	2^{-16}***
s(LMw:SeasWet)	8.875	8.995	40.35	6.52^{-6}***
s(IdCatr)#	221.856	242.000	2606.07	$< 2^{-16}$

R-sq.(adj) = 0.219 Deviance explained = 30.5 %; UBRE = 0.18173 Scale est. = 1

mately every two hours, usually separated by retreats into cover. As the night progressed, foraging periods become shorter and rests become longer, until the deer retired back into dense cover at sunrise. At night they move into more open forest areas or grasslands and cultivated area. These results were also similar to those recorded by Semiadi (2004) - individuals were occasionally seen on the beach in the southwest of the island or along the river at dusk until night period, but otherwise were rarely seen directly.

As for the activity patterns of red muntjac, the patterns appeared varies. In Taman Negara, Malaysia, Kawanishi and Sunquist (2004) noted that red muntjac tended to be diurnal, whereas in Gunung Leuser, Sumatra (van Schaik and Griffiths 1996) and also in East Java (S. Hedges *pers. comm.* 2008) showed cathemeral patterns, leading to a conclusion that there some variation between localities in balance of day and night activity.

Contrary to the prediction, the patterns of activity were discrepant with results from previous research by Blouch and Atmosoedirdjo (1978, 1987) and Semiadi (2004). Despite the small amount of data, the Bawean deer were significantly active throughout the day with a peak activity at 6 to 7h and 20 to 21h. The low hourly variation in the activity patterns of Bawean deer may be due to the lack of wild predators (Bonnot *et al.* 2016), except the large reticulated pythons (*Malayopython reticulatus*). However, pythons were not common and likely have little impact on the deer population (Blouch and Atmosoedirdjo 1987). Killing a young fawn may have occurred by wild boar or macaques in the study site, but no evidence has been found to support this (Blouch and Atmosoedirdjo 1987). People who hunting with their dogs were currently the greatest cause of mortality to this species, and were responsible for 9 out of the 11 deaths examined by Blouch and Atmosoedirdjo (1987) during October 1977 and May 1979. Hunters may hunt at any time of the day, although most of the hunting took place in the morning or late evening, between 7 to 10h and 16 to 18h, respectively.

Human-wildlife conflicts, fragmented landscape and predation by free-roaming dogs across the study site in Bawean Island were responsible for most mortality of

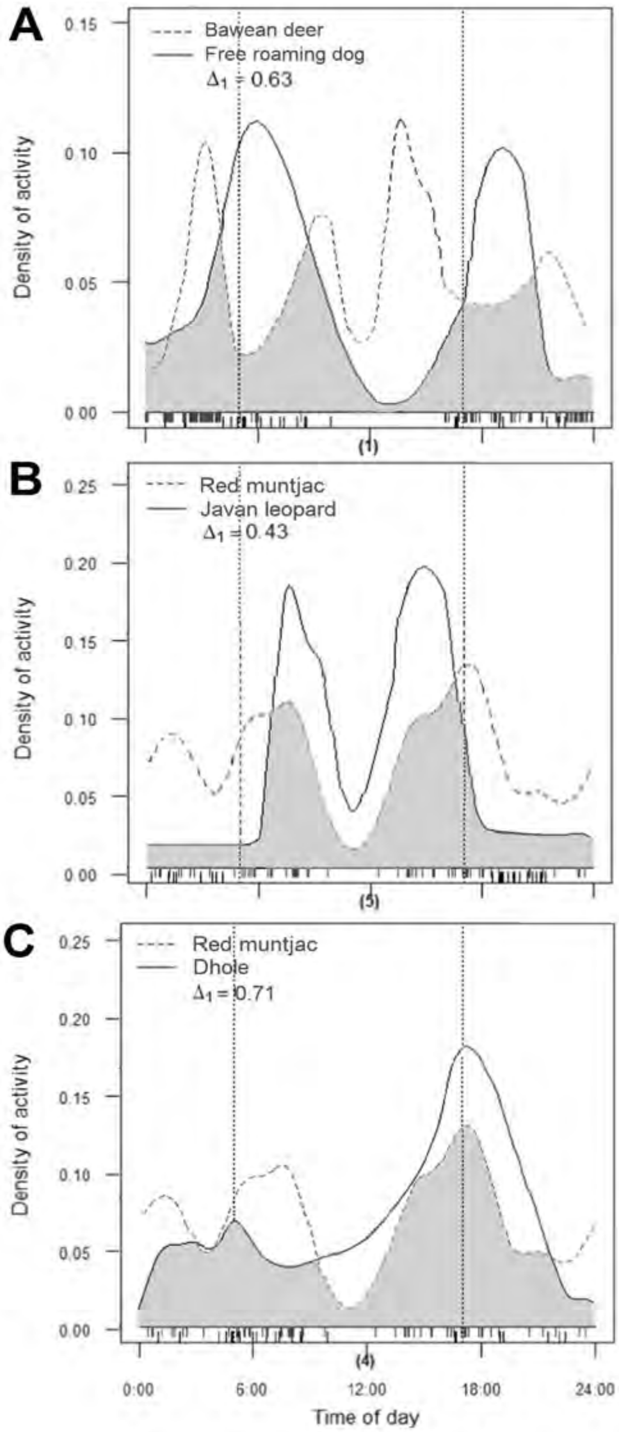

Figure 6. Daily activity patterns and overlap of A) Bawean deer- free-roaming dog, B) red muntjac-Javan leopard, C) red muntjac-dholes.

Table 3. Overlap coefficient (Δ_1 and Δ_4) between prey-predator activity patterns, 95 % confidence intervals (95 % Cis), and Mardia-Watson-Wheeler (MWW) test.

	Δ_1 and Δ_4 (95 % CI)	**MWW**	**P Value**
Bawean deer × Free-roaming dog	0.63 (0.52-0.74)	29.67	<0.0001**
Red muntjac × Javan leopard	0.43 (0.38-0.48)	42.75	<0.0001**
Red muntjac × Dholes	0.71 (0.68-0.74)	7.18	<0.05*

The *P* value of less than 0.05 indicate that the two sets of circular distributions come from different distribution.

Bawean deer. Most of the deaths occurred in area between the edge of protected areas and settlements or on cultivated areas. During this study, two cases of Bawean deer death were found, caused by free-roaming dogs, but this was much lower when compared with prior observation in the 1990s when hunting has led to high population declines in the past (Rahman *et al.* 2016, 2017). As a consequence, Bawean deer may be adopting a more flexible behavior, on the absence of natural predator and hunting activity by human. In addition, the presence of Bawean deer throughout the day in some harvested forest suggests that this species was able to tolerate selective logging. Previous study (Rahman *et al.* 2017) indicated that there was a tendency that the distribution of Bawean deer became closer to human settlements. However, a definitive testing of this assumption requires documenting Bawean deer activity patterns in other areas more exposed to human impact, such as those located outside and on the border of the Bawean Island. Therefore, reduced predator risk could enable Bawean deer to be active across the entire lunar cycle, without the need to avoid strong illuminated nights.

In Ujung Kulon, activity pattern of red muntjac seemed to be more related to the activity of predation by Javan leopard and dholes than hunting or habitat disturbance. In this study, the activity of red muntjac might be opposite toward their main predator activity pattern. The activity peaks for the red muntjac were found to be mostly diurnal, while dholes as the main predator were widely known as diurnal species with high activity in the afternoon and late afternoon (Nurvianto *et al.* 2015; Rahman *et al.* 2018). There were several images of the dholes captured during the day, indicated that the dhole's travelling activity pattern for hunting was during early morning to mid-day and with peak in the evening hours. As a carnivore, it is advantageous for the dholes to be most active during the hours when its prey is most active.

During the study, poaching was lower compared to the last 20 years ago, and thus may provide an opportunity for the red muntjac population to survive and continue to grow. However, there was no strong evidence that either hunting or habitat disruption were actually threats to the survival of populations except in the case of islands such as Singapore, where it is now extinct (Baker and Lim 2008). Peak densities of red muntjac were not in pristine forest (see Oka 1998; Laidlaw 2000; Azlan 2006; for habitat and ecology). In Danum Valley (Sabah, Borneo), an area with negligible hunting, the density of red muntjac was increased after logging (Davies *et al.* 2001), either strongly increased as reported by Davies *et al.* (2001) or weakly found by Duff *et al.* (1984). Nevertheless, even quite severe habitat disruption can increase ecological carrying capacity for this deer, but this trend must be considered with caution as it could be temporary at a small scale.

It has been known that the time dedicated by wild animals to search for and obtaining food is inversely proportional to its abundance (Chappel 1980). If this type of behavior was the main factor affecting the quantity of time spent active by Bawean deer and red muntjac, the lower activity level in wet season at each study site might be related to greater availability of food. In most tropic habitats, food is assumed to be uniform throughout the year (Foster 1973; Frankie *et al.* 1974), but become scarce over the dry season (Pontes and Chivers 2007). Study by Esparza-Carlos *et al.* (2011) might explain that during the wetter year, food resources became less important factor, while cover and visibility were better to explained deer habitat use. The fact that the number of captures by camera trapping were less for males and females of both deer in wet season is likely to be associated to the finding that food was abundant, and thus reduced their activity and movement. Alternatively, the maximum activity in dry season in both species, could also reflect the reproductive and distinct tactics in males and females of solitary deer (Blouch and Atmosoedirdjo 1987; Kitchener *et al.* 1990). The increased activity of females may be due to the greater energetic requirements to feed their weaned young. Breading season for both deer occurred from February to June (Blouch and Atmosoedirdjo 1987; Kurt 1990). Males may be induced to increase their level of activity to devote more time in activities related to the marking and maintenance of territory in response to the presence of dispersing juveniles (Oka 1998; Kitchener *et al.* 1990).

Bawean deer tended to be more active in bright nocturnal periods than when the night was dark in Bawean Island and this trend contradicts the hypothesis. Some authors have reported that moonlight may act indirectly on the behavior of ungulates by increasing their rate of movements in brighter nights when moonlight play an important role in sustaining relatively high levels of visual acuity to find food (Birkett *et al.* 2012; Prugh and Golden 2014). Deer movement increased when the moon directly positioned overhead or underneath. The moonlight effects on activity level was high enough to indicate that lunar cycles likely have a major impact on the foraging rates and habitat use, particularly for Bawean deer. An alternative explanation was associated with the trade-offs between food and safety from the risk of predation.

In 1950-2000, Bawean deer and warty pig hunting by dogs were widespread in Bawean Island, especially during the moonlight period. But, the current strict rules regarding the protection of both species caused this activity to be less frequent. Deer predation by dogs that happened recently is a natural behavior pattern of this carnivorous species, they benefit from detection of prey when the deer increase their activity during certain phases of the moon which provide good lighting at night.

Unlike Bawean deer, red muntjac activity did not vary with the level of moonlight. Being more active in bright night conditions makes red muntjac more visible and detectable by Javan leopards and dholes. Harmsen *et al.* (2011) showed that armadillos and pacas appeared to further reduce predation risk by lowering their activity during bright moonlight nights, as has been observed in many smaller rodent species (Daly *et al.* 1992; Kotler *et al.* 2004).

We believe that the differences between two similar-sized species, Bawean deer and red muntjac, can be explained by a difference in the exploitation of resources, habitat disturbance and behavior to avoidance of types of predator and their hunting tactics (Burkepile *et al.* 2013). Daily activity patterns can have consequences for an animal's predation risk (Kronfeld-Schor and Dayan 2003) and foraging success (Rijnsdorp *et al.* 1981). Predators are believed to have higher hunting success when prey are mobile (Avgar *et al.* 2011; Traill *et al.* 2016), so increased ungulate activity at dawn and dusk could provide predators with a useful means of enhancing predation success by investing hunting effort during periods of increased prey activity.

This paper presents the first daily activity data in relation to the lunar cycle and the predator activity patterns influencing tropical deer in Indonesia. The results suggested that moonlight may play an important role in sustaining relatively high levels of visual acuity on Bawean deer. Considering the extremely low numbers and limited geographic distribution of wild Bawean deer populations, conserving populations should ideally be developed by controlling the population of free-roaming dogs and the prohibition of hunting by humans using dogs.

The conservation of both protected areas should be considered a top priority because of high species endemism and conservation risk. Such knowledge may be essential for the future management plans and conservation of many unique areas, not only in Bawean Island but also in many areas in Indonesian tropical rainforest where they are facing the same threats.

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Literature Cited

Avgar, T., D. Kuefler, and J. M. Fryxell. 2011. Linking rates of diffusion and consumption in relation to resources. The American Naturalist 178:182–190.

Azlan, J. M. 2006. Mammal diversity and conservation in a secondary forest in Peninsular Malaysia. Biodiversity and Conservation 15:1013-1025.

Baker, N., and K. Lim. 2008. Wild animals of Singapore: a photographic guide to mammals, reptiles, amphibians and freshwater fishes. Draco Publishing and Distribution Pte Ltd and Nature Society. Singapore.

Batschelet, E. 1981. Circular statistis in biology. Academic Press. New York, U.S.A.

Beier, P., D. Choate, and R. H. Barrett. 1995. Movement patterns of mountain lions during different behaviors. Journal of Mammalogy 76:1056–1070.

Birkett, P. J., A. T. Vanak., V. M. R. Muggeo., S. M. Ferreira, and R. Slotow. 2012. Animal perception of seasonal thresholds: changes in elephant movement in relation to rainfall patterns. Plos One 7:e38363.

Blouch, R. A., and S. Atmosoedirdjo. 1978. Preliminary report on the status of the Bawean deer (Axis kuhli). Pp. 49-55, in Threatened deer: Proceedings of a Working Meeting of the Deer Specialist Group of the Survival Species Commission. Morges: IUCN.

Blouch, R. A., and S. Atmosoedirdjo. 1987. Biology of the Bawean deer and prospects for its management. Pp. 320-327, *in* Biology and Management of the Cervidae (Wemmer, C. M., ed.). Smithsonian Institution Press. Washington, U.S.A.

Blower, J. H, and A. P. M. Van Der Zon. 1977. Proposed Ujung Kulon National Prak including Gunung Honje, Pulau Peucang, and Pulau Panaitan Management Plan 1977-1981. Pp. 90, Field report UNDP/FAO. Nature Conservation and Wildlife Management Project INS/73/013. FAO.

Bonnot, N. C., N. Morellet., A. J. M. Hewison., J-L. Martin., S. Benhamou, and S. Chamaillé-Jammes. 2016. Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) adjust habitat selection and activity rhythm to the absence of predators. Canadian Journal of Zoology 94:385-394.

Bourgoin, G., M. Garel., P. Blanchard., D. Dubray., D. Maillard, and J. M. Gaillard. 2011. Daily responses of mouflon (*Ovis gmelini musimon* × *Ovis sp.*) activity to summer climatic conditions. Canadian Journal of Zoology 89:765-773.

Bowyer, R. T., and J. G. Kie. 2009. Thermal landscapes and resource selection by black-tailed deer: implications for large herbivores. California Fish and Game 95:128-139.

Buchholz, R. 2007. Behavioural biology: an effective and relevant conservation tool. Trends in Ecology and Evolution 22:401-407.

Burkepile, D. E., C. E. Burns., C. J. Tambling., E. Amendola., G. M. Buis., N. Govender., V. Nelson., D. I. Thompson., A. D. Zinn, and M. D. Smith. 2013. Habitat selection by large herbivores in a southern African savanna: the relative roles of bottom-up and top-down forces. Ecosphere 4:1-19.

Cain III, J. W., P. R. Krausman., S. S. Rosenstock, and J. C. Turner. 2006. Mechanisms of Thermoregulation and Water Balance in Desert Ungulates. Wildlife Society Bulletin 34:570-581.

Caravaggi, A., P. B. Banks., A. C. Burton., C. M. V. Finlay., P. M. Haswell., M. W. Hayward., M. J. Rowcliffe, and M. D. Wood. 2017. A review of camera trapping for conservation behaviour research. Remote Sensing in Ecology and Conservation 3:109–122.

Chappell, M. A. 1980. Thermal energetic and thermo-regulatory costs of small artic mammals. Journal of Mammalogy 61:278-291.

Contreras-Moreno, F. M., M. G. Hidalgo-Mihart, and W. M. Contreras-Sánchez. 2019. Daily traveled distances by the white-tailed deer in relation to seasonality and reproductive phenology in a tropical lowland of southeastern Mexico. Pp. 111-123, *in* Movement Ecology of Neotropical Forest Mammals. Springer, Cham, Switzerland.

Cozzi, G., F. Broekhuis., J. W. McNutt., L. A. Turnbull., D. W. MacDonald, and B. Scgmid. 2012. Fear of the dark or dinner by moonlight? Reduced temporal partitioning among Africa's large carnivores. Ecology 93:2590-2599.

Daly, M., P. R. Behrends., M. I. Wilson, and L. F. Jacobs. 1992. Behavioural modulation of predation risk: moonlight avoidance and crepuscular compensation in a nocturnal desert rodent, *Dipodomys merriami*. Animal Behaviour 44:1-9.

Davies, G., M. Heydon., N. Leader-Williams., J. MacKinnon, and H. Newing. 2001. The effects of logging on tropical forest ungulates. *In* The cutting edge: conserving wildlife in logged tropical forest (Fimbel, R.A., A. Grajal, and J.G. Robinson JG, eds.). Columbia University Press. New York, U.S.A.

Duff, A. B., R. A. Hall, and C. W. Marsh. 1984. A survey of wildlife in and around commercial tree plantation in Sabah. Malay Forester 47:197-213.

Esparza-Carlos, J. P., J. W. Laundré, and V. J. Sosa. 2011. Precipitation impacts on mule deer habitat use in the chihuahuan desert of Mexico. Journal of Arid Environments 75:1008-1015.

Fernández-Durán, J. J. 2004. Circular distributions based on nonnegative trigonometric sums. Biometrics 60:499-503.

Foster, R. B. 1973. Seasonality of fruit production and seed fall in a tropical forest ecosystem in Panama. Dissertation, Departement of Botany, Duke University, Durham, U.K..

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

Harmsen, B. J., R. J. Foster., S. C. Silver., L. E. Ostro, and C. P. Doncaster. 2011. Jaguar and puma activity patterns in relation to their main prey. Mammalian Biology 76:320-324.

Hommel, P. W. F. M. 1987. Landscape ecology of Ujung Kulon (West Java, Indonesia). Soil Research Institute. Wageningen, Netherlands.

Ikeda, T., H. Takahashi., T. Yoshida., H. Igota., Y. Matsuura., K. Takeshita, and K. Kaji. 2015. Seasonal variation of activity pattern in sika deer (*Cervus nippon*) as assessed by camera trap survey. Mammal Study 40:199-205.

Karanth, K. U., and J. D. Nichols. 1998. Estimation of tiger densities in India using photographic captures and recaptures. Ecology 79:2852-2862.

Kawanishi, K., and M. E. Sunquist. 2004. Conservation status of tigers in a primary rainforest of Peninsular Malaysia. Biological Conservation 120:329-344.

Kitchener, D. J., Boeadi., L. Charlton, and Maharadatunkamsi. 1990. The wild mammals of Lombok Island. Records of the Western Australian Museum 33:1-129.

Kolbe, J. A., and J. R. Squires. 2007. Circadian activity patterns of Canada lynx in Western Montana. Journal of Wildlife Management 71:1607-1611.

Kotler, B. P., J. S. Brown., A. Bouskila., S. Mukherjee, and T. Goldberg. 2004. Foraging games between gerbils and their predators: seasonal changes in schedules of activity and apprehension. Israel Journal of Zoology 50:255-271.

Kovach Computing Services. 2012. Oriana. Version 4.01. Kovach Computing Service, U.S.A.

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

Krop-Benesch, A., A. Berger., H. Hofer, and M. Heurich. 2013. Long-term measurement of roe deer (*Capreolus capreolus*) (Mammalia: Cervidae) activity using two-axis accelerometers in GPS-collars. Italian Journal of Zoology 80:69-81

Kurt, F. 1990. Muntjac deer. Pp. 137-139, *in* Grzimek's Encyclopedia of Mammals (Parker, S. P, ed.). McGraw-Hill. New York, U.S.A.

Laidlaw, R. K. 2000. Effects of habitat disturbance and protected areas on mammals of Peninsular Malaysia. Conservation Biology 14:1639-1648.

Lang, A. B., E. K. V. Kalko., H. Römer., C. Bockholdt, and D. K. N. Dechmann. 2006. Activity levels of bats and katydids in relation to the lunar cycle. Oecologia 146: 659-666.

Lashley, M. A., C. Chitwood., M. T. Biggerstaff., D. L. Morino., C. E. Morman, and C. S. DePerno. 2014. White-tailed deer vigilance: the influence of social and environmental factors. Plos One 9: e90652.

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

Manfredi, C., M. Lucherini., L. Soler., J. Baglioni., E. L. Vidal, and E. B. Casanave. 2011. Activity and movement patterns of Geoffroy's cat in the grasslands of Argentina. Mammalian Biology 76:313-319.

McLellan, M. L., and B. N. McLellan. 2015. Effect of season and high ambient temperature on activity levels and patterns of grizzly bears (*Ursus arctos*). Plos One 10:e0117734.

Meek, P. D., G. A. Ballard, and G. Falzon. 2016. The higher you go the less you will know: placing camera traps high to avoid theft will affect detection. Remote Sensing in Ecology and Conservation 2:204-211.

Meredith, M., and M. Ridout. 2014. Overlap: estimates of coefficient of overlapping for animal activity patterns. R package version 0.2.7. Available at: https://cran.r-project.org/web/packages/overlap/index.html. Accessed 24 August 2018.

Milchalski, F., and D. Norris. 2011. Activity pattern of Cuniculus paca (*Rodentia: Cuniculidae*) in relation to lunar illumination and other abiotic variables in the southern Brazilian Amazon. Zoologia 28:701-708.

Ministry of Environment and Forestry. 2014. Keputusan Menteri Kehutanan Republik Indonesia No. SK 3658/Menhut-VII/KUH/2014 tentang Penetapan Kawasan Hutan Taman Nasional Ujung Kulon. Sekretariat Kabinet RI. Jakarta, Indonesia.

Norris, D., F. Michalski, and C. A. Peres. 2010. Habitat patch size modulates terrestrial mammal activity patterns in Amazonian forest fragments. Journal of Mammalogy 91:551-560.

Nurvianto, S., M. A. Imron, and S. Herzog. 2015. Activity patterns and behaviour of denning dholes (*Cuon alpinus*) in a dry deciduous forest of East Java, Indonesia. Bulletin of Environment, Pharmacology and Life Sciences 4:45-54.

Oka, G. M. 1998. Factors affecting the management of muntjac deer (*Muntiacus muntjak*) in Bali Barat National Park, Indonesia. University of Western Sydney. Sydney, Australia .

Pipia, A., S. Ciuti., S. Grignolio., S. Luchetti., R. Madau, and M. Apollonio. 2008. Influence of sex, season, temperature and reproductive status on daily activity patterns in Sardinian mouflon (*Ovis orientalis musimon*). Behaviour 145: 1723-1745.

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

Porfirio, G., V. C. Foster., C. Fonseca, and P. Sarmento. 2016. Activity patterns of ocelots and their potential prey in the Brazilian Pantanal. Mammalian Biology 81: 511–517.

Pratas-Santiago, L. P., A. L. S. Gonçalves., A. J. A. Nogueira, and W. R. Spironello. 2017. Dodging the moon: The moon effect on activity allocation of prey in the presence of predators. Ethology 123:467–474.

Prugh, L. R., and C. D. Golden. 2014. Does moonlight increase predation risk? Meta-analysis reveals divergent responses of nocturnal mammals to lunar cycles. Journal of Animal Ecology 83: 504-514.

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org/.

Rahman, D. A., G. Gonzalez, and S. Aulagnier. 2016. Benefit of camera trapping for surveying the critically endangered Bawean deer (*Axis kuhlii*). Tropical Zoology 29:155–172.

Rahman, D. A., G. Gonzalez, and S. Aulagnier. 2017. Population size, distribution and status of the remote and critically endangered Bawean deer *Axis kuhlii*. Oryx 51:665–672.

Rahman, D. A., P. Rianti., M. Muhiban., A. Muhtarom., U. M. Rahmat., Y. Santosa, and S. Aulagnier. 2018. Density and spatial partitioning of endangered sympatric Javan leopard (Felidae) and dholes (Canidae) in a tropical forest landscape. Folia Zoologica 67: 207-219.

Ridout, M. S., and M. Linkie. 2009. Estimating overlap of daily activity patterns from camera trap data. Journal of Agricultural, Biological and Environmental Statistics 14:322–337.

Rijnsdorp, A., S. Daan, and C. Dijkstra. 1981. Hunting in th kestrel, *Falco tinnunculus*, and the adaptive significance of daily habitats. Oecologia 50:391-406.

Sanchez-Pinzon, K.., R. A. R. Hurtado, and N. F. Meyer. 2020. Moon light and the activity patterns of Tapirus bairdii in the Calakmul region, Southern Mexico. Therya 11:137-142.

Scheibe, K. M., A. Berger., K. Eichhorn, and J. W. Streich. 2001. Time and rhytm-environmental factors and biological structure. Aktuelle Arbeiten zur Artgemäßen Tierhaltung 407:64-75.

Schmidt, F. H., and J. H. A. Ferguson. 1951. Rainfall types based on wet and dry ratios for Indonesia. Djawatan Meteorologi dan Geofisika, Verhandelingen. Jakarta, Indonesia.

Semiadi, G. 2004. Sifat biologi rusa Bawean *(Axis kuhlii)*. Puslit Biologi LIPI Press. Bogor, Indonesia.

Theuerkauf, J., W. Jedrzejewski., K. Schmidt., H. Okarma., I. Ruczynski., S. Sniezko, and R. Gula. 2003. D aily patterns and duration of wolf activity in the Bialowieza Forest, Poland. Journal of Mammalogy 84:243-253.

Tjasyono, B. H. K. 2008. Meteorologi terapan. ITB press. Bandung, Indonesia.

Traill, L. W., J. Martin, and N. Owen-Smith. 2016. Lion proximity, not moon phase, affects the nocturnal movement behaviour of zebra and wildebeest. Journal of Zoology 299:221-227.

van Schaik, C. P., and M. Griffiths. 1996. Activity periods of Indonesian rain forest mammals. Biotropica 28:105-112.

Weitzman, M. S. 1970. Measures of the overlap of income distribution of white and negro families in the United States. Technical Report No. 22. US Department of Commerce, Bureau of the Census. Washington D.C, U.S.A.

Wood, S. N. 2006. Generalized additive models: An introduction with R. Boca Raton, Fl.: Chapman and Hall/CRC Press. Boca Raton, U.S.A.

Zar, J. H. 2010. Biostatistical analysis. Pearson Prentice-Hall. Massachusetts, U.S.A.

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Appendix 1

Covariates and dependent variable in the models

Name	Description covariates	Variable type
Elv	Elevation asl of camera trap station	Continuous
We-n	Week number	Continuous
Hab	For Bawean deer: primary forest (Pr_f), secondary forest (Se_f), teak forest (Tk_f) and Shrub (Shr) ; for red muntjac: primary forest (Pr_f), secondary forest (Se_f), beach forest (Bea_f), swamp and mangrove (SwMgr)	Factor
Seas	Wet (November to April), Dry (May to October)	Factor
IdCatr	Identity of the camera trap station	Factor (random term)
LMw	Lunar light received by week (see methods)	Semi-continuous
Name	Dependent variable	Variable type
wCR	Total deer captions by week	Continuous

Appendix 2

Importance of covariates for determining number of photographs/camera-week at 20 samples points, respectively for Bawean deer and red muntjac

Model	K	logLik	AIC_c	ΔAIC_c	w_i
Bawean deer					
IdCatr LMw*Seas Seas	16	-166.901	369.1	0	0.178
Elv IdCatr LMw*Seas Seas	16	-166.702	370	0.85	0.116
We-n IdCatr LMw*Seas Seas	17	-166.423	370.5	1.34	0.091
Hab IdCatr LMw*Seas Seas	17	-166.206	370.6	1.44	0.087
Hab Elv IdCatr LMw*Seas Seas	17	-166.219	370.6	1.45	0.086
We-n IdCatr LMw*Seas	13	-170.316	370.9	1.83	0.071
We-n Elv IdCatr LMw*Seas Seas	17	-166.244	371.2	2.11	0.062
We-n Elv IdCatr LMw*Seas	14	-170.140	371.5	2.39	0.054
Hab We-n IdCatr LMw*Seas Seas	18	-165.956	371.9	2.80	0.044
Hab We-n IdCatr LMw*Seas	14	-169.988	372.0	2.89	0.042
Hab We-n IdCatr Elv LMw*Seas Seas	18	-165.957	372.1	2.95	0.041
Hab We-n IdCatr Elv LMw*Seas	14	-169.977	372.3	3.16	0.037
IdCatr LMw*Seas	12	-173.232	373.1	3.97	0.024
Elv IdCatr LMw*Seas	12	-173.036	373.8	4.67	0.017
Red muntjac					
Hab IdCatr LMw*Seas	243	-6146.249	12797.4	0	0.287
Hab Elv IdCatr LMw*Seas	243	-6146.034	12797.6	0.17	0.264
Hab IdCatr LMw*Seas Seas	244	-6146.256	12799.5	2.10	0.101
Hab We-n Elv IdCatr LMw*Seas	244	-6146.255	12799.6	2.14	0.099
Hab Elv IdCatr LMw*Seas Seas	244	-6146.066	12799.7	2.27	0.093
Hab We-n Elv IdCatr LMw*Seas	244	-6146.032	12799.7	2.31	0.091
Hab We-n IdCatr LMw*Seas Seas	245	-6146.262	12801.7	4.23	0.035
Hab We-n ElvIdCatr LMw*Seas Seas	245	-6146.041	12801.8	4.41	0.032