





La portada

Chaetodipus siccus liaae is a microendemic subspecies from the Planes basin, in the south of the Baja California peninsula. The subspecies is associated with deep sandy terrain and it has been determined that despite its small distribution area it is a very abundant species (Photograph Sergio Ticul Álvarez Castañeda)

Nuestro logo "Ozomatli"

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



Volumen 15, número 2

mayo 2024

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DERECHOS DE AUTOR Y DERECHOS CONEXOS, año 15, No. 2, mayo-agosto del 2024, 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, <u>www.mastozoologiamexicana.org</u>, <u>therya@cibnor.mx</u>. Editor responsable: Dr. Sergio Ticul Álvarez Castañeda. Reservas de Derechos al Uso Exclusivo No. 04-2009-112812171700-102, ISSN: 2007-3364 ambos otorgados por el Instituto Nacional de Derechos de Autor. Responsable de la última actualización de este número, Unidad de informática de la Asociación Mexicana de Mastozoología A. C. Dr. Sergio Ticul Álvarez Castañeda. Instituto Politécnico Nacional 195. La Paz, Baja California Sur, C. P. 23096. Tel. (612) 123-8486, fecha de la última modificación 30 mayo 2024.

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Community of medium and large-sized mammals and functional diversity in a tropical rainforest of Southern México under different degrees of human pressure

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Functional diversity quantifies the distribution and range of functional traits of species that influence ecosystem processes. We evaluated the functional diversity of the medium and large-sized mammals in a tropical rainforest in Southern Mexico to determine which landscape characteristics associated with human activities affected the composition of mammal functional diversity and functional group abundances. We used camera-traps to document the diversity and composition of medium and large-sized mammals at four sites with different levels of protection and management. We estimated species richness, diversity, and functional diversity for each site, and compared these indices between sites to understand the effects of biodiversity loss in ecosystem dynamics and to detect ecological patterns driven by human perturbation. The density of human settlements was the main covariate related to low species richness and low abundance of large mammals with specialized diets and low population densities. Poaching and other human activities in forested areas near human settlements might have caused large herbivore and specialized carnivore populations to decrease, and populations of medium-sized rodent species to increase. Our results indicated that human perturbation in forested areas had also an impact at the functional level reducing the abundance of some functional groups, and this might have negative consequences for tropical rainforest functions in the long term. Effective management actions should be implemented in Protected Areas with a high density of human settlements nearby to prevent the decline of mammals' functional groups and negative consequences for tropical rainforest.

La diversidad funcional cuantifica las características funcionales de las especies que influyen en los procesos dentro de los ecosistemas. Evaluamos la diversidad funcional de los mamíferos medianos y grandes en una selva tropical del sur de México para determinar qué características del paisaje asociadas con las actividades humanas afectan la composición y abundancia de los grupos funcionales. Usamos trampas cámara en cuatro sitios con diferentes niveles de protección y manejo. Estimamos la riqueza de especies, la diversidad y la diversidad funcional y comparamos estos índices entre sitios para comprender los efectos de la pérdida de biodiversidad en la dinámica del ecosistema y detectar patrones promovidos por la perturbación humana. La densidad de asentamientos humanos estuvo relacionada con una baja riqueza de especies, y bajas abundancias de grandes mamíferos con dietas especializadas y bajas densidades poblacionales. La caza furtiva y otras actividades humanas en áreas cercanas a asentamientos humanos podrían promover una disminución de los grandes herbívoros y carnívoros especializados y un aumento de los roedores de tamaño mediano. La perturbación humana en áreas boscosas también tiene un impacto a nivel funcional, reduciendo la abundancia de algunos grupos funcionales, lo cual podría tener consecuencias negativas para el funcionamiento de estos ecosistemas a largo plazo. Se deben implementar acciones de manejo en Áreas Protegidas con una alta densidad de asentamientos humanos para prevenir las consecuencias negativas en las funciones ecológicas de los bosques tropicales debidas al declive de ciertos grupos funcionales de mamíferos

Keywords: Camera trapping; functional diversity; herbivores; carnivores; México; protected areas; payment for ecosystem services; mammals.

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Introduction

One of the main concerns of conservation managers is to understand the ability of the ecosystems to support and maintain their ecological capacities in response to anthropogenic disturbances (<u>Andreasen *et al.* 2001; Parrish *et al.* 2003). One way to evaluate how anthropogenic disturbances have impacted an ecosystem is through functional diversity which incorporates the role of species in ecosystem processes (<u>Hooper *et al.* 2002; Blaum *et al.* 2011; Roche and Campagne 2017</u>). Functional diversity quantifies the</u> distribution and range of species functional traits (morphological, physiological, or behavioral) that influence ecosystem processes (Tilman 2001; Petchey and Gaston 2006). This approach is useful to understand the effects of biodiversity loss on ecosystem dynamics and to prioritize the conservation of vulnerable functional groups (*i. e.*, a group of species with similar functional traits that play a similar role in ecosystem processes) to avoid the loss of their ecological functions (Chapin III *et al.* 2000; Tilman 2001; Flynn *et al.* 2009; Park and Razafindratsima 2019). Rainforest mammals perform key functions in ecosystem dynamics, such as seed dispersal, herbivory, and predation (<u>Terborgh 1992</u>; <u>Terborgh *et al.* 2001</u>; <u>Stoner *et al.*</u> 2007), and they are linked with other ecosystem processes, such as carbon storage and nutrient cycling (<u>Bello *et al.*</u> 2015; <u>Ripple *et al.* 2015; Sobral *et al.* 2017</u>). Therefore, they are crucial for maintaining the ecological integrity of rainforests in the long term.

Unfortunately, mammals remain one of the most vulnerable groups in tropical rainforests, mainly due to habitat loss and poaching (Carrillo *et al.* 2000; Cuarón 2000). The local extinction or severe widespread population decrease of medium and large-sized mammals has important consequences on ecosystem dynamics and resilience (Redford 1992: Wright *et al.* 2000; Redford and Feinsinger 2001; Dirzo *et al.* 2014), and the most vulnerable are those with large body size, specialized diets, and low population densities (Laurance 199191; Purvis *et al.* 2000; Cardillo *et al.* 2005; Stork *et al.* 2009). For instance, the decrease or extirpation of large herbivores has cascading effects on the structure of small mammal communities, plant recruitment, seed dispersal, and the carbon storage capacity of tropical rainforests (Galetti and Dirzo 2013; Bello *et al.* 2015; Bovendorp *et al.* 2019).

A large effort has been invested in understanding the effects of human activity on the diversity of rainforest mammals (Sampaio *et al.* 2010; Garmendia *et al.* 2013; Brodie *et al.* 2014: Beca *et al.* 2017; Hegerl *et al.* 2017; Boron *et al.* 2019), but less attention has been paid on how this activity affects functional diversity. Flynn *et al.* (2009) reported that the functional diversity of mammals decreased with land use intensification, and the species with unique functional traits were the first to disappear with landscape conversion. Other studies have shown a decrease in the functional diversity of mammals in fragmented landscapes (Ahumada *et al.* 2011; Magioli *et al.* 2015), however, landscape connectivity contributed to maintaining high functional diversity (Magioli *et al.* 2016).

Understanding the relationship between the functional diversity of mammals and anthropogenic activity is useful to assess the effectiveness of tools for conservation managers and prevent the loss of ecological functions of mammals (Chapin III et al. 2000; Hooper et al. 2002; Flynn et al. 2009). To date, Natural Protected Areas (NPA) are the most widely used conservation tool, and large protected areas are critical to safeguard populations of large mammals (Bruhl et al. 2003; Sampaio et al. 2010). Yet, NPA do not operate in isolation and are part of a complex landscape that affects the natural ecological processes within them (Defries et al. 2010). Areas outside NPA may include habitat patches that are important for biodiversity where other conservation tools are implemented simultaneously. One such additional conservation tool that has been implemented increasingly in the tropics over the last two decades is the Payment for Ecosystem Services (PES) program, which involves monetary compensation given by governments and other agencies to landowners (the providers of ecosystem services) to preserve natural forests on their lands (Wunder 2007; Carabias *et al.* 2013; Ezzinede-Blas *et al.* 2016), and PES has been an effective tool to preserving mammal species and their habitat (Hein *et al.* 2013; Chen *et al.* 2020). Both conservation tools have been reported to be effective in reducing forest loss (Geldmann *et al.* 2013; Costedoat *et al.* 2015; Sims and Alix-Garcia 2017; Izquierdo-Tort *et al.* 2019), but their ability to maintain the functional diversity of the mammal community has not been assessed yet.

Mammals play a vital role in the ecosystem and have diverse functional ecological roles (Lacher Jr. et al. 2019), which makes them an excellent group to evaluate the human influence on functional diversity. Previous studies have shown that the least impacted sites have a higher representation of certain functional groups such as carnivores, frugivore-herbivores, and arboreal mammals (Munguía-Carrara et al. 2019). On the other hand, highly fragmented sites have lower functional diversity compared to partially fragmented and continuous forests (Ahumada et al. 2011). Here, we evaluate the composition and functional diversity of medium and large terrestrial mammals using the camera trapping technique in the Selva Lacandona Region in Southern México. We determined which landscape characteristics associated with human activity (human pressure proxies) affected the composition, functional diversity, and functional group abundances of the rainforest mammal's assemblage, and we compared them between four different conservation areas. Using this approach, we evaluated mammalian diversity by incorporating their functional traits. We predicted that diversity and functional diversity decreased at sites with higher human pressure such as sites with persistent poaching or higher density of human settlements (Flynn et al. 2009; Sampaio et al. 2010; Ahumada et al. 2011; Magioli et al. 2015; Hegerl et al. 2017). Similarly, we predicted that the abundance of functional groups with large body sizes, specialized diets, and low population density would be lower at sites with persistent poaching or higher density of human settlements.

Materials and methods

Study area. This study was carried out in the Selva Lacandona Region, located in the state of Chiapas in Southern México. This is one of the largest tropical rainforest remnants in México and represents the area with the greatest biodiversity in the country (Medellín 1994). Originally, the region covered 1'800,000 ha of rainforest, but during the 1970s political colonization programs caused deforestation and landscape transformation from rainforest to croplands, cattle ranches, and, more recently, palm oil (*Elaeis guineensis*) plantations (Frías and de la Maza 2009; Izquierdo-Tort 2018). At present, only one-third of its original forest cover remains. Most of the preserved area is inside seven natural protected areas managed by the Federal Government through the National Commission of Natural Protected Areas (CONANP; Frias and de la Maza 2009). Our study area included different areas of the Selva Lacandona Region, each with different surface, management, and threats (Figure 1; Table 1):

Montes Azules Biosphere Reserve (hereafter Montes Azules), which was decreed in 1978 and spans over 331,200 hectares, most of them covered with tropical rainforest. A management plan for Montes Azules was implemented in 2000, which received recognition from the federal government, non-governmental organizations, and local communities to collaborate for the protection and management of the reserve (INE 2000). However, this NPA is currently facing a critical challenge due to deforestation caused by sociopolitical issues, and the situation is particularly severe in the northern and western parts of the reserve due to the population increase and the establishment of irregular settlements (INE 2000; Fernández-Montes de Oca et al. 2015). Our study specifically focused in Montes Azules on the Lacantún River basin near the Chajúl Biological Station, which is one of the most well-preserved and well-studied areas of the NPA, with extensive stretches of continuous vegetation in good state of conservation. However, in this area occur occasional poaching and wildlife trafficking (INE 2000).

Yaxchilán Natural Monument (hereafter Yaxchilán), which was decreed in 1992, is situated on the border with

Guatemala in the Usumacinta River covering an area of 2,621 ha of tropical rainforest. This NPA is part of the Lacandon Community, specifically of the Frontera Corozal Sub-Community section (Tejeda-Cruz 2009). The land ownership is communal, and the area is jointly administered by the National Commission of Protected Areas (hereafter CONANP) and the National Institute of Anthropology and History. This NAP has not witnessed any activities related to land use change, such as livestock and agriculture, but such activities occur in its periphery. The tropical rainforest of Yaxchilán is contiguous with other forest-covered areas like the La Cojolita Communal Reserve, the Sierra El Tornillo, and the Sierra Lacandón National Park on the other side of the Usumacinta River, in Guatemala (CONANP 2010). A management program for this NPA was established in 2010, and a surveillance camp operated during the study. Threats for this NAP included poaching and illegal wildlife extraction.

Chan-Kin Flora and Fauna Protection Area (hereafter Chan-Kin) was decreed in 1992 and is located near the border with Guatemala on the banks of the Usumacinta River, with an extension of 12,184 ha most of them covered with tropical rainforest. Chan-Kin is also part of the communal lands of the Frontera Corozal sub-community (Tejeda-Cruz 2009), and the settlements closest to this area are Frontera



Figure 1. a) Location of Selva Lacandona region in Mesoamerica; b) The four study areas in the Selva Lacandona region and the location of camera-trap stations (grey areas indicate other Natural Protected Areas nor included in the study).

Table 1. Description of the four areas sampled with camera-traps in Southern Mexico for one year (2015-2016).

Sampling site	Type of Protection	Description	Surface	Number of camera-trap stations used	Mean distance between camera- trap stations (km)	Camera- trap days	Information of illegal human activities in the study area	
Montes Azules	Protected area	In its southern section, the Lacantun river basin has plains and floodplains with an altitude	331,200 ha protected; ~	16	1.16	3.841	0 direct encounters with hunters	
		of about 200 m.a.s.l. (SEMARNAP 2000)	tropical rainforest			·	people with shotguns and/or dogs	
Chan-Kin	Protected area	Rolling hills and floodplains with an altitudinal range between	12,184 ha protected; ~ 11,884 ha with tropical rainforest	12,184 ha protected; ~ 11,884	14	1 27	3 3 1 8	4 direct encounters with hunters and Illegal loggers
Charl-Kin	The contract of the contract o	120 to 260 m.a.s.l. (CONANP 2018)				5,510	9 records in the camera-traps of people with shotguns and/or dogs	
		Karstic landscape with rugged	2,621 ha protected				0 direct encounters with hunters	
Yaxchilán	Protected area	altitude of 320 m.a.s.l. (Meave <i>et al.</i> 2008, CONANP 2010)	and with tropical rainforest.	and with tropical 12 rainforest.	0.89	3,973	7 records in the camera-traps of people with shotguns and/or dogs	
Marqués de Comillas	Payment for ecosystem	Altitudinal range between	~ 45 000 ba with				0 direct encounters	
	services	and plains, and without rocky outcrop (Carabias <i>et al.</i> 2013)	forest cover	14	0.92	2,824	6 records in the camera-traps of people with shotguns and/or dogs	

Corozal town and Benemérito de las Américas in México and several small localities in the other side of the border in Guatemala. This area is surrounded by pastures for livestock and agriculture, and apparently, no longer maintains structural connectivity anymore with other forested areas in both México and Guatemala. A management plan for this NAP was published by CONANP two years after we implemented the study in the area (CONANP 2018), although a surveillance camp operated during the implementation of the study. Among the threats identified within this NAP are land use change for livestock pastures and crops, poaching, and the illegal extraction of precious woods and other wildlife.

Marqués de Comillas region lies south of Montes Azules, with the Lacantún and Salinas rivers and the Guatemalan border acting as its boundaries. This area has undergone constant land use changes due to colonization in the mid-1970s by migrants from other regions of Chiapas and other states of México who established ejidos. Despite extensive deforestation, the region still preserves nearly 44 % of the municipality covered by tropical rainforests surrounded by a heterogeneous matrix of human settlements, cattle pastures, and crops of corn, beans, and oil palm. This study focused on four ejidos - Boca de Chajul, Playón de la Gloria, Flor del Margues, and Galacia - where tropical rainforest remnants are protected under PES. The PES is a conservation scheme used by the Mexican Government that provides economic compensation to local communities through the National Forestry Commission (CONAFOR) for conserving their land with natural forest. Since the implementation of the PES program in the region in 2007, land enrolled in the program has reduced its deforestation rates to less than 0.6 % (Izquierdo-Tort 2018). The program also has helped to regulate poaching, logging, and fishing in the ejidos enrolled in the program through internal agreements. Poaching and illegal wildlife extractions from forest remnants pose a significant threat to the biodiversity of this area.

Data collection. The study was focused on documenting the richness and diversity of medium and large terrestrial mammal species in the four sampled sites. The sampling design was developed for this study and has not been used before for any published study. The sampling design was such that the sampling effort was similar between the four sites. We used a total of 56 camera-traps (Cuddeback BlackFlash E3 and Long Range E2) to collect data from August 2015 to August 2016. Each of the four study sites was surveyed using 12-16 camera-trap stations that were all deployed in the forest using a grid of 1 km². Most camera-trap stations remained active during the year of sampling. However, some of the stations were active for less time due to camera-traps malfunctioning, humidity, theft, or removal due to flooding to prevent equipment damage. The camera-trap stations were placed at an average distance of 1.06 km from each other (Table 1, Figure 1), but some stations were at a shorter distance from each other due to poor accessibility to certain sites (0.8-2 km distance). This approach ensured that larger species with larger home ranges could be sampled with sufficient detection probability without over-spacing that may result in missing species with smaller home ranges (Rovero and Spilate 2016). Camera-traps were set 40 cm above the ground at places that showed potential mammal activity, such as feeding sites, natural or human trails, or water bodies such as ponds and small streams to maximize mammal captures. Camera-traps were placed at an angle where the detection of most species could be maximized, and the detection was verified with the mode camera test. Camera-traps were programmed with the fastest possible trigger speed, with a wide sensor sensitivity area, so that they would take a photo and a video with a duration of 20 seconds and operate 24 hours a day, with no delay between each detection. We checked the camera-traps every two months to change the batteries and recompile the information from the memory cards.

Data analysis. We carefully inspected all the videos obtained by the camera-traps and we identified the species recorded. For data analysis, we only used the camera-trap records of medium (weight > 1kg to < 10 kg) and large mammal species (weight > 10 kg; Robinson and Red-ford 1986; Medellín 1994). Data were filtered to exclude repeated records of the same species at the same camera-

trap station within a period of 24 h; for the analysis, we used only one record during this period as an independent event (Rovero and Spilate 2016). Group species were considered as only one record without considering the number of individuals recorded by the camera-traps. We estimated species richness using the non-parametric Chao2 estimator and used species accumulation curves with 1,000 random permutations, based on the number of species recorded in each study site and the number of camera-trap days, to compare species richness among the four study sites (Colwell and Coddington 1994; Tobler *et al.* 2008).

We calculated Shannon's diversity index for each camera-trap station to quantify community heterogeneity and used the Kruskal-Wallis test and a posthoc Dunn test with Bonferroni correction to evaluate differences among the study sites. To calculate Shannon's diversity index, we used the Relative Abundance Index (RAI), which was estimated as the number of independent events per 1,000 cameratrap days, as a proxy descriptor of the community composition (Rovero and Spilate 2016).

To determine functional diversity and abundance of functional groups, we selected animal functional traits, which were defined as measurable attributes of organisms that influenced their interactions with the ecosystem and their capacity to find and obtain resources (Flynn *et al.* 2009). We used four functional traits, which included body mass, trophic guild, sociability, and population density, to determine functional diversity and to determine functional groups. For each species, we reviewed published literature to obtain their functional traits (Table 2).

We calculated functional diversity using the functional dispersion index (*Fdis*), which measured the redundancy or complementarity of the functional traits within the community (Laliberté and Legendre 2010). *Fdis* was calculated for each camera-trap station using the package "FD" in R (Laliberté *et al.* 2015; R Core Team 2016). To determine differences in functional diversity among the four study sites we used the Kruskal-Wallis test and a post-hoc Dunn test with Bonferroni correction.

Functional groups were defined using hierarchical cluster analysis. We used the Gower dissimilarity distance matrix because functional traits were categorical and continuous variables (Pavoine *et al.* 2009), and the Ward clustering method to construct a functional dendrogram to differentiate branches of functional groups.

Then we estimated the abundance of functional groups using the <u>Royle and Nichols (2003)</u> model (RN-model). This model incorporates heterogeneity in detection probability at the site level to model abundance using a Poisson distribution (<u>Royle and Nichols 2003</u>; <u>Dénes et al. 2015</u>). We constructed a matrix with the detection history for each functional group with vectors 1s (detection) and 0s (nondetection) using their capture data, and we collapsed the sampling periods to 1-mo sampling occasions to increase the detection probability of the functional groups and to increase the independence of camera-trap records (Foster and Harmsen 2012). Malfunctions and theft of cameras between checks were treated as missing data (NAs) in the detection history matrixes. To model abundance we included covariates that might influence the detection probability (r), such as whether the camera was set on a trail or not (Trail), the presence of people on the camera-trap records (People), and the number of days that each cameratrap station was active during the 1-mo sampling occasion (Activity). We used the study sites (Montes Azules, Chan-Kin, Yaxchilán, and Marqués de Comillas) (Site) as covariates that might influence the local mean abundance (λ) to compare functional group abundance among study sites. We made covariate combination models and used the Akaike Information Criteria (AICc) to select the best candidate models. and then we calculated model-average coefficients of the models with $\triangle AICc < 2$ (Burnham and Anderson 2002). The predicted estimate parameter λ was used as relative abundance adjusted to detectability (Thorn et al. 2011). Analyses were conducted in "unmarked" and "MuMIn" packages in R (R Core Team 2016; Fiske and Chandler 2017; Bartoń 2019).

We used landscape covariates related to human activities (human pressure proxies) to evaluate if human activities affect the diversity and functional diversity at the landscape scale in our study area (Table 3). For this, we estimated for each camera-trap station the values of each landscape covariable evaluated. We represented all these explanatory landscape covariates as raster layers of 30 m resolution. We used as landscape covariates the Euclidean distance to rivers (Rivers), to paved or dirt roads (Roads), and to forest edges (Edge; see details in Table 3). Distance to rivers can be used as an indicator of human pressure, as navigable rivers provide poachers with access to certain areas. Several studies have shown that sites close to rivers tend to have a lower abundance of certain species, as these areas experience higher poaching pressure compared to conserved sites that are situated farther away from rivers (de la Torre 2009; Espinosa and Branch 2014). To determine the density of human settlements, we used a buffer of 15 km (Towns; circular moving windows with radii of 15 km; see details in Table 3). This distance range was chosen as most poaching activities occur within 10 to 20 km of human settlements (Koerner et al. 20167; Wright 2003). Additionally, we used a distance of 1 km to determine the percentage of forest cover (Forest; Table 3). This distance was chosen as it provides a good proxy to determine whether a habitat is continuous or not at the landscape scale (Defries et al. 2010; Sampaio et al. 2010). All these procedures were implemented using the geoprocessing tools in the software ArcGIS 10.2 software (ESRI 2013). We used the Pearson coefficient to evaluate correlation among the landscape-scale covariates (r< 0.7), and we did not include correlated covariates in the same model to avoid multicollinearity.

We performed Generalized Linear Models (GLM) to evaluate the effects of the landscape-level covariates at the community level. To perform the models, we used the species richness, diversity, and functional diversity values obtained for each camera trap station as response variables and the landscape covariates values obtained for each camera trap station as explanatory variables. For species richness, we used a GLM model with a Poisson error and log link function; for diversity and functional diversity, we used models with a normal distribution and an identity link function. We tested all combinations of model covariates and used the AIC to select the best candidate model (<u>Burnham</u> and Anderson 2002).

For each functional group, we employed the RN-model using the same covariates for *r* mentioned above and the landscape-level covariates for λ . All possible model combinations were tested, and we used AlC for model comparison. We used the model average with Δ AlC< 2 and calculated the relative importance of model covariates based on the sum of AlC weights. In this case, the model-averaged λ coefficient was used as a metric for landscape use by functional groups and, therefore, for the effects of human pressure (Brodie *et al.* 2015).

Results

We obtained 2,567 independent detections of medium and large mammals with a total sampling effort of 13,956 trap days including the four sites studied. The two species of opossums *Didelphis virginana* and *D. marsupialis* were classified as *Didelphis sp.* because it was not reliable to distinguish them with accuracy from the camera-trap records, and both species occurred in the study area (Medellín 1994). We recorded 20 medium and large mammal species in our four studied sites (Supplementary material Table S1). Central American agouti (Dasyprocta punctata), spotted paca (Cuniculus paca), and red brocket deer (Mazama temama) were the most recorded species, and anteater (Tamandua mexicana), striped hog-nosed skunk (Conepatus semistriatus), raccoon (Procyon lotor), margay (Leopardus wiedii), and jaguarundí (Puma yagouaroundi) were the least detected (Supplementary material Table S1).

Montes Azules and Marqués de Comillas were the sites with the highest species richness with 18 species (18 ± 0.48 and 18 ± 0.72, respectively), we recorded 17 species (17.5 ± 1.32) in Yaxchilán, and only 14 species (14 ± 0.48) in Chan-Kin according to the non-parametric Chao2 estimator (Figure 2). Chan-Kin was the site with the lowest diversity (*Shannon's diversity*) and functional diversity (*Fdis*). We found significant differences in Shannon's diversity (p <0.001, Kruskall-Wallis) between Chan-Kin and Yaxchilán (p <0.001, Dunn test). Further, there were significant differences (p < 0.05, Kruskall-Wallis) in functional diversity (*Fdis*) between Marques de Comillas and Chan-Kin (p < 0.05, Dunn test; Figure 3).

Based on the functional trait dendrogram, species were clustered mainly by trophic guild, body mass, and population density (Figure 4). The dendrogram was differentiated by five clusters demarcated by the trophic guild and nine subgroups: 1) Medium-size Frugivore-omnivores with



Figure 2. Species accumulation curves in the four study sites for the community of medium- and large-sized mammals detected by camera-traps.

Table 2. Functional traits of mammals present in the Selva Lacandona Region used for the analysis of functional diversity and the functional group cluster.

	_	Body				
Species	Common name	Massª	Trophic guild [®]	Population density ^c	Sociability	References
Tapirella bairdii	Baird's tapir	5.47	FH	Low	Solitary	1, 2, 3*
Odocoileus virginianus	White-tailed deer	4.6	HB	Low	Solitary	1, 2, 3*
Mazama temama	Red-brocket deer	4.41	FH	Low	Solitary	1, 2, 3*
Tayassu pecari	White-lipped peccary	4.45	FH	Low	Social	1, 2, 3*
Dicotyles tajacu	Collared peccary	4.24	FH	Medium	Social	1, 2, 4
Panthera onca	Jaguar	4.83	CA	Low	Solitary	1, 2, 5*
Puma concolor	Puma	4.56	CA	Low	Solitary	1, 2, 6
Leopardus pardalis	Ocelot	4.01	CA	Low	Solitary	1, 2, 7*
Puma yagouaroundi	Jaguarundí	3.69	CA	Low	Solitary	1, 2, 8
Leopardus wiedii	Margay	3.56	CA	Low	Solitary	1, 2, 6
Nasua narica	Coati	3.95	FO	High	Social	1, 2, 9
Procyon lotor	Racoon	3.94	FO	Medium	Solitary	1, 2, 10
Eira barbara	Tayra	3.59	FO	Medium	Solitary	1, 2, 11
Conepatus semistriatus	Striped hog-nosed	3.23	FO	High	Solitary	1, 2, 11
Dasypus novemcinctus	Nine-banded armadillo	3.54	Ю	Medium	Solitary	1, 2, 12
Tamandua mexicana	Anteater	3.62	MY	Medium	Solitary	1, 2
Didelphis spp	Opossum	3.01	FO	High	Solitary	1, 2, 13
Cuniculus paca	Spotted paca	3.91	FG	High	Solitary	1, 2, 14
Dasyprocta punctata	Central American Agouti	3.55	FG	High	Solitary	1, 2

^a Body mass (g) log-transformed

^b Carnivore (CA); Frugivore-Herbivore (FH); Frugivore-Granivore (FG); Frugivore-Omnivore (FO); Herbivore-Browser (HB); Insectivore-Omnivore (IO); Myrmecophage (MY).

^c Low population density (<2 individuals per km²); Medium population density (2<10 individuals per km²); High population density (>10 individuals per km²).

1. Jones et al. (2009). 2. Robinson and Redford (1986). 3. Naranjo and Bodmer (2007). 4. González-Marín et al. (2008). 5. de la Torre and Medellín (2011). 6. Ávila-Nájera et al. (2015). 7. de la Torre et al. (2016). 8. Kasper et al. (2016). 9. Gompper (1997). 10. Fritzell (1978). 11. Eisenberg et al. (1979). 12. Ferreguetti et al. (2016). 13. Kelly and Caro (2003). 14. Santos-Moreno and Pérez-Irineo (2013).

*Population density studies in the study area.

high population density (M-FO-High). 2) Medium size Frugivore-omnivores with medium population density (M-FO-Medium). 3) Frugivore-granivores with high population density (M-FG-High). 4) Medium-size insectivore species with medium population density (M-IN-Regular). 5) Large frugivore-herbivores with low population density (L-HF-Low). 6) Large frugivore-herbivores with medium population density (L-HF-Regular). 7) Large herbivore-browsers with low population density (L-HB-Low). 8) Medium size carnivores with low population density (M-CA-Low). 9) Large size carnivores with low population density (L-CA-Low; Figure 4).

Abundances of the functional groups varied among the sampling sites in six of the nine functional groups (Supplementary material Table S2). Montes Azules showed higher abundances of large frugivores-herbivores, large herbivores-browsers, and medium and large carnivores (Figure 5). On the other hand, we observed the opposite trend in Chan-Kin where there were very low abundances of all the functional groups that included large herbivores and carnivores, and a high abundance of medium frugivore-granivores (*i. e.,* rodents). In Yaxchilán we observed a higher abundance of medium insectivores. Marques de Comillas showed low abundances of all the functional groups (Figure 5).

There was no correlation between the landscape-level covariates (r > 0.7), therefore, all of them were included in the models evaluated. The relationship of richness, diversity, and functional diversity with the landscape-level covariates has different patterns. We observed a negative effect of the density of human settlements on species richness (p < 0.05; Figure 6a). For diversity, we observed a negative effect with the distance to roads (p < 0.01) and a positive effect with the distance to the forest edge (p < 0.01). On the other hand, we observed a negative effect of forest cover (p < 0.05) and distance to the forest edge (p < 0.01) on functional diversity (Table 4; Figure 6).

Response of functional group abundance to landscapelevel covariates also showed a different pattern. The abundance of medium frugivore-granivores (*i. e.*, rodents) was positively associated with the density of human settlements. Otherwise, abundances of large frugivore-herbivores (L-HF-Regular), large herbivore-browsers (L-HB-Low), and medium carnivores (M-CA-Low) were negatively associated with the density of human settlements (Table 5; Figure 7).

The abundance of medium insectivorous (M-IN-Medium) showed a negative response to the distance to rivers, but the abundance of large frugivore-herbivores (L-FH-Low)

Table 3. Landscape-level covariates used as human pressure predictors for the RN models and GLM analysis.

Covariate	Units	Description	Justification	Layer data-source
River	Km	Euclidean distance to the nearest navigable river	Rivers are access sources for hunters (Espinosa and Branch 2014)	(Tapia and Nunez 2008)
Forest	%	Percentage of forest cover estimated using a circular moving window with radii of 1 km.	Forest cover is a proxy measure of continuous habitat (Defries <i>et al.</i> 2010, Sampaio <i>et al.</i> 2010), and landscape configuration (Garmendia <i>et al.</i> 2013)	(Hansen <i>et al.</i> 2013)
Edge	Km	Euclidean distance to the nearest deforested patch $\leq 1 \text{ km}^2$	Forest edges are a proxy of disturbance since there are ecotones with different abiotic conditions (Asquith and Mejía-Chang 2005, Brodie <i>et al.</i> 2015, Garmendia <i>et al.</i> 2013, Sampaio <i>et al.</i> 2010)	(Hansen <i>et al.</i> 2013)
Towns	%	Density of human settlements estimated using a circular moving window with radii of 15 km.	Most hunting occurs near human settlements in a distance interval of 10-20 km (Koerner <i>et al.</i> 2016, Wright 2003)	INEGI (México) IDE-SEGEPLAN (Guatemala)
Roads	Km	Euclidean distance to the nearest paved or dirt road	Roads facilitate access for hunters (Cuarón 2000, Laurance <i>et al.</i> 2006, Vanthomme <i>et al.</i> 2013).	INEGI

showed a positive response to this landscape covariate. The abundance of large herbivore-browsers (L-HB-Low) was negatively affected by forest cover, and the abundance of medium-size frugivore-granivores (M-FG-High) was positively related to the distance to the forest edge (Table 5). Detection probability varied among functional groups. For large carnivores (L-CA-Low) and large herbivore-browsers (L-HB-Low), there was a positive relationship between the detection probability and the presence of trails, but for large frugivore-herbivores (L-FH-Low) and medium insectivores (M-IN-Medium), this relationship was negative (Table 5).



Figure 3. Differences between a) diversity (Shannon diversity Index), and b) functional diversity (Functional dispersion index) among the study sites.

Discussion

Forested areas with high human pressure showed low diversity and functional diversity of medium and large mammals and showed a low abundance of large mammals with specialized diets. The density of human settlements was the main driver of the low species richness and low abundance of several functional groups such as large frugivoreherbivores (L-HF-Medium), large herbivore-browsers (L-HB-Low), and medium carnivores (M-CA-Low). Our results also showed that in the forested areas with a high density of human settlements in their periphery, the abundance of large herbivore and specialized carnivore populations decreases, and populations of medium-sized rodent species increase. These results suggest that disturbance caused by human activities not only had an impact on diversity level but also would have an impact on ecosystem functions and may have important consequences for the resilience of tropical rainforests. Our results also showed that functional diversity is a valuable metric that can provide additional information about the status of mammal communities in tropical forests which can be used to improve conservation and management actions (Laméris et al. 2020).

Our prediction that the diversity and functional diversity of medium and large mammals decreased at sites with higher human pressure was supported by our results. Large mammals with specialized diets and low population densities were the most affected by human pressure. Other studies have shown the negative effect of human activity on frugivore and carnivore species because both groups were directly dependent on resource availability (Ahumada et al. 2011; Hegerl et al. 2017). Also, mammals with low population density and large body size have been reported to be severely affected by human activities because they are more susceptible to local extirpation and habitat destruc-

Table 4. Untransformed beta coefficients (B) of the response of richness, diversity, and functional diversity to the landscape variables.

	Landscape covariate	ß (SE)	t	p-value
Richness	Pop (-)	-54.62 (24.50)	-2.23	0.03
	Roads (-)	-0.02 (0.01)	-4.26	p < 0.01
Diversity	Edge (+)	0.06 (0.01)	4.4	p < 0.01
E an attace d	Forest (-)	-2.21x10 ⁻⁴ (9.02x10 ⁻⁵)	-2.45	0.02
diversity	Edge (-)	-3.22x10 ⁻³ (8.38x10 ⁻⁴)	-3.84	p < 0.01



Figure 4. Functional groups obtained from the cluster analysis. a) Medium-sized Frugivore-Omnivores with high population density (M-FO-High); b) Medium-size Frugivore-Omnivores with medium population density (M-FO-Medium); c) Medium-size Frugivore-Granivores with high population density (M-FG-High) d) Medium-size.

tion (<u>Chiarello 1999</u>; <u>Dirzo et al. 2014</u>; <u>Brodie et al. 2015</u>; <u>Hegerl et al. 2017</u>).

Comparison among study sites showed important differences in the composition of mammalian communities, and Chan-Kin was the site that showed the lowest species richness, diversity, and functional diversity. This might be due to the proximity of this protected area to several towns in its periphery, the rampant poaching and illegal logging, and the deficient protection and law enforcement carried on this protected area (obs pers.). In fact, during our fieldwork, we had several direct encounters with illegal loggers and poachers, and we recorded through camera-trap surveys several events of people with weapons or hunting dogs. Although events of this kind occur in other areas such as Marques de Comillas and Yaxchilán, they were more frequent in Chan-Kin than in any other site evaluated (Table 1). Other studies have documented that poaching is an important driver of species richness and diversity in mammal communities (Carrillo et al. 2000; Brodie et al. 2014; Hegerl et al. 2017). Further, in Chan-Kin we recorded low abundances of large mammals such as carnivores, which depend on prey availability (Karanth et al. 2004; Karanth and Sunguist 1995), and herbivores with low population densities, which are the main target of poachers (Dirzo et al. 2014; Ripple et al. 2015). Chan-Kin also showed an overabundance of medium frugivore-granivores (i.e., rodents),

which suggests a trophic release favored by the decrease of their natural predators and competitors (Galetti *et al.* 2015; Ripple *et al.* 2015). Both species of rodents evaluated in this study (*Cuniculus paca* and *Dasyprocta puntacta*) show high reproductive rates, and for then they would be more resistant to poaching pressure compared with large herbivores (Bodmer *et al.* 1997).

On the other hand, Montes Azules, Marqués de Comillas, and Yaxchilán exhibited similar species richness, diversity, and functional diversity. Continuous areas with forests usually sustain a high diversity and abundance of large mammals (Ahumada et al. 2011; Bruhl et al. 2003; Sampaio et al. 2010). This was the case of Montes Azules which maintains large extensions of primary forest with low human pressure, and Yaxchilán which maintains structural connectivity with other forested areas. In contrast, Marqués de Comillas, which is a fragmented landscape where the forest is mainly protected by the PES program, also exhibited high values of richness, diversity, and functional diversity probably due to a source-sink dynamic for its proximity to Montes Azules (the source), which has suitable habitat and stable mammal populations from where some individuals migrate to Marqués de Comillas (the sink; Naranjo and Bodmer 2007). Furthermore, the presence of heterogeneous landscapes, such as Marqués de Comillas, can foster interactions between forest-dependent species and more generalist ones, potentially explaining the greater diversity observed in this area (Medellín and Redford 1992). Further, rainforest remnants surveyed in Margues de Comillas were < 1,000 ha and maintain structural connectivity among them (Ahumada et al. 2011; Magioli et al. 2015), and previous studies have



Figure 5. Predicted abundances of functional groups in the study sites obtained with the RN occupancy models, with 95% confidence intervals. a) Medium-size Frugivore-Granivores with high population density (M-FG-High); b) Medium-size Insectivores with medium population density (M-IN-Medium); c) Large-size Frugivore-Herbivores with low population density (L-FH-Low); d) Large-size Herivores-Browsers with low population density (L-HB-Low); e) Medium-size Carnivores with low population density (M-CA-Low); f) Large-size Carnivores with low population density (L-CA-Low).

Table 5. Model averaged coefficients of RN models between human pressure predictor and detection covariates with values relative importance < 0.6.

Functional group	λ(Towns)	λ(Edge)	λ(River)	r(Effort)	r(Trail)
M-FG-High	10.29(10.15)	0.17(0.19)	·	0.06(0.01)	
M-IN-Medium			0.52(0.27)	0.04(0.03)	-0.53(0.55)
L-FH-Low	-16.96(15.25)		-0.56(0.29)		-0.79(0.47)
L-HB-Low	-52.42(34.29)				1.09(0.94)
M-CA-Low	-11.01(10.86)			0.05(0.01)	

shown that these forest remnants maintain high mammal diversity and are important for maintaining landscape connectivity among protected areas (Garmendia *et al.* 2013; de la Torre *et al.* 2017). This suggests that PES is an effective conservation tool for maintaining mammal diversity and the ecological functions of these species if forest patches protected by this conservation scheme are large enough and maintain structural connectivity with other large tracts of forest.

Our data suggests that some species would be favored by the habitat heterogeneity created in nearby sites of paved roads since we found a positive effect of roads on mammalian diversity in our study area. Other studies have shown that mammalian response to predictors of human pressure was highly variable; some species seem to be affected negatively, some seem to be favored by the habitat heterogeneity, and others had a neutral response (Goosem 2001; Fahrig and Rytwinski 2009). Further, some species



Figure 6. Predicted a) Richness, b) Diversity, and c) Functional diversity in response to human pressure predictors.

cross the roads indiscriminately, therefore main paved and dirt roads might not be a barrier for all mammal species in the region (McGregor *et al.* 2007; Fahrig and Rytwinski 2009). Conversely, we found that diversity increases in the forested areas located far away from the forest edge, but functional diversity increases in sites near the forest edge and in areas with a low percentage of forest cover. This pattern may be due to an edge effect, given that some species were more matrix-tolerant and were dominant closer to the forest edge, which resulted in the redundancy of functional traits (Brodie *et al.* 2015; Zimbres *et al.* 2017).

Response of functional groups to forest cover was diverse, for instance, large herbivore-browsers (L-HB-Low; *i. e., Odocoileus virginianus*) were more abundant at sites with low percentage forest cover in a surrounding area of 1 km; these landscape characteristics likely facilitated their food availability because this group fed on secondary vegetation that was more common in open spaces (Robinson and Redford 1986; Bodmer and Ward 2006; Corlett and Primack 2011). Both species of medium granivores (*C. paca* and *D. punctacta*; M-FG-High) were dependent on forests and were less abundant near forest edges (Medellín and Redford 1994; Zimbres *et al.* 2017; Boron *et al.* 2019). Distance to rivers covariate was found to have a significant effect on the abundance of certain functional groups.

Contrary to our expectations, however, the effect of distance from navigable rivers on functional groups varied, indicating that such distance is not always a reliable proxy of human disturbance. Large herbivores were more abundant near rivers, although medium insectivores exhibited the opposite trend. Near rivers, there are flooded plains rich in nutrients for palms and trees that provide fruits for several species like tapirs (*Tapirella bairdii*) and white-lipped peccaries (*Tayassu pecari*) with habitat requirements that include abundant food resources and permanent water bodies (Haugaasen and Peres 2005; Tejeda-Cruz *et al.* 2009). We suggest that medium insectivores (M-IN-Medium) may have been affected by seasonal flooding to their burrows and lower availability of food in the leaf litter (<u>Rueda-Delgado *et al.* 2006; Arteaga and Venticinque 2008).</u>

Implications for conservation and management. Protected areas play a vital role in the long-term survival of rainforest mammals and in maintaining ecosystem dynamics. However, to ensure protected areas and the biodiversity that they shelter in long-term, they should include effective management actions such as patrolling to reduce wildlife extraction and implementation of economic activities compatible with conservation in the local communities that inhabit their surrounding areas (Datta et al. 2008; Defries et al. 2010; Hegerl et al. 2017). This is crucial in protected areas



Figure 7. Predicted abundance response of functional groups to the density of human settlements.

with a high density of human settlements in its periphery, such as Chan-Kin, because human activities such as poaching or illegal logging would cause not only the reduction of the mammals' populations but also affect their ecological functions and this would lead to negative consequences for tropical rainforest functions.

Long-term conservation of large protected areas, such as Montes Azules, is also crucial to safeguard large mammal populations and their ecological functions because they preserve large extensions of suitable habitats with low human pressure. In the case of small protected areas, such as Yaxchilán, it is vital to ensure their connectivity with other large blocks of forest to avoid their isolation and maintain the diversity and functional diversity of mammal species that inhabit these areas. We urge the National Commission of Protected Areas (CONANP) to improve the patrolling strategies to detect illicit activities, especially in protected areas with a high density of human settlements in its periphery. We also urge the Federal Attorney for Environmental Protection (PROFEPA) to apply law enforcement due to the high incidence of poaching, illegal logging, and wildlife trade encouraged by the low presence of environmental activities and by the deficient law enforcement within protected areas with a high density of human settlements in its periphery.

PES conservation tool can be effective not only to reduce deforestation rates (Costedoat et al. 2015; Izquierdo-Tort et al. 2019), but also to maintain mammal diversity and functional diversity in areas where this conservation scheme is being implemented. Therefore, the Mexican Federal Government should assign a permanent and growing budget through the National Commission of Forestry (CONAFOR) to ensure the operations of this program in the long term. Through this economic incentive, local communities are encouraged to protect the forests within their land and to implement productive activities compatible with conservation and surveillance activities to prevent the illegal extraction of wildlife and poaching. Since poaching is probably the main threat for several species in the region, one action to preserve mammals and their ecological roles is to promote agreements with local communities to auto-regulate or ban poaching within and outside protected areas, especially for those species with large sizes and low population density such as white-lipped peccaries and Baird's tapirs.

Acknowledgments

We appreciate the financial support of the Commission of Natural Protected Areas (CONANP) and the WWF- Fundación Carlos Slim Alliance. We thank all the community monitors from Frontera Corozal, Boca de Chajúl, Flor del Marqués, Playón de la Gloria, Galacia, and the staff of the Chajúl Station for their hard work and assistance during the fieldwork. Also, we thank Marina Rivero, Kevin Lopez, Sebastian Arriaga, and Rodrigo León for their help with the fieldwork. We thank Julia Carabias, Francisco Botello, Santiago Izquierdo, and Horacio Bárcenas for their suggestions in the development of this study. We thank Laura Navarro and Mari Teniente from Bioconciencia A.C. and the staff of Natura y Ecosistemas Mexicanos A.C. for their technical support. We would like to thank Thomas A. Gavin, Professor Emeritus, Cornell University, for help with editing the English in this paper. In memory of Javier de la Maza, whose passion for Mexican biodiversity conservation continues to inspire us. His legacy lies in his lasting impact on the conservation of the Selva Lacandona Region.

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Supplementary material https://mastozoologiamexicana.com/therya/index.php/THERYA/article/view/5247/1415

MEDIUM AND LARGE-SIZED MAMMALS' FUNCTIONAL DIVERSITY IN SOUTHERN MEXICO

Arroyo-Gerala et al.

Non-volant mammals in the central Andes Yungas: the Pampa Hermosa National Sanctuary

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The Pampa Hermosa National Sanctuary (SNPH) is a Natural Protected Area (11,543 ha) that encompasses a large extension of central Yungas; however, despite its high priority status for the conservation of biodiversity, the non-volant mammals have been poorly documented. This study reports the non-volant mammals from the Yungas of the SNPH and its buffer zone, for the first time. We sampled four localities: Podocarpus (1,900 m), Los Cedros (1,600 m), Santa Isabel (1,450 m), and Nueva Italia (1,370 m), which encompass very humid premontane and montane forests. We used capture methods for small mammals; and direct and indirect records for medium and large-sized mammals. The capture effort was 6,860 traps-night (Sherman and Victor traps) and 490 buckets-night (pitfall traps). We recorded 31 native species: six marsupials, one cingulate, two primates, 18 rodents, two carnivores, and two artiodactyls, including the threatened species Lagothrix lagotricha tschudii and two endemic rodents. The most diverse orders were rodents and marsupials (77%). The analysis of true diversity (9D) showed that the montane forests of SNPH (1,600 to 1,900 m) had the highest values: Los Cedros ($^{\circ}D = 18.80$) when order q = 0; and Podocarpus ($^{1}D = 6.34$; $^{2}D = 4.99$) when order q = 1, 2. The rank-abundance curves indicated the same taxa group (*Hylaeamys yunganus, Akodon cf. A. aerosus*, and species of Neacomys) as the most abundant at all localities. The species Marmosa (Micoureus) constantiae, Hylaeamys yunganus, Rhipidomys gardneri, Euryoryzomys nitidus, Oligoryzomys microtis, and Neacomys macedoruizi are the first records for the region of Junín. One species of Neacomys and another of Oecomys might be new species to science. A beta diversity analysis showed that the Yungas of SNPH are more similar to the Manu National Park (21 %) and the Tingo María National Park (20 %); highlighting that dissimilarity is high even between relatively close communities. Our study indicates that the SNPH allow connectivity with other nearby Yungas, especially for medium and large-sized mammals that need pristine and preserved forests. Nevertheless, the real diversity of the region is still far from being completely inventoried; additional evaluations with a diverse array of methods are needed toward that goal. In addition, the local and regional governments should get involved in conserving these fragile forests where the changes in land use may affect many key and endemic species.

El Santuario Nacional Pampa Hermosa (SNPH) es un área natural protegida (11,543 ha) que abarca una gran extensión de yungas centrales; sin embargo, a pesar de su estatus de alta prioridad para la conservación de la biodiversidad, los mamíferos no voladores han sido poco documentados. Este trabajo reporta por primera vez a los mamíferos no voladores de las Yungas del SNPH y su zona de amortiguamiento. Muestreamos cuatro localidades: Podocarpus (1,900 m), Los Cedros (1,600 m), Santa Isabel (1,450 m) y Nueva Italia (1,370 m), que abarcan bosques muy húmedos premontanos y montanos. Utilizamos métodos de captura para mamíferos pequeños; y registros directos e indirectos para mamíferos medianos y grandes. El esfuerzo de captura fue de 6.860 trampas-noche (trampas Sherman y Victor) y 490 baldes-noche (trampas pitfall). Registramos 31 especies nativas: seis marsupiales, un cingulado, dos primates, 18 roedores, dos carnívoros y dos artiodáctilos, incluyendo a la especie amenazada Lagothrix lagotricha tschudii y dos roedores endémicos. Los órdenes más diversos fueron los roedores y marsupiales (77 %). El análisis de diversidad verdadera (ªD) mostró que los bosques montanos del SNPH (1,600 a 1,900 m) presentaron los valores más altos: Los Cedros (^oD = 18.80) cuando el orden q = 0; y Podocarpus (¹D = 6.34; ²D = 4.99) cuando el orden q = 1, 2. Las curvas de rango-abundancia indicaron el mismo grupo de taxones (Hylaeamys yunganus, Akodon cf. A. aerosus y especies de Neacomys) como los más abundantes para todas las localidades. Las especies Marmosa (Micoureus) constantiae, Hylaeamys yunganus, Rhipidomys gardneri, Euryoryzomys nitidus, Olygoryzomys microtis y Neacomys macedoruizi son los primeros registros para la región de Junín. Una especie de Neacomys y otra de Oecomys serían nuevas para la ciencia. Un análisis de diversidad beta mostró que las Yungas del SNPH son más similares con el Parque Nacional del Manu (21 %) y el Parque Nacional Tingo María (20%), destacando que la disimilitud es alta incluso entre comunidades cercanas. Nuestro estudio indica que el SNPH permite la conectividad con otras Yungas cercanas, especialmente para los mamíferos medianos y grandes los cuales requieren bosques prístinos y conservados. Sin embargo, la diversidad real de la región aún está lejos de ser completamente inventariada, por lo que se necesitan evaluaciones adicionales con diversos métodos para lograr ese objetivo. Además, los gobiernos locales y regionales deben involucrarse en conservar estos bosques frágiles donde los cambios en el uso del suelo pueden afectar a muchas especies claves y endémicas.

Keywords: Conservation status; diversity; endemic species; new records; non-volant mammals; central Yungas.

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Introduction

The Peruvian Yungas is one of the most diverse ecosystems where a great number of endemism occur (Young and León 1999; Myers et al. 2000; Pacheco 2002; Antón and Reynel 2004; Young 2007; Pacheco et al. 2009; Tovar et al. 2010). These ecosystems occupy 14 of the 24 political regions of Perú along the eastern and western slopes of the Andean Cordillera from Amazonas to Puno. They cover a total area of 12'765'993 ha, which represents 9.9 % of the total area of Perú (MINAM 2009). Moreover, there are three natural terrestrial ecoregions within the Yungas: Very Humid Pre-Montane Forests, Very Humid Montane Forests, and Montane Rain Forests (Britto 2017). In addition, the Yungas present a complex and steep orography with a great variety of altitudinal gradients and climates where high humidity conditions prevail, which favor a high diversity of flora and fauna. However, there are few studies documenting the diversity and endemism of various taxa throughout the Yungas, especially the non-volant mammals.

Protected Natural Areas (ANPs) have been recognized as places of protection of biological and genetic diversity (Joppa and Pfaff 2009), and almost a third of the total ANPs in Perú is located in the Yungas ecoregions (CDC-UNALM and TNC 2006). Peruvian laws have determined the scientific and cultural importance of ANPs, where their knowledge contributes to the country's sustainable development (Law No. 26834, DS 038-2001-AG, 2001). In the central Yungas, there are at least 11 ANPs with premontane and montane forest areas located in the regions Huánuco, Junín, Pasco, and Ucayali: The National Parks (NP) Otishi, Tingo María, Yanachaga-Chemillen, and Cordillera Azul; the Communal Reserves (CR) Ashaninka, Yanesha, "El Sira", and Amarakaeri; the Protection Forests Pui Pui and San Matias-San Carlos; and the Pampa Hermosa National Sanctuary (Tovar et al. 2010; Tovar 2021).

The Yungas of Ucayali, Huánuco, Junín, and Pasco are the regions with more than 70,000 hectares of deforested rainforest. Although the ANPs are places with intangibility categories, the diversity of these areas and their surroundings are strongly threatened by the change in land use for agricultural activities, mainly (SERFOR 2015; MINAM 2016). In this context, knowledge of diversity is highly urgent for the future conservation of species. Vivar (2006) evaluated the small volant and non-volant mammals' diversity in the central Yungas ANPs at the Yanachaga Chemillen NP; whereas Arias et al. (2016) evaluated the volant mammals' diversity at the Pampa Hermosa National Sanctuary. Likewise, Cossios and Ricra-Zevallos (2019), and Pillco Huarcaya et al. (2020) addressed the medium and large-sized mammals' diversity at "El Sira" CR and Tingo María NP. On the other hand, outside of ANPs, there are studies on small, medium, and large-sized mammals in various central regions (Pacheco et al. 2007; Medina et al. 2012; Pacheco and Noblecilla 2019; Cossios and Ricra-Zevallos 2019; Pillco Huarcaya et al. 2020; Aquino et al. 2022). Although Junín has ten ANPs, this region is one of the least studied (MINAM 2016).

The Pampa Hermosa National Sanctuary (SNPH) is a Natural Protected Area located precisely in the Central Yungas in the Region of Junín. To date, only one study assessed the diversity of non-volant mammals from SNPH but focused exclusively on medium and large species in the high-Andean regions (3,200 to 3,700 m; Melo-Dias et al. 2022). These authors recorded 11 species through camera traps, searching mammal tracks (e. g., feces, footprints, food consumed, marks on trees), and direct visualizations. Despite the importance of the Yungas of SNPH, which shelters and protects relict cedar forests Cedrela spp. in the central jungle of Perú, studies on the diversity of all groups of mammals are scarce. Considering that the central Yungas are priority conservation areas (Tovar et al. 2010) and that they function as an essential biological corridor between high Andean and Amazon forests (SERNANP 2012; Arias et al. 2016), the diversity of non-volant mammals is still poorly known.

For all the reasons mentioned above, this study aims: 1) Describe the alpha diversity and the composition of nonvolant mammals in the Yungas of SNPH between 1,370 and 1,900 m. 2) Evaluate the beta diversity of mammals in SNPH with other montane Peruvian forests in elevation ranges between 1,000 and 2,000 m. 3) Assess the conservation status and endemicity of the reported mammals.

Material and methods

Study area. We conducted the study in the Pampa Hermosa National Sanctuary (SNPH) and its buffer zone both near the Ulcumayo river basin, province of Chanchamayo, region of Junín, Perú (Figure 1). The area with a total extension of 11,543 ha is within the Yungas ecoregion (Brack-Egg 1986), also known as Montane Forests on the eastern slope of the Andes (Tovar et al. 2010), or as Very Humid Premontane and Montane Forests (Britto 2017). Four localities were evaluated: Podocarpus (1,900 m), Los Cedros (1,600 m), Santa Isabel (1,450 m), and Nueva Italia (1,370 m; Figure 1). Podocarpus and Los Cedros are located within the Pampa Hermosa National Sanctuary in the ecoregion named as "Very Humid Low Montane Tropical Forest" (Britto 2017). A primary forest with trees between 15 to 20 m high is present in both localities. The families Podocarpaceae and Clusiaceae are common in Podocarpus, whereas Meliaceae (mainly Cedrela spp.), Moraceae, Lauraceae, and arboreal ferns are commonly seen in Los Cedros. The localities Nueva Italia and Santa Isabel are in the buffer zone less than 1 km away from the SNPH and separated by Ulcumayo River in the ecoregion named as "Very Humid Premontane Forest" (Britto 2017). A dense understory with secondary forests is present in these localities, with trees and shrubs of the families Piperaceae, Solanaceae, and Cecropiaceae (Table 1). A detailed habitat description of all localities is found in Arias et al. (2016).

Mammals sampling. The localities of Podocarpus, Los Cedros, and Nueva Italia were evaluated during May and October 2011, whereas Santa Isabel was evaluated in May and June 2012. The sampling was carried out in two seasons, established by the presence of precipitation: the first in



Figure 1. Sampling localities in the Pampa Hermosa National Sanctuary (SNPH) and its Buffer Zone: 1) Podocarpus, 2) Los Cedros, 3) Nueva Italia, 4) Santa Isabel. See Table 1 for complete locality information.

May-June (wet season) and the second in October (dry season). The methodology consisted of using live (Sherman), snap (Victor), and pitfall traps to capture small mammals and records of direct and indirect evidence for medium and large mammals. All trapping methods were used following some modifications from the methods detailed by Jones *et al.* (1996), Voss and Emmons (1996), Woodman *et al.* (1996), and Sikes *et al.* (2011).

In each locality, two transects of 35 trapping stations separated by 10 m were set. Each trapping station consisted of one Sherman and one Victor trap; then, 140 traps were active each night. The bait for all traps was made of oatmeal, peanut butter, raisins, honey, vanilla essence, and unviable canary seeds. All traps were baited and activated during the morning and reviewed and re-baited the following day. In addition, a 90 m pitfall trap was installed in each locality (Voss and Emmons 1996), where 10 buckets of eight liters of capacity were buried and separated by about 10 m. The total trapping effort was calculated by multiplying the number of traps or buckets for the nights evaluated (Table 1).

The captured specimens were measured (total, tail, ear, and hind foot length), weighted, sexed (including reproductive condition), aged, and preliminary taxonomically identified in the field. All specimens were collected, except for some lactating or pregnant females that were released (making sure we already had a voucher for the species). The captured specimens were preserved as skins, skulls, and carcasses or complete bodies, fixed in 10 % formaldehyde for 10 days, and afterward preserved in 70 % alcohol. All specimens were identified, cataloged, and deposited in the scientific collection of the Mammalogy Department in the Museum of Natural History of the Universidad Nacional Mayor de San Marcos (MUSM).

The presence of medium and large-sized mammals was recorded through occasional records of direct and indirect traces. A direct record consisted of the observation of an individual (or a skull); meanwhile, indirect ones were feces, footprints, smell traces, or diggings. Furthermore, we surveyed the local people living near the sampling localities. In the interviews, people were presented with photos or illustrations of potential species and asked to confirm if they were familiar with some. Species, local names, and dates of observation were recorded. Because data of medium and large-sized mammals was qualitative, it was only used to update the list of species richness and beta diversity analyses. Analysis of data. Alpha diversity analyses were assessed only with quantitative data from small mammals and localities with dry and wet season sampling. Santa Isabel was sampled only once (in the wet season) and its results added to the overall richness. In addition, a rank abundance curve was constructed to measure the community structure of small mammals, calculating the base logarithm 10 of the relative abundance of species in each locality. This method lets us know the species richness and dominance or rareness in each sampled site or habitat (Feinsinger 2001).

The diversity of localities was estimated using the true diversity (^qD) proposed by Jost (2006). This measure is defined as the effective number of species (^qsp₋), which is a virtual species that is equally abundant as the species recorded on average (Tuomisto 2013). Hence, ^qD has properties intuitively of the diversity concept as the duplication property (Jost 2006). The superscript q is the order of the true diversity and indicates its sensitivity to rare and common species when q is less or greater than unity, respectively. To estimate ^qD, the order value "q" and the diversity estimators are considered (Moreno et al. 2011). The values of order "q" used in this study were: the diversity of order zero (°D) that is equivalent to species richness; the diversity of order one (1D), equal to the exponential of Shannon's entropy index; and the diversity of order two (²D), the inverse of Simpson's index (Tuomisto 2013). The richness estimators used consider the sample coverage as a measure of the completeness of the inventories, which represents the fraction of the total abundance of the community represented in the sample (Chao and Shen 2003). Thus, Moreno et al. (2011) recommend the following diversity estimators according to the value of "q": the non-parametric estimator ACE (Abundance-based coverage estimator) for ^oD; Bias-corrected Shannon diversity estimator for ¹D; and the MVUE estimator (Minimum variance unbiased estimator) for ²D. These analyses and their 95 % confidence intervals (CI) were calculated with the online program SpadeR (Chao et al. 2015).

Beta diversity was analyzed to assess the species' turnover of SNPH with other central and southern Peruvian Yungas in the elevation range of 1,000 to 2,000 m using qualitative data of the richness of small, medium, and largesized mammals based on presence-absence (Wilson and Shmida 1984; Magurran 1988; Villareal et al. 2006; Magurran 2011). This species' turnover was performed through a cluster analysis using Jaccard's similitude coefficient (I; Magurran 1988, 2004; Baev and Penev 1995; Samo et al. 2008). Data was compiled from similar forests such as the Manu National Park (Solari et al. 2006; Medina et al. 2012), the Yanachaga-Chemillén National Park (Vivar 2006), the Apurímac River Basin (Pacheco et al. 2007), Pozuzo Forests (Mena and Medellín 2010), the Middle Basin of the River Tambopata (Pacheco et al. 2011), Carpish Forests (Pacheco and Noblecilla 2019), the Yungas of Huánuco (Aquino et al. 2022), the Sira Communal Reserve (Pillco Huarcaya et al. 2020), the Tingo María National Park (Cossios and Ricra-Zevallos 2019), and Fundo La Genova in Chanchamayo (Guevara-Torres et al. 2021). This analysis was executed using PAST program 3.0.

Finally, the conservation status and endemism of all species recorded in all localities were reviewed following <u>IUCN</u> (2023), <u>DS 004-2014-MINAGRI (2014</u>), and <u>CITES (2023)</u>. In addition, including all species data from previous studies and the current from SNPH, their conservation status, and endemism, a list of potential mammal species in the central and southern Peruvian Yungas between 1,000-2,000 m was elaborated (see Supplementary material 1).

Results

Species composition. A total of 31 species of small, medium, and large-sized mammals were recorded in the four sampled localities in the SNPH and its buffer zone: six opossums (order Didelphimorphia), one armadillo (order Cingulata), two monkeys (order Primates), 18 rodents (order Rodentia), two carnivores (order Carnivora), and two even-toed ungulates (order Artiodactyla). According to size, 17 species were classified as small mammals: five opossums and 12 rodents. The remaining 14 species belong to the group of medium and large-sized mammals: the Andean White-eared Opossum, Didelphis pernigra; the Nine-banded Armadillo, Dasypus novemcinctus; the Tschudi's Woolly Monkey, Lagothrix lagothricha tschudii and the Black-headed Night Monkey, Aotus nigriceps; the Grey-headed Tayra, Eira barbara and the South American Coati, Nasua nasua; the Collared Peccary, Dycotiles tajacu and the Red Brocket, Mazama americana; and six large rodents (Table 2). An introduced small rodent,

 Table 1.
 Sampling localities in the Pampa Hermosa National Sanctuary and its Buffer Zone.
 VHMF: Very Humid Montane Forest, VHPF: Very Humid Premontane Forest, TN (trapsnight), BN (buckets-night).

Sampling	Ecoregion	Elevation	Geographical	coordinates	Live and Snap Tra	ps (Sherman/Victor)	Pitfa	ll traps
Locality		(11)	South	West	Number of nights	Capture effort (TN)	Number of nights	Capture effort (BN)
Podocarpus	VHMF	1,900	10° 59' 49.2"	75° 25' 57.1"	14	1,960	14	140
Los Cedros	VHMF	1,600	10° 59' 43.1"	75° 25' 37.2"	14	1,960	14	140
Nueva Italia	VHPF	1,370	10° 59' 18.6"	75° 25' 17.3"	14 1,960		14	140
Santa Isabel	VHPF	1,450	10° 58' 54.1"	75° 25' 53.3"	7	980	7	70
Total Effort Capture					6,860		490	

the Black Rat *Rattus rattus* (Muridae), was also recorded. For the nomenclature of the species, we follow the latest updated list of mammals from Perú (<u>Pacheco *et al.* 2021</u>).

The order Rodentia (18 species) and Didelphimorphia (six species) were the most diverse, reaching 77 % of the total richness. Within these taxonomic orders, the families Didelphidae and Cricetidae were the most representative, with six and 12 species, respectively. Other recorded orders accumulated seven species composing 23 % of the total richness, being Cingulata with a single species the least diverse. The records of these orders were mainly observations (individuals or skulls), vocalizations, feces, and footprints. In addition, the interviews conducted with park rangers were also considered reliable indirect records (see Table 2).

Species richness was different across localities and elevations. Nueva Italia (1,370 m) accumulated the highest richness with 17 species, followed by Podocarpus (1,900 m), Los Cedros (1,600 m), and Santa Isabel (1,370) each with 14 species. Analyzing by size, the richness of small mammals was very alike at all elevations. Medium and large-sized species were more frequently recorded at lower elevations mainly in Nueva Italia (Table 2).

Small mammals. We recorded 248 individuals from two orders, three families, 11 genera, and 18 species in the four sampled localities. Rodentia was the order with the greatest number of species (72.2 %) and number of records (87.1 %). Cricetidae, with 12 species and 215 individuals (86.7 %), was the most diverse family. In addition, six species were recorded with one single individual.

Noteworthy records. Several records are worth mentioning as it is the first report on the non-volant small mammals from the Yungas of the SNPH. Among our findings, new regional records and new species are revealed. The new records for the region of Junín are the following: the marsupial *Marmosa* (*Micoureus*) constantiae and the rodents *Hylaeamys yunganus*, *Rhipidomys gardneri*, *Euryoryzomys nitidus*, *Oligoryzomys microtis*, and *Neacomys macedoruizi* (Figure 5). Furthermore, *O. microtis* is captured for the first time in the habitats of Yungas. Previous studies only reported it in low forests (<u>Patton et al. 2000; Pacheco et</u> <u>al. 2007; Patton et al. 2015; Medina et al. 2016</u>).

The spiny rodent *Neacomys macedoruizi* highlights since it was described as a new species endemic to the region of Huánuco (<u>Sánchez-Vendizú *et al.* 2018</u>). Our record in Junín is represented by a single adult male (voucher number MUSM 45889) captured in the locality of Santa Isabel. Diagnostic characters observed on the specimen, that agree with the description of the species made by <u>Sánchez-Vendizú *et al.* (2018)</u> include a bicolored ventral fur with a gray basal area and the skull with: the anterior region of the nasal protruding sharply from the maxilla; the supraorbital region markedly divergent with slightly protruding ridges; a conspicuous and large subsquamosal fenestra; the petrosal bones (close to the auditory bulla) less exposed; the incisive foramina subrectangular and almost reaching the first upper molar; and the anterocone divided (Figure 2).

A probable new species of *Neacomys* captured in the four localities was also recorded in this study. After a thorough morphological examination of all specimens, a series of characters suggests that this might represent a new species, referred here to as *Neacomys* sp. "Junín". A relevant diagnostic character is the white ventral fur, not bicolored. A dorsal view of the skull shows a short rostrum; nasal with a width interorbital region, and the dorsal border of the foramen magnum usually rounded. Ventrally, the incisive foramina extend quite far from the plane of the first upper molar; the anterocone in the first upper molar is conspicu-



Figure 2. Dorsal, ventral, and lateral views of cranium and mandible of *Neacomys macedoruizi* MUSM 45889 (left), *Neacomys* sp. "Junín" MUSM 41292 (middle), and *Oecomys* sp. "Junín" MUSM 41305 (right). Bar = 10 mm.

MAMMALS IN THE CENTRAL ANDES YUNGAS

 Table 2.
 List of non-volant mammal species recorded by locality in the Pampa Hermosa National Sanctuary and its Buffer Zone. The number of captured individuals of small mammals and the type of record for medium and large-sized mammals is presented (O: observation, F: footprint, Fe: feces, and I: interviews with local people). ^a small, ^b medium, ^c large

			Locality	/	
Species	Common name	Podocarpus	Los Cedros	Nueva Italia	Santa Isabel
		(1,900 m)	(1,600 m)	(1,370 m)	(1,450 m)
ORDER DIDELPHIMORPHIA					
Family Didelphidae					
Didelphis pernigra ^b	Andean White-eared Opossum	-	-	-	0
Marmosa macrotarsus ª	Macrotarsus' Mouse Opossum	-	-	1	1
Marmosa constantiae ^a	Woolly Mouse Opossum	5	-	4	5
Marmosops bishopi ^a	Bishop's Slender Opossum	3	-	-	-
Marmosops caucae ^a	Cauca's Slender Opossum	4	1	-	-
Marmosops noctivagus ^a	White-bellied Slender Mouse Opossum	1	4	1	2
ORDER CINGULATA					
Family Dasypodidae					
Dasypus novemcinctus ^c	Nine-banded Armadillo	I	I	-	-
ORDER PRIMATES					
Family Cebidae					
Aotus nigriceps ^c	Black-headed Night Monkey	I	O, I	-	-
Family Atelidae					
Lagothrix lagothricha tschudii ^c	Tschudi's Woolly Monkey	0	0	-	-
ORDER RODENTIA					
Family Sciuridae					
Hadrosciurus pyrrhinus ^b	Junín Red Squirrel	-	0	-	-
Family Cricetidae ^a	·				
Akodon cf. A. aerosus	Grass Mouse	6	5	26	38
Euryoryzomys nitidus	Elegant Rice Rat	-	-	-	1
Hvlaeamvs vunaanus	Yungas Rice Rat	17	21	22	9
Neacomvs amoenus	"Northern" Ameno's Spiny Mouse	-	4	12	-
Neacomvs macedoruizi	Macedo Ruiz' Spiny Mouse	-	-	-	1
Neacomys sp. "Junín"	Spiny Mouse	10	3	6	12
Nephelomys keavsi	Keavs's Nephelomys	2	1	4	-
Oecomvs sp. "Junín"	White-bellied Oecomvs	- 1	1	1	1
Oligoryzomys destructor	Destructor Colilargo	-	-	-	8
Oligoryzomys microtis	Smell-eared Colilargo	-	-	1	-
Rhipidomys aardneri	Gardner's Rhipidomys	-	1	-	-
Rhipidomys leucodactylus	Great Rhipidomys	-	1	-	-
Family Erethizontidae					
Coendou bicolor ^c	Bicolor-spined Porcupine	-	-	-	0
Family Dinomyidae					0
Dinomys branickii °	Pacarana	-	-	-	0
Family Caviidae					-
Hvdrochoerus hvdrochaeris ^c	Capybara	-	-	0	-
Family Dasyproctidae				-	
Dasvprocta cf. D. kalinowskii b	Kalinowski Agouti	0	-	0	-
Family Cuniculidae	j				
Cuniculus paca ^c	Agouti, Spotted Paca	-	-	0	-
ORDER CARNIVORA	- goal, spolles , ala			0	
Family Mustelidae					
Fira barbara ^c	Tayra, Greybeaded Tayra	_	-	1	1
Family Procyonidae	,,,				
Nasua nasua ^c	South American Coati	-	-	0	-
ORDER ARTIODACTYLA				0	
Family Tayassuidae					
Dicotyles taiacu °	Collared Pecari	_	-	0	-
Family Cervidae				0	
Mazama americana c	Red Brocket	i i	-	F Fe	_
Total: 31 species		14	14	17	14
iotan Di species		17	17	17	17

We follow the latest updated list of mammals from Perú for the species names (Pacheco et al. 2021). Some species are shown in figures 2, 5, and 6.

ously divided, and the third upper molar has a distinct and conspicuous hypoflexus (Figure 2). A complete taxonomic review will be developed in a forthcoming manuscript.

Oecomys sp. "Junín" is probably another new species revealed in this study and recorded in the four localities. Our specimens were compared with all the species of the genus, and show many notable differences. *Oecomys* sp. "Junín" shows a small size and protruding hairs at the tip of the tail. In a dorsal view of the skull, the interorbital region is narrow and widely divergent, not beaded. Laterally, the postglenoid foramen is quite open; and the subsquamosal fenestra is greatly reduced. Ventrally, the incisive foramina are long and wide, reaching the anterior plane of the first upper molar or slightly posterior to it (Figure 2). Like the previous species reported as new, *Oecomys* sp. "Junín" will also be formally described in a forthcoming manuscript.

The grass mouse *Akodon* cf. *A. aerosus* was identified to this level due to some differences found when comparing it with the species *aerosus*. Our specimens differ from *aerosus* by exhibiting a narrower interorbital region; narrower incisive foramina that extend to the protocone of the first upper molar (versus extending to the anterocone); anterior edges of zygomatic-plates concave (versus straight); thicker hamular process; and upper incisors orthodont (versus slightly opisthodont). The anterior edges of zygomatic plates are concave (versus straight to slightly concave in *aerosus*, according to the description of <u>Pardiñas *et al.* 2015</u>). The complex taxonomy of *Akodon aerosus* was mentioned by Jiménez and Pacheco (2016), recommending more studies in the Peruvian Yungas, given that it could encompass more than one species.

Alpha diversity. For this analysis, the three localities sampled twice recorded 170 individuals and 14 species. Species richness (s) and number of individuals (n) were distributed as follows: Podocarpus (s = 9, n = 49), Los Cedros (s = 11, n = 43), and Nueva Italia (s = 10, n = 78).

The three localities had the same set of most abundant species according to the range-abundance curves: *Hylaea*-

mys yunganus, Akodon cf. *A. aerosus,* and *Neacomys* spp. Likewise, the curves of Los Cedros and Nueva Italia were similar, with few abundant species and many less abundant species. Furthermore, both localities showed at least one slope fall, meaning greater inequality between species. In contrast, Podocarpus showed a gradual decay indicating a greater diversity than the previous localities. On the other hand, species with one single individual (singletons) were placed in the base of curves, highlighting Los Cedros with the highest value (six singletons). *Oecomys* sp. "Junín" was the only singleton species recorded in each locality (Figure 3).

Results of true diversity analysis showed a decreasing pattern according to the increase in the number of order (^qD); however, the magnitude of that decrease was variable in the three sampled localities. Comparing the values of ^oD that indicate the estimated species richness, Los Cedros showed the highest value. Since ²D focuses on abundance, a more considerable difference between ²D and ⁰D means that locality is influenced by the dominance of one or more species. Thus, this difference was greater in Los Cedros, followed by Nueva Italia and Podocarpus with values of 15, 11, and 5 effective species (^qsp_e), respectively. On the other hand, considering the values of ¹D (which includes common, dominant, and even rare species) and ²D (focused mainly on dominant species), Podocarpus was ranked as the most diverse locality. Nevertheless, for each ¹D and ²D, the three localities differ by less than 1.5 ^qsp_r, in which we can assert these localities are relatively equally diverse. In addition, the completeness of each sampled locality was measured through the divide between the richness (S) and ^oD, ranking the localities in descending order as follows: Podocarpus (87 %), Nueva Italia (67 %) and Los Cedros (59 %) (Table 3).

Beta diversity. Using the Jaccard similarity index (I_j) to compare Yungas communities between 1,000 to 2,000 m, a low similarity was observed showing the highest values when comparing SNPH with the Manu National Park "PNM" $(I_j = 21 \%)$ and the Tingo María National Park "PNTM" $(I_j = 21 \%)$



Figure 3. Rank abundance curves for small mammals from localities sampled during two seasons in the Pampa Hermosa National Sanctuary and its Buffer Zone.

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Table 3. True diversity analysis performed in localities sampled during two seasons in the Pampa Hermosa National Sanctuary and its Buffer Zone. N: number of individuals, S: species richness, ^qD: true diversity of order q = 0, 1, 2.

Locality	Locality		6			True diver	sity (estimated)		
	N	3		٥D		٦ ^י		²D	
Podocarpus	49	9	10.30	(9.20-19.60)	6.34	(4.79-7.91)	4.99	(3.47-6.51)	
Los Cedros	43	11	18.80	(12.50-50.70)	5.77	(4.63-6.91)	3.60	(2.56-4.65)	
Nueva Italia	78	10	15.00	(10.80-42.10)	5.68	(4.91-6.44)	4.42	(3.66-5.18)	

20 %) sharing 16 and eight species, respectively. With the Yungas of Huánuco "YuHUA" and Sira Communal Reserve "SCR" the similarity values were the same ($l_j = 18$ %) sharing 10 species, while with the other communities, the values were less than 14 %. On the other hand, the Yanachaga-Chemillén National Park, and the forests of Carpish and Pozuzo formed a separate group (Figure 4). Species shared between SNPH, PNM, and PNTM included five orders, whilst between SNPH, YuHUA, and SCR shared six orders. The PNM is one of the communities with the most complete

and detailed studies on the richness of mammals (Solari et al. 2006; Medina et al. 2012). For this reason, its complementarity with SNPH is one of the lowest among all the communities analyzed. In addition, the greatest similarity between these two communities occurs mainly between the species of rodents and marsupials sharing 11 species (Table 4, Figure 4).

It is important to mention that in PNYCh (<u>Vivar 2006</u>) and in Pozuzo (<u>Mena and Medellín 2010</u>) only small mammals were evaluated. In contrast, in the Yungas of Huánuco



cc = 0.8699

Figure 4. Cluster analysis using the Jaccard similarity coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) of eleven communities with 'Yungas' habitats in the elevation rank of 1,000 to 2,000 m in the central and south of Perú. Localities that share more similarities with SNPH are in bold. cc=cophenetic correlation. The acronyms are detailed in Table 4.

Table 4. Jaccard similarity index (*I*) and complementarity values (*C*) of 11 study areas with Yungas in the elevation rank of 1,000-2,000 m in the central and south of Perú: SNPH (the Pampa Hermosa National Sanctuary), PNM (the Manu National Park), PNTM (the Tingo María National Park), SCR (the Sira Communal Reserve), YuHUA (the Yungas of Huánuco), MBRT (Middle Basin of the River Tambopata), ARB (the Apurímac River Basin), PNYCh (the Yanachaga-Chemillén National Park), CARP (Carpish Forests), POZ (Pozuzo Forests), and FLG (Fundo La Genova). Values in bold represent *I*, (decimals) and *C* (integers in %) between the SNPH and the other study areas.

Study Area	SNPH	PNM	PNTM	SCR	YuHUA	MBRT	ARB	PNYCh	CARP	POZ	FLG
SNPH	1	79	80	82	82	86	87	87	88	89	92
PNM	0.21	1	84	74	80	83	93	87	96	90	91
PNTM	0.20	0.16	1	66	70	95	93	97	100	100	81
SCR	0.18	0.26	0.34	1	70	85	90	96	100	100	83
YuHUA	0.18	0.20	0.30	0.30	1	89	91	96	98	100	90
MBRT	0.14	0.17	0.05	0.15	0.11	1	94	85	90	91	90
ARB	0.13	0.07	0.07	0.10	0.09	0.06	1	100	100	90	95
PNYCh	0.13	0.13	0.03	0.04	0.04	0.15	0.00	1	75	86	95
CARP	0.12	0.04	0.00	0.00	0.02	0.10	0.00	0.25	1	87	100
POZ	0.11	0.10	0.00	0.00	0.00	0.09	0.10	0.14	0.13	1	100
FLG	0.08	0.09	0.19	0.17	0.10	0.10	0.05	0.05	0.00	0.00	1

(Aquino et al. 2022), Sira Communal Reserve (Pillco Huarcaya et al. 2020), and The Tingo María National Park (Cossios and Ricra-Zevallos 2019), only medium and large-sized mammals were considered. However, all these studies were considered to assess the similarity between communities and elaborate a list of potential mammals in Yungas' habitats in central and southern Perú between 1,000 and 2,000 m (see Supplementary material 1).

Medium and large-sized mammals. We recorded 14 medium and large mammal species in all sampling localities (Table 2). Order Rodentia was the most diverse group, with six taxonomic families and six species. Orders Primates, Carnivora, and Artiodactyla each presented two families and species. Didelphimorphia and Cingulata orders each had only one species (Table 2).

One of the most noteworthy records was the sightings of the Tschudi's Woolly Monkey, *Lagothrix lagothricha tschudii*. Their troops were observed in the first hours of the morning, almost during all trapping sampling for small mammals into the SNPH, in both Los Cedros y Podocarpus (Figure 6). Furthermore, the Black-headed Night Monkey, *Aotus nigriceps* was listened to, observed, and confirmed by local people in interviews.

Among the large rodents, we highlight the agouti Dasyprocta cf. D. kalinowskii. Our records included the sighting of an individual and a skull that was thoroughly reviewed to be identified at this taxonomic level (Figure 6). After analyzing specimens from Junín which included *D. variegata* reviewed by Teta and Reyes-Amaya (2021) and one specimen of D. kalinowskii (MUSM 38533), a series of characters suggests that our record might represent D. kalinowskii. Our specimen is close to *D. kalinowskii* and differs from *D*. variegata (characters in parenthesis) by exhibiting in a dorsal view of the skull, a short and thin sagittal crest (versus elongated and thickened). Laterally, the maxillary foramen is rounded rectangular (versus oval and shorter). Ventrally, the septum in the maxilla is thick and the mesopterygoid fossa has a spine (versus thin septum and mesopterygoid fossa without a spine). According to these characters and

added to the fact that it was only contrasted with a single specimen of *D. kalinowskii* from the MUSM, we decided to identify our specimen as *Dasyprocta* cf. *D. kalinowskii*.

Other groups also recorded by sightings were, the Andean White-eared Opossum *Didelphis pernigra*, the South American Coati *Nasua nasua*, and the Collared Peccary *Dicotyles tajacu*. Footprints and feces of the Red Brocket *Mazama americana* were recorded. The Nine-banded Armadillo *Dasypus variegatus*, and the Grey-headed Tayra *Eira barbara* were the only records by interviews with local people.

Endemic species. Two rodent species were recorded as endemic to Perú: The Macedo 'Ruiz' Spiny Mouse *Neacomys macedoruizi* and the Kalinowski Agouti *Dasyprocta* cf. *D. kalinowskii.* One single specimen of *N. macedoruizi* was captured in the premontane forests of Santa Isabel and is a range extension of 185 km south from the previous report from Huánuco (Sanchez-Vendizú *et al.* 2018). On the other hand, *Dasyprocta* cf. *D. kalinowskii* was sighted in the montane forests of Los Cedros and in the premontane forests of Nueva Italia. Records of this large rodent are significant, because if future studies confirm that it is indeed *D. kalinowskii*, new records and information about this poorlyknown species will be added.

Conservation status. Seven species recorded in this study are included in some conservation category according to Peruvian (DS 004-2014-MINAGRI 2014) or international legislation (IUCN 2023, CITES 2023).

The Tschudi's Woolly Monkey Lagothrix lagothricha tschudii is the most threatened species, listed as Endangered (EN) by Peruvian legislation (DS 004-2014-MINAGRI 2014), Vulnerable (VU) by IUCN (2023), and included together with the Black-headed Night Monkey Aotus nigriceps into the Appendix II of CITES (2023). Three rodents are also species with some threat status: the Pacarana Dinomys branickii, listed as VU by Peruvian legislation; the Kalinowskii Agouti Dasyprocta cf. D. kalinowskii, and the Junín Red Squirrel Hadrosciurus pyrrhinus, both listed as Data Deficient (DD) by IUCN and Peruvian legislation. Both IUCN and Peruvian legislation also list the Red Brocket Mazama


Figure 5. Small mammals in the Yungas of Pampa Hermosa National Sanctuary, Junín-Perú. (A) Akodon cf. A. aerosus; (B) Hylaeamys yunganus; (C) Neacomys amoenus "Northern"; (D) Marmosa (Micoureus) constantiae; (E) Marmosops (Marmosops) noctivagus; and (F) Marmosops (Sciophanes) bishopi. Photos by V. Pacheco.

americana as DD, and Appendix II of CITES includes the Collared Peccary *Dicotyles tajacu*. Appendix II lists species that are not necessarily currently threatened with extinction, but that may become so unless trade is closely controlled (<u>CITES 2023</u>). The remaining medium and large-sized mammals, and all small-sized mammals are not included in some threat status. However, the population trend of almost all medium and large mammals except the Collared Peccary is decreasing or unknown, and more studies are needed to know their actual conservation status of threat (<u>IUCN 2023</u>).

In addition, we surveyed the local people and asked them about the uses of many mammals that live there. Thus, the Spotted Paca *Cuniculus paca*, the Pacarana *Dinomys branickii*, the Kalinowskii Agouti *Dasyprocta* cf. *D. kalinowskii*, and the Red Brocket *Mazama americana* were reported as animals hunted for food. Some people stated that medium and large-sized mammals could affect their coffee crops. Since most of the records of these mammals were in the buffer zone of SNPH, it would be essential to inform local people about the conservation status and importance of mammals for the maintenance of the Yungas where they live.

Discussion

We document for the first time the non-volant small, medium, and large-sized mammals in the central Yungas of the Pampa Hermosa National Sanctuary (SNPH). This assessment reports a high diversity of species composed of six marsupials, one cingulate, two primates, 18 rodents, two carnivores, and two artiodactyls, which supports the recognition of the area as protected for the government (<u>MINAM 2009</u>) and highlights the biogeographic, ecological, systematic, and conservation value of the mammals of the central Yungas of Perú.

Our report provides several new records of the presence and range extension of rodents such as *Neacomys macedoruizi*, *Oligoryzomys microtis*, and *Euryoryzomys nitidus*, which were previously reported only in the lowland forests of the departments of Loreto and Pasco (Patton et *al.* 2000; Pacheco et al. 2009; Patton et al. 2015). The record of the marsupial *Marmosa* (*Micoureus*) constantiae, previously known for Perú as *M.* (*Micoureus*) demerarae (Silva et *al.* 2019), is also the first for the region of Junín (Pacheco et *al.* 2020). In addition, the species *Neacomys* sp. "Junín" and *Oecomys* sp. "Junín" are likely new species for science. These new records indicate that the diversity of the Yungas is still poorly known.

The SNPH and natural areas with habitats of Yungas have an ecological interest, because they promote faunal connectivity between remaining pristine forests, which are also influenced by changes in land use due to agricultural and livestock activities (SERFOR 2015; MINAM 2016). La Torre-Cuadros *et al.* (2007), in their study of tree flora in SNPH, mention that the preserved forests can function as

biological corridors due to the great diversity and tree density. Likewise, studies of tree flora in the Yanachaga Chemillén National Park "PNYCH" and further south in the Manu National Park "PMN" (MINAM 2012); Farfan-Rios et al. 2015), report a great diversity, biomass, and tree volume, being indicators of a good condition of the forests in Yungas. Considering our records in common with close forests such as Chanchamayo Forests, 12 km away from SNPH (Guevara-Torres et al. 2021), the PNYCh, 78 km away (Vivar 2006), and Yungas of Huánuco, 140 km away (Aquino et al. 2022), the species recorded in these Yungas require large extensions of forests both in upper strata (e. g., Lagothrix lagothricha tschudii, Aotus spp.) and in lower strata (i.e. Dasypus novemcinctus, Dinomys branickii, Cuniculus paca, Eira barbara, Nasua nasua, Dicotyles tajacu). These records support the statement of La Torre-Cuadros et al. (2007), that connectivity between forests promotes gene flow in mammalian populations. In addition, the SNPH, being a natural protected area, allows the maintenance of suitable ecosystems for various species of arboreal habitats. Hence, our study reveals how meaningful is the close relationship between mammal diversity and the good condition of the Yungas.

Besides the importance of connectivity between forests, it is crucial to know each species' ecological role in the ecosystem to propose feasible conservation plans. For example, the species of the genus Lagothrix have a frugivorous diet which makes them responsible for maintaining the balance and complexity of the tropical forest (Luna 2013; López 2020). Large rodents such as Dasyprocta and Cuniculus also show frugivorous diets mainly of arboreal species (Dubost and Henry 2006; McWilliams 2009; Martinez 2017). Small rodents of the genera Akodon and Oligoryzomys may also include some fruits and seeds in their diets, and together with species of the tribu Thomasomyini, they may disperse viable seeds that can potentially regenerate forests (Cervantes 2014; Sahley et al. 2015). Although this study does not include a diet analysis, it is likely that Akodon cf. A. aerosus, two species of Oligoryzomys, and two species of Rhipidomys (tribu Thomasomyini), reported here in SNPH, could also be fruit consumers and seed dispersers. Since a frugivorous diet appears to be prevalent, the 17 species of small mammals recorded in SNPH likely have different food habits, including fruits, to avoid competition (Cervantes 2014). For this reason, future studies on the diet



Figure 6. Medium and large-sized mammals recorded in the Yungas of Pampa Hermosa National Sanctuary. (A, B, C) Lagothrix lagothricha tschudii; (D) Coendu bicolor; (E) dorsal, ventral, and lateral views of skulls of Cuniculus paca; and (F) Dasyprocta cf. D. kalinowskii. Skulls belonging to the local people.

of these mammals are recommended to know the ecological roles they play and the functional group they take part (<u>Córdova-Tapia and Zambrano 2015</u>).

Abundance. The abundance range curve allowed us to differentiate the dominance or rarity of one single species or a group of species (Feinsinger 2001). Thus, a set of abundant species was observed, including Hylaeamys yunganus, Akodon cf. A. aerosus, and Neacomys spp. in an elevational range <1,370 to 1,900 m> in SNPH. This same species complex is also noticed in Yungas of Valley of Pozuzo (Pasco) at 1,200 m with high relative abundance (Mena and Medellín 2010). Other studies in Yungas show variable relative abundances of similar species, such as Hylaeamys perenensis, Akodon aerosus, and Neacomys spinosus in the Apurimac River basin at 805 m and in Cusco at 1,050 m (Pacheco et al. 2007). Likewise, in the middle basin of the Tambopata River, between 1,200 and 1,985 m in Puno, the set of species H. perenensis, Akodon baliolus, and Neacomys spinosus was also reported (Pacheco et al. 2011). All these results suggest a species pattern, including genera Hylaeamys, Akodon, and Neacomys in Yungas below 2,000 m. Among these three genera, Akodon had the highest frequency in most sampled localities in Yungas, mainly recorded at ground level (Pacheco et al. 2007; Mena and Medellín 2010; Pacheco et al. 2011; Pacheco and Noblecilla 2019). Furthermore, it should be noted that H. yunganus lives in sympatry with H. perenensis (Percequillo 2015), and both have been recorded at ground level (Voss et al. 2001).

Regarding the species with the lowest abundances, such as Marmosops (Marmosops) noctivagus, Nephelomys keaysi y Oecomys sp. "Junín", similar records were observed between 950 and 1,600 m in the middle basin of the Tambopata River in Puno (Pacheco et al. 2011) and at 1,200 m in the Pozuzo valley in Pasco (Mena and Medellín 2010). It is prevailing for M. (Marmosops) noctivagus to be recorded at ground level (Patton et al. 2000; Voss et al. 2001; Hice and Velazco 2012) and its habits are associated with primary, secondary, and disturbed forests (Tirira 2007). Similarly, Nephelomys keaysi exhibits these same habits and tolerates disturbed areas such as cattle pastures (Mena and Medellín 2010). In contrast, Oecomys is rarely found in disturbed areas (Hice and Velazco 2012) and is primarily recorded in canopy traps (Carleton and Musser 2015). Therefore, for greater representativeness in future research, it is recommended to equate the effort in installing traps at ground level and in specific heights in the trees or bushes.

Alpha diversity. Alpha diversity indicates a reduction in ^qD_a as "q" increases for each locality, revealing a high degree of dominance (Jost 2006). This ^qD_a reduction agrees with the range-abundance curves' results, especially for Los Cedros and Nueva Italia, which have steep slopes. Jost (2007) recommends using ¹D_a when abundances are somewhat relevant and in compliance with the fifth property of alpha diversity (D_a < D_y). It would be affirmed that Nueva Italia is the least diverse locality with 4.90 ¹sp_F compared to Podo-

carpus and Los Cedros with 6.34 ¹sp_e and 5.34 ¹sp_{e'} respectively. This difference may be because more than 80 % of the small mammal individuals in Nueva Italia belong to the three most abundant species (*Hylaeamys yunganus, Akodon* cf. *A. aerosus*, and *Neacomys* spp.). Another factor that affects diversity is the type of habitat and the geographical location within the SNPH. The montane forests of Los Cedros and Podocarpus located in higher elevations (1,600 to 1,900 m) and far from anthropic influence, are related to a greater species diversity. On the other hand, the proximity of human activities and the presence of local people in the lower areas of Nueva Italia (1,370 m) confirm that the first effects of tropical defaunation are the reduction of the richness and local diversity of wild vertebrate communities (Mendoza and Camargo-Sanabria 2019).

Beta diversity. The similitude method of Jaccard to compare diversity in Yungas between 1,000 to 2,000 m shows a low similarity between communities. Despite these low values, the cluster analysis indicates that the SNPH is more similar to three Natural Protected Areas. Regarding species richness, SNPH was slightly similar to the Manu National Park (PNM) sharing 16 species, contrasting with communities out of natural protected areas that form an aggrupation (see Figure 4). Since the National Parks and Sanctuaries are intangible protected areas with high diversity, the pairwise comparisons were closer between SNPH with PNM and PNTM. This moderate beta diversity has also been noted when contrasting other studies of nonvolant mammals in Yungas, but above 3,000 m (Pacheco et al. 2013). Although nearby communities were dissimilar in this research, the greatest similarity was observed when comparing it with the PNM (Pacheco et al. 2013). Moreover, in the study of the diversity of bats in the same SNPH, a high beta diversity is also observed (Arias et al. 2016). The great richness of species in these Yungas, many even endemic (Young and Valencia 1992; Pacheco 2002), only confirms these habitats' tremendous relevance in biodiversity conservation.

One of the most noticeable records was Tschudi's Woolly Monkey, Lagothrix lagothricha tschudii due to recent studies that differentiate its distribution from L. lagothricha cana (Ruiz-García et al. 2019). Fooden (1963), in the first and the most comprehensive taxonomic revision of the genus Lagothrix, recognized three subspecies of L. lagothricha, with only L. l. cana inhabiting in Perú. Grooves (2001) and Pacheco et al. (2009) considered L. cana a full species. Nonetheless, molecular studies suggest that L. lagothricha would be composed of five different subspecies, with L. lagothricha cana and L. lagothricha tschudii distributed in center and southern Perú respectively (Aquino et al. 2019; Ruiz-García et al. 2019). Recent studies have recorded L. l. tschudii to the north of SNPH, in the montane forests of Ucayali, Pasco, and Huánuco (Aquino et al. 2019, 2022), and to the south in the cloud forests and low jungles of the Manu National Park (Solari et al. 2006). Regarding large rodents, the agouti Dasyprocta kalinowskii is a poorly known endemic species of Yungas (Teta and Reyes-Amaya 2021). This species is distributed between 1,000 and 2,000 m (Patton *et al.* 2015) and is nearly sympatric with *D. variegata* which inhabits mainly lowland rainforest, but also reaches upper forest up to 1,800 m (Tschudi 1845; Teta and Reyes-Amaya 2021). Although our record of a skull in Nueva Italia suggests that could be *D. kalinowskii*, the cranial morphology of the few specimens in scientific collections is quite variable. Teta and Reyes-Amaya (2021) assert that individuals of *D. kalinowskii* stored at the AMNH (the American Museum of Natural History) present mostly indistinguishable characters from *D. variegata*. Hence, an adequate characterization of *D. kalinowskii* is needed to differentiate it from the other sympatric species such as *D. variegata* and even *D. fuliginosa* (Teta and Reyes-Amaya 2021).

Conservation status. Yungas is one of the most fragile ecosystems where a great diversity of ecotones and forest patches can be found (Pacheco 2002), and where fragmentation due to deforestation by agriculture and grazing is one of the major threats (Tovar et al. 2010, Tejedor et al. 2012). Yungas of SNPH is not exempt from this issue because the forests could suffer this threat mainly in their buffer zone, close to human settlements. This issue was observed in Nueva Italia and Santa Isabel, areas surrounding the sanctuary that are currently being affected by crops, mainly coffee. Although this crop is one of the primary sources of income for the inhabitants, it is also among the five most important in causing the highest rates of deforestation in Perú (SERFOR 2015). This issue directly affects the conservation status of many species, as revealed by the current decreasing trends of the population of medium and large-sized mammals recorded in this study (IUCN 2023). For this reason, local and regional governments must initiate sustainable management that includes the conservation of Yungas forests and the species of mammals and other vertebrates they keep in these central jungle regions. In addition, the knowledge and diffusion of the key species for conservation play a relevant role in the care, protection, and conservation of the species in the SNPH.

Although we managed to record the non-volant mammals in this Yungas poorly known, the number of mammalian species of SNPH is likely to be much larger than that recorded in this study, and further assessments will be needed to better understand the real diversity. In addition, a more exhaustive statistical analysis will be necessary to know if there may be changes in diversity on a temporal and spatial scale.

Finally, we urge future researchers and all decision stakeholders of the SNPH itself to encourage more research in this area. Future studies on mammals living in SNPH should focus on 1) functional diversity, 2) the ecological role of each species, 3) the current conservation status of the species, and 4) the population trends and habitat requirements of each species. These are key questions to produce conservation plans for the species and the area.

Acknowledgments

To SNPH for granting us the collection permits of specimens, Resolution N° 02-2011-SERNANP-DGANP-J/SNPH; likewise, to the "Dirección General de Flora y Fauna Silvestre" of Ministry of Agriculture and Irrigation for the collection permits outside protected areas, Resolution N° 0272-2012-AG- DGFFS-DGEFFS. A very special thanks to José Álvarez, Anthony Almeyda, Jaime Pacheco, Melisa Del Alcazar, David Aybar, and Angie Uturunco who participated in the fieldwork. To Elena Vivar for her support in the identification of the marsupials. To Pamela Sánchez for sharing phylogenetic data and her inquisitive support in the identification of some rodent species. To Dennisse Ruelas for sharing phylogenetic data that allowed us to pinpoint the identifications of some complex species. To Oscar Apari for the elaboration of the map. To the Aguilar family from the locality of Nueva Italia and especially to our local guide Fernando Aguilar for his support in the fieldwork. We thank our funding sources: "Vicerrectorado de Investigación" from Universidad Nacional Mayor de San Marcos, projects CON/CON N° 111001031, 121001061, and Idea Wild for the donation of field equipment given to Edith Arias.

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Associated editor: Monica Diaz Submitted: July 28, 2023; Reviewed: August 4, 2023 Accepted: November 22, 2023; Published on line: January 14, 2024

Supplementary material 1 https://mastozoologiamexicana.com/therya/index.php/THERYA/article/view/6124/1418

MAMMALS IN THE CENTRAL ANDES YUNGAS

Group structure and diurnal behavior in a large colony of Mimon cozumelae in Yucatán, México

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The Phyllostominae is a Neotropical subfamily of bats that include species considered sensitive to habitat disturbance, but that are the poorly known. The Cozumelan Golden Bat, *Mimon cozumelae*, is a rare phyllostomine that inhabits forests and semi-deciduous tropical forests from central México to western Colombia. This study describes for the first time, the social organization and diurnal behavior of *M. cozumelae* to provide basic information about the social relationships of this elusive species. We captured and marked individuals inhabiting a cave in Yucatán, México in 2020 and 2021. Observations were carried out filming its behavior inside the cave in two seasons, breeding (March-July) and non-breeding (August-January). We constructed an ethogram and classified the observed behaviors into states and events in each season. A total of 103 individuals (47 females and 56 males) of *M. cozumelae* were captured. The sex ratio was not different from 1:1. The size of the groups did not vary throughout the seasons. The most frequent behaviors performed by *M. cozumelae* were resting, followed by flight, self-grooming, wing extension, and social grooming. The group composition most common in both seasons was multi-male, ruling out the typical formation of harems in the breeding season for this species. Copulation and maternal grooming were recorded for the first time in the reproductive season. This bat exhibits social grooming, which in theoretical terms could categorize it as a species that forms societies. This study contributes updated information regarding group size and composition, and especially diurnal behavior of *M. cozumelae*. It is suggested that this work serves as a baseline to investigate its social systems (behavioral ecology) in depth, and when carrying out conservation plans for this species.

Los Phyllostominae son una subfamilia neotropical de murciélagos que incluyen especies consideradas como sensibles a la alteración del hábitat, pero que son las pobremente conocidas. *Mimon cozumelae*, es un raro filostomino que habita en bosques y selvas tropicales semideciduos desde el centro de México hasta el oeste de Colombia. Este estudio describe por primera vez la organización social y el comportamiento diurno de *M. cozumelae* para proporcionar información básica sobre las relaciones sociales de esta especie. Capturamos y marcamos individuos que perchan una cueva en Yucatán, México en 2020 y 2021. Se realizaron observaciones filmando su comportamiento dentro de la cueva en dos temporadas, reproductiva (marzo-julio) y no reproductiva (agosto-enero). Se construyó un etograma y se clasificaron los comportamientos observados en estados y eventos en cada estación. Se capturaron un total de 103 individuos (47 hembras y 56 machos) de *M. cozumelae*. La proporción de sexos no fue diferente de 1:1. El tamaño de los grupos no varió a lo largo de las estaciones. Los comportamientos más frecuentes registrados para *M. cozumelae* fueron de descanso, seguido del vuelo, el aseo personal, la extensión de alas y el aseo social. La composición de grupo más común en ambas épocas fue la de multimachos, descartando la típica formación de harenes en época reproductiva para esta especie. La cópula y el aseo materno se registraron por primera vez en la época reproductiva. Este murciélago exhibe acicalamiento social, lo que en términos teóricos podría categorizarlo como una especie formadora de sociedades. Este estudio aporta información actualizada en cuanto a la descripción del tamaño y composición de los grupos y en especial, del comportamiento diurno de *M. cozumelae*. Se sugiere que este trabajo sirva como línea base para investigar a profundidad sobre sus sistemas sociales (ecología del comportamiento), y realizar planes de conservación para esta especie.

Keywords: Cave-roosting; diurnal activities; group composition; Phyllostominae; Yucatán Peninsula.

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Introduction

Bats are considered mammals with a high degree of sociability because they form large groups of hundreds to millions of individuals. In addition, some species of bats present social interactions considered cooperative with a social component (<u>Wilkinson 1984; Ripperger et al. 2019</u>). All behaviors carried out by bats in their daily activity, or during their life cycle, are important for the survival and reproduction of individuals, but those behaviors that imply some type of interaction between the members of a group can sometimes be more relevant, since they can reveal temporal patterns that describe the social structure of the species (Kerth *et al.* 2003). Social behavior includes the interaction of two or more individuals, and these associations can form groups that allow them to obtain benefits such as avoiding predation, preventing the spread of diseases by parasites, or obtaining energy benefits due to social thermoregulation (Kerth 2008; Cárdenas-Canales *et al.* 2022).

The groups of individuals that display social bonds and engage in cooperative behaviors and can recognize each other individually or as members of the same group, are known as bat societies (Kerth 2008). Some species that have been classified under these parameters and considered as bat societies are: Eptesicus fuscus, Noctilio leporinus, and Rhynchonycteris naso, belonging to different taxonomic families but showing similar social interactions (Bradbury and Vehrencamp 1977; Brooke 1997; Willis and Brigham 2004; Kerth 2008). Kerth (2008), added to this classification a sublevel within societies, which he called complex societies, but which briefly involves "complex interactions" within roosting sites. Some species of tropical bats that have been classified as complex societies are Phyllostomus hastatus, Trachops cirrhosus, and Desmodus rotundus, due to their social bonds and interactions displayed in their roosting sites (McCracken and Bradbury 1981; Wilkinson 1988; Wilkinson and Boughman 1998; Flores et al. 2020; Razik et al. 2022). Another aspect to consider is that these species live in roosting refuges and can change their social structure not only over time but also throughout the day (Chaverri and Kunz 2010; Wilkinson et al. 2019). Daytime roosts are one of the most important places for the performance of individual and social behaviors, and several reports suggest that most species interact and communicate with their conspecifics at roosting sites, presenting several complex behavioral interactions (Kerth et al. 2003; Chaverri and Kunz 2006; Ortega and Maldonado 2006; Prat et al. 2016; Carter 2021; Crisp et al. 2021).

Tropical bats of the family Phyllostomidae often form groups throughout the year, and hence are considered to be more stable groups, while sociability in temperate zone species is restricted to certain times of the year (i. e., hibernation-- Bradbury and Vehrencamp 1977; Brooke 1997; McCracken and Wilkinson 2000; Kerth et al. 2011). Studies of sociability in bats of the subfamily Phyllostominae have been addressed to a lesser extent compared to other subfamilies of these tropical bats, mainly due to the fact that the members of this subfamily tend to form colonies of very few individuals, in addition to which their abundance in their tropical habitat is lower than that of other phyllostomids. The Cozumelan Golden Bat (Mimon cozumelae) it is considered rare and scarce throughout its distribution, with few reports regarding its roosting sites (Ortega and Arita 1997). Bats of this species roost principally in caves, mines, culverts, and hollow logs (Tuttle 1976), inhabiting a great variety of tropical habitats including disturbed and undisturbed sites (Fenton et al. 1992). The few reports on group size in M. cozumelae showed that the maximum number of individuals recorded to date is 15 to 20 bats, sometimes sharing the refuge with Trachops cirrhosus and segregating their refuge roosting sites (LaVal 1977). M. cozumelae is considered an opportunistic carnivorous species since it occasionally includes vertebrates in its diet, but mainly feeds on invertebrates (Ortega and Arita 1997; Gual Suárez and Medellín 2021).

We recorded data from a cave located in southeast México, in the state of Yucatán, with approximately 90 individuals of *M. cozumelae* in a single refuge, being the largest colony recorded to date. Our main goal was to investigate the social organization of this group and the behavior of *M. cozumelae* in the wild, as well as carrying out behavioral observations to provide basic information about the social relationships of this elusive species.

Materials and methods

Our study was conducted in January 2020 and from January to June 2021, recording data during four days on each visit. In January 2020 we located the cave, established all the necessary logistics for the work, and obtained behavioral data but we could not sample during the following months due to COVID-19 restrictions. The site is located in the state of Yucatán, México, situated in the municipality of Uayma (20.7003° N, -88.2918° W), in the northeast part of the state. The cave is located within the Hacienda X'conzuk, surrounded by tropical deciduous forest and patchy areas of cropland. The site was selected during our previous visit due the continuous presence of a large colony of the Cozumelan Golden Bat (*Mimon cozumelae*).

The cave has three small simple chambers, with two small but accessible entrances. The largest chamber harbored a large colony of *Mimon cozumelae* (*ca.* 90 individuals) and included scattered individuals of *Diphylla ecaudata* (*ca.* 10 individuals) that temporarily use the roost and one or two individuals of *Micronycteris microtis*. Dimensions of the chamber were 5.70 m by 3.65 m, with an average height of 2.5 m. Individuals of the tropical porcupine (*Sphiggurus mexicanus*) and a big-eared climbing rat (*Ototylomys phyllotis*) were occasionally detected within the cave.

Captures were made in the first two visits by placing two mist nets of 3 and 6 m long, located at the entrance of the second and third chambers of the cave, in order to capture the largest number of individuals. Hand nets were also used inside the cave to capture individuals. The captured bats were kept in individual cloth bags for later processing. They were weighed, measured, sexed and their reproductive status was determined. Individuals were considered adults when presenting ossified epiphyses and subadults when having cartilaginous joints (Brunet-Rossinni and Wilkinson 2009). We corroborated this characterization by measuring body mass using a spring scale with a precision of 0.1 g, and measuring the length of the forearm with an electronic caliper with a precision of 0.1 mm. Reproductive conditions were scrotal or abdominal testes for adult males and pregnant, lactating or without signs for adult females. The reproductive or breeding season was defined as extending from April to June when > 80 % of adult females had inflamed vulva or were pregnant or lactating (Figure 1). From January to March, most adult females showed no signs of reproductive condition.



Figure 1. Reproductive condition of females of *Mimon cozumelae* in the Uayma refuge, during the reproductive and non-reproductive season during the two-year study. Females were captured to determine the reproductive condition. In 2020 we did not sample bats after January due to COVID-19 restrictions.

Bats were marked with a metallic ring on the forearm (Nacional de Identificación, S. A. de C. V.). The metal ring was placed on the left forearm for females and the right forearm for males, which allowed us to distinguish the sex of individuals visually and in the recordings. The handling of the individuals was carried out in accordance with the guide of the American Society of Mammalogists for the use of wild mammals in research (Sikes *et al.* 2016). This project used collection permits issued by SEMARNAT (Secretaría del Medio Ambiente y Recursos Naturales, SGPA/DGVS/9032/19) to MCMG.

For the recordings, a 4K AX33 video camera was used in Night Shot mode (Sony Inc. Japan). A fast-scanning method was implemented with the camera, which allowed us a panel exploration of the main chamber where the M. cozumelae individuals were found, with an intrusion into the system lasting no more than five minutes to not disturb the structure of the groups (Martin and Bateson 2007). This procedure was done twice a day (6:00 and 16:00). The scan shots were later visualized in the laboratory and with them an estimate of the group sizes and their composition could be obtained. To consider the size of the group, the number of animals observed during the panning of the fast-scanning recordings was calculated. The composition of the group was tallied according with the number of animals that had the metallic ring on the left forearm and for those that had it on the right forearm.

Once the fast-scanning recordings were completed, the camera was placed inside the cave, focusing on as many bats as possible in an open panel position. The location of the camera was kept fixed for four hours to make the focal recordings. The first 15 minutes of the recording were not measured in the results because the bats changed positions due to the cave intrusion. We established this period of time since the subsequent analysis of the focal recordings showed that the bats stop moving and stayed in stable positions after 15 minutes (Olson and Barclay 2013). Focal recordings were filmed during four hours of observation per day (6:00 to 8:00 and 16:00 to 18:00 h). These times were selected since they are critical points in which there is the possibility of greater activity in bats (two hours after returning to the shelter and two hours before the emergence).

The ad libitum method was used since opportunistic observations are recorded without restrictions in the measurement time in which the different activities were carried out by one or several individuals (Martin and Bateson 2007). This method is useful for the first behavioral description in a new system for the observer, or at the stage of preliminary observations. The disadvantages of this sampling are that behaviors that are easier to detect are generally recorded and others that could be included in a systematic sample are underestimated (Altmann 1974). Ad libitum observations were made in real time by observing them with the help of a computer (MacBook Pro, Apple). The observer was positioned with the monitoring equipment near the entrance, between the second and third chambers of the cave. For the monitoring equipment, an HDMI cable with an extension of approximately 10 meters was used, connected to the video camera (4K AX33 in Night Shot mode, Sony Inc.). The camera was placed on a tripod, with a video translator (Blackmagic Design UltraStudio Mini Recorder-Thunderbolt), and a Thunderbolt cable for computer input,

enabling the observer to mitigate the disturbance caused by being in their roost.

For the construction of the ethogram, the behavioral units defined by Muñoz-Romo (2006) were used as a reference, combined with other studies on behavior in bats (Muñoz-Romo et al. 2008). The observed behaviors were classified into states and events (Table 1). We measured the frequencies of the behaviors classified as events, and resting was the only state that was measured as such. Subsequently, the frequencies of each of the behaviors were recorded for each observation for each season. For non-breeding season, we analyzed recordings carried out in January 2020 and January 2021, whereas for breeding season we analyzed recordings from March to June 2021. Absolute and relative frequencies of the behaviors were obtained for each season and for sex of the performer. All analyses and graphs were carried out in RStudio version 4.1.1 and in GraphPad 9.0.1. To analyze the results of the sex ratio of bats, a binomial test was performed. To obtain the behavior accumulation curve, the number of behaviors that were observed for each field trip were considered, then they were graphed to obtain the accumulation curve. A generalized linear model was performed to analyze the behaviors that bats performed between seasons and sexes of the performer and if these behaviors differed in frequency. Normality tests (Shapiro-Wilk) were applied to all data. Mann-Whitney U tests were performed to explore statistically significant behavioral differences: 1) between sexes. 2) Between the two seasons. 3) Comparing the behavioral activities carried out after arrival of the forager, when the individual had expenditure energy, against the activities carried out in a brief period of time prior to their nocturnal departure, after resting and sleeping all day.

Results

A total of 24 days of recordings were made in the study site in the period from January 2020 to June 2021, of which 15 days were in the non-breeding season and 13 days in the breeding season. A total of 103 individuals (47 females and 56 males) of *M. cozumelae* were captured. A total of 61 (59.23%) of the individuals were captured in the first visit (January 2020), and the rest in the January 2021. The sex ratio was not different from 1:1 (n = 103, $x^2 = 0.62$, P = > 0.05). A total of 59 individuals were marked with metal rings and were considered our study subjects. During the January 2020 visit, we noticed that metal rings on juveniles harm them, so we stopped tagging individuals in this age group.

We recorded 30 observations with the fast-scanning method, each with a duration of five minutes throughout all sampling days between the two seasons. Twelve observations were made for the breeding season and for the non-breeding season 18 observations were recorded. The size of the groups did not vary throughout the seasons, despite the fact that individuals moved during our observations. No statistically significant differences were obtained between the average size of groups observed between seasons (breeding season 1.66 ± 1.0 groups vs. non-breeding season 1.85 \pm 1.20; *P* = 0.96, Mann-Whitney U = 4). However, it was observed that the highest average group size was with the presence of offspring (31.75 \pm 15.77), in addition to the fact that movements between sites were reduced exponentially, leaving more structured stable groups. Within groups we observed a greater number of males than females in both the breeding and nonbreeding seasons. On average, in the breeding season there were 1.67 \pm 1.18 females vs. 3.5 \pm 2.26 males. In the non-breeding season, there were 1.87 ± 0.59 females vs. 2.75 ± 1.87 males. Individuals roosting alone were occasionally observed, but these events were rare because most of the bats were observed in large clusters.

A total of 79.4 hours of filming was obtained. Of the total number of hours, 55 were recorded with the *ad libitum* method and the rest were reviewed by the focal method. The 79.4 hours included the breeding season (40.4 hours) and the non-breeding season (39 hours). A total of nine behavioral activities were identified within our *ad libitum* observations. The most frequent behaviors performed by

Table 1. Definition of the behavioral activities most frequently observed in Mimon cozumelae with the ad libitum method.

Behavior	Definition
Self-grooming	Grooming was recorded when an individual passed their tongue over different areas of their own body. This behavior was recorded each time an individual licked its legs up to the abdomen with short, rapid, ascending movements on the same point once or several times. Self-grooming behavior was also recorded when the individual groomed their back, head, and wings.
Social grooming	These behaviors can be performed between individuals of the same sex, different sex, or from an adult to a child (maternal grooming), performing the same behavior patterns described in self-grooming.
Flight	The individual spreads its wings, freeing the hind legs from the wall and moves in the air by repeatedly flapping its wings, the individual that ends the flight approaches to the wall and holds on to perch with hind legs (hanging).
Wing Extension	A roosting individual extends one or both wings in a stretching manner.
Resting	This behavior is recorded when an individual remains motionless on the perch site with eyes open or closed.
Vocalization	It is recorded when paused and continuous guttural sounds are heard, but are audible on the recording.
Alert	It is described when the individual is perched but moving his head from side to side in observation of what is happening inside the cave.
Aggression	When an individual approaches another and performs flapping behaviors, bites and short chases.
Copulation	Copulation behavior was recorded when the adult male mounted one female and mated for several seconds.

M. cozumelae was resting, followed by flight, self-grooming, wing extension, and social grooming. The least frequent behaviors were being alert, vocalizations, aggression, and copulation; these four were not considered in our analysis due to their low frequency of occurrence. For the generalized linear model, the most frequent behaviors were considered. The analysis showed that the behaviors were statistically different between seasons, between behaviors, and in the interaction of the season (breeding versus nonbreeding) with the type of behavior (behaviors P < 0.001, season P < 0.001, behavior-season P < 0.001). To obtain the frequencies of the behaviors of males and females in the breeding and non-breeding season, only marked individuals were considered. The behaviors with the highest frequencies were included in the analysis. The behaviors were significantly different among themselves (P < 0.001), between males and females (P < 0.001), but not in the interaction of behaviors and sex (P < 0.001).

We performed paired comparisons among the most frequent behaviors between sexes and breeding/nonbreeding seasons (Figure 2a and 2b). Social grooming was not statistically significant between males and females (P = 0.98 – Figure 3), and was also similar when comparing the two seasons (P = 0.39). It was possible to document 22 observations of social grooming between pairs of individuals, the most frequent of which was performed by mothers towards their offspring, lasting longer than the same behavior observed among adults. Self-grooming performed by males and females was not significantly different (P = 0.9403). However, self-grooming was significantly lower during the reproductive season compared with the non-breeding season (P = 0.0023). Resting time was similar between both sexes (P = 0.8022). Resting time showed lower numbers during breeding season compared with non-breeding season and those numbers were statistically different (P = 0.01). Wing extension behavior was similar in number for both sexes and seasons and did not differ significantly (P = 0.24). Finally, the number of flights made by males and females to change roosting sites inside the chamber were not statistically significant between the different categories of adults (P = 0.6395), and were similar when both seasons were compared (P = 0.75). Some marked females with offspring were observed, in which the mothers made short flights inside the chamber carrying the young.

We analyzed paired comparisons among the previously described behaviors between observations made in the hours after arrival versus the hours prior to departure. Bats showed a pattern of more persistent behavioral activities during the hours prior to their nightly departure compared with activities performed at dawn after foraging. Resting behavior was consistently most frequently observed in the period after arrival compared to the period before leaving the cave (P = 0.04, Figure 4). Self-grooming was also a very occasional activity after arrival at the cave, compared with the frequency of occurrence observed at sunset (P < 0.001,

Figure 4). All the other behavioral activities did not show significant values of difference between the two periods of the day.

Discussion

This study reports the largest colony of Mimon cozumelae in a single refuge, with 103 individuals captured in a period of two consecutive years (2020 to 2021). It is considered that the number of individuals that have used the refuge is greater, because in the samplings carried out throughout the study individuals without marks were observed in the recordings. In general, the number of individuals observed in the cave ranged between 60 to 90. The species showed fidelity to the refuge since in all our visits, M. cozumelae was be found roosting at our study site. The unmarked individuals that were observed during the recordings exceed the number of the marked ones, which suggests that they come from nearby roosts, since during this study, other roosts were found nearby, one of them located approximately 2 km away. The home range for this species is unknown, so we do not know how far these individuals could move to other refuges. In other bat species such as Tadarida brasiliensis and Myotis velifer, the percentage of recaptured individuals suggested that they have low fidelity to the refuge and was attributed to the availability and proximity of other refuges (Lewis 1995). However, the individuals of *M. cozumelae* showed high fidelity to the refuge despite the proximity of other available refuges, since we



Figure 2. Relative frequencies of most common behaviors performed by *Mimon cozumelae* during the reproductive season, a) versus non-reproductive season b) in the cave of Uayma, Yucatán, México. For definition of each behavior see Table 1.



Figure 3. Average time spent on social grooming (s) by different observed pairs of *Mimon cozumelae* during the reproductive season (blue: male-male; orange: female-male; gray: mother-offspring).

recorded the presence of the colony in very similar numbers in every month during the study. The composition of the colony was mixed groups of adults of both sexes in the non-breeding season, adding offspring and juveniles during the reproductive season. The rearing season coincided with the dates previously reported for the species in the Yucatán Peninsula (Jones *et al.* 1973).

Opportunities to study behavioral activities in bats have been limited due to their nocturnal habits, crypticity, and the difficulty in accessing their shelters (Kerth and Dechmann 2009). However, there are several studies on certain species considered common such as hematophagous bats (Desmodus rotundus) or pollinators (Glossophaga mutica), which have been studied with the use of PIT Tags (Integrated Passive Transponders), light remotes, proximity sensors, radio transmitters and infrared video recordings, combining these tools to allow more detailed studies of social behavior in bats (Ripperger et al. 2019, 2020; Rose et al. 2020). Day roosting sites are among the most important places for the performance of individual and social behaviors. Scientific reports suggest that most bat species interact and communicate with their conspecifics at perch sites (Chaverri and Kunz 2010; Prat et al. 2016). Self-grooming or social grooming, copulation, alloparental care, and nursing seem to occur exclusively at resting sites (Wilkinson 1984; Kerth et al. 2003; Chaverri and Kunz 2006; Ortega and Maldonado 2006). Studies have reported that roost attributes

exert a significant influence on bat social systems (<u>Hodg-kison et al. 2003</u>; <u>Campbell et al. 2006</u>; <u>Lausen and Barclay 2006</u>; <u>Chaverri et al. 2007</u>, 2008).

In the breeding and non-breeding seasons, the number of groups of *M. cozumelae* did not vary significantly. Our results suggest that M. cozumelae does not form harems in the reproductive season, since our data obtained from the composition of the groups of M. cozumelae included several males. Observations suggest that most of the females captured and tagged during the study left the cave, possibly to another nearby refuge, and that only a few females remained during the breeding season, which were surrounded by one or more males, which also suggests that it does not form maternity colonies. However, it is necessary to study this species in other refuges to rule out this possibility as other species of phyllostomines such as Phyllostomus hastatus do form harems (McCracken and Bradbury 1981). Our data and further samplings in roosting sites of *M. cozumelae* in the region show that this species has a seasonally monoestrous reproduction. Females give birth a single pup and offspring become adults by the end of August. From September to March all individuals captured in the region are adults, with no signs of reproductive activity (MCMG, personal observation).

Copulation events are difficult to register in the wild, particularly in tropical areas where roosting sites are not accessible for filming purposes, coupled with the elusive

behavior of the species. During our study, we registered a behavior that we classified as a copulation event (Supplementary Material, Video1). A female was roosting in the cave and a male arrived and positioned himself behind the female for about 15 sec. After this, both individuals adopt a face-to-face and female opened the wings in an embrace that lasted about 28 sec. After this behavior, the male flew away with a loud squeal. The whole interaction between female and male lasted about 43 sec. A couple of seconds later a male returned and positioned himself next to the female and both began to clean themselves. Although we cannot be certain, we suspect that it was the same male that returned to the visual field of the camera. Recent copulation events recorded in other phyllostomids such as Centurio senex had a similar duration (38 sec), although behavior between female and male of C. senex show different pattern as in this species females select and approach to a male for copulation (Rodríguez-Herrera et al. 20210).

Rest is one of the behaviors that lasts the longest in bats, and one of its functions is to conserve energy. Measuring this behavior can be confusingly complicated because when the organism is immobile it is difficult to differentiate whether it is completely sleeping or just inactive. The amount of time bats spends sleeping varies by day and between species but it has been recorded that some cave species can spend more than three quarters of their time resting (Fleming *et al.* 1998; Hengjan *et al.* 2017). Our observations indicated that not all individuals rested at the same time, at least one individual remained active, usually the individual separated from the group. This suggests that a certain level of vigilance may be necessary for bats to successfully occupy roosts (Muñoz-Romo 2006). Although the individuals of *M. cozumelae* were inactive most of the diurnal rest time, this was an interrupted phenomenon since the individuals did not sleep continuously for more than 20 minutes. This observation has also been reported for the fruit bat *Artibeus lituratus* (Muñoz-Romo 2006).

Flight in bats is part of locomotion and is one of the behaviors that is difficult to record, since it is necessary to use radio telemetry or light remote sensors to track individuals (Ripperger et al. 2020). In cave bats, this activity is possible to record in frequencies of short periods within the refuge, which is spotted when individuals change social groups (Ortega et al. 2010). Prior to flight, stretching of the wings could contribute not only to maintaining the optimal condition of the flight muscles and joints, but also to avoid hypothermia that could occur during rest periods in cold climates (Kunz 1982). In M. cozumelae short flights were observed inside the refuge near dusk and dawn. Particularly, at dusk a few individuals were observed leaving the roost for shorts periods and returning, which probably help to identify the exact moment of emergence for the rest of the group, which come out one by one every few seconds or minutes. The whole group left the roost 30 minutes after this initial recognition flights carried out by a few individuals.

Self-grooming performed by bats also has a personal hygiene character and this behavior is the most common behavior reported in bats (Fleming *et al.* 1998). In the case



Figure 4. Relative frequencies of the most common behavioral activities observed during the morning versus the afternoon. For definition of each behavior see Table 1.

of social grooming, the vampire bat (D. rotundus) is a species that spends a lot of time in this behavior compared to other bat species (Carter and Leffer 2015; Rathinakumar et al. 2017). However, the social grooming performed by the vampire bat is not always intended to help another individual because sometimes its response to their own need (Carter and Leffer 2015). Social grooming might be a consequence of licking a groupmate to gather sensory information, also a bat might preen due to the needs of the recipient (Narizano and Carter 2020). In maternal grooming it might be expected that mothers' grooming towards young offspring might be more recipient-driven (attuned to offspring's needs) compared to offspring's grooming towards mothers, which might be more recipient-based in the need of the offspring (Narizano and Carter 2020). In the case of *M. cozumelae*, maternal grooming was recorded, and in the case of one pup, grooming was carried out not only by the mother, but also by another unmarked individual. We were unable to determine whether this individual was a male or another female. Further investigations are needed to document whether this behavior is frequent in the species.

Night activity patterns, mainly foraging behaviors, showed a peak of activity around midnight for insect gleaning bats (Lee and McCracken 2001; Berková and Zukal 2009). Bats expend a great deal of energy at night because they carry out a great number of activities to find their food. Nocturnal activities are closely correlated with the time of year, reproductive activity and even the age of the bat, so that in times of food scarcity, the search effort is greater (Lee and McCracken 2001). It is common to observe that many species of bats return to their roosting site at dawn, after having carried out intense foraging activities. We found that *M. cozumelae* return to the roost site at dawn, but that a large amount of time after arrival is devoted to resting, which contrasts with the increased activity in the time span before setting out to forage in the evening.

Finally, it is important to mention that M. cozumelae seems to be a species sensitive to disturbance as our observations showed that after several days visiting the cave, individuals tend to move to another roosting site. However, all individuals of M. cozumelae returned to the site two to three weeks after. The movement of individuals in a colony of bats due to the presence of researchers has been reported for other species of bats such as the vespertilionid Corynorhinus rafinesquii in which after a few days after young bats were marked, the mothers took their offspring and moved to a nearby refuge, but after a few days they returned to the initial roosting site (Pearson et al. 1952). Another species with a similar behavior is C. mexicanus, which abandoned its refuge near Pachuca, Hidalgo, México, after this site started to be visited by tourists (Aguilar, M. personal communication).

In general, it had been previously reported that phyllostomines are particularly sensitive group to disturbance of their environments (Fenton *et al.* 1992). This study contributes with updated information regarding the description of the size and composition of groups and, especially diurnal behavior of *M. cozumelae*. It is suggested that this work may serve as background to consider both for works that delve into the subject of social systems (behavioral ecology), and when carrying out conservation plans for this species, due to the alterations that are changing its habitat, with the potential megaprojects that are currently being developed in the Yucatán Peninsula.

Acknowledgments

First author would like to thank Consejo Nacional de Ciencia y Tecnología, México, for its financial support with master's degree scholarship number 977727 and Centro de Investigaciones Tropicales, Universidad Veracruzana for the field's work financial support. We also like to thank IDEA WILD for the grant for equipment. We thank Dr. G. Chaverri for her comments and suggestions on the thesis that derived this manuscript. We thank the colleagues, students and local guides that helped with the fieldwork and the authorities of the Cuxtal Reserve and landowners that granted the permit to sample in their sites.

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Associated editor: Robert Owen Submitted: July 7, 2023; Reviewed: September 4, 2023 Accepted: October 10, 2023; Published on line: XX xx, 2023

Supplementary material

Video1. Copulation event of Mimon cozumelae in the Yucatán, México: https://doi.org/10.5281/zenodo.7178551.

DIURNAL BEHAVIOR OF THE COZUMELAN GOLDEN BAT

Social arenas in the open habitat: the social role of waterholes for saiga antelope

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In wild animals, specific locations may function as 'social arenas' playing a significant role in various aspects of intraspecific interactions. The emergence of such social arenas is assumed to be particularly important under conditions of low visibility and density of individuals typical for forest dwelling species. In the forest, open areas with a high probability of meeting conspecifics such as waterholes and mineral licks can favor socialization. The paucity of studies on the open-habitat species limits our understanding of whether forest habitat is an essential condition for the emergence of social arenas. Here we address this issue by investigating whether resource hotspots function as social arenas in a species adapted to open habitat. The social behaviour in groups of saiga antelopes (Saiga tatarica tatarica) was recorded at large permanent waterholes (serving also as mineral licks) and away from them in the steppe environment. The frequency and variety of social interactions between the group mates were compared with a special effort made to minimize the potential impact of other factors besides the location. Also the focal individuals, interacting or not interacting with conspecifics at the waterholes, were traced continuously to explore the duration of their visit at the waterhole area. Finally, we recorded the number of individuals in the groups entering and leaving the waterhole. Active social interactions between group members occurred more often and were more varied near the waterholes as compared to the areas away from them. The participation in social interactions influenced the duration of individual waterhole visits. Males that interacted with others shortly after arrival stayed significantly longer compared to those who did not. Females whose calves engaged in social interactions with age-mates spent more time at the waterholes than females whose calves did not. The comparison of mean group sizes showed that saigas left waterhole areas in larger groups than they entered them. Our findings, together with previous studies, suggest that resource hotspots can function as social arenas in mammals regardless of the habitat type (open or closed). The emergence of specific locations having particular social significance may be especially important for highly dispersed species with large home ranges. The results of the study emphasize the significance of large permanent waterholes and mineral licks used by many individuals year after year for the social behaviour of saiga antelopes.

Para animales silvestres, sitios específicos pueden funcionar como "arenas sociales" que desempeñan un papel importante en varios aspectos de las interacciones intraespecíficas. Se sugiere que el surgimiento de tales espacios sociales es particularmente importante en áreas de baja visibilidad y con una densidad baja de individuos, condiciones típicas en especies que habitan en los bosques. En el bosque, las áreas abiertas con una alta probabilidad de encontrarse con congéneres, como pozos de agua y saladeros de minerales, pueden favorecer la socialización. La escasez de estudios sobre especies de hábitat abierto limita nuestra comprensión de si el hábitat forestal es una condición esencial para el surgimiento de espacios sociales. Aquí abordamos este tema investigando si los puntos críticos de recursos funcionan como arenas sociales en una especie adaptada al hábitat abierto. El comportamiento social en grupos de antílopes saiga (Saiga tatarica tatarica) se registró en grandes pozos de agua permanentes (que sirven también como fuentes de minerales) y lejos de ellos en las estepas. Se comparó la frecuencia y variedad de interacciones sociales entre los compañeros del grupo con un esfuerzo especial para minimizar el impacto potencial de otros factores además de la ubicación. También, los individuos focales, que interactuaban o no con sus congéneres en los pozos de agua, fueron rastreados continuamente para explorar la duración de su visita al área del pozo de agua. Finalmente, registramos el número de individuos de los grupos que entraban y salían del pozo de agua. Las interacciones sociales activas entre los miembros del grupo ocurrieron con mayor frecuencia y fueron más variadas cerca de los pozos de agua en comparación con las áreas alejadas de ellos. La participación en interacciones sociales influyó en la duración de las visitas individuales a los pozos de agua. Los machos que interactuaron con otros poco después de su llegada permanecieron significativamente más tiempo en comparación con los que no lo hicieron. Las hembras cuyas crías participaban en interacciones sociales con sus compañeros de edad pasaban más tiempo en los abrevaderos que las hembras cuyas crías no lo hacían. La comparación de los tamaños medios de los grupos mostró que los saigas abandonaron las áreas de los pozos de agua en grupos más grandes de los que entraron en ellas. Nuestros hallazgos demuestran que los puntos críticos de recursos pueden funcionar como arenas sociales en los mamíferos independientemente del tipo de hábitat (abierto o cerrado). La aparición de lugares específicos que tengan un significado social particular puede ser especialmente importante para especies muy dispersas con grandes áreas de distribución. Los resultados del estudio enfatizan la importancia de los grandes pozos de agua permanentes y saladeros de minerales utilizados por muchos individuos año tras año para el comportamiento social de los antílopes saiga.

Keywords: Mineral lick; open landscape; social behavior; socialization; ungulate; waterhole.

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Introduction

In many animals, the social interactions between conspecifics are not evenly distributed throughout the species' habitat (e. g., Fishlock et al. 2008; Brightsmith and Villalobos 2011). There are specific places providing the enhanced probability of meeting conspecifics, maximizing social opportunities for both solitary (Klaus-Hügi et al. 2000) and group-living species (Turkalo and Fay 1995). For example, leks and resource hotspots are strategically used to increase the possibility of encountering opposite-sex individuals in the mating context (Hardenberg et al. 2000; Klaus-Hügi et al. 2000; Bro-Jørgensen 2003). Some of these areas may serve as so-called 'social arenas', i. e. social hotspots promoting inter-individual interaction and playing a significant role not only in mating but in various aspects of social behaviour. Social arenas are supposed to emerge in places which provide limited and highly dispersed nutritional resources, such as specific food (e. g., Egbert and Stokes 1976) or mineral-rich soil and water (e. g., Fishlock et al. 2008). Apart from nutritional benefits, resource hotspots have been found to function as lodestones for social contacts and communication in birds (Brightsmith and Villalobos 2011; van Overveld et al. 2020) and mammals (Couturier and Barrette 1988; Klaus-Hügi et al. 2000; Fishlock and Lee 2013). Social opportunities may motivate visits to the salt licks on a par with mineral consumption (vVan Strien 1985; Turkalo and Fay 1995; Clayton and MacDonald 1999; Brightsmith and Villalobo 2011). Potential social benefits of licks and other resource hotspots used as gathering places include the establishment of new relationships or the maintenance of the existing ones, exchange of social information between individuals and different social units, socialization of younger individuals, etc. (Fraser and Hristienko 1981; Fishlock et al. 2008, Gilev and Karenina 2015). For researchers, such places provide unique opportunities for detailed investigation of social behaviours which are difficult to observe otherwise (Clayton and MacDonald 1999; Fishlock and Lee 2013; Hii 2017; Giljov et al. 2019).

Places with a high probability of meeting conspecifics, such as resource hotspots, may be particularly important for forest dwelling species (e. g., moose, Alces alces, Couturier and Barrette 1988; forest elephants, Loxodonta cyclotis, Turkalo and Fay 1995; babirusa, Babyrousa babyrussa, Clayton and MacDonald 1999; bongo antelope Tragelaphus eurycerus, Klaus-Hügi et al. 2000; forest buffalo, Syncerus caffer nanus, Melletti et al. 2007; Asian elephants, Elephas maximus, Fishlock et al. 2008, Fishlock and Lee 2013, Hii 2017; Baird's tapir, Tapirus bairdii, Reyna-Hurtado and Arias-Domínguez 2024). Under conditions of low visibility and low density of individuals in the forest environment, animals can use open areas as social arenas facilitating interactions among individuals. For example, forest elephants form large, dynamically changing social gatherings in natural forest clearings offering nutritional resources (Klaus et al. 1998; Fishlock and Lee 2013). Forest clearings can be used by elephants to interact, to establish and maintain the dominance hierarchy, and learn social skills (Turkalo and Fay 1995; Turkalo et al. 2013). Individual elephants maximize their social opportunities in the clearing and significantly increase their mean visit duration when they take the opportunity to associate with individuals outside their ranging party. Both inter-and intrasexual interactions occur within the clearing. Social benefits are further maximized by joining large groups, often including older elephants, or, for males, parties with females (Fishlock and Lee 2013).

On the other hand, little effort has been made to investigate the presence of social gathering places in species inhabiting open landscapes. Mineral licks are supposed to have some social significance for large herbivores in African savanna landscapes (Weir 1969; Ruggiero and Fay 1994; Merte 2010) but no focused research has been conducted to test this. It is widely assumed that the social function of waterhole/mineral lick clearings can be explained by the high visibility contrasting with dense forest (Klaus et al. 1998; Melletti et al. 2007). It is proposed that in areas with high visibility, visual contact between herd members is easier and probably more group members can see each other simultaneously. Greater choice of partners for interaction may also play some role. The paucity of studies on the open landscape species restricts our understanding of whether closed habitat is, in fact, a necessary condition for the emergence of social arenas. In the present study, we tested the alternative hypothesis by investigating whether resource hotspots function as social arenas in open landscapes, particularly for saiga antelope (Saiga tatarica tatar*ica*) – an ungulate strongly adapted to the open habitat.

The saiga antelope is a nomadic bovid inhabiting arid areas of central Asia and the northwestern pre-Caspian (Bekenov *et al.* 1998). A drastic decline in the global population has led to a Critically Endangered listing on the 2002 IUCN Red List (Milner-Gulland *et al.* 2003). There are two subspecies, *S. t. tatarica* (in the present study), the nominate subspecies studied, in Kazakhstan and Russia, and the morphologically distinct *S. t. mongolica* in Mongolia.

In the middle of the 20th century, the population of the saiga antelope in the northwestern pre-Caspian region reached over 800,000 individuals. At the beginning of the 21st century, the population dropped to only 8,000 to 9,000 individuals in response to a combination of multiple negative factors (Neronov *et al.* 2012). Nowadays, the remaining population of the saiga antelope inhabit an area of about 2000 to 3000 km², with the core habitat lying within the two contiguous protected areas within the northwestern pre-Caspian region of Russia, Stepnoi State Nature Sanctuary in Astrakhan Oblast and Biosphere Reserve "Chernye Zemli" in the Republic of Kalmykia (Karimova and Lushchekina 2018).

An adult male saiga typically defends a harem of about 10 to 30 females during the rut which takes place in December (Bekenov *et al.* 1998; Sokolov and Zhirnov 1998). Once the rut is over, the social structure of saigas becomes loose and highly variable. Group size and composition can vary significantly depending on the weather conditions, food availability, migratory activity, population size and other factors (<u>Sokolov and Zhirnov 1998</u>; <u>Karimova et al. 2020</u>). Saigas occasionally occur in large aggregations of several thousand but the typical group size is referred to as 30 individuals on average (<u>Bekenov et al. 1998</u>). Within the study area in the northwestern pre-Caspian region of Russia, small (21 to 200 individuals) and very small (1 to 20 individuals) saiga groups constitute 80 to 90 % of the population (<u>Karimova and Lushchekina 2018</u>). Mixed-sex groups are common throughout the year, while male- or female-only groups and large aggregations occur seasonally (<u>Bekenov et al. 1998</u>). The current knowledge of group composition, stability, and hierarchy in saigas remains very limited.

The comparability between the open and closed habitat species studied is especially important considering the very small number of species investigated in terms of the presence of social arenas so far. Most extensively, the social arenas at resource hotspots have been studied in forest elephants (Fishlock and Lee 2013). The saiga has similar behavioral characteristics that resemble those of the forest elephants, such as fluid social structure and large range areas (Bekenov *et al.* 1998; Sokolov and Zhirnov 1998). Thus, saiga antelope can serve as a model to compare with closed habitat species the social function of resource hotspots between different types of environments.

In the arid steppes of the northwestern pre-Caspian region, during the warm season, natural water bodies are scarce and surface water is mainly found in artificial waterholes fed by artesian wells drilled more than two decades ago. Moreover, many of them became fenced for the livestock use in the recent. The remaining large permanent waterholes are extensively used by the local saiga population for drinking, geophagia (deliberate soil ingestion) and cooling down during the summer heat (Gilev and Karenina 2015). Concentration around permanent water sources is typical behaviour of antelopes living in arid areas (Blank and Li 2022).

We conducted a study to determine if large permanent waterholes, which also serve as mineral licks, act as social arenas for saigas. We compared the frequency and variety of social interactions within saiga groups at waterholes versus away from them. We hypothesized that if waterholes promote social interactions, we would see more varied social behaviors at these sites. Additionally, we explored whether engaging in social interactions influenced the length of time saigas spent at waterholes. We aimed to understand if saigas adjust their behavior based on social cues, with individuals who enter interactions staying longer at waterholes to maximize their social opportunities. Finally, to test whether visiting waterholes favors social grouping in saigas, the sizes of the groups entering and leaving a waterhole area were compared.

Material and methods

Study area. The data for this study were collected at Stepnoi Nature Sanctuary (45° 57' 28.8" N, 46° 33' 6.1" E) which includes well-preserved native pastures and several major watering places attracting saigas throughout the year. The sanctuary includes semi-desert steppes characterized by a dry continental climate and even landscape with low vegetation. In this region, saigas exist under natural predation pressure, with wolves assumed to be a major predator (Karimova and Lushchekina 2018). Human disturbance within the sanctuary has been kept to a minimum for the last decade. Poaching, vehicle traffic and cattle grazing have been eliminated.

Saigas display a variety of active social interactions on the banks of the waterholes that inspired this study. Flat areas lacking vegetation which surround waterholes provide good visibility and enable continuous observations of focal individuals (Gilev and Karenina 2015). For conservation and research purposes, small (2.4 x 1.5 m) observation hides have been built by the main saiga watering places (Figure 1). The construction of partly underground hides (only 50 cm above the ground level) covered by camouflage nets minimizes disturbance and saigas casually approach them at very close distances even when there are humans inside. The data for the present study were collected from the hides at the two largest permanent waterholes in the sanctuary (perimeters 1,200 m and 460 m), which are situated more than 14 km away from each other. On average, about four hundred saigas including single individuals, same-sex and mixed-sex groups visited the waterholes daily. On particularly hot days, more than 1,500 individuals per day have been recorded. As a nomadic species, saigas almost constantly move around the sanctuary and the nearby territories (Karimova and Lushchekina 2018); therefore, many different individuals visited the study sites during the period of data collection. Study areas away from the waterholes (at least 1 km away) were flat steppe grasslands dominating in the sanctuary. Because of the flat nature of the steppe in this saiga habitat, saiga antelopes were visible with binoculars up to approximately 10 km away from the hide.

Data collection and analysis. The data collection was carried out at the beginning of summer (May-June) when social activity in saiga groups is prominent and diverse for several reasons: daytime heat is still moderate, food has been plentiful for a while, and the most of the groups are mixed-sex and mixed-age. Social interactions were recorded during the peak period of saiga activity in the morning (between 5:30 and 11:00 a.m.) in 2019 (31 days) and 2020 (20 days). Morning hours were used for the data collection because this is when the great majority of saiga visits to the waterhole occur (Gilev and Karenina 2015). Two researchers entered the hide well before the beginning of data recording (before dawn) to minimize the potential disturbance of animals.

Several questions were addressed to investigate the social significance of waterholes for saiga antelopes, and the process of data collection varied accordingly.

The comparison of within-group social behaviour at waterholes and away from waterholes. Since waterholes attract many groups of saiga antelopes, the general level

SOCIAL WATERHOLES OF SAIGA

of social activity at the waterholes may be increased simply because of a higher number of individuals and/or antagonism and competition between strangers. This potential confounding factor was avoided by studying only withingroup interactions.

Within-group social behaviour of saigas was investigated by the waterholes (within a 30-m perimeter from the shoreline) and more than 1 km away from them. The procedure of data collection was similar at the waterholes and away from them. The groups with similar characteristics were investigated to minimize the potential impact of other factors besides the location. The typical size of saiga groups is supposed to be around 30 individuals (<u>Sokolov</u> and <u>Zhirnov 1998</u>); therefore, we chose the groups consisting of 25 to 35 adults for the data collection. Mixed-sex and mixed-age groups were observed as the most socially active. Basic parameters of the group composition were balanced: each group included at least three males (more than one year old) and at least 70 % of females with calves. In addition, for these observations, we applied a narrower time window between 5:30 and 8:30 a.m. (which is the saiga activity peak at the waterhole) to avoid the influence of the rising air temperatures on the intensity of social activities. Overcast and rainy days (very rare) when saiga activity at the waterholes significantly decreased were excluded. To avoid any systematic impact of the changing weather (*e. g.*, increasing average air temperatures as the summer progressed), data recording at the waterholes and away from them were alternated (one day the 'waterhole' observations followed by one day 'away', etc.).



Figure 1. Study site. Bottom right: a digtal map of the northwestern pre-Caspian with a map marker showing the position of the Stepnoi Nature Sanctuary. Top left: an aerial view of the WH1 waterhole. Top right: a camouflaged observation hide.

For further comparison, the behaviour of saiga groups at the waterholes and away from them was video recorded for standardized periods of time. At both types of locations, the groups of antelopes which were at least 100 m away from other saigas were filmed continuously for 10 minutes. Each group was filmed just once. The groups were defined as individuals moving in the same direction and staying together longer than 20 minutes before the beginning of data recording (Sokolov and Zhirnov 1998, Karimova and Lushchekina 2018). In addition, only the animals arriving at the waterhole together were considered as a group (in the case of observations at the waterholes). The distance between the closest individuals within a group typically did not exceed 50 m, except for a short-term separation of adult males chasing each other.

Both at the waterholes and away from them, we only filmed saiga groups showing no signs of disturbance, *i. e.* not running, walking fast or jumping in a particular manner (Sokolov and Zhirnov 1998). Calm slow movement was the most common type of activity recorded. In addition, data recording started at least 10 minutes after the arrival of the group at the waterholes to minimize the impact of arousal associated with drinking. During the further analysis, all clearly distinguishable active interactions between the group members, e. g., male fights, male-female chases, and playful interactions between calves were recorded (Figure 2; see Table 1 for a full list of interactions). The number of occurrences of each social interaction was recorded. Non-obvious subtle social interactions (e. g., approaching, sniffing, side-by-side walking) which cannot be accurately recorded in relatively large and tight groups typical for saigas were excluded. To test whether waterholes facilitate active social interactions within groups, we compared the frequency and variety of social interactions at waterholes and away from them. No interactions between the antelopes from different groups were included in the analysis to avoid the impact of the concentration of unfamiliar individuals at the waterholes.

The impact of social interactions on the staying time at waterholes. Another question we aimed to address was whether utilizing social opportunities influences the duration of saiga visits to waterholes. The focal individuals at the waterholes were traced continuously and their active interactions with conspecifics (Table 1) were recorded. The data were collected only when 60 or more saigas and all sex/ age classes (adult females, one-year-old males, adult males and calves) were present at the waterhole, that is, the individual's social opportunities were abundant. In the case of males, each observed individual was photographed using a camera with a long-focus lens for individual identification to avoid repeated observations of the same individuals. Photo-identification was based on a set of natural markings such as scars, and individual features in colouration, shape and size of horns as described elsewhere (Giljov et al. 2019). Younger (one-year-old) and adult (\geq 2 years old) males recognized by the horn size were considered separately because of the likely differences in their social behaviour. In females, reliable individual identification was not possible except for a few individuals with remarkable colouration features. Only females with calves were included as they significantly prevailed in the population during the study period. Females with calves rarely engaged in active social interactions with other individuals besides their calves, therefore we recorded not their own interactions but the interactions of their calves.

To test the influence of the social experience on the visit duration we compared the time spent at the waterhole in males which interacted and did not interact with others during the first three minutes at the waterhole. It is plausible that the longer the individual stay the more chances to interact. We, in contrast, aimed to test whether the individuals interested in socializing choose to stay longer. Therefore, the time for the recording of the social interactions was standardized (three minutes) to avoid the effect of the duration of the stay on the probability of social interactions. However the total time spent at the waterhole by focal individuals was scored. In the case of females, the duration of the visit was compared between the females whose calves interacted with other calves and the females whose calves did not interact with age-mates during the first three minutes after arrival at the waterhole.

The comparison of group sizes when entering and leaving the waterholes. Finally, we tested whether visits to the waterholes favor social grouping. The number of individuals in the groups entering and leaving the waterhole area was recorded. The group sizes were compared. The cases when only one group was present at the waterhole were not included in the analysis as there was no opportunity to form a larger group.

In addition, we investigated whether the drinking itself works as a factor favoring spatial aggregation of individuals at waterholes. Solitary individuals may be particularly vulnerable during drinking when they are not able to monitor the surroundings and must group with mates

Table 1. The types of active social interactions recorded within saiga groups.

Behaviour	Interactors		
Fight	male-male		
Chase	male-male		
Jump	male-male		
Snout touch	male-male		
Displace from the resting place	male-male		
Jump	female-female		
Snout touch	female-female		
Displace from the resting place	female-female		
Play-chase	calf-calf		
Jump	calf-calf		
Inspection	calf-calf		
Chase	male-female		
Snout touch	female-calf		
Inspection	male-calf		

to rely on their vigilance. We recorded whether a single individual approached a group prior to starting drinking or stayed alone. The following situations were observed: a single individual arrived at the waterhole when a group of 15–35 individuals was already present by the water. Smaller groups could potentially be not enough for social attraction, while larger groups may occupy a significant part of the shoreline and leave the arriving individual not much spatial choice. We included only the single individuals who approached the waterhole from a different direction (the movement directions of the group and the solitary individual forming 90 to 270° angle) than did the already present group to minimize the chances that this individual is a member of the same group. Approaching was defined as drinking at 20 m or less from the closest individual. Whether single individuals preferentially started drinking near the group of saigas was tested with a binomial z test.

The normality of continuous data was assessed using a Shapiro-Wilk test. Since the data deviated significantly from normality, a non-parametric Mann-Whitney U test was used for comparisons. The statistical analyses were conducted in JAMOVI v2.3.28 software <u>https://www.jamovi.org</u> (Gallucci 2019). The α level was set at P < 0.05.

This purely observational study was conducted in several study sites in the protected nature reserve using longlasting stationary camouflaged hides for animal observation and population monitoring.

Ethical statement. The ethical permission for the study was obtained from the St. Petersburg State University ethical committee (permit no. 131-03-3). Work was conducted with the approval of Stepnoi State Nature Sanctuary authorities. The study was purely observational. The observations were conducted from the hide that kept the disturbance of the antelopes to a minimum.

Results

For the comparison of saiga social behaviour by the waterholes (two sites) and more than one km away from them (two sites), 2 513 within-group social interactions were recorded in 204 groups of saiga antelopes (see Supplementary material Tables). Analysis of the frequency and variety of active social interactions between the group members near the waterholes (112 groups) and away from them (92 groups) revealed significant differences. Interactions occurred more often near the waterhole (WH) than away from it (near: median = 15, IQR = 24.0, away: median = 5, IQR = 7.25; Mann-Whitney *U* = 2263, *P* < 0.001). This difference was revealed for both waterholes (near: median = 18, IQR = 27.0 vs. away: median = 4, IQR = 8.0, Mann-Whitney U = 621, P < 0.001, WH1; and *near*: median = 7.5, IQR = 5.5, *away*: median = 5, IQR=6.0, Mann-Whitney U = 479, P = 0.028, WH2). Similarly, a greater variety of social interactions were recorded near the waterholes than away from them (Mann-Whitney U = 2629, P < 0.001) with both waterholes showing the same pattern (WH 1: Mann-Whitney U = 784, P < 0.001;

Continuous observations of focal individuals showed that engagement in active social interactions affected the duration of saiga visits to the waterhole. One-year-old males (n = 114) which interacted with conspecifics stayed at the waterhole significantly longer (median time = 13 min, IQR = 14.5) than those who did not (median time = 7 min, IQR = 9.5, Mann-Whitney U = 1000, P = 0.002). In adult males (n = 72) also, the individuals which engaged in social interactions spent more time at the waterhole (median time = 10 min, IQR=17.0) than males who did not interact with others (median time = 6.5 min, IQR = 6.3; Mann-Whitney U = 394, P = 0.030). In females with calves (n = 53), the influence of calves' social involvement was found. Females whose calves participated in social interactions with other calves stayed at the waterhole significantly longer (median time = 8.0 min, IQR = 6.0), than the females whose calves did not interact with age-mates (median time = 5.5 min, IQR = 4.5; Mann-Whitney U = 183, P = 0.028).

The comparison of the number of individuals in any group entering (n = 214) and leaving (n = 141) the waterhole area showed significant differences. The groups of leaving saigas were larger than the groups of arriving individuals at both WH1 (arriving: median group size = 29 individuals, *IQR* = 55.3: leaving: median group size = 75 individuals, *IQR* = 132.0; Mann-Whitney U = 5517, P < 0.001) and WH 2 (arriving: median group size = 8 individuals, *IQR* = 10.0; Mann-Whitney U = 636, P = 0.033).

Analysis of the behaviour of solitary saigas coming at the waterholes showed that, in the presence of a group of conspecifics by the water, such individuals did not demonstrate a preference to approach the group for drinking (WH 1: 39 out of 52, binomial z = 1.26, P = 0.208; WH2: 16 out of 23, z = 0.96, P = 0.337).

Discussion

The results of the present study suggest that in saiga antelopes, large permanent waterholes serving also as mineral licks facilitate social interactions, at least among group mates. Both the frequency and variety of active social interactions within groups were increased at the waterholes, as compared to the steppe environment away from the water. A special effort was made to minimize the potential impact of other factors besides the location: data collection at the waterholes and away from them was conducted in the same season and time of the day, while the social opportunities were balanced. The greater frequency and variety of interactions between the individuals cannot be explained by the novel or increased social opportunities at the waterholes as only intragroup interactions, *i. e.* interactions



Figure 2. Saiga antelopes at the waterhole (A) A group of males drinking and licking mineral-rich soil on the lacking vegetation banks of the waterhole. B) 'Male-male fight': two males fighting with their horns crossed. C) 'Calf-calf jump': a calf standing on its hind limbs and trying to jump on the other calf's back. D) 'Male-male jump': a male standing on its hind limbs and trying to jump on the other calf's back.

between group members, were included in the analysis. The groups of similar size and composition were observed at the waterholes and away from them, that is, the revealed difference in the social activity is not a result of the greater number and variety of social partners at the waterholes. Since we only analyzed the interactions between saigas which came to the waterhole together as a group, the elevated frequency of interactions cannot be explained by the antagonism between strangers or competition between different groups.

There are several plausible explanations for why waterholes facilitate social interactions between group members. First, the physical characteristics of the steppe waterholes may provide the optimal conditions for the manifestation of social behaviours. The water mirror is surrounded by relatively large areas of flat hard ground lacking vegetation (due to the soil salinity), which provide good visibility of both conspecifics and potential predators. In addition, hard substrate lacking obstacles facilitates fast movements, such as running and bouncing, common during saiga interactions. Another positive factor may be the physical condition of the saigas themselves. Arriving at the waterhole, saigas usually drink and lick minerals first and then start interacting while keeping drinking and licking from time to time until leaving. Having satisfied the need for water and minerals and staying close to their source (to get more if needed), saigas may be more prone to participate in active physically demanding social interactions. The artesian well water coming from the underground and the breeze over the water surface could help saigas to lower body temperature in the absence of shade. This could serve as an additional positive factor for the manifestation of social activity.

Whether the individual entered into social interactions may influence the duration of saiga visits to the waterholes. Both young and adult males that interacted with others during the first three minutes at the waterhole stayed significantly longer than those who did not. In females with calves, a more sophisticated version of this tendency was found: the mothers whose calves engaged in social interactions with age-mates in the first three minutes after arrival stayed at the waterhole significantly longer than the mothers whose calves did not interact with other calves during this time interval. All individuals were observed in contexts with abundant social opportunities, that is, the absence of interaction cannot be explained by the absence of social opportunities. Therefore, our results suggest that saigas modify their visit duration according to their social incentives, *i. e.* the individuals that are prone to participate in social activity stay longer to maximize their social opportunities, while those who do not – leave earlier. In the case of females with calves, mothers may choose to stay longer to favor their calves' socialization.

Much like saiga antelopes, forest elephants form social gatherings at places offering them scarce nutritional resources (Klaus et al. 1998; Turkalo et al. 2013). In line with the results on saigas, forest elephants modify the duration of their visits to forest clearings to facilitate their involvement in social interactions. Thus, despite ecological and behavioural differences, saigas and forest elephants display a similar tendency to the management of social opportunities at resource hotspots. In elephants, the novelty of the social opportunities plays a key role: individuals increase their visit duration when they associate with conspecifics outside their ranging party (Fishlock and Lee 2013), while in saiga antelopes, the participation in social interactions with members of their group may be enough to motivate the individuals to stay longer. Intriguingly, recent study on Baird's tapir, Tapirus bairdii, revealed that water bodies (aguadas) serve as important place for various social interactions of this predominately solitary species. Taking into account that Malayan tapirs gather near salt licks which facilitate male-female social behaviour (Tawa et al. 2021), further research is needed to determine whether these watering/salt-licking sites can be utilized by tapirs as social arenas (Reyna-Hurtado and Arias-Domínguez 2024).

During the period of study, saigas visited the waterholes intensively and, in many cases, several groups were simultaneously present, providing the opportunity to form larger aggregations. The comparison of mean group sizes showed that saigas left waterhole areas in larger groups than they entered them. That is, group merging took place at the waterholes. In nomadic species with large home ranges, such as saiga antelope, places with a high probability of meeting conspecifics could be particularly important providing aggregation opportunities. Long-lasting resource hotspots such as waterholes and mineral licks may potentially play an important role in the fission-fusion social dynamics of otherwise highly dispersed groups.

The tendency for social attraction was not evident in individuals coming to the waterholes alone. In the presence of a group of conspecifics, many solitary individuals did not approach the group for drinking. As saiga antelope is a highly social species with most individuals in the study population living in groups (Karimova and Lushchekina 2018), the absence of social attraction in solitary individuals could be a byproduct of a specific condition and/or status of these animals. For example, a significant portion of individuals coming at the waterholes alone could be sick or exhausted which prevents them from joining the group. Alternatively, their low social status may explain their hesitance to interact with others. Unfortunately, too little is known about the saiga social hierarchy to provide a more confident interpretation of this result.

Social benefits provided by social arenas can vary according to species-specific social requirements. If saigas visiting social arenas may potentially benefit from both socialization and group merging, forest elephants appear to use gatherings at forest clearings specifically to develop important social relationships that would otherwise be lacking. Groups formed in the clearing were larger than ranging groups, and individuals were specifically attracted to these large groups (Fishlock and Lee 2013). However, unlike saiga antelopes, forest elephants did not form larger groups when leaving the clearing, as most animals exited the clearing with those same conspecifics with whom they arrived. This could be explained by the difference in social organization, in particular a greater stability of group composition in forest elephants.

Our findings support the hypothesis that resource hotspots may function as social arenas in species inhabiting open landscapes. Among mammals, resource hotspots such as mineral licks have been found to be used for socialization in, e. g., moose (Alces alces) in the forests of North America (Couturier and Barrette 1988), Asian elephants (Elephas maximus) in the forests of South East Asia (Hii 2017), babirusa (Babyrousa babyrussa) in the forests of Sulawesi (Clayton and MacDonald 1999), bongo antelope (Tragelaphus eurycerus; Klaus-Hügi et al. 2000), forest elephants (Turkalo and Fay 1995, Fishlock et al. 2008; Fishlock and Lee 2013) and forest buffalo (Syncerus caffer nanus; Melletti et al. 2007) in the forests of Central Africa. Since such social arenas have been found predominantly in forest dwelling species, it may be hypothesized that the social significance of mineral licks and other resource hotspots, typically situated in the forest clearings, is specifically associated with higher visibility, contrasting with the forest environment and favoring interactions between conspecifics. Our results in saiga antelopes emphasize that a closed habitat is not a necessary condition for the emergence of such social arenas. The emergence of specific locations having particular social significance may be important for highly dispersed species with large home ranges irrespective of the habitat type (open or closed). In addition, the increased level of social activity at waterholes and mineral licks may be associated with the improved physical and emotional condition of animals, which has satisfied their needs for water and minerals.

The results of the study emphasize the significance of large permanent waterholes and mineral licks used by many individuals year after year for the social behavior of saiga antelopes and likely many other nomadic ungulates inhabiting open landscapes. Their presence and condition should be considered as important factors for the success of conservation efforts such establishment and management of protected areas for threatened species (Klaus-Hügi et al. 2000; King et al. 2016).

Acknowledgements

We would like to thank Anna Lushchekina and the staff of Stepnoi State Nature Sanctuary, and especially Vladimir Kalmykov and Galina Kalmykova, for their valuable organizational support and assistance during data collection. This work was supported by the Russian Science Foundation (grant no. 22-24-00403).

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Associated editor: Rafael Reyna Submitted: December 3, 2023; Reviewed: January 8, 202 Accepted: March 27, 2023; Published on line: May 7, 2024

Supplementary material

https://mastozoologiamexicana.com/therya/index.php/ THERYA/article/view/5908/1423

Molecular cytogenetics markers reveal the existence of a cryptic complex of *Mazama temama* species

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Mazama temama, commonly named Central American red brocket deer, was described in Veracruz, east-central México. Cytogenetic studies have characterized differentiated karyotypes observed in captive individuals of the species with a diploid number (2n) = 49 to 50, in a recently collected neotype with a diploid number (2n) = 44 and fundamental number (FN) = 70, and in specimens collected in Campeche in the southeast of México with a diploid number (2n) = 47 and fundamental number (FN) = 70. Then, we used BAC probes derived from cattle genome aiming to describe the chromosomal differences in *M. temama* karyotypic variants. We compared three individuals from Campeche (CAM) and the Veracruz neotype (VER). A total of 38 clones have been mapped by fluorescent *in situ* hybridization onto the chromosomes of both variants and a comparative map has been established. We assessed Cytochrome b (Cytb) gene sequences to perform phylogenetic analyzes including *M. temama* individuals from these localities and other Neotropical deer. The integrated analysis of hybridization results showed the real and surprising differences between the specimens. Besides the morphological similarities between the *M. temama* specimens, the results showed a difference of 10 chromosomes involved in rearrangements that separate their karyotypic composition, associated with tandem and centric fused chromosomes. Bayesian Inference tree evidenced Campeche and Veracruz individuals in two separated subclades within *M. temama* clade. The observed chromosomal and genetic differences are a substantially evidence of a reproductive isolation mechanism between the Veracruz and Campeche individuals suggesting the existence of a cryptic complex of species under *M. temama* nomenclature. Therefore, conservation strategies should be considered separately for each population of central American red brocket deer.

Mazama temama es conocido comúnmente como temazate rojo y fue descrito en Veracruz en el centro-este de México. Estudios citogenéticos han caracterizado cariotipos diferenciados observados en individuos de cautiverio con un número diploide 2n = 49 a 50, en el neotipo recientemente colectado con un número diploide 2n = 44 y número fundamental NF = 70 y en los especímenes colectados en Campeche en el sudeste de México con un número diploide 2n = 47 y FN = 70. De tal forma, utilizamos sondas BAC derivadas del genoma de bovino con el objetivo de describir las diferencias cromosómicas entre las variantes cariotípicas de *M. temama* comparando tres individuos de Campeche (CAM) con el neotipo de Veracruz (VER). Fueron mapeados un total de 38 clones a través de hibridación fluorescente *in situ* en los cromosomas de ambas variantes y de esa forma, un mapa comparativo fue establecido. Se accedió a secuencias de gene Citocromo b (Cytb) para realizar análisis filogenéticos incluyendo individuos *M. temama* de esas localidades y otros cérvidos neotropicales. Los resultados del análisis integrado de hibridación mostraron las diferencias reales y sorprendentes entre los especímenes. A pesar de las semejanzas morfológicas entre los individuos *M. temama*, fue observado que 10 cromosomas diferentes están involucrados en rearreglos que separan la composición cariotípica de ambos grupos. Tales rearreglos se corresponden con una fusión en tándem y fusiones céntricas. El árbol de Inferencia Bayesiana evidenció a los individuos de Campeche y Veracruz en dos subclados separados dentro del clado *M. temama*. Las diferencias observadas son una evidencia sustancial de un mecanismo de aislamiento reproductivo entre los individuos de Veracruz y Campeche revelando la existencia de un complejo de especies crípticas bajo la nomenclatura *M. temama*. Por tal motivo, deben ser consideradas estrategias de conservación para cada población de temazate rojo centroamericano.

Keywords: BAC probe; cervids; fluorescent in situ hybridization; karyotype; Central American red brocket deer.

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Introduction

Cytogenetic analyzes have been a very important tool for species delimitation of Neotropical deer, especially those belonging to the genus *Mazama*. This is associated with the existence of cryptic species complexes, which are almost exclusively differentiated by chromosomal changes (Duarte et al. 2008). Several studies consider the morphological similarities among *Mazama* as adaptative convergence that is not correlated with karyotypic a molecular

variation (<u>Duarte et al. 2008; Abril et al. 2010;</u> <u>Cifuentes-</u><u>Rincón et al. 2020;</u> <u>Peres et al. 2021</u>).

The great chromosomal variation in the group was first studied by <u>Duarte and Jorge (1996)</u> revealing the cryptic diversity of *M. americana* species and describing karyotypical variants in Brazil. Thus, it allowed the identification of cytotypes from two distinct chromosomal lineages (<u>Abril et al. 2010</u>). The analysis of the *M. americana* neotype showed its karyotypic pattern with considerably chromosomes dif-

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ferences from the Brazilian cytotypes and subsequently, recognized that these variants should be considered as different species (<u>Cifuentes-Rincón et al. 2020</u>, <u>Peres et al. 2021</u>).

Cytogenetic techniques also contributed with the taxonomic differentiation of *Subulo gouazoubira* and *Passalites nemorivagus*, showing a high frequency of B chromosomes and a fused X-autosome that formed the multiple sexual chromosome system in *P. nemorivagus* (Resende *et al.* 2012). Also, Abril and Duarte (2008) considered that centric fusions and quantities of B chromosomes variation are involved in the eight different karyotypes described for *M. nana*. The authors suggested that the species has not reached its optimal karyotypic evolutionary state accumulating rearrangements by selective pressure (Abril and Duarte 2008).

Thus, cytogenetic differences in the genus *Mazama* are a surprising characteristic especially when correlated with its small morphological diversification (<u>Duarte *et al.* 2008</u>; <u>Abril *et al.* 2010</u>). The chromosomal variation is closely related to speciation within the group, since karyotype differences are efficient reproductive barriers and isolate potentially sympatric populations (<u>Cursino *et al.* 2014</u>; <u>Salviano *et al.* 2017</u>). Differences greater than two pairs of chromosomes between individuals have a significant impact on the offspring fertility, that when crossed referenced with each other, producing infertile individuals or with serious fertility problems, although they cannot be morphologically distinguished (<u>Cursino *et al.* 2014</u>; <u>Salviano *et al.* 2017</u>; <u>Galindo *et al.* 2021a, b).</u>

In this context, the recognition of *M. temama* as a valid species was also based on karyotypical analyses. Jorge and Bernishke (1977) described cytogenetics features of three captive specimens with known origin in Tamaulipas, México. In these specimens the observed diploid number was (2n) = 49 to 50 and fundamental number (FN) = 70contrasting with the 2n = 68 and FN = 74 of *M. americana* (Taylor et al. 1969) from whom it was considered subspecies (Hershkovitz 1951). The classical cytogenetic characterization of a recent neotype of *M. temama* from Veracruz, México, allowed the description of a karyotype with 2n = 44and NF = 70 and detected variant karyotypes with 2n = 46to 47 and NF = 70 from specimens collected in Campeche, in the southeast of México (Sandoval et al. 2022). The authors associated the karyotypical differences with two fused acrocentric pairs in the Veracruz neotype compared with the Campeche specimens, considered as more ancestral karyotype, and with an additional heterozygotic centric fusion in pair 11 of Campeche's female (Sandoval et al. 2022). Understanding this karyotypical divergence could be relevant in a context in which recent studies suggest the morphological and ecological differences between the M. temama from Guatemala and México, compared with populations from Nicaragua, Honduras, Costa Rica and Panamá (Escobedo-Morales et al. 2023).

To confirm those rearrangements, the association of the G Band with molecular cytogenetics is a promissory technique since the karyotypical study of *Mazama* species requires high quality bandings and, even in this condition, the classical cytogenetic analyzes can lead to misunderstanding (Bernegossi et al. 2022). Mapped cattle chromosome probes have been described as a strategy to optimize chromosomal analysis and its efficiency in Neotropical deer species was already confirmed (Galindo et al. 2021a). The Whole Chromosome Probes from bovine fluorescently labeled have been employed to characterized inter-chromosomal rearrangements, and the region-specific probes derived from microdissection or DNA clones as in Bacterial Artifical Chromosomes (BAC) provides information on intra-chromosomal rearrangements (Frohlich et al. 2017; Romanenko et al. 2017). Recently, Bernegossi et al. (2022, 2023) characterized both types of probes derived from the entire set of cattle chromosomes in S. gouazoubira and demonstrated that this species had the same pattern of rearrangements proposed by Dementyeva et al. (2010) and Frohlich et al. (2017) for Cervidae. Moreover, mapped cattle chromosomes were used to compare M. americana neotype and Paraná cytotype, supporting the description of M. rufa as a distinct species with at least 15 rearrangements with *M. americana* species (Peres et al. 2021).

Then, we used BAC probes derived from cattle genome aiming to describe the chromosomal differences in *M. temama* karyotypic variants, comparing the male neotype from Veracruz and three individuals from Campeche. We also analyzed the mitochondrial Cyt-b gene phylogeny including individuals for these Mexican populations.

Material and methods

Animal samples and metaphases preparation. The chromosomal preparation was obtained from the cell culture procedures described in <u>Sandoval et al.</u> (2022) from *M. temama* samples of specimens collected in México, permission for collection SGPA/DGVS number 06821. The identities and localities of collection of each sample used for molecular cytogenetic also follows <u>Sandoval et al.</u> (2022): A) a male neotype (T366) from Veracruz, collected in a location called San José Acualco, coordinates 18° 38' 53" N and -96° 56' 57" W. B) a male (T363) and a female (T362) adult from Campeche, collected in Zoh Laguna, near the Calakmul Reserve, coordinates 18° 35' 32.2" N and -89° 24' 55.3" W. C) a male fetus (T364) from Zoh Laguna, Campeche.

Probe acquisition and labeling. Fluorescence *in situ* hybridization (FISH) using BAC probes was performed to characterize the homologies between the karyotype of *M. temama* from Veracruz (VER) and *M. temama* from Campeche (CAM).

Bacterial Artifical Chromosomes clones acquisition followed the procedure described by <u>Bernegossi *et al.*</u> (2022), for each selected probe from the cattle CHORI-240 library based on the NCBI ARS-UCD1.2. Assembly data and obtained from BACPAC Genomics, Emeryville, CA, USA (Table 1). After DNA extraction, probes were purified using a Wizard[®] Plus SV Minipreps DNA Purification Systems kit, and BioPrime[®] Array CGH Genomic Labeling (Invitrogen, Carlsbad, CA, USA) kit was used to labeled BAC DNA with Green-DdUTP (Abbott, IL, USA), biotin 16-dUTP or digoxigenin-11-dUTP (Roche, Mannheim, Germany).

Fluorescence in situ hybridization. FISH was performed using the BAC probes on karyotypes of the *M. temama* specimens studied here. FISH was performed as presented in <u>Vozdova et al. (2019</u>), according to this protocol, the slides with metaphase spreads were incubated in 2×SSC at 72 °C, denatured in 0.07M NaOH, and dehydrated in ethanol series. The probe mixtures were denatured separately and applied on the denatured slides. A Zeiss AxioCam MRm camera attached to an Olympus BX60 microscope, equipped with appropriate fluorescence filters for the visualization of FISH results, was used.

Phylogenetic analysis. We assessed the mitochondrial gene Cytochrome B (Cyt-b) sequences for Neotropical deer available in the Genbank, including *M. temama* individuals from Veracruz and Campeche localities in México (Table 2). All sequences were aligned using the Clustal X program (Thompson *et al.* 1997). We followed the Akaike information

criterion AICc (<u>Akaike 1973</u>) and selected the best molecular evolution model using the jModelTest v. 0.1.1 (<u>Posada</u> and <u>Crandall 1998</u>). Bayesian inference (BI) analysis was performed using MRBAYES 3 (<u>Huelsenbeck and Ronquist</u> 2001) with 10,000,000 generations, sampling every 1,000 generations and a variance of <0.01. A burn-in of 25 % was used and the consensus tree was constructed. Tree topology was recovered using FigTree v.1.4.0 (<u>Rambaut 2012</u>).

Results

The karyotypes of the male neotype from Veracruz and both adult specimens from Campeche locality are shown in Figure 1. Due to the similarity of diploid and fundamental number between the fetus and adult male from Campeche, we only show the karyotype of the adult specimen. We observed that these *M. temama* male individuals (2n = 46) differ from the female of the same locality (2n = 47) by one chromosomal translocation (Figure 1B, 1C).

The comparative analysis by FISH confirmed a karyotypic distinction among *M. temama* individuals from Campeche. Specifically, both males presented a centric fusion in a homozygous state, forming pair 3, while the

Table 1. List of BAC clones from the CHORI-240 cattle (BTA) library and their correspondence in *Mazama temama* neotype from Veracruz (VER) and *M. temama* individuals from Campeche (CAM) chromosomes.

VER	САМ	BTA chromosome	BAC clone (position Mb)	VER	САМ	BTA chromosome	BAC clone (position Mb)
1p	8p	25	89A17(21.27-21.51)	9p	9p	2	124N14 (96.51-96.71)
							437C7 (135.51-135.71)
1qa	8q	12	68F22 (11.10-11.33)	9q	9q	6	46O3 (61.12-61.34)
1qb	6q	11	98M8 (3.70-3.92)	10p	6р	16	140117 (80.23-80.49)
2p	1p	19	50L8 (34.35-34.57)	10q	10p	5	78L8 (70.86-71.06)
2qa	1qa	7	57013 (1.36-1.56)	11p	15	23	77G24 (28.26-28.47)
2qb	1qb	17	63H8 (3.90-4.12)	11q	2р	22	155B17 (59.06-59.29)
3р	3р	18	105L6 (65.58-65.76)	12p	11p	1d	69G2 (57.29-57.48)
3q	3q	3	106P15 (98.75-98.97)	12q	11q	1p	109118 (116.68-116.91)
			24H18 (4.38-4.62)			1t	273F5 (154.36-154.55)
4p	4p	8d	504A4 (112.10-112.29)	13	12	26/28	47G11(24.54-24.77)/ 108O21 (24.74-24.94)
4q	4q	4	259C9 (119.29-119.48)	14	13	21	377F11 (33.89-33.98)
5p	10q	15	122B6 (5.29-5.51)	15	14	1c	106N15 (2.08-2.25)
			121K12 (41.87-42.09)				
5q	2qb	10	214M18(103.02-103.24)	16	17	9р	78C10 (60.39-60.60)
6р	5р	5	411D6 (5.81-5.99)	17	18	6t	200F18 (117.34-117.56)
			56D20 (55.50-55.75)				
6q	5q	2	110M8 (44.41-44.65)	18	19	8p	71G4 (62.73-62.93)
7р	16	24	205G22 (0.61-0.82)	19	20	29	103L15 (28.99-29.16)
7q	2qa	20	189H8 (5.16-6.89)	20	21	27	126M6 (41.90-42.14)
8p	7р	13	114C2 (42.80-43.05)	21	22	9t	90A6 (103.38-103.59)
8q	7q	14	319C15 (0.87-1.06)				159016 (23.04-23.22)
							67P21 (33.77-34.00)
				Х	Х	Х	311B9(47.76-47.97)
							316D2 (68.49-68.68)
							40H2 (74.95-75.12)



Figure 1. Mazama temama specimens analyzed cytogenetically. A) Male neotype from Veracruz, México (2n = 44 + 4-5Bs, FN=70 ID: T366). B) female from Campeche (CAM; 2n = 47 + 1-4Bs, FN = 70), ID: T362). C) male from Campeche (CAM; 2n = 46 + 1-4Bs, FN = 70 ID: T363). Groups of chromosomal relative lengths: A - large biarmed autosome; C - small biarmed autosomes; D - large acrocentric autosome; E - small acrocentric autosome). Adapted from <u>Sandoval et al. 2022</u>.

female displayed it in heterozygous (Figure 2A). All other chromosomes, including the composition of sex chromosomes, were homologous in all Campeche individuals.

Upon comparing the *M. temama* neotype (VER) with individuals from Campeche (CAM), various rearrangements were identified in the formation of their karyotypes. We observed that 10 pairs of chromosomes are involved in different rearrangements between VER and CAM karyotypes, involving both tandem and centric fusions (Figure 2): the proximal region of VER1 was homologous to CAM8 (Figure 2B), and the distal region to the shorter arm (q) of CAM6 (Figure 2C); the q arm of VER5 showed homology to the distal region of CAM2 q arm, while the shorter arm (p) of VER5 was homologous to the q arm of CAM10 (Figure 2D);

the p arm of VER7 was homologous to CAM16 while the q arm was homologous to the proximal region of CAM2 q arm (Figure 2E); the p arm of VER10 was homologous to the p arm of CAM6 while the q arm was homologous to the p arm of CAM10 (Figure 2c); finally, the p arm of VER11 was homologous to CAM15 and the q arm to the CAM2 p arm (Figure 2F). The remaining chromosomes in VER were homologous to a sole chromosome in CAM, encompassing the two-armed pairs VER2, 3, 4, 6, 8, 9, 12 and the acrocentric pairs VER13, 14, 15, 16, 17, 18, 19, 20, 21. The composition of sex chromosomes was conserved and homologous between VER and CAM. A summary of these differences is highlighted in schematic Figure 3 by the colored chromosomes pairs. It is important to note that besides B chromosomes have been observed in all *M. temama* individuals, we did not represent it in schematic Figure 3 due to the intra and inter individual variation characteristic of this supernumerary chromosomes.

The Bayesian Inference tree of mitochondrial gene Cyt-b showed *Mazama temama* individuals forming a monophyletic clade separated from the other species of the genus with 98% of branch value (<u>Sandoval et al. 2022; Escobedo-</u><u>Morales et al. 2016</u>). Within *M. temama* clade, the tree topology recovered two subclades (100 % branch value), one formed by the Mexican individuals from Veracruz and the other, by Campeche individuals of the study.

 Table 2. List of mitochondrial gene Cytochrome b (Cytb) used in the phylogenetic analysis.

Species	Access number	Origin
Mazama temama	KP954719	Veracruz, México
Mazama temama	MZ350864	Veracruz, México
Mazama temama	OP712670	Veracruz, México
Mazama temama	MZ362858	Campeche, México
Mazama temama	MW047255	Campeche, México
Mazama americana	MZ350856	Juina, Mato Grosso, Brazil
Mazama americana	MZ350857	Cayenne, French Guiana
Mazama americana	JN632656	French Guiana
Mazama americana	MZ488872	Roraima, Brazil
Mazama rufa	MZ488852	Parana, Brazil
Mazama nana	MZ350863	Paraguay
Mazama nana	DQ789210	Parana, Brazil
Mazama jucunda	MZ350859	Brazil
Mazama rufina	JN632661	Colombia
Odocoileus virginianus	KM612278	México
Odocoileus virginianus	JN632671	French Guiana
Odocoileus hemionus	JN632670	USA
Odocoileus pandora	BK062825	México
Odocoileus Pandora	OQ731410	Campeche, México
Subulo gouazoubira	MZ350858	Paraguay
Subulo gouazoubira	KJ772514	Brazil
Passalites nemorivagus	MT008225	French Guiana
Passalites nemorivagus	MZ350861	Brazil
Ozotoceros bezoarticus	MZ350860	Brazil
Ozotoceros bezoarticus	JN632681	Uruguay
Blastocerus dichotomus	OQ196442	Brazil
Pudu puda	JN632692	Chile
Hippocamelus antisensis	JN632646	Argentina
Rangifer tarandus	KM506758	China

Discussion

Mazama temama species was first named by <u>Kerr (1792)</u> from <u>Hernández (1651)</u> descriptions of Mexican temamaçame (temazate) with type locality designated in El Mirador, Veracruz in east-central México (<u>Hershkovitz 1951; Miller</u> and <u>Kellogg 1955; Cabrera 1960</u>). However, the distribution ranges of the species have been reported from northeastern México to western Colombia, with records in Nicaragua, Guatemala, and Nariño state in Colombia (Allen 1915; Gutiérrez et al. 2017; Ramírez-Chávez et al. 2021). Across its wide distribution, a previous study showed karyotypical variants in México based on a neotype from Veracruz compared with specimens from Campeche, at southeast of México, with more than two pairs of central fused acrocentric chromosomes (Sandoval et al. 2022). Here, the BAC probes derived from cattle genome allowed to record the chromosomal differences between these two populations (Veracruz and Campeche) and confirmed the efficiency of using this technique in Neotropical deer species (Galindo et al. 2021a; Bernegossi et al. 2022). The integrated analysis of hybridization of both karyotypical variants showed the real differences among the specimens with 10 chromosomes involved in rearrangements between them. The karyotypical differences were one tandem fusion, four centric fusions formed by different chromosomes and a heterozygotic centric fusion. Although the occurrence of chromosomal rearrangements has been highly described among Neotropical deer species, mainly centric fusion in heterozygosis, its accumulation has been discussed as decreasing the reproductive fitness by leading to errors in meiosis and recombination suppression (Dobigny et al. 2017; Galindo et al. 2021a; Peres et al. 2021). Other studies have considered the difference of one tandem fusion as deleterious between karyotypes related to the fertility reduction of balanced gamete (Salviano et al. 2017, Galindo et al. 2021). Based on that, the evidence of at least 15 rearrangements, involving centric and tandem fusions, between M. americana and M. rufa supported the recognition of the later as a valid species within Mazama (Peres et al. 2021). Similar to this, the enormous karyotypic differences between Mexican red brockets from Veracruz and Campeche may be efficient reproductive barriers compromising the meiotic pairing between individuals of these populations and leading to the discussion of the biological species concept.

When compared with the karyotype described for captive individuals in San Diego Zoo with probable origin from Tamaulipas in México (Jorge and Bernishke 1977), it is important to consider that the authors misidentified four B chromosomes as acrocentric pairs 23 and 24. A later edition by Duarte and González (2010) allocated one pair of acrocentric autosomes as B chromosomes, however, the variation described between female and male in the previous study together with the small size of the pair 23 indicate that the karyotype should be newly edited to designate these two small acrocentric as B chromosomes. With this reorganization, the diploid and fundamental number of the red brocket deer from Tamaulipas will be corrected to 46 and 72, respectively. This karyotype differs with one more acrocentric pair and one less bi-armed chromosomes pairs from the Campeche individuals of our study. It is important to note that authors have discussed a 2n = 70 as the hypothetic ancestral karyotype of Cervids (Neitzel 1987;


Figure 2. FISH results revealing distinctive chromosomal rearrangements among *Mazama temama* individuals from Veracruz (VER) and Campeche (CAM), as highlighted by the BAC clones specified on the right. A) Chromosomal polymorphism observed in individuals from CAM; T362 displays a fusion in heterozygosity, while T363 exhibits in homozygosity, as demonstrated by BAC105L6 (in pink). Chromosomal correspondence between. B) VER 1 proximal and CAM 8 revealed by BAC 89A17 (in green) and 68F22 (in pink). C) VER 1 q arm and CAM 6 revealed by BAC 98M8 (in pink), and between VER 10 and CAM 6 p arm revealed by BAC 140I17 (in green). D) q arm VER 5 and q arm CAM 2 highlighted by BAC 214M18 (in green), and between p arm VER 5 and CAM 10 p arm revealed by BAC 122B6 (in pink). E) q arm VER 7 and q arm CAM 2 indicated by BAC 189H8 (in pink), and between p arm VER 7 and CAM 16 revealed by BAC 205G22 (in green). F) q arm VER 11 and p arm CAM 2 revealed by BAC 155B17 (in green), and between p arm VER 11 and CAM 15 demonstrated by BAC 77G24 (in pink). The positions of centromeres are demarcated by dashed white lines.

Fontana and Rubini 1990; Dementyeva et al. 2010). Based on this, karyotypic evolution in Cervids is established by chromosomal fusions, such as Robertsonian translocations and tandem fusions, leading to a reduction in the number of chromosomes and changes in the fundamental number of chromosomal arms (Neitzel 1987; Fontana and Rubini 1990). Thus, it may suggest that a different rearrangement, probably a centromeric shift, an inversion of one acrocentric pair, or a tandem fusion between one acrocentric and a bi-armed chromosome, differentiates these karyotypes. FISH study should be performed in metaphasic cells of the Tamaulipas karyotypes described by Jorge and Bernishke (1977) to certainly identify the chromosomal divergences with the Campeche and Veracruz karyotypes. In any case, our results confirm that as other red brockets, M. temama evolved from successive tandem and centric fusions, in complex chromosomal rearrangements involving more than one chromosome (Jorge and Bernishke 1977; Sarria-Perea 2012; Sandoval et al. 2022). It is important to consider that accumulation of chromosomal rearrangements causes meiotic segregation errors with chromosome pairing limitations (Villagómez and Pinton 2008; Dobigny et al. 2017).

Specifically, the difference of more than two pairs of chromosomes has been described as a reproductive barrier in *M. americana* cytotypes, with tandem fusion as the mean causes of reduction in hybrid fertility considering the chance of successful reproduction in a backcross breeding with animals from the parent populations (Cursino et al. 2014; Salviano et al. 2017; Carranza et al. 2018). A recent study evaluated the meiotic segregation of hybrids with one heterozygotic tandem fusion in the *M. americana* com-

plex, including a hybrid between the Carajás and Paraná cytotypes obtaining a rate of ~30 % gametic unbalance (Galindo *et al.* 2021b). Thus, the difference of one tandem fusion between populations is considered an efficient post-zygotic reproductive barrier and it was the mean key to suggest the validation of *M. rufa* species as separated from *M. americana sensu stricto* and Carajás cytoype (Galindo *et al.* 2021b; Peres *et al.* 2021).

The great morphological similarity between the neotype from Veracruz (east-central México) and Campeche individuals (southeast México) analyzed here, contrasted with the chromosomal differences among them. Our findings suggest that central American red brocket should be considered a cryptic complex with morphologically indistinguishable characterization and considerable cytogenetic diversity. The karyotypical rearrangements between *M. temama* populations analyzed here were greater than those that allowed the recognition of *M. temama* and *M. bororo* as valid species (Jorge and Bernishke 1977; Duarte and Jorge 2003).

These karyotypical differences among populations could be associated with geographic isolation array that may have occurred by habitat fragmentation that promoted genetic differences between populations (Abril et al. 2010). Sarria-Perea (2004) considered that chromosome and evolution rearrangements of *Mazama* species occurred at a faster rate than the molecular changes. Thus, the first phylogeographic study of *M. temama* also showed differences in genetic diversity of two Mexican populations of the species, with individuals from Oaxaca with a lower number of polymorphic sites compared to the population of Vera-



Figure 3. Comparative cytogenetics analysis of *Mazama temama* neotype from Veracruz (VER; 2n = 44 + 0 - 4 Bs, FN = 70) and *M. temama* specimens from Campeche (CAM; 2n = 46-47 + 1 - 4 Bs, FN = 70). The colored chromosomes indicate the karyotypes differences, each color representing one homologous chromosome in VER and CAM.



Figure 4. Bayesian inference tree of Cytochrome b (Cytb) gene from several Neotropical deer including *Mazama temama* individuals from Veracruz and Campeche localities in México. Odocoleina subtribe represented by: *M. americana, M. jucunda, M. nana, M. temama, M. rufina* and the genus *Odocoleus*. Blastocerina subtribe represented by *Subulo gouazoubira, Blastocerus dichotomus, Hippocamelus antisensis, Ozotoceros bezoarticus, Passalites nemorivagus,* and *Pudu puda*. Outgroup: *Rangifer tarandus*. The value above the clade represents the posterior probability of the analysis.

cruz (Serna-Lagunes et al. 2021). The authors hypothesized that populations underwent a genetic bottleneck and rapid population expansion, evidencing that geographic and reproductive isolation is due to the low gene flow between the two studied *M. temama* populations (Serna-Lagunes et al. 2021). Our phylogenetic results, in addition to confirm the monophyletic clade of Mexican red brocket within the genus Mazama (Escobedo-Morales et al. 2016, 2023; Sandoval et al. 2022), the Cyt-b tree presented here revealed the existence of potentially genetic structure between Veracruz and Campeche populations with high branch support. A similar result was observed in the phylogenetic analysis of partial ND5 and COI genes that positioned Campeche individuals in a separate subclade relative to one individual from Veracruz, however, the low support values preclude the recognition of existence of genetic structure (Sandoval et al. 2022). The distinction among *M. temama* populations was also discussed when Mexican and northern Central American populations were analyzed, in which a recent study suggested to keep M. t. temama (Kerr 1792) and M. t. reperticia (Goldman 1913) for the two morphological and ecologically differentiated groups based on craniometrical and environmental niche differences (Escobedo-Morales et al. 2023).

The existence of genetic variants between *M. temama* populations could be confirmed by molecular cytogenetic markers as shown in this study, demonstrating they are an efficient tool to study the evolution of brocket deer (Abril et al. 2010; Galindo et al. 2021a, b; Bernegossi et al. 2022). Although the number of samples is limited, our results suggest that central American red brocket populations have differentiated karyotypes. The possibility of crossing these M. temama individuals with these karyotypic differences could produce hybrids carrying tandem fusion and centromeric fusion, both in heterozygosis resulting in increased rates of unbalanced gametes (Galindo et al. 2021a). Thus, based on cytogenetic and molecular data, we assert that these M. temama populations should be individually considered from a management and conservation perspective, but is imperative to classify and taxonomically delimit these species. Therefore, it is important to increase cytogenetic sampling within the entire area of occurrence in order to understand this variation at the population level, and the limits of distribution of each karyotype and species.

The BAC probes were efficient to assist in chromosomal differentiation of *M. temama* individuals. The morphological similarity of the specimens contrasted with great chromosomal rearrangements between karyotypes from both localities, suggesting that *M. temama* could be a cryptic species complex with karyotypical differences ultimately resulting in a reproductive barrier. Conservation strategy efforts should be considered separately for each population of red brocket in México and Central America.

Aknowledgment

We thank Dirección General de Vida Silvestre de Secretaría del Medio Ambiente y Recursos Naturales, México for the official permission SGPA/DGVS/04214/15 to collect them and also deer hunters in Campeche (Zoh Laguna), team from Zongolica and UMA Bellreguart, Sochiapa, in the municipality of Tenampa, Veracruz, México (specially for the help in contacting hunters of its owner Carlos Ros). *This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.* Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided financial support. We also thank João Airton Boer for his support for the laboratory work at the NUPECCE/UNESP.

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Associated editor: Eduardo Mendoza

Submitted: June 21, 2023; Reviewed: August 21, 202 Accepted: February 21, 2023; Published on line: May 16, 2024 CYTOGENETIC OF Mazama temama

Land-use change and habitat fragmentation of *Leopardus pardalis* in Highlands of Puebla, Mexico

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Ocelots are relatively tolerant to habitat modification. However, it has been observed that they may be sensitive to habitat loss and fragmentation as a result of human development. The Sierra Norte de Puebla is considered a potential habitat for ocelots and other tropical felines. However, it has suffered heavy loss and fragmentation of its natural ecosystems, which considerably affects habitat availability and quality. This study analyzed the land-use changes and habitat fragmentation in the distribution range of the ocelot in the Sierra Norte de Puebla, Mexico, from 1993 to 2020. Habitat suitability was determined using potential distribution models and vegetation and land-use maps from 1993, 2003, and 2020, obtained using supervised classification of Landsat images. The resulting maps were reclassified in terms of the habitat suitability for Leopardus pardalis according to their quality. Land-use changes and habitat loss were quantified with a transition matrix, and fragmentation was assessed using the Morphological Spatial Pattern Analysis tool of the program GUIDOS. These habitat fragments were quantified using the integral index of connectivity with the CONEFOR Sensinode program. We estimated that 22.2 % of the study area has suitable conditions for ocelots. From 1993 to 2020, the area covered by natural vegetation decreased 16 % at an annual rate of -2.4 %. During this period, the mean fragment size and shape index decreased; on the other hand, the number of fragments and the Euclidean distance between fragments increased. The percentages of edge, branch, and islet vegetation dropped from 1993 to 2003 but increased in 2020. The connectivity analysis indicated that two habitat fragments showed high values of the integral index of connectivity. The increase in anthropogenic cover and the habitat loss for L. pardalis between 1993 and 2020 affected mainly vegetation fragments considered optimal for the persistence of this species. In these ecosystems, agricultural and livestock practices are expanding vigorously, increasing edge habitats and decreasing the core area of habitat fragments. The areas that recorded the presence of ocelots have optimum conditions to serve as biological corridors in the Sierra Madre Oriental, particularly in the portion of the Sierra Norte de Puebla. The fragmentation of the ocelot habitat is of particular concern and should be addressed strategically for the long-term conservation of the ocelot and regional biodiversity.

Los ocelotes presentan cierta tolerancia a la modificación de su hábitat, sin embargo, se ha observado que pueden ser sensibles a la pérdida y fragmentación como resultado del desarrollo humano. La Sierra Norte de Puebla se considera con potencial para la distribución del ocelote y otros felinos tropicales; no obstante, la pérdida y fragmentación de sus ecosistemas es alta y genera un efecto significativo en la disponibilidad y calidad del hábitat. En este estudio se analizó el cambio de uso de suelo y la fragmentación del hábitat del ocelote en la Sierra Norte de Puebla, México de 1993 a 2020. Se determinó la idoneidad de hábitat del ocelote mediante modelos de distribución potencial y mapas de vegetación y uso de suelo de 1993, 2003 y 2020, obtenidos mediante una clasificación supervisada de imágenes Landsat. Los mapas derivados fueron reclasificados según la aptitud de hábitat de Leopardus pardalis de acuerdo con su calidad. Se cuantificó el cambio y pérdida de hábitat mediante una matriz de transición y se evalúo la fragmentación con la herramienta Morphological Spatial Pattern Analysis del programa GUIDOS. A estos fragmentos de hábitat se les cuantificó el Índice Integral de Conectividad con el programa CONEFOR Sensinode. 22.2 % de la superficie total de la zona de estudio tiene condiciones para la presencia del ocelote. De 1993 a 2020 la superficie de vegetación disminuyó 16% a una tasa de cambio de -2.4 %. Durante este periodo el tamaño medio del fragmento e índice de forma disminuyeron; en cambio, el número de fragmentos y la distancia euclidiana entre fragmentos se incrementaron. El porcentaje de borde, rama y relicto disminuyeron de 1993 a 2003, aunque aumentaron en 2020. El análisis de conectividad indicó que dos fragmentos de hábitat presentaron valores altos del índice de conectividad integral. El incremento en la superficie de las coberturas antrópicas y pérdida de hábitat de L. pardalis entre 1993 y 2020, se concentró en fragmentos de vegetación considerados óptimos para la persistencia de esta especie. En estos ecosistemas el avance de las prácticas agrícolas y ganaderas es alto, lo cual contribuye a un incremento en el hábitat de borde y una disminución en el área interior de los fragmentos de hábitat. Las áreas en las que se registró la presencia de ocelotes tienen las condiciones ideales para que éstas funcionen como corredores biológicos en la Sierra Madre Oriental, particularmente en la porción de la Sierra Norte de Puebla. La fragmentación del hábitat del ocelote es especialmente preocupante y necesita abordarse estratégicamente para la conservación a largo plazo, no solo del ocelote, sino de la biodiversidad de la región.

Keywords: Carnivores; connectivity; conservation; deforestation; optimal habitat; landscape; habitat loss.

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Introduction

Deforestation and fragmentation are among the main threats to natural ecosystems worldwide, with adverse effects on biotic communities (Armenteras *et al.* 2003; Lindenmayer and Fischer 2006; Haddad *et al.* 2015). Longterm fragmentation increases population isolation and decreases connectivity, with consequences for species survival, abundance, and dispersal, as well as for functional connections between habitat fragments (Ewers and Didham 2006; Scolozzi and Geneletti 2012; Gao *et al.* 2013). The functional diversity of wild mammals has been observed to decrease as land-use has become more intensive, and 70 % of species respond negatively to fragmentation (Flynn *et al.* 2009; Thornton *et al.* 2011), to the extent that one-quarter of all mammal species are at risk of extinction (Ceballos and Ehrlich 2002; Baillie *et al.* 2010).

Among mammals, carnivores are one of the groups most vulnerable to habitat loss, fragmentation, and alteration (Fahrig 2003; Michalski and Peres 2005; Crooks *et al.* 2011; Dotta and Verdade 2011) due to their low population density, high habitat requirements, and low reproductive potential. Consequently, populations of terrestrial carnivores have experienced major declines, and their ranges have shrunk (Ripple *et al.* 2014). Particularly, felines face a high risk of extinction as a result of habitat fragmentation and loss (Treves and Karanth 2003; Zanin *et al.* 2015). According to the IUCN Red List (2012), habitat loss and fragmentation affect all 36 species of wild felines and are the main threats to 21 species.

The ocelot is a forest species (Harveson et al. 2004; Jackson et al. 2005; Haines et al. 2006) that can use multiple habitats depending on availability (De Oliveira et al. 2010; Fusco-Costa et al. 2010), including disturbed and undisturbed habitats (Kolowski and Alonso 2010), although it prefers areas with closed canopy and dense vegetation (López-González et al. 2003; Harveson et al. 2004; Jackson et al. 2005; Haines et al. 2006; Martínez-Calderas et al. 2011; Torres-Romero et al. 2017; Galindo-Aguilar et al. 2019). Several studies indicate that the presence of the ocelot is adversely affected by local disturbances, such as land-use changes and forest fragmentation (Garmendia et al. 2013; Cruz et al. 2018; Wang et al. 2019; Lombardi et al. 2022). Habitat fragmentation can influence the home range of the ocelot (Cruz et al. 2019) by reducing the area of habitat fragments (Garmendia et al. 2013; Lombardi et al. 2020). It alters the connectivity between populations by increasing the isolation of habitat fragments and reducing the availability of corridors (Tewes and Everett 1986; Jackson et al. 2005; Haines et al. 2005; Galindo-Aguilar et al. 2019), which contribute to the demographic instability of the species (Reed 2004; Janecka et al. 2014).

In Mexico, the ocelot is distributed in the coastal areas of the Pacific and the Gulf of Mexico, in semi-arid regions with xeric and semitropical scrubland, tropical and subtropical forests, and temperate forests (<u>Aranda 2005</u>; <u>Martínez-Calderas et al. 2011</u>). In Puebla, its presence has been docuCastillo et al. 2020); Ramírez-Bravo et al. (2010a) confirmed the presence of ocelots in the Sierra Norte, in tropical evergreen and sub-evergreen forests, which may indicate the mobility of the species across the Sierra Madre Oriental. This region has been identified as critical for the dispersal of the ocelot and other tropical felines and is considered a priority biological corridor from north to south (Ramírez-Bravo et al. 2010b; Hernández-Flores et al. 2013; Dueñas-López et al. 2015; Cacelin-Castillo et al. 2020). However, the Sierra Norte de Puebla (SNP) experienced a 30 % habitat loss and fragmentation between 1980 and 2000 due to the growing human population and agricultural expansion (Guevara-Romero 2011), affecting the presence of several species of felines, including the ocelot (Ortega-Huerta and Peterson 2008; Ramírez-Bravo et al. 2010b; Villordo-Galván et al. 2010; Galindo Aquilar et al. 2019). However, there is insufficient information on the effects of land-use change and fragmentation of the ocelot habitat on the SNP. Therefore, analyzing the fragmentation and loss of connectivity between the habitats of Leopardus pardalis will allow the preservation of potential displacement routes for ocelots in this region of Mexico. This study aimed to analyze in landuse changes and morphological fragmentation patterns of the L. pardalis habitat in the Sierra Norte de Puebla. This approach was used to identify and classify morphological fragmentation types based on habitat availability for the species and to recognize their variation in 1993 to 2003 and 2003 to 2022. We hypothesized that due to the land-use changes in the SNP, the anthropic use matrix will determine the structure and availability of the remaining habitat fragments suitable for L. pardalis; therefore, isolated fragments will contribute marginally to maintaining the overall landscape connectivity.

mented in Sierra Negra (Galindo-Aquilar et al. 2016; Cacelin-

Materials and methods

Study area. The SNP is located at 20° 1′ 48" N, 97° 52′ 18" W (Figure 1) and comprises 64 municipalities. It is part of three morphotectonic provinces: Sierra Madre Oriental, Trans-Mexican Volcanic Belt, and Gulf Coastal Plain (INEGI 2005a). Climates in the region are varied, including warm sub-humid Aw2 and humid Am(f) in the north, humid semi-warm (A) C(fm) and humid temperate C(m) in the central area, and temperate sub-humid C(w2), semi-cold sub-humid C(E) (w2), and temperate semi-dry climates BS1k(w) in the south (INEGI 2005b). The mean annual temperature is 14 °C in the high-altitude regions and 20 °C in the lowlands. The total annual rainfall ranges between 1,000 mm and 3,500 mm, with March being the driest month and September experiencing the highest precipitation (INEGI 2005b). The region is undergoing a heavy deforestation process and has large areas of rainfed agriculture and cultivated pastures, as well as urban areas. However, it still has areas covered by mountain cloud forest, oyamel forest, oak forest, juniper forest, high and medium evergreen forest, and pine and pine-oak forests (INEGI 1997Rzedowski 1990; INEGI 199).

Image processing. Vegetation and land use data were integrated and analyzed using Landsat 4 (TM, October 1993), Landsat 7 (ETM, January 2003), and Landsat 8 (Oli Tirs, January 2020) imagery obtained from the Global Land Cover Facility. Supervised classification of satellite images for each year was carried out to obtain spectral information by estimating maximum likelihood (Lillesand *et al.* 2015). The classification of the 1993 image used 250 reference points from vegetation and land use maps Series I, scale 1:250 000 (INEGI 1997). The 2003 image was classified using 250 reference points based on vegetation and land use maps Series III, scale 1:250 000 (INEGI 2005c). The classification of the 2020 image used 300 randomly defined checkpoints recorded during fieldwork from February to May 2020, supported by Google Earth imagery[®].

Selection of habitat fragments. Habitat suitability for Leopardus pardalis was determined through a potential distribution model, as this allows for the identification of areas or fragments with a high probability of ocelot presence, which can be considered suitable habitat fragments (Pascual-Hortal and Saura 2008; Decout *et al.* 2012). The model was based on 70 records of *L. pardalis* from 2005 to 2011 in Hidalgo, San Luis Potosí, Veracruz, and Sierra Norte de Puebla in the Sierra Madre Oriental region. These records were obtained from the National Biodiversity Information System (CONABIO 2020), the Global Biodiversity Information Facility (GBIF 2021), and the database of Neotropical Carnivores (Nagy-Reis *et al.* 2020). To reduce spatial bias, ocelot records were processed with the Spatial Thin module (spatial filtering; <u>Aiello-Lammens *et al.* 2015</u>), which consists of a thinning function of data points to filter those that are at a short distance from an adjacent record; this distance was used to define the home range of the ocelot (mean 12 km²; <u>Dillon and Kelly 2007</u>; <u>González-Borrajo *et al.* 2016).</u>

Records of ocelot presence and environmental variables were processed in the program Wallace 1.0.6.1 in R (Kass *et al.* 2018). Environmental variables were selected based on knowledge of the distribution of the ocelot in the Sierra Madre Oriental (Martínez-Calderas *et al.* 2015; Cacelin-Castillo *et al.* 2020). We used 14 climatic variables (BIO 1, BIO 2, BIO 3, BIO 4, BIO 5, BIO 6, BIO, BIO 7, BIO 8, BIO 13, BIO 14, BIO 15, BIO 16, and BIO 17), with a 1 km² resolution, obtained from the WorldClim version 1.4 database (Hijmans *et al.* 2005). The following default settings were selected: maximum number of background points = 10,000; regularization multiplier = 1; replicates = 20; replication run type = boot; convergence threshold = 0.00001; and



Figure 1. Borders of the study area in the Sierra Norte de Puebla, Mexico.

maximum number of iterations = 10,000. Seventy percent of the occurrence data were used as a training dataset and 30% as a test dataset. The variables were evaluated using a Jackknife test, which compares the models with all possible combinations of environmental variables by measuring the importance of the variable. The model validation considered the weight of the omission and commission errors for the area under the curve (AUC; <u>Hernández *et al.* 2006</u>).

The vegetation and land use layers of each year were reclassified considering the habitat suitability for Leopardus pardalis into three categories (inhospitable, hospitable, and habitat), according to the habitat guality following the method by Tischendorf et al. (2003), Rayfield et al. (2010) and Correa-Ayram et al. (2014). Human settlements, pine forests, and coniferous forests were classified as inhospitable (values from 0 to 30) as these areas are unsuitable or marginally suitable for the ocelot. Plant covers of anthropic origin, such as induced pastures, crops, and agroforestry plantations, were classified as hospitable (values of 31-60), *i. e.*, areas where ocelots may be present or in transit but inadequate to maintain a stable population. The highest interval (60-100) indicated areas with the greatest habitat suitability, corresponding to medium evergreen forests, mountain cloud forests, and oak-pine forests (Tischendorf et al. 2003; Di Bitteti et al. 2008; Dde Oliveira et al. 2010; Rayfield et al. 2010; Martínez-Calderas et al. 2015; Cacelín-Castillo et al. 2020; Lambardi et al. 2022).

Layers of slope, elevation, distance from roads, distance from human settlements, and road density were integrated. Each variable was assigned a value from 0 to 100 based on the available information on the habitat requirements of Leopardus pardalis (De Oliveira et al. 2010; Ramírez-Bravo et al. 2010b; Cruz-Rodríguez et al. 2015; Gil-Fernández et al. 2017; Cruz et al. 2018; Wang et al. 2019). Values close to 100 were considered to indicate highly suitable areas in terms of habitat quality for ocelots, and vice versa (Tischendorf et al. 2003; Rayfield et al. 2010). Regarding altitude, values from 0 to 1,500 m were rated as most suitable since ocelots have been observed between 300 m and 1,300 m in this region. Altitudes above 2,100 m were classified as less suitable (Nowell and Jackson 1996; Cacelin-Castillo et al. 2020; Lambardi et al. 2022). Regarding slope, the difficulty of movement was considered. Likewise, high values of distance to roads correspond to the potential impact on the species; high values of distance to human settlements estimate the capacity of ocelots to adapt to and use urban and suburban areas, and low values of road density were interpreted as indicating areas of greater habitat suitability. All layers were standardized to homogenize the pixel resolution at 30 meters and processed with QGIS version 3.4.

Raster layers of potential distribution, reclassified plant covers, and land use, slope, altitude, and disturbance were added up using map algebra with the program QGIS version 3.4., to approximate the final potential distribution model to the area corresponding to the actual niche of the species (<u>Sánchez-Cordero et al. 2005</u>; Peterson et al. 2006).

The resulting map values were split into quartiles, with the lower interval classified as poor habitat (absence of habitat), the next interval as suboptimal, and the upper interval as optimal habitat (<u>Tischendorf *et al.* 2003</u>; <u>Correa-Ayram *et al.* 2014</u>). Fragments classified as optimal with a size of less than 100 ha were reclassified as suboptimal. This area has been observed to correspond to the highest recorded home range for the ocelot in Central America, South America, and Mexico (Ludlow and Sunquist 1987; Murray and Gardner 1997). In the definition of the final habitat fragments, only those of the optimal category were considered, and a new binary map (Optimal–Non-optimal) was generated using the QGIS version 3.4 program.

Land-use change. Changes in the ocelot habitat cover in 1993, 2003, and 2020 were analyzed using ArcView version 3.2 (ESRI 1999). Land-use change was estimated using a transition matrix to quantify the gain, loss, and persistence of change trajectories between the covers analyzed (Pontius *et al.* 2004). The annual rate of change (*r*) proposed by Puyravaud (2003) was calculated using the formula: r =(100/t₂-t₁) x ln (A₂/A₁)... Where A₁ is the coverage area at the beginning of the period, and A₂ is the coverage area at the end of the period; t₁ is the start year of the analyzed interval and t₂ is the end year.

Morphological Classification of Spatial Patterns of Habitat Patches (MSPA). The morphological types of habitat fragmentation for Leopardus pardalis were identified and classified using the program GUIDOS (Vogt et al. 2006). MSPA is a sequence of mathematical morphological operators that describe the geometry and connectivity of the components of an image; it uses a binary method of image classification based on the geometry and shapes of the elements to classify the fragmentation patterns into seven morphological categories: core area, edge, loop, perforation, bridge, branch, and islet (Vogt et al. 2007; Soille and Vogt 2009; Saura et al. 2011; Table 1).

Analysis of landscape connectivity. The connectivity of the habitats of Leopardus pardalis at the landscape level was analyzed in 1993, 2003, and 2020, using the integral index of connectivity (IIC) (Saura and Torné 2009), with the program CONEFOR Sensinode version 2.5.8 (Pascual-Hortal and Saura 2006; Saura and Pascual-Hortal 2007). The IIC integrates habitat attributes and landscape connectivity into a single connectivity value (Decout et al. 2012). In this way, habitat fragments are represented as nodes and the connections between them as links. IIC values range from 0 to 1, which increase as connectivity improves. A value of 1 is reached in the hypothetical case that the landscape is completely occupied by the habitat. The importance of each landscape element or change was determined by the delta values of this index (dIIC) (Pascual-Hortal and Saura 2006). The data were ranked from lowest to highest and then divided into five categories: very high, high, medium, low, and very low. Only those fragments of very high importance for connectivity were considered fragments of interest (Saura and Rubio 2010).

Table 1. Categories and Ecological Implications of Morphological Spatial Patterns of Fragmentation (MSPA).

Morphological Type	Ecological Meaning
Core area	Core areas habitat fragment that are relatively far from the edges between covers classified as habitat and those classified as non- habitat, also considering areas that have not been degraded by the edge effect and fragmentation.
Edge	Habitat zones spanning the width of the boundary between forest and non-forest cover with relatively large spaces corresponding to the outer boundary of a core area.
Perforation	Zones defined by the boundaries between core areas and relatively small gaps within the habitat. Perforation occurs within the edge zone along the boundary between the gap and the non-fragmented area.
Bridge	Narrow fragments contiguous without core area that connect at least two different core areas at their ends. They correspond to structural connectors or corridors that link different forest core habitat areas. Therefore bridges make reachable a higher amount of habitat for those organisms that dwell in any of the two linked cores and that can effectively disperse through these corridors.
Loop	Similar to bridges but with the ends of the element connecting to different parts of the same core area. Therefore, their presence does not increase the amount of core habitat that can be reached by a particular organism.
Branch	Connects bridges, loops, perforations, or islets, but not core areas. They correspond to contiguous elongated forest clusters that emanate from one forest area and do not reach any other forest area at another end.
Islet	Small, isolated fragments degraded due to the edge effect, which do not contain core areas because of their small size.

Results

The area under the curve (AUC) for the potential distribution model of *Leopardus pardalis* was 0.87. The variables with the highest percent contribution were precipitation (driest month, driest quarter, and warmest quarter), temperature seasonality, annual temperature range, and mean temperature of the driest quarter. The potential distribution model indicated that 22.2 % of the study area (189,337



Figure 2. Potential distribution areas and records of Leopardus pardalis in the Sierra Norte de Puebla. The blue and purple polygons indicate the borders set for Priority Land Regions.

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ha) has conditions compatible with the presence of the ocelot. It also shows areas with potential distribution with no records for the species, such as the Lakes and Volcanoes of Anahuac subprovince. In addition, it showed potential areas within the Bosques Mesófilos de la Sierra Madre Oriental and Cuetzalan Priority Land Regions (Figure 2).

Process of habitat change of *Leopardus pardalis*. From 1993 to 2020, the annual rate of change was -0.18 %. During this period, the area covered by natural vegetation decreased from 63 % to 47 %, while anthropic coverage increased from 36 % to 52 % (Table 2). The mountain cloud forest lost area at an annual rate of -2.4 %, while the medium evergreen for-

Table 2. Area (ha) and percentage of natural and anthropogenic cover in the *Leopardus pardalis* habitat in the Sierra Norte de Puebla, Mexico, for the years 1993, 2003, and 2020.

		1993		2003		2020		Overall change 1993–2020
	Cover Classes	Area (ha)	%	Area (ha)	%	Area (ha)	%	
Natural covers	Mountain cloud forest	14,591	7.47	10,855	5.28	7,623	4.52	-2.40
	Mediumn evergreen forest	71,660	36.68	105,187	51.11	48,697	28.89	-1.43
	Oak forest	26,709	13.67	17,288	8.40	14,033	14.26	-2.38
	Pine-oak forest	1,113	0.01	1,735	0.84	1,775	2.83	1.73
	Coniferous forest	1,236	0.12	918	0.45	1,301	0.77	0.19
	Oak-pine forest	9,964	5.10	10,765	5.23	15,374	15.05	1.61
	Total percent		63.06		71.31		66.33	
Anthrogenic covers	Rainfed agriculture	3,226	1.65	3,950	1.92	4,280	0.59	1.05
	Induced pastures	20,197	10.34	1,339	0.65	15,780	0.94	-0.91
	Agroforestry crops	47,521	24.33	52,149	25.34	77,041	32.06	1.79
	Human settlements	1,222	0.63	1,594	0.78	2,130	0.08	2.06
	Total percent		36.94		28.69		33.67	-0.18

est and the oak forest decreased in area at annual rates of -1.4 % and -2.3 %, respectively. On the other hand, agroforestry crops, rainfed agriculture, and human settlements recorded an expansion in area during this period, of 1.79 %, 1.0 %, and 2.06 %, respectively (Table 2).

Among the processes of plant cover change in the habitat of Leopardus pardalis, the greatest transformation was recorded in the medium evergreen forest from 1993 to 2003, which was converted to agroforestry crops, rainfed agriculture, and induced pastures (representing a 27 % loss). Five-point two percent of the mountain cloud forest area was converted mainly to agroforestry crops and induced pastures. Oak forests were replaced by induced pastures and agroforestry crops (3.3 % loss of total area; Table 3). From 2003 to 2020, 24 % of the area of medium evergreen forest was replaced by agroforestry crops and induced pastures, 4.3 % of the mountain cloud forest area was replaced by rainfed agriculture and agroforestry crops, 6.5 % of the area of oak forests was converted to agroforestry crops, and 1.9 % of oak-pine forests was replaced mainly by rainfed agriculture and agroforestry crops (Table 3).

The greatest natural vegetation recovery from 1993 to 2003 was recorded in medium evergreen forest, mountain cloud forest, and oak forest. From 2003 to 2020, the greatest recovery was observed in medium evergreen forest, mountain cloud forest, oak forest, and oak-pine forest (Table 3).

Structure of *Leopardus pardalis* habitat fragments. From 1993 to 2003, the number of optimal habitat fragments dropped from 639 to 533; however, the habitat area (200,174 ha to 208,459 ha) and the mean fragment size increased (Table 4). Between 2003 and 2020, the number of fragments increased, although the habitat area and mean fragment size decreased (Table 4). As for fragments with areas greater than 100 ha, 12 fragments were quantified in 1993; this number increased in 2003 (18 fragments) and then decreased in 2020 (16 fragments). An average Euclidean distance between fragments of 7.3 km (\pm 10.5 km) was calculated for 1993; subsequently, it increased to 9.4 km (\pm 9.1 km) in 2003 and then decreased to 6.6 km (\pm 9.8 km) in 2020 (Table 4).

Spatial fragmentation patterns of the optimal habitat of *Leopardus pardalis*. The results of the MSPA analysis of habitat fragments between 1993 and 2020 indicate a decrease in the percentage of habitat covered by the loop (yellow), bridge (red), and perforation (blue) categories. In contrast, the percentage of edge, branch, and islet decreased from 1993 to 2003 (Figure 3a, b), although they increased in 2020 (Figure 3c). The same change trend was observed regarding the number of elements in the morphological categories. The number of core area, loop, branch, and islet elements decreased from 1993 to 2003 and subsequently increased in 2020. From 1993 to 2020, the number of loop, perforation, and bridge elements decreased (Table 5).

Table 3. Transitions of *Leopardus pardalis* habitat covers, expressed as a percentage, in the Sierra Norte de Puebla during 1993 to 2003 and 2003 to 2020. Mountain cloud forest (Mcf), medium evergreen forest (Mef), pine-oak forest (Pof), coniferous forest (Cf), oak forest (Of), oak-pine forest (Opf), agroforestry crops (Agro), rainfed agriculture (Ra), induced pastures (lp), human settlements (Hs). The percentage of area that remains unchanged from one year to the next is indicated in bold. Values above the diagonal indicate area losses, and values below the diagonal indicate gains.

	1993-2003	Mcf	Mef	Of	Pof	Cf	Opf	Ra	lp	Agro	Hs	Total loss
Natural covers	Mcf	0.355	0.607	0.169	0.004	0.018	0.328	0.045	0.687	3.364	0.060	5.28
	Mef	0.508	12.659	0.602	0.001	0.026	0.073	1.811	6.101	18.359	0.239	27.21
	Of	0.387	0.215	3.036	_	0.006	0.672	0.376	1.244	0.891	0.118	3.31
	Pof	0.023	0.103	0.096	0.001	—	0.047	0.004	0.033	0.110	0.008	0.20
	Cf	0.029	0.064	0.093	—	0.005	0.080	0.002	0.020	0.209	0.005	0.32
	Opf	0.511	0.951	0.284	—	0.010	0.842	0.252	0.605	1.322	0.075	2.25
Anthropogenic	Ra	4.123	2.685	0.336	—	—	0.338	0.232	0.431	0.075	0.042	0.55
covers	lp	0.038	1.167	0.179	0.001	0.002	0.068	0.016	0.170	0.052	0.027	0.08
	Agro	2.483	7.416	3.432	0.001	0.072	1.327	0.082	1.583	13.94	0.068	0.07
	Hs	0.042	0.211	0.243	_	0.002	0.055	0.034	0.187	0.037	0.050	
	Total recovery	8.146	12.812	4.663	0.002	0.086	1.787	0.133	1.770	0.037		
	2003-2020											
Natural covers	Mcf	0.290	0.584	0.453	_	0.013	0.242	0.121	0.050	2.791	0.050	4.30
	Mef	0.199	19.480	0.726	0.041	0.020	0.642	0.340	5.206	17.816	0.027	24.82
	Of	0.632	0.979	1.060	_	0.064	0.314	0.062	0.060	6.037	0.048	6.59
	Pof	0.157	0.427	0.443	0.014	0.011	0.250	0.189	0.043	0.217	0.048	0.76
	Cf	0.080	0.297	0.063	0.007	0.009	0.068	0.015	0.015	0.201	0.010	0.31
	Opf	0.467	0.854	1.973	0.030	0.020	0.906	0.692	0.112	0.964	0.153	1.92
Anthropogenic covers	Ra	1.016	1.342	0.087	0.001	_	0.051	0.047	0.007	0.057	0.014	0.08
	lp	0.052	2.499	0.106	0.009	0.001	0.039	0.035	0.019	0.127	0.034	0.16
	Agro	1.573	6.891	1.307	0.131	0.280	2.526	0.194	0.082	14.262	0.047	0.05
	Hs	0.006	0.023	0.012	_	_	0.004	0.007	0.003	0.003	0.019	
	Total recovery	4.182	13.312	3.990	0.178	0.300	2.619	0.235	0.085	0.003		

Contribution of *Leopardus pardalis* habitat connectivity to the SNP landscape. From 1993 to 2020, the most important habitat fragment for connectivity had a dllC value of 99.7, decreasing in 2003 (dllC = 97.7), and then increasing in 2020 (dllC = 99.9) (Figure 4). The same trend was observed with the area of this fragment, increasing from 1993 to 2003 (from 230,152 ha to 240,439 ha) and decreasing in 2020 (197,595 ha). From 1993 to 2003, seven fragments were recorded in the very low and low categories, respectively, with dllC values of less than 1 % (Figure 5a, b). In 2020, 15 fragments were recorded in the very low category. Two fragments were also quantified in the high category for connectivity during 1993 (2 %); from 2003 to 2020, no fragments were observed in this category (Figure 5b, c).

Discussion

The distribution patterns of *Leopardus pardalis* in the study area indicate that it is located along one of the most important mountain ranges in the state of Puebla, the Sierra Madre Oriental, and one of the most extensive plains that covers part of Veracruz, the Gulf of Mexico Plain, which includes the portion of the Carso Huasteco and Chiconquiaco physiographic subprovinces. Ocelots have been reported in some areas of Hidalgo and Puebla (Ramírez-Bravo et al. 2010b; Hernández-Flores et al. 2013), which may indicate their mobility through the Sierra Madre Oriental. Ceballos-González et al. (2006) point out that the sites where favorable conditions for the presence of ocelots most probably exist are found in the Pacific Coastal Plains and the Gulf of Mexico, as well as throughout the south of México in the humid tropics (Di Bitetti et al. 2006; Dillon and Kelly 2007); however, ocelots can also be found in sub-humid climates (Trolle and Kerry 20036; Maffei et al. 2005). These results are consistent with our findings, where the potential distribution model indicates that precipitation (driest month, driest guarter, and warmest guarter) contributes to explaining the potential distribution of the ocelot in the study area. However, no records were obtained in the Lakes and Volcanoes of Anahuac subprovince, so the potential distribution of the ocelot was low in this region (probability less than 0.1). This finding is probably consistent with the fact that this region has arid climates with extreme temperatures and sparse vegetation, characteristics that are unfavorable for the presence of the species (Martínez-Calderas et al. 2011).

Change of *Leopardus pardalis* habitat cover. The optimal habitat still maintains 25 % of medium evergreen forest, 7 % of oak forest, and 4 % of mountain cloud forest, although



Figure 3. Morphological classification of spatial fragmentation patterns (MSPA) of the optimal habitat of *Leopardus pardalis* in 1993 (a), 2003 (b), and 2020 (c) in the Sierra Norte de Puebla.

the latter vegetation may be mostly secondary because about 90 % of the vegetation in the Sierra Norte de Puebla has been altered (Galván et al. 1999). Evangelista-Oliva et al. (2010) state that there were still 15.1 % of areas with tropical forest and mountain cloud forest in this region in 2003, although they included advanced-stage secondary vegetation, coinciding with the increase in vegetation loss in recent decades (Mass et al. 2004; Rosete-Vergés et al. 2014). It is estimated that more than 50 % of mountain cloud forest area in México was lost between 1968 and 2011 (Ochoa-Ochoa et al. 2017). The data obtained in the present study is consistent with this estimate, since, from 1993 to 2020 in the distribution range of L. pardalis, the area of mountain cloud forest underwent a 54 % decrease at an annual rate of change of -2.4 %, a figure similar to that observed for Latin America (-2.9 %; Armenteras and Rodríguez-Eraso 2014). It has been pointed out that the main cause of deforestation of tropical forests in Mexico is their conversion to pastures (Williams-Linera et al. 2002; Cayuela et al. 2006); however, in the study area, these forests were mainly replaced by shade coffee plantations.

In the study area, shaded coffee plantations are the main source of income for the agricultural sector (Evangelista-Oliva et al. 2010). The coffee register indicates an area of 61,460 hectares cultivated with coffee, distributed in 548 localities in 46 municipalities of the state of Puebla (SIAP 2005). In addition, in 2017, this productive activity covered 18.86 % of the region, making this system crucial for the conservation of biodiversity (Moguel and Toledo 1999; Williams-Linera et al. 2002; Pineda-López et al. 2005; Redo et al. 2009; Perfecto and Vandeermer 2010). Shade coffee plantations have been considered biodiversity refuges with slightly modified land use, since they are generally established in such a way that only the understory is replaced by coffee trees, maintaining elements of the native vegetation; sometimes, some fruit and timber trees are also included (Moguel and Toledo 1999). Although some studies question the biodiversity conservation aspect of these production systems (Rappole et al. 2003), others point out that diversified shade coffee plantations are a habitat that may have less impact on biodiversity compared to other activities such as cattle ranching and seasonal or permanent crops,

although they may not have the same species richness as native forests (Greenberg *et al.* 1997; Cruz-Lara *et al.* 2004).

The greatest recovery in vegetation cover from 1993 to 2020 was recorded in medium evergreen forest, mountain cloud forest, and oak forest. This recovery may be related to the abandonment of agricultural areas, the shift in productive activities, proper forest management, and the implementation of reforestation programs (TuiránTuiran 2002; García-Barrios et al. 2009). The abandonment of farmland in the study area is likely due to the decrease in subsidies for agriculture and livestock raising implemented by the Mexican government since 1994 (De Janvry et al. 2001; Pascual and Barbier 2007). It was also observed that part of this recovery of vegetation cover occurred along with the decrease in coffee plantations. Espinoza-Guzmán et al. (2020) points out that from 1993 to 2017, shaded coffee plantations in Veracruz were abandoned and mostly evolved into secondary vegetation, which is related to low coffee productivity due to pests and diseases, among other factors. Some research indicates that the abandonment of agricultural land, the growth of secondary forests, and the recovery of forest cover could influence the decrease in the rate of change (Muñoz-Villers and López-Blanco 2008; García-Barrios et al. 2009; López-Barrera et al. 2014).

Structure and configuration of *Leopardus pardalis* habitat fragments. In recent decades, the ocelot habitat has **Table 4.** Values of changes (1993, 2003, and 2020) in the structure and configuration of *Leopardus pardalis* habitat fragments in the Sierra Norte de Puebla.

	1993	2003	2020
Number of optimal habitat fragments ≤ 100 ha	639	533	904
Number of optimal habitat fragments ≥ 100 ha	12	18	16
Euclidean distance between fragments (m)	6,682	7365	9,423
Fragment Shape Index	11	9.4	7.3
Mean Fragment Edge (m)	169	260	301
Mean Fragment Size (ha)	310	391	214
Total habitat area (ha)	197,445	217,838	188,038

been affected by a progressive fragmentation of vegetation patches due to their shrinking area associated with land-use change. It has been suggested that the conversion of carnivore habitats is related to population declines (Ripple et al. 2014). Likewise, local disturbances, such as land-use changes, can influence the home range of the ocelot, becoming a potentially more sensitive indicator of the impact of disturbances (Cruz et al. 2019). For example, some studies indicate that ocelots prefer closed-canopy areas and avoid open areas (López-González et al. 2003; Harveson et al. 2004; Martínez-Calderas et al. 2011; Torres-Romero et al. 2017; Galindo-Aguilar et al. 2019). Cruz et al.



Figure 4. Integral Index of Connectivity (dIIC) of Leopardus pardalis optimal habitat fragments larger than 100 hectares during 1993, 2003, and 2020 in the Sierra Norte de Puebla.

(2018) point out that ocelots prefer better-preserved areas, whereas smaller felines are more prevalent in suboptimal habitats (Nowell and Jackson 1996; De Oliveira *et al.* 2010; Di Bitetti *et al.* 2010). In addition, in areas with less tree cover, medium and large prey, which are important for the ocelot, are less abundant (Cruz et al. 2018). In addition, these areas pose greater threats (presence of dogs, higher road density, and others), which may also negatively affect the presence of this species in open areas (Tewes and Everett 1986; López González et al. 2003; Haines *et al.* 2005; Cruz *et al.* 2019).

In the present study, the largest number of ocelot records were obtained in areas with agricultural activity. Some studies describe that ocelots tolerate fragmentation (<u>Gil-Fernández et al. 2017</u>) and move across disturbed vegetation (crops, pasture, scrub, and secondary vegetation) (<u>Cruz-Rodríguez et al. 2015</u>). According to <u>Bisbal (1991</u>), ocelots are relatively tolerant to habitat modification compared to other large felines, such as jaguars and pumas, because their home range is smaller and their prey are abundant, including rodents, reptiles, and birds. However,

other studies indicate that ocelots can use different habitats, both disturbed and conserved (Kolowski and Alonso 2010), according to their availability (Fusco-Costa *et al.* 2010), which are frequently used to move towards more structurally complex and closed fragments (López González et al. 2003; Cruz *et al.* 2018).

The percentage of large habitat fragments with core area decreased from 2003 to 2020, while the number of smaller fragments increased. <u>Galindo-Aguilar et al.</u> (2019) also observed a decrease in the size of *Leopardus pardalis* habitat fragments in the Sierra Negra de Puebla and considered that the remaining fragments are insufficient to sustain an ocelot population in this region. In this sense, it has been observed that medium and small felines are adversely affected by habitat loss and that ocelots are most affected by this decrease in vegetation cover, compared to smaller species with which it coexists, such as *Leopardus weidii* and *Herpailurus yagouaroundi* (Cruz *et al.* 2019). Nowell and Jackson (1996) point out that some feline species depend on large, preserved fragments, and others are more tolerant of degraded habitats because they are more flexible regard-



Figure 5. Changes in integral index of connectivity (dIIC) values and importance of *Leopardus pardalis* habitat fragments in the functional connectivity of the landscape of the Sierra Norte de Puebla during 1993 (a), 2003 (b), and 2020 (c).

ing habitat requirements. Land-use change, in addition to modifying natural vegetation, also alters the spatial configuration and quality of the remaining fragments, resulting in smaller fragments being more exposed to human interference (Fischer and Lindenmayer 2004; Bennett and Saunders 2010), which could have major implications for the conservation of the *L. pardalis* habitat (Ludlow and Sunquist 1987; Dillon and Kelly 2007; Lambardi et al. 2022).

The percentage of islet categories decreased in 2003 and then increased in 2020, which could indicate that the habitat of Leopardus pardalis includes a high number of small habitat fragments, isolated by the fragmentation process, with a gradual loss of core area. The increase in islet elements coincides with the increase in habitat fragments smaller than 100 ha, which may be insufficient to maintain ocelot populations in the study area (Moreno et al. 2012; Torres-Romero et al. 2017). On the other hand, it could be a gain in transit habitat fragments, which reduces the resistance of the anthropic matrix. These could function as intermediate elements, temporary shelters, and transit sites between larger habitat fragments (Correa-Ayram and Mendoza-Cantú 2013). Smaller fragments could play a key role in promoting connectivity for the ocelot and other species in the study area (Saura et al. 2014; Diniz et al. 2021).

The classes categorized as connectors, bridge, and loop elements decreased between 1993 and 2020. This decrease is consistent with the increase in Euclidean distance from habitat fragments, which could be related to the fragmentation and loss of the Leopardus pardalis habitat in the study area. Correa-Ayram and Mendoza-Cantú (2013) state that the decrease in bridge and loop connectors may negatively affect habitat connectivity and force ocelots to take a longer and more expensive route to cover the same habitat fragment. By facilitating dispersal and sustaining long-distance movements, loop and bridge elements allow species to colonize suitable new fragments and expand their range (Saura et al. 2014), which is particularly important when considering land-use changes in the region. Maintaining landscape connectors and transit paths could be critical for L. pardalis and other widely distributed carnivores in fragmented landscapes (Beier and Noss 1998; Crooks et al. 2011; Cruz-Rodríguez et al. 2015; Khosravi et al. 2018; Ashrafza-

 Table 5. Number of elements of the morphological categories of the Leopardus pardalis optimal habitat in the Sierra Norte de Puebla in 1993, 2003, and 2020.

C ategories	Number of elements					
	1993	2003	2020			
Core area	2,254	1,893	2,231			
Edge	1,282	1,110	1,436			
Loop	3,158	3,034	2,482			
Perforation	5,435	5,416	3,950			
Bridge	2,686	2,333	2,272			
Branch	7,688	7,626	8,244			
lslet	4,826	2,878	4,291			

deh et al. 2020). For example, <u>Cruz et al. (2018)</u> observed that ocelots are adversely affected by the distance between fragments. These authors also mention that the presence of felines in small fragments could depend on the presence of other nearby populations; therefore, connectivity between fragments is essential for small and medium-sized felines in anthropic landscapes.

Elements considered branches are areas derived from fragmentation processes due to the rupture of bridges connecting two habitat fragments, particularly islets, but not core areas (Soille and Vogt 2009; Correa-Ayram and Mendoza-Cantú 2013). In the present study, an increase of this element was observed in the habitat of Leopardus pardalis during the period analyzed, which could indicate an increasing rupture of bridges between smaller fragments, thus decreasing the connectivity between them. Fragmentation can cause loss of connectivity with adverse effects on the persistence of other carnivores, increasing the risk of extinction (Saura and Pascual-Hortal 2007; Cavalcanti and Gese 2009; Reding et al. 2013). In addition, the loss of structural connectivity can also alter the movement patterns of felines; by increasing the isolation between fragments, species consumed as prey become less abundant (Zemanova et al. 2017).

Regarding the habitat fragmentation processes of Leopardus pardalis, an increase in the percentage and number of edge elements was observed between 1993 and 2020. The increase in edge fragments of the ocelot's habitat may be related to the constant extraction of wood for fuel and the conversion of small areas for agriculture (Ochoa-Gaona 2001; López-Barrera et al. 2014). The increase of edges leads to a higher risk of mortality for those species specialized in the habitat inside the fragment (Murcia 1995), increasing the probability of local extinction. For example, large-sized felines are more abundant in fragments with large core areas, making them susceptible to edge effects, where they conflict with livestock and humans (Luskin et al. 2017; Cruz et al. 2018). Lombardi et al. (2022) observed that ocelots avoid areas with edge habitats and are more prevalent in core areas of the fragment (Wang et al. 2019). Other felines with more flexible diets are more tolerant of edge habitats or even become more abundant in degraded habitats because they adapt to the changing prey availability near the edges (Prugh et al. 2009; Delibes-Mateos et al. 2014; Gil-Fernández et al. 20157; Cruz et al. 2018).

The number of perforation elements in the habitat increased from 1993 to 2020. It should be considered that the perforation is one of the initial stages in the habitat fragmentation process and begins when anthropogenic activities such as deforestation, produce small clearings that act as gaps within habitat fragments, which increase in area until they split the fragment, with the consequent reduction in its area (Forman 1995). In Mexico, some vegetation fragments are deforested from the interior to the edge, resulting in fragments without defined edges and a smaller core area; this could affect the conservation value of the

remaining vegetation (<u>López-Barrera *et al.* 2014</u>). Although different studies point to the plasticity of ocelots to use different habitats according to their availability (<u>Fusco-Costa *et al.* 2010</u>; <u>Kolowski and Alonso 2010</u>; <u>Cruz-Rodríguez *et al.* 2015; <u>Gil-Fernández *et al.* 2017</u>), their preference for more conserved habitats has also been recorded (<u>López-González *et al.* 2003</u>; <u>Cruz *et al.* 2018</u>). Habitat loss and fragmentation favor certain feline species and adversely affect others, depending on the predominant matrix type, as species differ in their environmental plasticity and ability to use suboptimal habitats (<u>Ripple *et al.* 2014</u>; <u>Zanin *et al.* 2015</u>).</u>

Contribution of Leopardus pardalis habitat fragments to connectivity. The integral index of connectivity (dIIC) of the L. pardalis habitat was high compared to values obtained in other feline studies. For instance, Correa-Ayram et al. (2014) recorded dIIC values of 0.15 to 85 for the Linx rufus habitat between 1975 and 2008. Other studies of functional connectivity in terrestrial mammals in the mountain cloud forests of Veracruz indicate that low dIIC values indicate a serious threat to their persistence in fragmented landscapes. Pascual-Hortal and Saura (2008) consider that dIIC is a good indicator of habitat availability because it integrates the connectivity network and the area of fragments as an attribute. The fragment with the highest dIIC during the period analyzed is located in the central part of the study area (Figure 5), being the fragment with the largest surface area that could function as a bridge between ocelot populations of Puebla and those living in the north of Oaxaca. Cacelin-Castillo (2020) points out that ocelots are likely to use the lowest-quality habitat (northern Puebla, western Veracruz) only to disperse; therefore, the survival of the ocelot in the Sierra Madre Oriental and most of the eastern region of Mexico would depend on its ability to move in inhospitable fragments and get resources (Gil-Fernández et al. 2017). In addition, some studies have stressed the need to establish the Sierra Madre Oriental as a priority area as a biological corridor connecting ocelot populations between northern and southern Mexico (Grigione et al. 2009; Ramírez-Bravo et al. 2010b).

It was also observed that the best performance in the connectivity of the habitat of *Leopardus pardalis* occurred in fragments $\ge 2,000$ ha. A minimal fragment size was previously identified as important for landscape connectivity (Pascual-Hortal and Saura 2007). In addition, it has been recorded that fragments of potential use as habitats that are most important for the overall connectivity of medium and large feline habitats are generally those fragments with the largest surface area (Ramírez-Reyes *et al.* 2016). Other studies report that large fragments are more important in the landscape since they can function as transit fragments and habitat simultaneously (Saura and Pascual-Hortal 2007; Pascual-Hortal and Saura 2008; Saura and Rubio 2010).

The increase in anthropogenic cover and the loss of natural vegetation in the habitat of *Leopardus pardalis* between 1993 and 2020 is associated with economic activities in the region. One of the ecosystems most affected by these activities is the mountain cloud forest. In these ecosystems, the advance of agricultural and livestock practices is high and jeopardizes the persistence of this type of vegetation. In addition, these alterations contribute to the increase in edge habitats and a decrease in core areas of the remaining habitat fragments of *L. pardalis*. However, the recovery of the medium evergreen forest from 1993 to 2003 stands out, attributable to factors that allowed the growth of secondary vegetation and the recovery of forest cover, such as land abandonment, changes in productive activities, and the implementation of reforestation programs.

The areas that recorded the presence of ocelots have optimum conditions to function as biological corridors in the Sierra Madre Oriental, in the portion that comprises the Sierra Norte de Puebla. In addition, Leopardus pardalis tends to prefer areas covered with primary vegetation, or at least with a similar structure and good connectivity, although they are able to move through the anthropic matrix. However, the study area shows continued growth of human settlements, which may exacerbate habitat loss. Areas with human settlements could affect the movements of L. pardalis because they generate greater resistance, unlike transit areas such as agroforestry crops. In this regard, components with buffer capacity, such as shade coffee plantations, include corridors and fragments with significant core areas habitat, which should be prioritized in conservation strategies, as they serve as core reserves and maintain the functional connectivity of the study area. However, a few habitat fragments located in the central part of the Sierra Norte de Puebla, corresponding to the Sierra Madre Oriental, were categorized as important and very important. Therefore, preserving these fragments would allow the functional connectivity within the region to be largely preserved.

We recommend systematically monitoring the areas adjacent to the study area to determine how anthropogenic activities affect the distribution of ocelots in the Sierra Madre Oriental. In addition, recording ocelots in contiguous areas provides relevant information that will support the development of a more robust habitat connectivity model to better understand the south-to-north dispersal of ocelot populations. The fragmentation of the Sierra Norte de Puebla is an issue that should be addressed strategically for the long-term conservation of the regional biodiversity in general and the ocelot in particular.

Acknowledgments

Research carried out thanks to UNAM-PAPIIT IA206221 program. S. Lemus Rincón is grateful for the bachelor's degree scholarship, awarded by the UNAM-PAPIIT IA206221 program, scholarship number: 438421.

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Associated editor: Mircea Gabriel Hidalgo Mihart Submitted: December 23, 2023; Reviewed: January 21, 2023 Accepted: April 29, 2024; Published on line: May 29, 2024

Morphological variation in the Cerralvo Island pocket mouse Chaetodipus siccus from the Baja California Peninsula, México

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Chaetodipus siccus is a microendemic species with a restricted distribution and two geographically isolated populations, one on Cerralvo Island and its counterpart on the Baja California Sur peninsula, each associated with dissimilar environmental conditions. The hypothesis to test is that each population is adapted to its distinct environment and has its own evolutionary trend that can be used to differentiate them. The analysis of the skull shows that each population has a differential development of the region associated with chewing muscles. The statistical analyses of the Procrustes (shape) and Mahalanobis (size) distances confirm these differences statistically (P < 0.05). The set of differences in skull shape associated with the chewing muscles, craniodental measurements, external morphological traits, genetic isolation, and association with a different environment support the hypothesis that each population has its own evolutionary tendency.

Chaetodipus siccus es una especie microendémica de distribución restringida con dos poblaciones aisladas geográficamente, en la isla Cerralvo y a su contraparte en la península de Baja California Sur y cada una se asocian a condiciones ambientales disimiles. La hipótesis para probar es que cada una de las poblaciones se ha adaptado a su propia condición específica y tiene su propia tendencia evolutiva que puede usarse para distinguirlas. Los análisis del cráneo muestran que cada una de las poblaciones tiene un desarrollo de una región diferencial asociada a los músculos de la masticación. Los análisis estadísticos de las distancias Procrustes (forma) y Mahalanobis (talla) confirman estas diferencias de manera estadística (P< 0.05). El conjunto de diferencias en la forma del cráneo asociada a la musculatura usada para masticar, medidas craniodentales, características morfológicas externas, aislamiento genético y asociación a ambiente diferente, permiten considerar que cada una de las poblaciones tiene su tendencia evolutiva propia.

Keywords: Chaetodipus; Baja California Peninsula; endemic; México; new subspecies; Rodentia.

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Introduction

A revision of Chaetodipus arenarius specimens collected across the geographic range of this species demonstrates that it is a species complex that includes three species endemics to the Baja California Peninsula (Álvarez-Castañeda and Rios 2011; Hafner 2016). The three species share very similar morphological characteristics, including the skull, measurements, fur pattern, and external traits; before the study of <u>Álvarez-Castañeda and Rios (2011</u>), these were considered a single species (Lackey 1991; Williams et al. 1993; Patton and Álvarez-Castañeda 1999). The three species can only be differentiated from one another through direct comparison by a specialist with solid experience in the subfamily Heteromyinae, based primarily on morphological characteristics such as rump bristle width and length and fur texture; however, an easier methodology is genetic analyses (Álvarez-Castañeda and Rios 2011). Chaetodipus arenarius and C. ammophilus (sensu Rios and Álvarez-Castañeda 2013) are sympatric in a small area within their distribution range. C. arenarius lives from La Paz isthmus to the USA-México border, including Magdalena Island; C. ammophilus thrives from the Magdalena plains to the cape region, including Margarita Island (Hafner 2016). C. siccus is parapatric with the other two species and its range is restricted to Los Planes Basin and Cerralvo Island (<u>Álvarez-Castañeda and Rios 2011</u>). These three species can be considered the C. arenarius species complex.

The Cerralvo Island Pocket Mouse, *Chaetodipus siccus*, can be differentiated from the other two species within the *C. arenarius* complex by being larger than *C. arenarius* (not statistically significant) and with very weak or rarely present rump bristles (Álvarez-Castañeda and Rios 2011). The cranium is markedly larger and heavier; the mastoids are somewhat larger (not significant) and have broad ascending branches of the supraoccipital ridge (Osgood 1907; Álvarez-Castañeda and Rios 2011); no measurements differ significantly and, thus, cannot be used to separate the species.

Chaetodipus siccus can be considered a microendemic species of the Baja California peninsula. It was originally described from Cerralvo Island as a subspecies of C. penicillatus (Osgood 1907) and later as C. arenarius (Nelson and Goldman 1929). C. siccus has a very restricted distribution (~1.7 km²) in Cerralvo Island and is seriously threatened by the presence of feral cats (Lorenzo *et al.* 2010). Genetic studies of the C. arenarius species complex (Álvarez-Castañeda and Rios 2011) found a second population of C. siccus inhabiting a small basin (Los Planes basin) in front of the Cerralvo Island, a population that was unknown within the C. arenarius distribution range (~200 km²). The two known populations of C. siccus are associated with different environmental conditions based on the local vegetation, being considered two different regions (Reimann and Ezcurra 2007).

Associated with different environments that can produce a differential adaptive effect between populations, the variation in the bite force provided by the masticatory apparatus, including the zygomatic arch (masseter muscle, mandible elevation) and braincase (temporalis muscle, bite force; <u>Becerra et al. 2014</u>; <u>Cox et al. 2012</u>) is considered to comprise elements that provide functional information on the variation between populations (<u>Gomes Rodrigues</u> <u>et al. 2023</u>).

Since *C. siccus* was recognized as a different species (<u>Álvarez-Castañeda and Rios 2011</u>), Álvarez-Castañeda has surveyed the whole distribution range (~202 km²) in both the mainland and Cerralvo Island to understand its distribution, genetic structure, geographic boundaries, and ethology (<u>Aguilera-Miller 2011</u>; <u>Aguilera-Miller et al 2018a, b</u>; <u>Aguilera-Miller and Álvarez-Castañeda 2019</u>).

Los Planes is a U-shaped basin that drains into the Gulf of California, characterized by flat sandy lowlands with deep soils (average depth >100 cm), sedimentary rocks, Regosols with a high organic matter content (León de la Luz et al. 2000; INEGI 2010). The local vegetation is a sarcocaulescens shrubland with a very dense cactus forest (Pachycereus pringlei), in addition to Jatropha cinerea, Cylindropuntia cholla, Prosopis articulata, Stenocereus gummosus, Parkinsonia microphyllum, Fouquieria diguetii, and Cyrtocarpa edulis (León de la Luz et al. 2000; León de la Luz in lit.). Los Planes basin is surrounded by rocky slopes that are not suitable habitats for C. siccus; consequently, this mouse thrives only in the basin lowlands where it can be found in sympatry with C. rudinoris and C. spinatus (Aguilera-Miler 2016).

At Cerralvo Island, C. siccus has been found only in the small sandy areas of the western central portion, including the coastal plain and the sandy bed of streams. The rocks are igneous intrusive and the main soil types are Leptosoil (mean depth >15 cm) and Eutric Arenosol with a low organic matter content (Carreño and Helens 2002; Hernandez Ramirez 2004; INEGI 2010). These sandy areas originated from the accumulation of sand with a different granulometry as a result of sea waves and ocean currents (Hernandez Ramirez 2004) and in stream beds due to erosion. There are no large suitable areas for the species in the rest of the island. The vegetation is a sarcocaulescens shrubland composed mainly of Pherocactus diquetii, Olneya tesota, Cyrtocarpa edulis, Jatropha cuneata, Lysiloma candida, Fouquieria diguetii, Acaciella goldmanii, Mimosa xanti, Euphorbia leucophylla, and Pachycereus pringlei, and with at least one endemic species, Mammillaria cerralboa (Blazquez et al. 1997; León de la Luz et al. 2000). A large part of the island has very steep slopes and is stony (> 90 %). Only two other native mammals are known from the island: Peromyscus avius, which is endemic to the island, and Lepus californicus, introduced to the islands by fishers as a source of fresh meat (Lorenzo et al. 2010), in addition to two endemic reptiles (Teiidae: Aspidoscelis ceralbensis, and Colubridae: Chilomeniscus savagei; Case et al. 2002).

The original revision of the C. arenarius species complex using genetic data focused primarily on differences at the species level, and no detailed analyses within the C. siccus species were conducted (Álvarez-Castañeda and Rios 2011). C. siccus is a species with two isolated populations in two areas with different environmental conditions and with no genetic flow between them because the Gulf of California acts as an effective barrier. Under these conditions, the hypothesis is that each population has adapted to its particular environment, with skull modifications related to variations of the chewing muscles. Each species has its own distribution range and evolutionary trend, which can be used to differentiate them. The alternative hypothesis is that as the species has a discontinuous distribution between the two known populations, the geographic variations will involve only size, which can be statistically significant, but not the morphology as an adaptation to the local environment. This study aimed to describe the disparities between the two populations in the study area and whether these reflect geographically defined patterns of character variation.

Material and methods

The specimens studied were collected between 1991 and 2014 from Los Planes Basin (mainland) in the Baja California Peninsula and Cerralvo Island (Figure 1). In all cases, animals were handled according to the recommendations of the American Society of Mammalogists (Sikes *et al.* 2016) and the Norma Oficial Mexicana NOM-126-ECOL-2000 (NOM 2001). Voucher specimens were deposited in the Collection of Mammals at Centro de Investigaciones Biológicas del Noroeste (CIB), La Paz, B.C.S., México (Appendix 1). To explore potential differences between the two disjoint populations of *C. siccus*, we examined 129 specimens collected throughout the distribution range of the species in the Baja California Peninsula and Cerralvo Island.

Age criteria. We categorized age classes using the numerical scores defined by <u>Genoways (1973)</u> based on molar wear. Only adult specimens were included in the analyses (<u>Álvarez-Castañeda and Rios 2011</u>).

Non-geographic variation. To examine sexual dimorphism, we performed generalized least-squares analyses of ventral and lateral views of specimens from each population. *P*-values for the Procrustes and Mahalanobis distances were tested for 1,000 permutations in the MorphoJ program. As a result, sexes were pooled in all analyses.

Cranial shape analyses. Two cranium views were photographed, and only complete craniums with no apparent damage were digitized for each view: ventral surface (n =129) and lateral surface (n = 129; Figure 2). Photographs were captured with a Canon EOS 50D camera by the same photographer (Carmen Gutierrez) using standard settings (Canon EF 50 F/1. 8 STM), at the same lens-to-cranium distance (15 cm) and including a scale bar in all photographs to estimate the centroid size based on a scale factor. Most



Figure 1. Distribution of *Chaetodipus siccus* in Cerralvo Island (1) and Los Planes Basin (2), Baja California Sur, México.

landmarks were Type 1 (where the intersection of bony sutures is locally defined) or Type 2 (by the tip of a structure; Bookstein 1991).

Nineteen ventral landmarks were used, as follows: 1, posterior most point of the occipital and interorbital suture; 2, posteriormost point of the occipital condyle; 3, outermost point of the auditory meatus; 4, outermost point of the mastoid; 5, posteriormost inner point of the zygomatic arch; 6, innermost point of the interorbital breadth; 7, posteriormost point of the rear of the last molar; 8, outermost point of the first molar; 9, innermost point of the first molar; 10, anteriormost point of the premolar alveolus; 11, anteriormost point of the zygomatic arch at the level of the inner margin of the zygomatic arch; 12, joint of the zygomatic and maxillary suture; 13, uppermost point of the incisor alveolus; 14, anteriormost point of both incisors; 15, anteriormost point of the incisive foramen; 16, posteriormost point of the palatal; 17, anteriormost point of the auditory bulla; 18, posteriormost point of the auditory bulla; 19, anteriormost point of the foramen magnum.

The 14 landmarks in the lateral cranium are as follows: 1, posteriormost point of the occipital; 2, uppermost point of the braincase at the level of the auditory meatus anterior margin; 3, dorsal extremity of the joint of the frontal and parietal suture; 4, dorsal extremity of the joint of the frontal and nasal suture; 5, anteriormost point of the suture between the nasal and the premaxilla; 6, anterior tip of the nasal; 7, anteriormost point of the premaxillary; 8, uppermost point of the incisor alveolus; 9, lowermost point of the incisor alveolus; 10, anteriormost point of the premolar alveolus; 11, posteriormost point of the rear of the last molar; 12, outermost point of the mastoid; 13, posteriormost point of the zygomatic arch; 14, posteriormost point of the occipital condyle.

Landmarks were digitized from images using TPSDig 2.16 (Rohlf 2010) and superimposed using Procrusts to remove the effects of rotation, translation, and size; these were then projected into a Euclidean tangent space (Rohlf and Slice 1990). With the landmarks, a wireframe diagram was drawn and used to compare the differences in cranium shape. Procrustes coordinate residuals (PC) were calculated by subtracting the mean or consensus shape, after which a Principal Component Analysis (PCA) was performed on the covariance matrix of the residuals (Dryden and Mardia 1998). Shape variation was analyzed with a PCA based on the covariance matrix of symmetric and asymmetric components in the entire dataset for females and males. Cross-validation was performed with a CVA used to calculate partial deformation scores and uniform components, extracting the canonical variations of such scores to generate a plot of the distribution of points. Changes in cranium shape between groups were visualized with a relative deformation grid in MorphoJ 1. 6d (Klingenber 2011).

Cranium size analysis. The size variable used was centroid size, a geometrically based measure of size calculated as the square root of the sum of the squared distances of the landmarks to their centroid (Bookstein 1991). Centroid size is independent of the variability in landmark shape (in the absence of allometry), so it can serve as a primary size variable (Yazdi and Alhajeri 2018).



Figure 2. Morphological landmarks defined for A) ventral and B) lateral views of the cranium of *Chaetodipus siccus* (CIB 5616).

Morphological analyses. We obtained four conventional external measurements from specimen labels (total length [ToL], tail length [TaL], hindfoot length [LHF], ear length [LE]), and weight (WG). Also, we recorded 18 linear measurements of cleaned skulls (*sensu* Best 1978) from adult specimens (*sensu* Genoways 1973) of the two populations using digital calipers (to the nearest 0.01 mm). Craniodental characters included greatest skull length (GLS), occipitobasal length (OBL), palatal length (PL), palatinal length (PIL), postpalatal length (PPL), mandibular length (MTR), maxillar tooth row (MXR), nasal length (NAL), anterior nasal width (ANW), posterior nasal width (PNW), bulla width (BW), mastoidal width (MW), M1 width (M1W), width across M1 (WM1), diastema length (DL), interparietal width (IW), interparietal length (IL), and cranium depth (CD).

Differences between the two geographic groups (Figure 1) were explored with a two-sided unequal variance Student's *t*-test (Welch's *t*-test) between the two independent geographic regions (mainland versus island) for each of the 18 cranial variables using JMP^M (ver. 3.1.6.2; <u>SAS</u> Institute Inc. 1997). The data were tested for normality and homoscedasticity with the Shapiro-Wilk and Bartlett's tests, respectively.

A multivariate principal component analysis (PCA) and a canonical variate analysis (CVA) were performed to distinguish specimens belonging to each physiographic region. The statistical significance of the principal components was assessed using the broken-stick method (Peres-Neto *et al.* 2005). The independence of the variables was evaluated with a correlation analysis; in those pairs of variables with a correlation greater than 0.8, one was excluded from the analyses. Both multivariate analyses were implemented using log-transformations of the original variables in Statistica (StatSoft Inc. 1984–1998 ver. 6) or SAS ver 8.2 (SAS Institute Inc. 1997). We excluded external measurements from our multivariate analyses because an unknown proportion of their variance is due to differences in measuring methods.

Discriminant function analyses. These analyses were used to evaluate the similarity in the cranial shape between the different species. Each analysis separated pairs of species for each view. The results are reported as the percentage of specimens of one species that can be considered of the other species. The analyses were carried out using only the Procrustes coordinates. For the conventional cranium measurements, the Wilk's lambda discriminant function analysis was used and the percentage of misidentifications for each group was recorded.

Pelage coloration pattern. Four areas of the specimens were sampled: the mid-dorsal surface of the nape, the central part of the back, the belly, and the dark line at the back of the tail; these were evaluated with two different methodologies. The first was recorded through a direct visual comparison with Munsell Soil Color Charts (Munsell Color Co., 1975) under uniform light conditions. We used the color

names and keys in the charts. For each color, we noted the chart, hue, and chroma (i. e., 10YR 8/4, chart hue/chroma). The second was the same area used by Rios and Álvarez-Castañeda (2012). The pelage color of the specimens was determined with an X-Rite Digital Swatchbook spectrophotometer (X-Rite, Inc., Grandville, MI, USA); data were compared to the Commission Internationale d'Eclairage (International Commission on Illumination) with the standard Illuminant F7 for fluorescent illumination, which represents a broad-band daylight fluorescent lamp (6500 K). This standard was used because all the measurements were performed indoors under fluorescent ambient lighting. The instrument provides the reflectance spectrum (390 nm-700 nm) of the object being measured, plus tristimuli color scores (CIE X, Y, and Z). Color was measured on each form; five separate measurements were recorded and averaged. The sample area of the X-Rite Digital has a 3 mm-diameter port. Only adult specimens of both sexes were analyzed (n = 246).

The variation in pelage was evaluated by adding the three-color scores (CIE X, Y, and Z). When brightness is represented by the sum of the three variables, pelage of very different colors (hue) may produce the same brightness. A Student's *t*-test was used to test for differences in pelage brightness between localities. The analyses were carried out with StatisticaTM ver. 5.0 (StatSoft, Inc., Tulsa, OK, USA).

Nomenclature statement. A life science identifier (LSID) number was obtained for the new subspecies *Chaetodipus siccus* detain: urn:lsid:lsid:zoobank.org:pub:7AE730F4-95C2-4ADD-B3D1-7E25AE9FA8A4.

Results

The analyses performed on the two landmark sets of the *Chaetodipus siccus* cranium yielded similar results. The ventral and lateral cranium datasets did not show significant differences between sexes in overall morphological variability (shape) and size (centroid). Consequently, the data for both sexes were pooled for subsequent analyses. The two views show that the marked variations in the skull between the two populations are related to the zygomatic arch, the dorsal area of the braincase and the occipital region.

Cranium shape analysis. The PCA showed significant differences (as per the broken-stick test) between the island and mainland populations in the first two principal components of the ventral view (PC1 = 16.7 %; PC2 = 10.6 %) and lateral view (PC1 = 19.1 %; PC2 = 13.8 %; Figure 3, Table 1).

In the ventral view, the PC1 shows differences between Cerralvo Island and Los Planes Basin specimens in the braincase width and the anterior area of the nasal and rostrum. The PC2 also shows significant differences (as per the brokenstick test); the greatest difference between the skulls refers to the proportion in the extension of the zygomatic arches in the Cerralvo Island population relative to Los Planes. In



Figure 3. Principal Component Analyses for the ventral and lateral views. The wireframe diagram depicting areas of ventral and lateral cranial differentiation highlighted comparing specimens from the mainland at Los Planes Basin (dask line, orange) and Cerralvo Island (solid line, blue). A) Ventral view and B) lateral view.

the lateral view, the PC1 shows that Los Planes has a higher braincase, a more developed occipital region, and a different landmark 13 (posterior area of the zygomatic arch). In the PC2, the differences are related to braincase depth and the position of the back part of the arch with a displacement towards the center of the skull (Figure 3). Combining the wireframe of the two views of the two populations, there are

Table 1. Loadings of the first three components of a Principal Component Analysis
of 15 measurements of Chaetodipus siccus from Cerralvo Island and Los Planes Basin in
the Baja California Peninsula. Specimen classes 4 and 5. Trait abbreviations are provided
in the text.

		Principal Component				
Character	1	2	3	4		
OBL	-0.846	0.162	-0.058	0.014		
PL	-0.632	0.297	0.144	0.138		
PIL	-0.603	0.181	0.176	0.055		
PPL	-0.595	0.288	-0.067	0.046		
MTR	-0.760	-0.014	-0.048	-0.020		
MXR	-0.172	-0.651	0.242	0.247		
ANW	-0.034	0.190	-0.762	0.103		
PNW	-0.563	-0.135	-0.056	0.378		
BW	0.016	-0.850	-0.168	-0.121		
MW	-0.759	-0.382	-0.130	-0.173		
M1W	-0.524	0.047	0.293	-0.571		
WM1	-0.751	-0.029	0.251	0.105		
IW	-0.274	0.232	-0.607	0.099		
IL	-0.138	-0.442	-0.041	0.568		
SD	-0.328	-0.435	-0.395	-0.492		
Eigenvalues	4.379	2.021	1.428	1.198		
% of Variance	4.380	2.022	1.428	1.198		
Cumulative %	0.292	0.135	0.095	0.080		

differences in the muscle insertion areas associated with the chewing process, mainly the temporalis and masseter muscles. Los Planes population has a higher upper part of the braincase, which accommodates a larger temporal muscle, while in the Cerralvo Island population, the insertion areas of the masseter muscle are more developed. These results point to a differential bite-force capacity, in which the Los Planes population would be stronger than the Cerralvo Island population. The statistical analyses of Procustes and centroid distances for both populations yield significant differences as per the broken-stick test (Table 2).

Canonical Variate Analysis. The morphological variability of *Chaetodipus siccus* across the whole sample was explored with CVA analyses, which are highly sensitive to differences between populations. The CVA analysis to evaluate the variation in shape showed that the island and mainland populations show a low overlap in both views. The results of the CVA are consistent with those of the PCA.

 Table 2.
 Statistical analyses of Procustes and centroid distances observed from the analyses of mainland and Cerralvo island specimens.

	Ventral	Lateral					
	Procustes distances						
Ms	398,158	134,100					
F	38.55	28.37					
Ρ	0.001	0.001					
	Centroid distances						
Ms	0.001	0.0003					
f	6.32	4.49					
p	0.001	0.001					
Mainland <i>n</i>	73	72					
Island <i>n</i>	56	57					

Cranial disparity between populations. Differences in ventral and lateral cranial shape are illustrated in Figure 3, a biplot of PC variate scores for the first two axes. To the right and left of these plots, we display the wireframe diagrams that compare the resulting differences in shape between the mainland and Cerralvo Island populations, with most disparate samples aligned on the CP2 axis. Figure 3a shows the variation in the ventral view, and Figure 3b in the lateral view.

The first two CV axes of the Procrustes coordinates combined explain 89.8 % of the variances in the ventral view, with Mahalanobis distances of 2.68 (P < 0.001) and Procrustes distances of 0.0149 (P < 0.001). The lateral view explains 72.5 % of the variances, with Mahalanobis distances of 2.08 (P < 0.001) and Procrustes distances of 0.0158 (P < 0.001). The combination of CV1 scores and the centroid size (log_nCS; Figure 4) cleanly separates the mainland population from the island population, with significant differences (predicted: 3.96 %; P < 0.02) in the ventral view but not in the lateral view.

Morphological analyses. Los Planes Basin specimens showed smaller values than Cerralvo Island specimens in all the craniodental and somatic measurements. The *t*-test showed statistically significant differences at $\alpha = 0.05$ or $\alpha = 0.01$ for one somatic and 15 craniodental traits (Table 3), with similar variances and a standard normal distribution.



Figure 4. Linear regression of canonical variable (CV1) scores on log centroid size (log_CS). Crosses indicate mean values. Mainland at Los Planes Basin (orange) and Cerralvo Island (blue). A) Ventral view and B) lateral view.

Table 3. Mean values for four external and 18 craniodental characters for the holotype, followed by values for the characters of each of the two geographic groups: specimens from the mainland and from Cerralvo Island. *P*-values and significance levels for comparisons between samples using the Student's *t*-test are shown. *n* = sample sizes, μ = population average, and SD = standard deviation. (**P* < 0.05; ***P* < 0.01).

Measurements	Туре	Cerralvo Island (n = 33)		Los Planes	Basin (<i>n</i> = 48)		
		μ±SD	max - min	μ±SD	max - min	t-value	Р
Greatest length	24.77	25.49 ± 0.68	(27.06 - 24.20)	24.75 ± 0.48	(25.87 - 23.92)	6.415	0.01 **
Occipitobasal length	21.49	22.68 ± 0.64	(24.28 - 21.11)	21.98 ± 0.54	(22.97 - 20.92)	1.934	0.06
Palatal length	12.44	13.62 ± 0.47	(14.56 - 12.40)	13.18 ± 0.47	(13.81 - 12.24)	5.753	0.01 **
Palatinal length	10.16	10.35 ± 0.37	(11.08 - 9.55)	9.99 ± 0.32	(10.59 - 9.33)	5.070	0.01 **
Nasal length	10.05	10.34 ± 0.46	(11.32 - 9.51)	9.72 ± 0.30	(10.19 - 9.04)	7.951	0.01 **
Postpalatal length	6.8	7.10 ± 0.26	(7.76 - 6.64)	6.83 ± 0.24	(7.25 - 6.35)	2.723	0.01 **
Mandibular length	10.29	10.78 ± 0.40	(11.53 - 10.11)	10.47 ± 0.34	(11.11 - 9.72)	4.565	0.01 **
Maxillar tooth row	2.87	3.10 ± 0.13	(3.53 - 2.95)	3.11 ± 0.25	(3.52 - 2.79)	3.164	0.01 **
Anterior nasal width	1.93	1.82 ± 0.12	(2.03 - 1.56)	1.84 ± 0.12	(2.06 - 1.67)	5.455	0.01 **
Posterior nasal width	2.06	2.26 ± 0.13	(2.58 - 2.05)	2.13 ± 0.14	(2.37 - 1.88)	2.137	0.04 *
Bulla width	4.03	3.84 ± 0.18	(4.19 - 3.59)	3.96 ± 0.18	(4.33 - 3.65)	4.297	0.01 **
Mastoidal width	13.3	13.14 ± 0.36	(14.01 - 12.54)	12.97 ± 0.29	(13.58 - 12.49)	5.084	0.01 **
M1 width	1.01	1.07 ± 0.03	(1.14 – 1.00)	1.02 ± 0.05	(1.11 - 0.89)	3.967	0.01 **
Width across M1	2.18	2.29 ± 0.14	(2.66 - 2.08)	2.12 ± 0.08	(2.31 - 1.96)	3.293	0.01 **
Diastema length	6.22	6.20 ± 0.23	(6.63 - 5.61)	5.93 ± 0.19	(6.40 - 5.59)	3.772	0.01 **
Interparietal width	7.2	7.06 ± 0.42	(7.71 - 6.06)	6.81 ± 0.35	(7.63 - 6.16)	-3.697	0.01 **
Interparietal length	3.49	3.57 ± 0.23	(4.01 - 3.22)	3.52 ± 0.21	(3.88 - 3.16)	1.570	0.12
Cranium deep	8.53	8.30 ± 0.15	(8.77 - 7.99)	8.29 ± 0.15	(8.53 - 7.98)	0.857	0.39
Total length	167	177.20 ± 8.03	(190 - 158)	167.20 ± 6.06	(177 - 157)	1.617	0.11
Tail length	99	96.30 ± 4.92	(107 - 85)	91.21 ± 3.25	(99 - 85)	2.316	0.02 *
Hind foot length	21	22.70 ± 1.18	(24 - 20)	20.60 ± 1.17	(23 - 19)	1.391	0.17
Ear length	8	9.32 ± 0.68	(10 - 8)	7.92 ± 0.72	(9 - 7)	0.541	0.59
Weight	15	14.96 ± 1.77	(20.0 - 11.5)	14.55 ± 1.72	(18.0 - 10.0)	1.165	0.25

The first three principal components of the cranial measurements in the PCA explained 51.37 % of the total variation (Figure 5). The loadings of the variables analyzed along components 1, 2, and 3 are shown in Table 1. All variables showed a positive loading on the first axis (except BW), thus indicating a general variation in size, where OBL, BW, and ANW had relatively large loadings. Meanwhile, LIF, DI, and DL (positives) on the second axis and BZP (negative) on the third axis had relatively large loadings. GLS, LN, and PL were excluded from the PCA and CVA because these measurements showed a correlation greater than 0.8 with other measurements. Individual scores for the components (PC1 and PC2) are plotted in Figure 5. Two major groups of specimens can be identified, one clustering individuals from Cerralvo Island and the other grouping specimens collected in Los Planes Basin. Both groups overlap marginally (Figure 5).

Discriminant analyses. The differences in the ventral and lateral views between means using Procrustes and Mahalanobis distances between the Cerralvo Island and mainland populations were statistically significant (P < 0.001; Table 3). For cranium measurements, the discriminant function analysis revealed a significant variation between the Los Planes Basin and Cerralvo Island populations (Wilk's lambda = 0.28; P < 0.0001) and showed a clear separation between them (Figure 3b). The first discriminant function accounted for 63.9 % of the variance. The most influential variables for the first and second functions were WM1 and MXR, respectively. The percentage of misidentifications was zero (0) for each group. Pelage coloration pattern. Specimens from the mainland and Cerralvo Island populations have a noticeably distinct coloration. The specimens from Los Planes showed darker greyish shades, while those of Cerralvo Island were lighter in brownish shades. The underparts, particularly the belly, display a somewhat different but still very similar coloration that cannot be used to differentiate between specimens from any of these areas (Table 4). In mainland specimens, the dorsal and lateral pelage is grizzly, very dark greyish brown (10YR 3/2) mixed with very pale brown (7.5YR 8/1); a very dark gray (7.5YR 3/1) dorsal part of the tail, and light gray (7.5YR 3/1) underparts. On the other hand, the specimens inhabiting Cerralvo Island have a black (7.5YR 2.5/1) upper and lateral pelage mixed with brown (7.5YR 5/3); dark brown (7.5YR 3/2) tail dorsum, and pink (7.5YR 7/3) underparts.

The pelage color using the X-Rite Digital Swatchbook spectrophotometer shows significant differences between Cerralvo Island and Los Planes populations in all the color measurements: nape (P < 0.01), back (P < 0.05), and tail strip (P < 0.01; Table 4). No significant differences were found in the ventral coloration.

Discussion

Non-geographic variation. The analyses between sexes did not show statistically significant differences in shape in any cranial views. However, significant differences between the mainland and Cerralvo Island populations were found in size, cranium shape, and fur color. In addition, the genetic analyses revealed that the Cerralvo Island population is



Figure 5. Specimen scores of 18 craniodental measurements of adult individuals of *Chaetodupus siccus*. Specimens from the mainland at Los Planes Basin (orange) and Cerralvo Island (blue). A) For Principal Components 1 and 2 generated from the correlation matrix and B) for Canonical Variants extracted from a discriminant function analysis. The percentage of the total variation explained by each axis is indicated.

within the *C. siccus* clade and has unique haplotypes that differ from those of *C. siccus* inhabiting Cerralvo Island (Álvarez-Castañeda and Rios 2011).

Cerralvo Island populations (C. siccus) were isolated from mainland ones during the Pleistocene by a sea channel impassable for the vicariant arenarius complex (Carreño and Helens 2002; Álvarez-Castañeda and Rios 2011; Aguilera-Miller 2011). These barriers between islands and the mainland persisted in the Last Glacial Maximum (LGM), while land bridges connected many islands to the adjacent mainland until about 18,000 years ago, when the sea level was 120 m lower than today (Fairbanks 1989). However, the least depth between the islands and the mainland is about 235 m (Case et al. 2002), so Cerralvo Island populations have remained isolated since the LGM and currently comprise one endemic species of mammals (Peromyscus avius, Cornejo-Latorre et al. 2017) and two of reptiles (Aspidoscelis ceralbensis, Chilomeniscus savagei, Case et al. 2002). Under these circumstances, insular populations of C. siccus have remained isolated from mainland ones. However, Cerralvo Island harbors species introduced by fishers, such as jackrabbits (Lepus californicus), cats (Felis sylvestris), and goats (Capra aegagrus), for use as sources of fresh meat in the islands during the fishing season (Lorenzo et al. 2010).

The results show that the specimens from populations inhabiting Los Planes basin (mainland) and Cerralvo Island differ in external characteristics, including the coloration pattern and size. The skull shape is different in both populations, each showing areas associated with the main chewing muscles with differential development. The Cerralvo Island population has more developed zygomatic arches (where masseter muscles are attached) and near to the central axes of the skull. This position to the center of the skull allows for a greater distance with the jaw and a stronger muscle. On the other hand, Los Planes specimens have a higher braincase related to a stronger temporal muscle (bite force). These conditions have been recorded in relation to the bite force in species of the superfamily Geomyoidea (Lessa and Stein 1992; Cox et al. 2020). Both populations have been isolated without a generic flow at least from the LGM due to the presence of the Gulf of California. Each population inhabits a different environment (soil characteristics, vegetation composition, and physiography) and can be identified as geographic units with a sustained evolutionary divergence (sensus Patton and Smith 1990:107) with its own evolutionary tendency. Under these conditions, our findings support the hypothesis that each population has been adapted to its own habitat and has its own evolutionary tendency. Based on the skull anatomical differences associated with the chewing muscles, fur color, and size, each population can be considered taxonomically different at the subspecies level (Lidicker 1962); therefore, a new name is proposed for the currently unnamed subspecies inhabiting Los Planes Basin in the Baja California Peninsula.

Chaetodipus siccus (Osgood, 1907)

Perognathus penicillatus siccus (Osgood, 1907). Perognathus arenarius siccus (Nelson and Goldman, 1929). Chaetodipus siccus Álvarez-Castañeda and Rios (2011).

Holotype. U. S. National Museum, Biological Survey Collection 146,890 (USNM), an adult male collected by E. W. Nelson and E. A. Goldman on February 13, 1906, at Ceralbo [Cerralvo] Island, Lower California [Baja California Sur], México. The specimen consists of a stuffed museum study skin with accompanying cranium and mandibles.

Paratypes. 10 specimens (no catalog number is given).

Common name. Cerralvo Island Pocket Mouse, ratón de abazones de Isla Cerralvo.

Distribution. Chaetodipus siccus is known only from the southwestern part of Cerralvo Island in the Gulf of California, Baja California Sur, México. *C. siccus* inhabits only areas with deep sandy soil.

Diagnosis and description. From the original description (Osgood, 1907:20). Size larger than in *Perognathus arenarius* [*Chaetodipus arenarius*]; very weak rump bristles rarely present; color dimorphic, buff phase slightly darker than in *C. arenarius*; gray phase markedly different; cranium large and heavy; mastoids relatively large.

Comparisons. For a comparison between *C. siccus* from Cerralvo Island and the Los Planes population, refer to the description of the latter.

Ecology. Chaetodipus siccus is not abundant; it is only found in the southwestern part of the island and on the sandy bed of streams. The local vegetation where it thrives is sarcocaule scrubs, dominated by plants of the families Euphorbiaceae, Cactaceae, and Leguminosae (León de la Luz et al. 1996).

Remarks. From the original description (Osgood 1907:20), "Careful examination reveals a very weak rump bristle in several species of *siccus*, though they are not found in other members of the *penicillatus* (sensu *arenarius*; Nelson and Goldman 1929) series".

Etymology. From siccus/sicca/siccum, latin. Refers to the physical state of any object that lacks moisture, so it can be translated as "dry" or "arid". Pocket Mouse of arid land.

Conservation status. The species is listed as Threatened by the Mexican regulations based on its restricted distribution range.

Chaetodipus siccus liaae, new subspecies

Holotype. CIB 5616, an adult male collected by Edgar Martínez-Agama (original number 106) on October 1, 2000, at Ensenada de Muertos, Baja California Sur, México, 23.9992° N, -109.8269° W; Figure 1). The specimen consists of a stuffed museum study skin with accompanying cranium and mandibles (Figure 6) housed at the Collection of Mammals of Centro de Investigaciones Biológicas del Noroeste (CIB).

Paratypes. CIB 5600-5615, 5617-5647.

Common name. Lia Pocket Mouse, ratón de abazones de Los Planes.

Distribution. Chaetodipus siccus liaae is currently known only from Los Planes Basin lowlands (*ca.* sea level to 250 m), 40 km south of La Paz, Baja California Sur. *C. s. liaae* inhabits only the basin lowlands, where the soil is sandy and deep; it is less abundant at slopes as the soil becomes stonier, where it coexists with *C. spinatus*. The latter is another pocket mouse likely endemic to Los Planes Basin but thriving mainly in slopes with stony soil.

Diagnosis. A member of the *C. arenarius* complex, *C. s. liaae* is characterized externally by a small body. The general pelage coloration is light gray; the dorsum and sides are grizzly, very dark greyish brown (10YR 3/2) mixed with white (7.5YR 8/1); lateral lines usually absent, very light when present; tail very dark gray (7.5YR 3/1) and crested dorsally, light gray (7.5YR 7/1) ventrally; a very dark gray ring around the eye (7.5YR 3/1); ears light brown (7.5YR 6/4) with a very dark gray edge (7.5YR 3/1); cheeks, neck, and underparts white (7.5YR 9/1); dorsal parts of feet white (7.5YR 8/1). Cranium: *C. s. liaae* has shortened face and nasal bones; smaller diastema; branch of the mandible relatively small; cheektooth relatively small and narrow; braincase flattened and relatively narrow; bulla elliptical.

Description and comparisons. Chaetodipus siccus liaae differs externally from C. s. siccus — a pocket mouse that inhabits Cerralvo Island. The former is restricted to Los Planes Basin by its smaller size (mean total length 163.7 mm for C. s. liaae vs 177.3 mm for C. s. siccus) and smaller somatic and cranium measurements (Table 1). The dorsal and lateral pelage is grizzly, very dark greyish brown (10YR 3/2) mixed with very pale brown (7.5YR 8/1) in C. s. liaae vs black (7.5YR 2.5/1) mixed with brown (7.5YR 5/3) in C. s. siccus; in addition, the dorsal part of the tail is very dark gray (7.5YR 3/1) in C. s. liaae vs dark brown (7.5YR 3/2) in C. s. siccus; ventrally, the pelage is light gray (7.5YR 3/1) in C. s. liaae vs. pink (7.5YR 7/3) in C. s. siccus; all other pelage areas are similar in color in these two subspecies.

Ecology. Chaetodipus siccus liaae is an abundant pocket mouse, frequently being the dominant species and the only Chaetodipus mouse in the basin lowlands and salty soils. Specimens were captured in large numbers in the basin lowlands, characterized by a slope <1.6 % and deep soils, and were absent in slopes where stony and shallow soils prevail. The dominant vegetation in the basin lowlands is sarcocaule scrub dominated by plants of the families Euphorbiaceae, Cactaceae, and Leguminosae (León de la Luz et al. 1996), including Pachycereus pringlei (cardón), probably the largest plant species thriving in the Baja California Peninsula. Chaetodipus s. liaae occurs in agricultural fields and a small area with salty soils near the coastline. Data from the samples shows that reproduction is seasonal with two peaks, the first in April-May and the second mostly from August to October, although the latter period may vary according to the precipitation pattern in the rainy season (hurricanes). Juveniles were collected in early March and from August to October.

Remarks: The sequences of 153 specimens of *C. siccus* show 56 Cytochrome-b haplotypes, three being unique to *C. s. siccus* and 53 unique to *C. s. liaae*; only one of the most frequent haplotypes occurs in both subspecies (Aguilar Miler et al. 2011; Álvarez-Castañeda and Rios 2011, refer to Figure 3). Other small mammals collected at Los Planes Basin were Notiosorex crawfordi, Thomomys nigricans anitae, Ammospermophilus leucurus extimus, Chaetodipus spinatus peninsulae, C. rudinoris extimus, Dipodomys merriami melanurus, Peromyscus eva eva, and Neotoma bryanti bryanti.

Conservation status. The upper portion of Los Planes Basin is a currently inactive mining area; however, there are gold deposits across the area, so mining could be reactivated at any time. Agriculture is practiced in the basin lowlands, and coastal areas include tourist, recreational, and



Figure 6. Dorsal, lateral, ventral, and mandibular views of the holotype skull of *Chae-todipus siccus liaae*. Adult male (CIB 5616) from Ensenada de Muertos, Baja California Sur, 23.9992° N, -109.8269° W.

residential uses. These conditions will involve growing conflicts with wildlife that are likely to increase in importance and magnitude because many parties with strong opinions clash over management objectives, with adverse impacts on wildlife (Delibes-Mateos 2015). Besides, climate change is likely to modify the habitat and distribution of microendemic species due to rising temperatures and changes in precipitation (Sántiz et al. 2016). The potential distribution range of this subspecies is 280 km², without considering areas with current human activities.

Etymology. The name proposed honors Lia C. Méndez Rodríguez, Ph. D., for her outstanding career in toxicology and mammalogy, and Lia Montserrat Álvarez Méndez, MSc. Both have provided invaluable support for decades.

Acknowledgements

Appreciation is expressed to P. Cortés for their valuable assistance in the field; to C. Gutiérrez for laboratory assistance; to E. Ríos, for assisting in specimen measurements; to the Associate Editor and two anonymous reviewers who contributed many valuable comments that helped improve the manuscript; and to María Elena Sánchez-Salazar for editing the English manuscript. Financial support was provided by the Consejo Nacional de Ciencia y Tecnología (CONACYT, grants J28319-N, 39467Q, 263069, and 151189).

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Associated editor: Lazaro Guevara

Submitted: Agosto 18, 2023; Reviewed: October 21, 2023 Accepted: April 25, 2024; Published on line: May 29, 2024

Appendix 1

Specimens Examined:

Chaetodipus siccus liaae (n = 596): 11.8 km S, 0.6 km E Los Planes (23.8598, -109.9300; n = 4); 11.5 km S, 8.7 km W Los Planes (23.8635, -110.0209; n = 1); 11 km S, 2.5 km E Los Planes (23.8674, -109.9113; n = 1); 10.8 km S, 3.7 km W Los Planes (23.8703, -109.9711; n = 1); 11 km S, 3.7 km W Los Planes (23.8703, -109.9711; n = 10); 10.4 km S, 5.5 km W Los Planes (23.8708, -109.9663; n = 22); 11 km S, 8 km W Los Planes (23.8712, -110.0136; n = 9); 10.4 km S, 6 km W Los Planes (23.8728, -109.9914; n = 21); 10.6 km S, 3 km W Los Planes (23.8729, -109.9906; n = 11); 10.3 km S, 7.4 km W Los Planes (23.8742, -110.0091; n = 20); 9.2 km S, 3.1 km W Los Planes (23.8828, -109.9663; n = 14); 9.1 km S, 2.9 km W Los Planes (23.8850, -109.9073; n = 2); 8.7 km S, 8 km W Los Planes (23.8893, -110.0183; n = 8); 7.4 km S, 8.6 km W Los Planes (23.8990, -110.0218; n = 5); 7.5 km S, 7.6 km W Los Planes (23.9005, -110.0121; n = 21); 7 km S, 8 km W Los Planes (23.9044, -110.0167; n = 9); 5.98 km S, 0.83 km E Los Planes (23.9130, -109.9272; n = 2); 6 km S, 2.8 km E Los Planes (23.9130, -109.9073; n = 31); 6 km S, 2.8 km W Los Planes (23.9130, -109.9073; n = 25); 5.7 km S, 8 km W Los Planes (23.9155, -110.0164; n = 15); 4.9 km S, 1.7 km W Los Planes (23.9232, 10.0164; n = 15); 4.9 km S, 1.7 km S-109.9535; n = 10); 5 km S, 1.6 km W Los Planes (23.9232, -109.9535; n = 17); 5 km S, 1.6 km W, Los Planes (23.9232, -109.9535; n = 12); 4.6 km S, 1.7 km E Los Planes (23.9248, -109.9170; n = 2); 4.5 km S, 1.9 km E Los Planes (23.9254, -109.9073; n = 18); 5.6 km S, 1.6 km W Los Planes (23.9254, -109.9170; n = 46); 4.3 km S Los Planes (23.9283, -109.9358; n = 55); 4.24 km S, 400 mts W Los Planes (23.9326, -109.9466; n = 4); 3.7 km S, 1 km E Los Planes (23.9334, -109.9480; n = 7); 3.1 km S, 4 km W Los Planes (23.9371, -109.8971; n = 3); 3 km S, 7 km W Los Planes (23.9440, -110.0060; n = 27); 2.5 km S Los Planes (23.9446, -109.9363; n = 1); 1.3 km S, 0.8 km W Los Planes (23.9549, -109.9278; n = 3); 1.2 km S, 1.7 km W Los Planes (23.9569, -109.9529; n = 7); 0.5 km S, 6.1 km W, Los Planes (23.9625, -109.9978; n = 4); 5 km S, 6 km W Los Planes (23.9625, -109.9978; n = 9); 3 km N, 2.6 km W Los Planes (23.9959, -109.9616; *n* = 10); Ensenada de Muertos (23.9992, -109.8269; *n* = 49); 1 km W Ensenada de Muertos (23.9993, -109.8371; n = 1); 5.3 km N Los Planes (24.0152, -109.9334; n = 18); 6 km N, 2 km E Los Planes (24.0207, -109.9175; n = 21); 6 km N, 8 km W Los Planes (24.0226, -110.0146; n = 19); 7 km N, Los Planes (24.0261, -109.934; n = 2); 8.1 km N, 6.3 km E Los Planes (24.0377, -109.8729; n = 4); Brisamar, 25 km W La Paz (24.1486, -110.5427; n = 5); 1.5 km S, 1.6 km W Los Planes (24.1694, -110.3714; *n* = 10).

Chaetodipus siccus siccus (*n* = 68): Isla Cerralvo (24.1554, -109.8692; *n* = 31); Playa Los Viejitos, Isla Cerralvo (24.1527, -109.8697; *n* = 37).

Rediscovery of Oryzomys fulgens: implications for the taxonomy of Oryzomys

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The rodents belonging to the genus Oryzomys are among the most widely distributed groups inhabiting North America. Currently, 13 species with continental, peninsular, or insular distributions are known. However, this genus also faces significant conservation challenges, primarily due to climate change and other human-related activities. One such enigmatic species is Oryzomys fulgens. Its main challenge lies in the fact that it was originally described from an imprecise type locality, simply labeled as "Mexico." Fortunately, recent research has delimited its range to the Basin of México. Nonetheless, the lack of comparison between its holotype and other specimens of the genus in México has hindered the accurate assignment of its name to any known taxon, despite being one of the oldest names within the genus, O. fulgens has rarely been used since its original description. During our research, we collected two specimens of O. fulgens from Xochimilco, México City, a remnant wetland situated within the Basin of México, nestled within one of the largest and most urbanized cities globally. To properly assess its taxonomic status, we compared our specimens with the holotype of O. fulgens, as well as with other species from southern and western México. The considerable morphological differences we observed in O. fulgens, coupled with its distinct and unique habitat as the sole species of the genus occupying temperate regions at high elevations, lead us to propose that it is indeed a distinct species, separate from O. mexicanus and O. albiventer. Historically, its distribution was likely limited to the Basin of México. Today, O. fulgens faces serious conservation problems, mainly due to the restriction of its current distribution, as far as we know, confined to Xochimilco. Although the ancestral agricultural system known as "chinampas" might provide an ideal environment for sustaining populations of this species, it remains under significant pressure from various human activities, predators, and competition with invasive species like the black rat. Urgent conservation efforts are needed to safeguard the unique habitat of O. fulgens and protect this enigmatic species from further decline.

Los roedores del género Oryzomys son uno de los grupos más ampliamente distribuidos que habita Norteamérica. Actualmente se conocen por 13 especies con distribución continental, peninsular o insular; asimismo, también es uno de los géneros con más problemas de conservación, por el cambio climático y otras actividades relacionadas con el humano. Oryzomys fulgens es una especie desconocida y enigmática, su principal problema es que fue descrita de una localidad tipo poco precisa, sólo como "Mexico", aunque recientemente se delimitó a la Cuenca de México. Además, el holotipo nunca se ha comparado con otros ejemplares del género en México, esto ha impedido que el nombre se asigne correctamente a algún taxón conocido y, desde su descripción, prácticamente no fue utilizado a pesar que es uno de los nombres más antiguos del género. Nosotros recolectamos dos ejemplares de O. fulgens dentro de la región de Xochimilco, en un humedal remanente dentro de la Cuenca de México e inmerso dentro de una de las ciudades más grandes y urbanizadas del mundo. Para evaluar adecuadamente su estatus taxonómico, nuestros ejemplares fueron comparados con el holotipo de O. fulgens y otras especies del sur y oeste de México. Por las marcadas diferencias morfológicas y por ser la única especie del género que habita regiones templadas y en grandes elevaciones, proponemos que sea una especie distinta de Oryzomys, y por lo tanto, diferente de O. mexicanus y O. albiventer. Su distribución histórica probablemente se limitaba a la Cuenca de México. Oryzomys fulgens tiene serios problemas de conservación al limitar su distribución actual, hasta donde nosotros sabemos, dentro de Xochimilco. A pesar que el sistema agrícola ancestral llamado "chinampas" pueden ser ideal para mantener las poblaciones de la especie, sigue estando bajo mucha presión por actividades humanas, depredadores exóticos y la competencia con especies invasoras, como la rata negra. Se necesitan esfuerzos de conservación urgentes para salvaguardar el hábitat único de O. fulgens y proteger esta enigmática especie.

Keywords: Basin of México; conservation; Oryzomys; taxonomy; Xochimilco.

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Introduction

The tribe Oryzomyini is one of the most diverse groups of rodents on the planet, with 33 genera and over 152 species (Weksler 2015; Pardiñas et al. 2017). However, it is also one of the taxa that has undergone numerous recent taxonomic changes. These proposals include the split of some genera into distinct new genera, new species, and the revival of old names (Weksler 2006; Percequillo et al. 2011; Pine et al. 2012; Brito et al. 2019; Brito et al. 2020; Caccavo and Weksler 2021; Prado et al. 2021; Semedo et al. 2021). Recent phylogenetic studies, combined with detailed morphological and morphometric analysis, have revealed previously hidden diversity of rodents within the tribe Oryzomyini. The recognized diversity is expected to continue growing, particularly in the tropical regions of South America, where these rodents have been historically understudied, but are known to be highly diverse. Within the tribe Oryzomyini, the genus Oryzomys has undergone significant reorganization in the last two decades, with morphological and molecular analyses leading to its division into at least 11 different genera (Weksler 2006; Weksler et al. 2006). Nevertheless, it remains one of the most widely distributed genera within the family, found from the southeastern United States of America to Colombia and Venezuela, including several islands (Hall 1981; Musser and Carleton 2005; Weksler 2015).

Oryzomys, after being redefined, has also undergone recent taxonomic changes, including the placement of taxonomic forms that were previously considered subspecies at the species level (Carleton and Arroyo-Cabrales 2009; Hanson et al. 2010; Indorf and Gaines 2013). Since of the revision of Musser and Carleton (2005), four species were recognized within the genus (O. couesi, O. dimidiatus, O. gorgasi, and O. palustris), later an ancient species from the island of Jamaica, O. antillarum, was confirmed (Morgan 1993; McFarlane et al. 2002; Weksler et al. 2006). Through a morphological and morphometric study, three additional species (O. nelsoni, O. peninsulae, and O. albiventer) were recognized (Carleton and Arroyo-Cabrales 2009). Subsequently, Hanson et al. (2010) conducted a molecular phylogenetic analysis of the genus in Central America and North America, leading to the separation of O. texensis and O. mexicanus. The taxonomic situation of O. argentatus is also complex and widely discussed, but current evidence indicates that it is a distinct species facing serious conservation problems (Goodyear 1991; Wang et al. 2005; Indorf and Gaines 2013). Actually, at least 11 species of the genus Oryzomys are known, with two additional species, one from Panama and other from Costa Rica, pending formal descriptions (Hanson et al. 2010).

The species *Oryzomys couesi* (Alston, 1877) is the most widely distributed species of the genus, found on the coastal plains of the Gulf of México from Tamaulipas in México to northern Colombia, including most of the Yucatán Peninsula, as well as on the Cozumel Island (<u>Reid 1997</u>; <u>Hanson et al. 2010</u>; <u>Vázquez-Domínguez et al. 2020</u>). *Oryzomys mexicanus* Allen, 1897 distributes in tropical regions of the coastal plains of the Mexican Pacific from Sonora to at least to El Salvador (Hanson *et al.* 2010; Percequillo 2015). Ramírez-Pulido *et al.* (2014), after formally restricting its type locality to the "Valle de Mexico", proposed that this species must be called *O. fulgens* Thomas, 1893 because this name has priority name over *O. mexicanus*.

Oryzomys fulgens is an enigmatic form known from only a few specimens. The main problem is that it was described with a single specimen without a precise type locality, only as "México", and the holotype has never been compared with other specimens from México (Merriam 1901; Goldman 1918; Carleton and Arroyo-Cabrales 2009); these facts have prevented the name from being correctly assigned to any known population and as such contrasted with populations of other taxa, making it a name that practically have not being used, despite the fact that the name is one of the oldest associated to the genus. We interpreted that the authors of the past visualized the species, or in its case the name, in at least three different ways after its description. Many researchers accepted it with caution as a distinct species, awaiting future research that would delineate it geographically and morphologically (Merriam 1901; Goldman 1918; Hall 1981). Other authors consider it taxonomically dubious (Anderson 1977), assuming that it is nomenclaturally unavailable. Most of the times it was recognized as a subspecies of "O. couesi", lacking a clearly defined geographical distribution as expected for a subspecies (Musser and Carleton 1993, 2005). Thus it was used for more than 40 years; sometimes it was used as conspecific of O. crinitus (e. g., Ramírez-Pulido et al. 2005), another subspecies of "O. couesi" that was also described from the Basin of México and therefore theoretically occupied the same distribution area.

Rice rats of the genus *Oryzomys* usually live in close relationship with water or flooded areas and wetlands, both in inland wetlands and in mangroves and marshes (Wolfe 1982, 1985). However, although they can be found in other environments (Genoways and Timm 2005; Engstrom *et al.* 1989; Poindexter *et al.* 2012a, b), but that periodically include semi-aquatic microhabitats (Benson and Gehlbach 1979). Therefore, we considered that the region Xochimilco region could be the last possible area in the Basin of México with the ecological conditions where a population of *O. fulgens* could still exist. We sample on that area and at the light of results based on the captured specimens we made taxonomic considerations on the distinction of *O. fulgens*.

Material and methods

The area of the Xochimilco lake is a valuable remnant wetland within one of the most highly urbanized regions in México. It is located within the Basin of México (also known as Valle de México) in the southeastern of México City. It comprises an area of 2,657 hectares. It is internationally recognized as a Zone of Historical Monuments, Protected Natural Area, a World Natural and Cultural Heritage Zone, and a RAMSAR wetland (<u>Wigle 2010</u>). Xochimilco comprises a complex lake system consisting of interconnected channels, permanent, and temporary lagoons. The depth of these water bodies varies, with some channels reaching 60 cm and the San Gregorio Atlapulco lagoon reaching depths of 2 to 4 m. The entire system is estimated to have an approximate length of 203 km, and currently relies on artificial feeding through treated water sources (Figure 1).

Marginal aquatic vegetation in the Xochimilco region is diverse, consisting of emerged vegetation such as tulares (Typha latifolia) and floating vegetation including water lilies (Eichornia crassipes) and lentils (Lemna minuscula and L. trisulcata) found in channels and lagoons. In non-flooded areas, there are cultivated lands, occasionally interspersed with different pastures such as Agrostis semiverticillata and Pennisetum clandestinum. Trees in the region comprises mixed forests consisting of native species like pines (Pinus sp.), cedars (Cedrus sp.), ahuehuetes (Taxodium sp.), and willows (Salix sp.). However, there are also introduced species like casuarinas, eucalyptus, and pirules (Schinus sp.) (Jiménez-Gutiérrez 2016). The Xochimilco region has a temperate sub-humid climate characterized by summer rains. The average annual rainfall is around 620 mm and the average annual temperature is 16 °C (Jiménez-Gutiérrez 2016).

During our study on the wild mammals that inhabit the Xochimilco Lake Zone, aimed at understanding the diversity, distribution, and abundance in the region, we conducted 12 monthly samples from February 2013 to February 2014. For rodents, we used Museum Special, Victor, and Sherman traps baited with oats. In total, 2,517 trap-nights were set across all types of plant associations.

Most of the collected rodents were released at the site of capture, while some individuals were prepared as museum specimens and deposited in the Mammal Collection of the Metropolitan Autonomous University (UAMI); no tissue sample was salved. Additionally, reference and comparison specimens, unless explicitly stated, are housed in the same collection. Collected specimens were morphologically compared with the holotypes of *Oryzomys fulgens* and *O. couesi* by two of us (NGR and JAC), which are preserved at the Natural History Museum (BMNH) in London, England.

External dimensions, in mm, were transcribed from skin tags: total length (TOTL); tail length (TL); hindfoot length (HFL); and ear length (EL). Other 17 cranial variables were measured to 0.01 mm using digital calipers, and taken according to the specifications of Carleton and Arroyo-Cabrales (2009). These measurements and their abbreviations are: total length (TOTL), tail length (TL), hindfoot length (HFL), ear (pinna) length (EL), occipitonasal length (ONL), greatest zygomatic breadth (ZB), breadth of rostrum (BR), interorbital breadth (IOB), breadth of braincase (BBC), depth of braincase (DBC), breadth of bony palate (BBP), breadth of zygomatic plate (BZP), length of diastema (LD), breadth across incisive foramina (BIF), width of the upper first molar (WM1), length of rostrum (LR), length of bony palate (LBP), postpalatal length (PPL), length of incisive foramen (LIF), coronal length of maxillary tooth row (CLM), and length of auditory bulla (LAB). All specimens examined are adults according to the criteria of Carleton and Arroyo-Cabrales (2009).



Figure 1. Region associated with the distribution of *Oryzomys fulgens*. Left: Limits of the Basin of Mexico with the Xochimilco region (in red). Right: Lake Xochimilco within the urban area of Mexico City. The blue dots show the localities of *O. fulgens*: 1) Probably the type locality "Tepepan" (Boucard 1894), 2) type locality of *O. crinitus* of "Tlalpan" (Merriam 1901), 3) "Xochimilco" (González-Romero 1980), and 4) 2 km N, 3.9 km W of San Gregorio Atlapulco, 2,240 m (this study).
Results

With 2.517 trap-nights a total of 269 rodent specimens were collected, resulting in a capture success rate of 9.4%. Collected specimens belong to 17 species, including Reithrodontomys megalotis, Baiomys taylori, Mus musculus, Rattus sp., Microtus mexicanus, and Oryzomys fulgens. Among them, R. megalotis was the most abundant species, accounting for 54.6 % of the collected rodents, while Oryzomys fulgens was the least abundant (two specimens), comprising only 0.7 % of the specimens. Invasive species were also present in our samples, with Mus musculus representing 8.9 % and Rattus sp. accounting for 6.7 % of the collected rodents. It is worth noting that despite the genus Peromyscus being one of the most diverse and abundant in North America (Carleton 1989), no species from this genus was trapping sites during this study. However, it is important to mention that two species of the genus (P. gratus and P. melanotis) were previously recorded in the study area more than 40 years ago (González-Romero 1980).

In the locality of 2 km north and 3.9 km west of San Gregorio Atlapulco (19.2758° N, -99.0940° W), we collected two female specimens of Oryzomys fulgens. One specimen was preserved as a skin and skull and deposited under number UAMI 17346, while the other specimen was released at the collection site (Figure 2C, D). Both individuals were captured in the canals surrounding a chinampa in February 2014. The collection site was situated on the banks of a canal where there was a high abundance of tulares (Typha latifolia; Figure 2B). These areas also contained other types of vegetation, including crops. Land cover of this locality also exhibited an abundance of grass (Pennisetum clandestinum), while the tree layer consisted of ahuehuetes (Taxodium sp.), along with some eucalyptus and pirules. Additionally, Baiomys taylori, Reithrodontomys megalotis, and Rattus sp. were found in the same area. Oryzomys fulgens was the only rodent species exclusively found within the tulares, but nearby other species are found, primarily R. megalotis.



Figure 2. Photographs of the Xochimilco region and specimens of Oryzomys fulgens. A) General view of the Xochimico wetland. B) Precise site where we collected the two specimens of O. fulgens. C) A female specimen (UAMI 17346) and D) A second female specimen (released at the collection site) of O. fulgens.

Discussion

Taxonomy. Oryzomys fulgens was originally described based on a single male specimen (Holotype: British Museum Natural History, BMNH 70.6.20.3) collected by Adolphe Boucard in México. The precise locality of the specimen is unknown, as it was only recorded as "Mexico" (Thomas 1893a). Adolphe Boucard, a naturalist, along with Auguste Sallé, collected several species of amphibians, reptiles, invertebrates, and particularly birds in central and southern México between 1865 and 1867 (Flores-Villela et al. 2004). Many of these specimens did not have specific localities documented (see Papavero and Ibanez-Bernal 2001). Thomas (1893b) suggested that the type specimen of O. fulgens was likely collected alongside the holotype of the gopher Geomys merriami [Cratogeomys merriami], which is primarily found in the Basin of México. This assumption prompted Merriam (1901) and later Goldman (1918) to designate the type locality as "southern Mexico, probably in or near the Valley of Mexico [= Basin of México]." Finally, Ramírez-Pulido et al. (2014) provided additional evidence and formally restricted the type locality to the "Valle de México" (Gardner and Ramírez-Pulido 2020). Additional evidence supporting that the type specimen of O. fulgens was collected within the Basin of México is that A. Boucard, during his extensive travels throughout México, journeyed from Acapulco to México City (Boucard 1894:72). Within the Basin of México, he passed through [San Miguel] Topilejo, which is approximately 19 km from the collection location of our O. fulgens specimen from Xochimilco. He also reached Tepepa[n], which is 9.7 km from our collection site. It is plausible that it was in Tepepan where he collected both the type specimens of Cratogeomys merriami and O. fulgens, as in the past, it likely had suitable conditions for both species. However, presently, Tepepan is integrated into the urban area of México City. Defining the type locality was crucial because O. fulgens, after O. couesi and O. palustris, is the third oldest name among all species and subspecies associated to the genus Oryzomys, and then is of much relevance to name any lineage of Oryzomys present in México.

We had the opportunity to compare the type of O. fulgens with the specimen collected at Xochimilco. Although the holotype skull is missing most of braincase and the zygoma is broken, they are very similar, except that the type has the interorbital region not completely convergent anteriorly and the frontal extension of the zygomatic plate is more pronounced (Figure 3). Some of these differences and, even more, the similarities, had already been noticed by Merriam (1901) when interpreting the O. fulgens description and comparing it with the holotype of O. palustris crinitus. In fact, this author mentioned that they are "strongly marked species appears to be closely related" (see also Goldman 1918). Our specimen is female and the type of O. fulgens is male, which is relevant given that the species of this group present sexual dimorphism (Carleton and Arroyo-Cabrales 2009). In addition, even when the type misses its posterior part of the skull, we can interpret that the two specimens are very similar and likely belong to the same species. We also compared the skulls of O. fulgens with those of O. albiventer and O. mexicanus, the other species southern and western México to understand and morphologically define the characteristics of the species of the genus Oryzomys in México. In the comparison of O. fulgens with O. mexicanus we observed that the former has the previous enterostyle of the first upper molar; the upper molars are noticeably larger and more robust, particularly the anterocone is larger (Figure 4); the posterior edge of the incisive foramen reaches the posterior edge of the upper first molars (in most specimens of O. mexicanus it does not reach that edge); the lateral extension of the lacrimal bone forms only a small notch whereas in O. mexicanus it is well developed; the interorbital region convergent anteriorly but with a contraction in the proximal part, in O. mexicanus this contraction regularly does not occur (Figure 3); the posterior exposure of the parietal is large and square while in O. mexicanus it is small and triangular. In addition, the hair is long, it has a fine, dense and silky coat, while in O. mexicanus it is short and harsh. In O. fulgens, unlike O. albiventer, the mesopterygoid fossa extends forward before the middle of the pterygoid fossa, while in albiventer it extends beyond the middle of the pterygoid fossa; the posterior part of the rounded occipital and nape extends behind the condyles of the foramen magnum, in O. albiventer it is flat and extends further behind the condyles (Figure 1); the interparental bone thick and elongated versus small and narrow. O. albiventer is notably larger in overall skull and body size (Table 1; see also Carleton and Arroyo-Cabrales 2009). The dorsal hair of O. fulgens is long and silky like that of O. albiventer, possibly as an adaptation of the two species to temperate regions at higher elevations (see below; Figure 3).

Craniodental measurements are also similar between the holotype of *O. fulgens* and the Xochimilco specimen, although the latter is slightly larger in the breadth of zygomatic plate and length of bony palate (Table 1), but these small differences could be interpreted as sexual dimorphism. However, the two *O. fulgens* specimens are consistently larger in all variables than the average *O. mexicanus* specimen, and both species are markedly smaller than *O. albiventer* (Table 1).

Due to the morphological differences of *O. fulgens*, and its inhabitant of highlands and temperate climate that strongly contrasts with the habitats of other species of the genus *Oryzomys*, we consider that *O. fulgens* represents, as originally proposed by <u>Thomas (1893a)</u>, a distinct species of the genus. We agree with the taxonomic proposal of <u>Ramírez-Pulido et al. (2014)</u> but with the difference that we consider that *O. fulgens* that restrict to the Basin of México. Therefore, the populations of the Pacific coastal plains, distributed from Sonora to El Salvador, correspond to *O. mexicanus* Merriam, 1901 as it is the oldest name in the region (see also <u>Carleton</u> and Arroyo–Cabrales 2009; Hanson *et al.* 2010).



Figure 3. Lateral, ventral and dorsal view of some of the specimens examined. A) Holotype, a young adult male, of *Oryzomys fulgens* (BMNH 70.6.20.3). B) Young adult female *O. fulgens* collected in Xochimilco at 2 km N, 3.9 km W San Gregorio Atlapulco, 2240 m (UAMI 17346). C) Old adult male *O. mexicanus* from El Salto, 4 km WSW Minatitlán, Colima (UAMI 16023). D) Young adult male *O. albiventer* from 3 km W Venustiano Carranza, Michoacán (UAMI 999).

Our taxonomic hypothesis should be further tested with the study of additional specimens and the analysis of genetic variation. The later would also allow testing the phylogenetic position of *O. fulgens*.

Distribution and habitat. The first locality or region where O. fulgens was recognized is the "Valle de México," collected between 1865 and 1867 (Boucard 1894; Ramírez-Pulido et al. 2014; Gardner and Ramírez-Pulido 2020). The second locality of the species corresponds to the type locality of O. palustris crinitus "Tlalpam [Tlalpan], Federal District [Ciudad de México], Mexico," collected by E. W. Nelson and E. A. Goldman in 1892 (Merriam 1901; Goldman 1918). The third location was almost 90 years later, González-Romero (1980), in his work on rodent pest from México City, mentions O. couesi crinitus from "Xochimilco" without a precise location. However, a one specimen of the rice rat was collected within the chinampera zone of Xochimilco, but it was not preserved in a scientific collection (A. Gonzalez-Romero, pers. comm. 2019); however, two photographs were obtained of this specimen that were later published in other works (Ceballos and Galindo 1984; López-Medellín and Medellín 2005). It is necessary to clarify that Ceballos and Galindo (1984) included in the report of *O. palustris crinitus* a specimen of "Xochimilco (UAMI)"; however, no such specimen is cataloged in the Mammal Collection of the Autonomous Metropolitan University (UAMI).

Until now, O. fulgens is recorded at three localities: the type locality of O. fulgens is in the Basin of México, the type locality of O. palustris crinitus in Tlalpan, and our record of O. fulgens from 2 km N, 3.9 km W of San Gregorio Atlapulco, 2,240 m. Based on the known localities of O. fulgens, its distribution can be extended to México City, or more likely, to the broader Basin of México (Figure 1). Davis (1944) and later Hall and Kelson (1959), recorded a locality from Tlaxcala, "8 km SW Tlaxcala, 7,500 ft." (see also Hall 1981; Fernández et al. 2015), which occurs outside the Basin of México. Although we did not have the opportunity to review the specimen from Tlaxcala, it is probable that it corresponds to O. fulgens for four reasons: 1) the Sierra Nevada, which separates the populations of the Basin of México from those of Tlaxcala, is formed by many volcanoes with an average altitude of 3,479 m (Tlaloc, Telapón, Iztaccihuatl, Popocatépetl, among others). The oldest of those volcanoes formed approximately 1.8 million years



Figure 4. Occlusal views of right upper molar of some of the specimens examined. The specimens are the same as in figure 2.

ago, and the most recent ones formed around 0.33 million years ago (Macías et al. 2012; Siebe et al. 2017; Martínez-Abarca 2023). This suggests that the population of Tlaxcala likely separated recently from that of the Basin of México before the formation of the Sierra Nevada. 2) Additionally, the locality of Tlaxcala shares similarities in terms of climate, vegetation, and altitude with those from the Basin of Mexico, 3) furthermore it was captured under very similar conditions to our example, at the edge of a swamp (Davis 1944). 4) Moreover, it also exhibits large molars (Davis 1944), which are one of the diagnostic characteristics of *O. fulgens*. However, further studies are need to evaluate the taxonomic status of the Tlaxcala populations.

The genus *Oryzomys* generally distributed in tropical regions from evergreen tropical forests, deciduous tropical forest, lowland forests, coastal vegetation, mountain cloud forests to and wetlands in the north of the distribution, but never inhabits temperate climates and forests (<u>Sánchez-Cordero and Valadez 1989</u>), although they only occasion-ally reach mesic habitat in the case of *O. albiventer* (<u>Carleton and Arroyo-Cabrales 2009</u>). In this sense, *O. fulgens* completely apart from the genus pattern, it is the only species that inhabits temperate climates but is associated with halophilous and semi-aquatic vegetation. It is the species of *Oryzomys* that inhabits at the highest elevation between

2,192 to 2,267 m; the species that follows it is *Oryzomys albiventer* (1,249 to 1,760 m) whole all other inhabit at altitudes lower than 1,380 m (Figure 5) where tropical regions are normally associated.

The distribution of O. fulgens is confined to the Basin of México, with its populations isolated from the species of the O. couesi group, especially O. mexicanus and O. albiventer, to which O. fulgens may be related. The Basin of México is surrounded by volcanoes; on the western side, there is the Sierra de la Cruces with several volcanoes (Volcán La Bufa, Catedral, Iturbide, Chimalpa, Salazar, San Miguel), on the east by the Sierra Nevada (Tlaloc, Telapón, Papayo, Iztaccihuatl, and Popocatépetl volcanoes), to the north, the Sierra de Guadalupe, and to the south, the Sierra de Ajusco-Chichinauhtzin (Wallace and Carmichael 1999). The Sierra de Ajusco-Chichinauhtzin is of particular interest because it geographically isolates O. fulgens from the southern populations of O. mexicanus in the nearby states of Morelos, southern Puebla, and Guerrero (Hall 1981). It is a mountainous system of Sierra de Ajusco-Chichinauhtzin that limits the southern part of the Basin of México, formed by more than one hundred volcanoes, including Chichinauhtzin, Tláloc, Xitle, Cerro Pelado, Cuauhtzin, and with an average height of 3,400 m (Figure 1; Bloomfield 1975; Vázquez-Sánchez et al. 1989). This Sierra was formed between 700,000 and 400,000 years ago (Bloomfield 1975; Márquez et al. 1999; Vázquez-Sánchez et al. 1989). As it developed, it physically and ecologically impeded faunal exchange towards the southern tropical regions, thus resulting in the isolation and refuge of O. fulgens in a temperate region with altitudes >2,200 m within the Basin of México. As said, this scenario should be tested with formal historical biogeographic analyses.



Figure 5. Variation in the elevational distribution of species of *Oryzomys* in southern and western México. Oa = *Oryzomys albiventer*, Om = *O. mexicanus*, Of = *O. fulgens*, Op = *O. peninsulae*, On = *O. nelsoni*. Most altitudinal records were obtained from Carleton and Arroyo-Cabrales (2009).

Conservation. The abundance of *O. fulgens*, measured by the number of specimens collected over the past 120 years or more, is remarkably low, despite México City and the Basin of México being extensively studied historically (Villa-Ramírez 1953; Ceballos and Galindo 1984; Hortelano-Moncada and Cervantes 2011), only three individuals are known to exist. Species that are rare, localized, and restricted to specific ranges are particularly vulnerable to demographic and environmental changes, such as reduced reproductive capacity and increased susceptibility to catastrophic events like floods and fires (Kunin and Gaston 1993; Gaston and Gaston 1994; Flather and Sieg 2007).

The Xochimilco region is the only known habitat where *O. fulgens* likely persists. This area is entirely surrounded by human densely populated regions (Figure 1), making it highly susceptible to human pressures, especially concerning resource exploitation, construction activities within the region, conversion of chinampas areas for tourism, recreation, and sports facilities, among other factors. Although further studies might reveal additional populations within the Basin of México, they would likely face similar pressures due to their association with México City and other large cities within the Basin of México.

Predators pose a significant threat to the rice rat population. Invasive predators, particularly cats and dogs, have been highly detrimental to global biodiversity (<u>Doherty et</u> <u>al. 2016</u>). Within the collection points of *O. fulgens*, several predator species, including domestic or feral dogs (*Canis lupus domesticus*), domestic or feral cats (*Felis catus*), rattlesnakes (*Crotalus polystictus*), and other types of snakes are frequently encountered. Although we do not currently have direct evidence of these predators preying on *O. fulgens*, their presence in abundance at the site could contribute to the extinction risk faced by the rice rat.

Invasive rodent species, notably the black rat (R. rattus) and Norway rat (R. norvegicus), are well-known for their association with the extinction or decline of native vertebrate populations, including reptiles, birds, and mammals, particularly other rodents (Harris 2009; Stokes et al. 2009; Harris and Macdonald 2007). In the same area where O. fulgens was collected, Rattus sp. were also frequently captured (capture success rate >6.7%), suggesting possible interactions between the two species, potentially due to overlapping spatial and habitat usage (Stokes et al. 2009). The abundance of the invasive black rat may intensify competition between it and the native rice rat, as observed in Florida, and has been implicated in the extinction of several Oryzomys species on various islands, including the Galapagos Islands and the Antilles (Goodyear 1992). Despite extensive evidence of rodent extinction due to the black rat on oceanic islands (Álvarez-Castañeda and Cortés-Calva 1996; Álvarez-Castañeda and Ortega-Rubio 2003; Harris 2009), the Xochimilco region can be considered an urban island, completely surrounded by urban areas, making the impact of the black rat on O. fulgens potentially similar to what occurs on oceanic islands.

The conservation problems of *O. fulgens* and its probable risk of extinction are summarized due to a variety of factors, including low population abundance, a restricted area of distribution, urbanization, and habitat destruction, as well as the impact of introduced predators and invasive rodents.

Xochimilco and conservation of Oryzomys fulgens. In past, the Basin of México featured a landscape dominated by five large lakes, which would merge during the rainy season into one continuous water body, all surrounded by imposing mountains that impeded water drainage (Candiani 2014; Manley *et al.* 2022). The major lakes in the area were Lake México-Texcoco, Lake Xochimilco, and Lake Chalco, with Zumpango and Xaltocan being smaller in size. Until pre-Hispanic times, the region surrounding these lakes likely provided favorable conditions for the survival of *O. fulgens*, which might have been distributed in association with the lakes and their tributaries, spanning from the southernmost in Xochimilco to the northernmost in Zumpango.

This five-lake water system has undergone countless anthropogenic alterations over two thousand years (Narchi 2014; Manley et al. 2022). Humans have occupied the Basin of México in the last 20,000 years BC, and the population grew significantly with the rise of Tenochtitlan and even more with the arrival of the Spanish from the 13th to the 17th centuries. Later, there was accelerated growth in the middle of the 20th century (Narchi 2014; Voss et al. 2015), until it became the fifth largest urban area in the world with more than 22 million people, concentrating a large part of México's industrial, commercial, and political activity (Manley et al. 2022; UN 2018). This rapid urban growth has multiple consequences at different scales, including: 1) Reduction of the original vegetation cover area, substituted by urban areas or cultivated fields (Ezcurra et al. 1999; Merlín-Uribe et al. 2013). 2) Alteration of precipitation patterns, changes in groundwater levels and drying (Bojorguez Tapia et al. 1998; Tortajada and Castelán 2003), and 3) Decrease in biodiversity (Rodríguez-Franco 2001; Ezcurra et al. 2001; Narchi 2014). Currently, some lakes no longer exist, particularly the remnants of Lake Zumpango, Texcoco, and Chalco, surrounded by urban, peri-urban areas and, in the least of cases, cultivated fields, so their surroundings do not seem to be the appropriate habitats for O. fulgens. However, Oryzomys are not of the lake itself, is more in relation to the wet areas and the marsh areas associated to these lagoons. Lake Xochimilco is a remnant water system of the Basin of México lakes composed of channels, small lakes, marsh areas, and temporary wetlands surrounded by urban regions (Pérez-Belmont et al. 2021; Merlín-Uribe et al. 2013a). Most of Xochimilco is made up of small artificial islets that ancestrally were built as farm sites called chinampas, which have the optimal condition for the species of Oryzomys.

The chinampas have been cultivated for at least six centuries and reached their maximum development between Table 1. External and craniodental measurements (in mm) of some specimens of *Oryzomys* from south-central México. The measurements correspond to holotype of *Oryzomys fulgens* (probably the type locality "Tepepan", BMNH 70.6.20.3), specimen of *O. fulgens* collected in Xochimilco (2 km N, 3.9 km W San Gregorio Atlapulco, UAMI 17346), specimens of *O. mexicanus* (Michoacán: Las Campanas 6 km N Maruata, UAMI 16025; 2 km NW Presa Pucuato, UAMI 16026; El Capiro 20 km SSW Nueva Italia, UAMI 16027. Colima: El Salto 4 km WSW Minatitlán, UAMI 16023), and specimen of *O. albiventer* (Michoacán: 3 km W Venustiano Carranza, UAMI 999). See materials and methods for variable abbreviations.

Variable	<i>O. fulgens</i> (holotype)	<i>O. fulgens</i> (this work)	O. mexicanus	O. albiventer
TOTL	-	262.0	280.3	344.0
			(260.0-344.0)	
TL	-	139.0	148.7	181.0
			(142.0-181.0)	
HFL	-	36.0	31.7	39.0
			(30.0-39.0)	
EL	-	18.0	16.7	19.0
			(15.0-20.0)	
ONL	-	32.7	30.8	36.5
			(29.6-36.5)	
ZB	-	17.7	16.5	19.9
			(15.7-19.9)	
BR	6.6	6.6	5.9	7.4
			(5.6-7.4)	
IOB	4.8	5.0	4.9	5.3
			(4.7-5.3)	
BBC	-	13.8	13	14.3
			(12.6-14.3)	
DBC	-	11.0	10.3	11.4
			(9.4-11.4)	
BBP	5.9	6.2	5.7	6.7
			(5.5-6.7)	
BZP	3.7	4.4	3.5	4.0
			(3.2-4.1)	
LD	8.9	8.5	7.8	9.5
			(7.2-9.5)	
BIF	2.2	2.5	2.9	3.5
			(2.3-3.5)	
WM1	1.5	1.4	1.4	2.0
			(1.4-2.0)	
LR	10.2	11.1	10.2	12.4
			(9.9-12.4)	
LBP	-	6.6	6.1	6.8
			(5.9-6.8)	
PPL	-	11.2	10.6	20.7
			(10.1-20.7)	
LIF	7.0	6.7	6	7.1
			(5.5-7.1)	
CLM	5.4	5.3	4.7	5.5
			(4.6-5.5)	
LAB	-	7.0	5.6	6.3
			(5.46.3)	

1400 and 1600 (Merlín-Uribe *et al.* 2013b). This ancestral agricultural technique has been used since the Mexicas and is currently considered one of the most sustainable and

productive production methods in the world (Rojas 1983; Alcántara 2020; Pérez-Belmont et al. 2021), with the addition that this system preserves great parts of the original vegetation on all three strata and the marginal subaquatic vegetation. This network of canals and vegetation produces a landscape that probably positive directly impacts the quality and availability of habitat for the rice rat O. fulgens. We believe that the ecological characteristics of the chinampas have maintained, although with low abundance, the populations of O. fulgens and promote its possibilities for study and conservation, unlike other Oryzomys species that have become extinct under similar pressures. However, even though the chinampas area is an ideal habitat for the rice rat, it is exposed to anthropogenic factors that can severely decimate their populations (see above). The anthropic effect of Xochimilco have a dual effect in the O. fulgens. On one hand the chinampas, use, build, and conservation increase the optimal habitat for the species, for which need to be abundant, but in the other hand this same activity increase the exotic species that can cause the extinction of the species (e.g., feral cats and dogs; Doherty et al. 2016). Unfortunately, at present this area of Xochimilco is constantly subjected to anthropogenic pressures that endanger the native vegetation where these rodent live, so its long-term permanence is uncertain. The rarity and limited population data for these species make them particularly vulnerable to threats, such as habitat loss, fragmentation, and invasive and exotic species. Conservation efforts are crucial to protect these unique rodents and their habitats and prevent further decline in their populations.

Acknowledgments

To the authorities of the Protected Natural Area "Ejidos de Xochimilco y San Gregorio Atlapulco." The ejidal commissioner of the irrigation district, Francisco Vega Jiménez, and the ejidal commissioner of San Gregorio Atlapulco, Eng. Julio Rosas, we extend our gratitude for granting us the necessary permits and access to the Xochimilco region. Special thanks to Mr. Leonardo Medina of the Cuahilama Wildlife Conservation Management Units (UMA) for generously allowing us to conduct our work on his land and for his remarkable cooperation. We express our appreciation to Professor Fernando Arana Magallón, coordinator of the Cuemanco Biological and Aquaculture Research Center (CIBAC), for providing us access to the CIBAC facilities during our field sampling. We acknowledge the Master's Degree in Biology program at the Autonomous Metropolitan University-Iztapalapa for enabling the development of the ARJ-G thesis, from which this work stems. Furthermore, we are grateful to the National Commission for the Knowledge and Use of Biodiversity (CONABIO) for partial financial support through project DE020. We extend our thanks to Paula Jenkins of the Department of Zoology at The Natural History Museum (London) for her valuable assistance during our visit to the Mammal Session. Our sincere appreciation goes to Diana López and Ángel López from the Mammal Collection of the Autonomous Metropolitan University-Iztapalapa, who kindly assisted us in creating the figures and capturing photographs of the skulls of the *Oryzomys* species. Two anonymous reviewers provided comments on an earlier version of this contribution.

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Associated editor: Guillermo D'Elia

Submitted: October 27, 2023; Reviewed: August 21, 202 Accepted: May 10, 2024; Published on line: May 30, 2024

Activity patterns of the white-tailed deer (Odocoileus virginianus) in a neotropical dry forest: changes according to age, sex, and climatic season

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Mammalian daily activity is shaped by a combination of intrinsic and extrinsic factors. Age influences activity rhythms due to energy requirements, while physiological and reproductive traits cause differences between genders. In ecosystems with marked climatic seasonality, such as the seasonally dry tropical forest (SDTF), activity patterns adapt to extrinsic factors like resource availability and environmental stress. This study investigates how intrinsic factors, specifically age and sex, influence the white-tailed deer's (Odocoileus virginianus) daily activities, and how these vary between dry and rainy seasons. Between 2015 and 2018, we conducted a camera trapping study to monitor the daily activity of a population of white-tailed deer in the Arenillas Ecological Reserve, southwestern Ecuador. We estimated individual daily activity based on four parameters: total and diurnal relative abundance index (RAI), activity directionality, and activity overlap between groups. We used generalized linear models to evaluate the changes in RAI based on age-classes and sex of the individuals. The Watson test was employed to assess differences in directional patterns during activity hours, while the Wald test was utilized to evaluate significant variations in activity overlap. The same analyses were also performed to assess changes in daily activity between the dry and rainy seasons. The daily activity patterns of white-tailed deer varied by age and sex. Fawns were predominantly diurnal, whereas adults displayed continuous activity throughout the 24 hours of the day, with males being more active during the night than females. Females did not show significant differences in the activity pattern compared to fawns and juveniles. The daily activity pattern of white-tailed deer varied between seasons. Overall, there was an increase in daily activity during the dry season, but significant only for males. Females were the only group that showing seasonal variation in activity directionality, with more morning activity during the dry season. The daily activity patterns of white-tailed deer in the dry forest exhibit slight differences compared to those observed in other ecosystems, with extended daily activity periods. During the rainy season, reproductive and post-reproductive behaviors, rather than resource abundance, predominantly shaped the white-tailed deer's daily activity patterns. In contrast, the dry season presented a notable rise in overall activity and daily activity, accompanied by partition between groups. This partition likely stems from diminished resource accessibility and increased intraspecific competition. Given the slight differences in the white-tailed deer's daily activity from those noted in other regions, these insights are crucial for formulating management and conservation strategies tailored to specific environmental conditions.

Los patrones de actividad diaria de los mamíferos están determinados por diferentes factores. La edad puede determinar diferentes ritmos de actividad como respuesta a requerimientos energéticos, mientras que las características fisiológicas y reproductivas marcan diferencias entre sexos. En ecosistemas con una marcada estacionalidad climática, los ritmos de actividad pueden variar temporalmente en respuesta a cambios en la disponibilidad de recursos o factores de estrés ambiental. Este estudio analiza en qué medida los factores intrínsecos, como la edad y el sexo, determinan los patrones de actividad diaria del venado de cola blanca (Odocoileus virginianus), y si estos patrones cambian como consecuencia de la estacionalidad climática del bosque tropical estacionalmente seco (BTES). Entre 2015 y 2018 se monitoreó mediante fototrampeo la actividad diaria del venado de cola blanca en la Reserva Ecológica Arenillas, Ecuador. Se usaron modelos lineales generalizados para evaluar cambios en el índice de abundancia relativa (RAI) según la edad y el sexo del individuo. Se utilizó la prueba de Watson para evaluar diferencias en la direccionalidad en las horas de actividad y la prueba de Wald para evaluar diferencias en el solapamiento de la actividad entre grupos y entre estación seca y lluviosa. Los patrones de actividad diaria de O. virginianus variaron según la edad y sexo. Los cervatillos fueron predominantemente diurnos, mientras que en la etapa adulta la actividad se distribuyó durante todo el día. Además, el patrón de actividad diaria cambió entre estaciones, siendo los machos significativamente más activos durante la estación seca. Las hembras mostraron diferencias en la direccionalidad de la actividad diaria entre estaciones, con una mayor actividad durante las primeras horas de la mañana en la época seca. El patrón de actividad diaria de los venados en el BTES es ligeramente distinto al observado en otros ecosistemas, con patrones de actividad diaria más extendidos a lo largo del día. En el BTES, los cambios en la disponibilidad de recursos y de comportamiento de los venados entre estaciones generan cambios en el patrón de actividad diaria. Durante la época lluviosa los patrones de actividad diaria estuvieron definidos por el comportamiento reproductivo y post-reproductivo antes que por la alta disponibilidad de recursos. En la época seca hubo un aumento general de la actividad y de los periodos de actividad diaria, con una partición en los períodos de actividad entre grupos, lo que podría explicarse como una consecuencia de la reducción del acceso a los recursos y el incremento de la competencia intraespecífica. Considerando que la actividad diaria de O. virginianus puede diferir ligeramente de los patrones observados en otros ecosistemas, estos hallazgos son importantes para plantear medidas de manejo y conservación adaptadas a las condiciones propias de la localidad.

Keywords: Activity patterns; camera trapping; circadian rhythms; seasonality; seasonally dry tropical forest.

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Introduction

The daily activity patterns of species are natural responses to various biological, physiological, behavioral, and survival processes (Hut *et al.* 2012). For many species, these activity cycles follow patterns called circadian rhythms, repeating every 24 hours, allowing anticipation of and response to biotic and abiotic conditions (Halberg 1960; Dodd *et al.* 2005; Bradshaw and Holzapfel 2010; Libert *et al.* 2012; Spoelstra *et al.* 2016). Understanding these cycles is essential for managing vulnerable species or those that are key to the ecosystem.

The activity patterns exhibited by a species result from a complex balance between processes that maximize species success and reduce energy expenditure. Studies have shown that daily activity patterns can be determined by intrinsic factors such as sex, age, and physiological state, or by external factors such as forage availability and habitat quality (Beier and McCullough 1990; Main et al. 1996; Yearsley and Pérez-Barbería 2005; Fuller et al. 2020). Among intrinsic factors, changes in activity patterns at the demographic level enable adaptation to specific activities of different age groups, such as searching for shelter, food, or mates (Beier and McCullough 1990; Scheibe et al. 1999; Berger et <u>al. 2002</u>). In herbivores, activity patterns are closely related to feeding behavior since digestive processes impose cyclic activity patterns of less than 24 hours (Scheibe et al. 1999, 2009). These patterns result from the interaction between daily behavioral rhythms and digestive physiology (Scheibe et al. 1999, 2009; Berger et al. 2002; Owen-Smith and Goodall 2014). In large gregarious herbivores, having different activity patterns helps distribute their temporal niche for resource consumption and meet their energy needs, avoiding the energy expenditure that intraspecific competition can entail (Fortin et al. 2004; Valeix et al. 2007). The activity peaks of male and female ungulates may differ mainly due to their energy requirements (Beier and McCullough 1990). Males have higher energy needs; therefore, they cover more territory. However, their activity during the day and throughout the year may vary depending on the climatic conditions of each season (Webb et al. 2010; Gallina and Bello 2010; Massé and Côté 2013). In the case of fawns, the activity patterns are similar to those of their mothers since they depend on them for feeding, at least in the first months of the offspring's life (Ozoga and Verme 1986).

Furthermore, studies focused on deer species indicate that daily activity patterns change in response to extrinsic factors, such as environmental conditions (Mandujano and Gallina 1995; Sánchez-Rojas *et al.* 1997; Gallina and Bello 2010). It is known that in the tropics, ungulates carry out most of their activities at twilight (dawn and dusk), when climatic conditions are favorable, and it is possible to avoid energy and water loss due to high temperatures (Leuthold and Leuthold 1978; Beier and McCullough 1990; Galindo Leal and Weber 1998; Owen-Smith 1998; Gallina and Bello Gutierrez 2014). Since these conditions can vary between seasons, it is expected that these activity patterns will change according to climatic conditions. It has been reported that during the rainy season, deer are active throughout the day due to an increase in food availability, while during the dry season, when the availability and quality of food and water are very low (Arceo *et al.* 2005; <u>Gallina-Tessaro 2019</u>), they are more active during twilight hours (Mandujano and Gallina 1995; Sánchez-Rojas *et al.* 1997; <u>Gallina and Bello 2010</u>). Finally, changes in activity patterns between seasons could also be a consequence of mating behavior. Thus, during the breeding season, which matches with the rainy season, greater activity is expected than in the post-breeding period, which coincides with the dry season (<u>Holzenbein and Schwede 1989</u>; <u>Beier and</u> <u>McCullough 1990</u>; <u>Fuller *et al.* 2020</u>).

This work studied the daily activity patterns of whitetailed deer (Odocoileus virginianus), a widely distributed ungulate mammal species in the American continent. These animals have significant ecological and cultural importance (McShea 2012). Besides being an important prey for predators, they play a fundamental role in seed dispersal and forest regeneration (Crawford et al. 2019; Jara-Guerrero et al. 2018; Rooney and Waller 2003). Therefore, conserving this species is paramount for maintaining ecosystem health and preserving biodiversity. By understanding their circadian rhythms and associated moments of activity, appropriate management strategies can be recommended, promoting their conservation and ensuring the balanced functioning of the species and the ecosystems they inhabit. Understanding the activity patterns will allow informed decisions on deer habitat management, hunting control, and prevention of threats that may affect their survival.

With this background, we are interested in understanding to what extent the age class and sex of white-tailed deer determine daily activity patterns, and if these change as a consequence of the climatic seasonality characteristic of the seasonally dry tropical forest (SDTF). This ecosystem is characterized by strong climatic seasonality, with a dry season of six to eight months, during which more than 70 % of woody species lose their leaves, and the herbaceous layer disappears (Sierra 1999). This condition implies a reduction in resource availability for different herbivore species (Davis 1990; Mandujano et al. 2004; Arceo et al. 2005). It is proposed that the activity pattern of the white-tailed deer would be a consequence of the balance between physiological constraints, reproductive and post-reproductive behaviors, and intraspecific competition for resources. During the rainy season, activity patterns among groups could show temporal partitioning or overlap depending on which factor is dominant, resource availability or behavior. Thus, i) if the balance favors resource availability, daily activity patterns should differ among groups as a consequence of intraspecific competition (Owen-Smith and Goodall 2014). The greater availability of resources would allow a stratification of activity hours that minimize potential conflicts between different age groups. ii) Conversely, if reproductive and post-reproductive behavior is more important,

we expect daily activity among groups to be similar. The interaction between males and females should increase as a consequence of the mating behavior that occurs at the end of the rainy season (Mandujano and Gallina 1996). While the activity between the initial development stages (juveniles and fawns) and their mothers should be more coincident because the breeding season coincides with the rainy season (Hawkins and Klimstra 1970; Mandujano and Gallina 1996).

In the dry season, the temporal partitioning or overlap is expected to depend on the balance between physiological constraints due to drought and intraspecific competition for resources. Thus, iii) if physiological constraints influenced by drought predominate, the daily activity patterns of different groups should align. These patterns would aim to minimize energy expenditure and water loss, maximizing activity during twilight hours as a strategy to cope with water stress (Mandujano and Gallina 1995; Sánchez-Rojas *et al.* 1997; Gallina and Bello 2010), thereby reducing differences in activity patterns among groups. Conversely, iv) if the availability of resources is the dominant factor, a temporal partition in the daily activity of the groups is expected. The dry season implies a significant reduction in resources for these animals (Davis 1990; Arceo *et al.* 2005), potentially increasing intraspecific competition (<u>Bowyer</u> 2004; <u>Donohue *et al.* 2013</u>), leading different groups to exploit resources at distinct times.

Materials and methods

Study area. The study was conducted in the Arenillas Ecological Reserve (REAr), a key remnant of Ecuador's seasonally dry forests, located in El Oro province, southwestern Ecuador (Espinosa 2012; Sierra 1999; Figure 1). Spanning 131.7 km² (Ministerio del Ambiente 2014a), the reserve lies between 3° 27' 30.94" and 3° 39' 37.49" S latitude and 80° 9' 18.65" to 80° 9' 47.93" W longitude, with elevations ranging from 4 to 160 meters above sea level (Luna-Florin et al. 2022). It has an average annual temperature of 25.9 °C and precipitation of 661 mm, featuring a distinct rainy season from January to May and a dry season from June to December during which precipitation does not exceeds 40 mm (Espinosa et al. 2018; Figure 1). The area is characterized by deciduous forests and shrublands (Luna-Florin et al. 2022), rich in Fabaceae family plants (Espinosa 2012), which are crucial to the diet of white-tailed deer (Arceo et al. 2005; Vasquez et al. 2016), especially their fruits during the dry season (Arceo et al. 2005; Jara-Guerrero et al. 2018).

The REAr is surrounded by a matrix of rural human settle-



Figure 1. Location of study area. The forest remnants in 2022 are highlighted in green (Geoportal MAATE), and the border of the Arenillas Ecological Reserve is shown as a black line. A black spot represents the location of each camera trap. The climate diagram was elaborated using the meteorological station data from Arenillas (1965 to 2012), provided by the Instituto Nacional de Meteorología e Hidrología de Ecuador (INAMHI; Espinosa et al. 2018).

ments and farmlands. Unlike other dry forest areas where livestock farming is common, no human activities such as livestock are conducted within the reserve (Jara-Guerrero et al. 2019). Although there are instances of illegal hunting, these are limited due to its status as a protected area. Despite human pressures, the reserve maintains high ecological integrity, with significant diversity and no evidence of pressures that could be filtering specific mammal groups (Espinosa et al. 2016). The vegetation cover and structure meet the criteria for natural forests proposed by Jara-Guerrero et al. (2019) for the SDTF, with low to no human disturbance inside the reserve.

Data collection. Sixteen trail cameras were installed, including eight Bushnell model M-990i and eight Moultrie model Trophy Cam HD Max cameras. They were active from July 2015 until January 2018 and were placed on natural trails within a grid, with approximately 1000 meters separating each camera (Rovero and Marshall 2009).

The cameras operated continuously throughout the day, set to take three photos per trigger with a 5-second reload time (<u>Ahumada *et al.* 2013</u>). Cameras were checked monthly for battery replacement and memory card exchange.

Data Processing. Images were uploaded and processed using the free Wild.ID software, which extracts the date and time from each photo. Images were categorized by climatic season, with January to May as the rainy season and June to December as the dry season (Espinosa et al. 2018; Figure 1). Deer photos were classified by age class based on morphological characteristics: juveniles were identified by longer limbs relative to body size, smaller and less branched antlers; fawns by white spots on their fur, smaller body size, proportionally larger ears, and always being near their mother. When fawns appeared with their mother, presence data were recorded for both age classes. Adults were differentiated by the presence of testicles and antlers, visible in the images (Gaillard et al. 2000; Monteith et al. 2009; Flinn et al. 2015). Photos where sex or age class couldn't be identified were excluded.

To minimize overestimation of individual abundance, consecutive images separated by at least 30 minutes were considered independent records. From these, the Relative Abundance Index (RAI) was calculated to standardize data to records per 100 trap days. This was done by dividing the number of independent records by the number of camera activation days and multiplying by 100. (Balme *et al.* 2010). RAI served as a measure of overall activity throughout the day for each group.

Statistical Analyses. To analyze the activity patterns of the white-tailed deer, three metrics were utilized: total RAI and diurnal RAI, directionality, and overlap. Total RAI sums all independent records across the day, while diurnal RAI calculates the daytime vs. nighttime records proportion. Directionality measures average daily activity per hour. Overlap quantifies the daily activity overlap between two groups. Changes in total and diurnal RAI across age classes and seasons were examined using generalized linear models. As RAI is continuous but not normally distributed, a Gamma error distribution and log link function were applied. Additionally, differences in diurnal activity probability among age classes were assessed using a generalized linear model with a binomial error distribution. Record counts during day (6:00 to 18:00 hrs.) and night for each camera were compared using pairwise tests with Bonferroni correction via the "emmeans" package (Lenth *et al.* 2023).

Changes in the directionality of daily activity among all pairs of age classes, genders, and climatic seasons were tested using the two-sample Watson homogeneity test. This test assesses whether two samples come from populations with the same mean, based on the t-test statistic, which compares the means of the two samples and evaluates if there is sufficient evidence to reject the null hypothesis that the means are equal (Agostinelli and Ulric 2022).

To evaluate the overlap between different age classes, the "overlap" package was used, calculating the overlap coefficient Δ as a descriptive measure of the degree of overlap between two density curves. This coefficient fits the camera trap data to a non-parametric circular kernel density function and estimates probabilities of temporal activity overlap, which can range from 0, indicating no overlap in activity patterns, to 1, indicating complete overlap in activity patterns (<u>Ridout and Linkie 2009</u>; <u>Rowcliffe et al. 2014</u>; <u>Lashley et al. 2018</u>; <u>Meredith et al. 2018</u>).

Overlap was defined as the area under the curve, determined by taking the minimum value of two density curves at each time point. We used the $\Delta 4$ estimator for sample sizes comparing categories (sex, age, and between seasons) greater than 50 observations (Ridout and Linkie 2009; Meredith et al. 2018; Saisamorn et al. 2019). To assess the reliability of the $\Delta 4$ estimator and estimate a 95 % confidence interval, we performed 1,000 iterations of smoothed bootstrapping (Meredith et al. 2018). The "activity" package was used to compare activity level estimates and determine significant differences in activity patterns using the Wald Test, which assesses if the differences between category estimates are significantly different from 0 (Rowcliffe et al. 2014; Rowcliffe and Rowcliffe 2016). All statistical analyses were conducted using R Studio (R Core Team 2022)

Results

Over 32 months of sampling and 14,272 trap-days, 7,633 deer records were obtained: 5,446 adults, 1,085 juveniles, 1,047 fawns, with 55 unclassified records. Among adults, 3,335 were females and 2,111 males.

Generalized linear models indicated age stage significantly affected relative abundance indices, with fawns significantly lower than other stages (Figure 2a). Diurnal activity was highest in fawns and lowest in adult males, with only fawns showing significantly different diurnal activity (Figure 2b).



Figure 2. Activity index (a) and proportion of diurnal activity (b) for different sex, and age classes of white-tailed deer. The pairwise test with Bonferroni correction is shown in letters and with dark cyan color bar.

Watson tests revealed significant daily activity mean differences between males and females, males and juveniles, and juveniles and fawns (*P* value < 0.05; Figure 3). Females peaked in activity between 15:00 to 18:00 hrs, differing in directionality from males, who had a more distributed activity pattern. Juveniles were most active in the late afternoon (16:00 to 18:00 hrs), and fawns in the morning (6:00 to 12:00 hrs). Circular kernel analyses showed significant activity pattern overlaps between age classes, notably between juveniles and fawns, females and juveniles, and males and juveniles (Figure 3).

Circular kernel analysis revealed significant differences in daily activity pattern overlap between age classes, specifically between juveniles and fawns, females and juveniles, and males and juveniles (Wald test; Figure 4).

Seasonal changes. Overall, increased activity was observed during the dry season, except for fawns, which were more active during the rainy season. The only statistically significant difference was found in males (deviance 2.067, P = 0.036), showing less activity in the rainy. Significant differences in activity among age class states were only found during the dry season, with fawns being significantly less active than other categories (Figure 5a, c). No significant differences were observed in diurnal activity across seasons or within each season for any group, although males were less diurnal during the rainy season and juveniles during the dry season (Figure 5b, d).

Directionality of daily activity patterns between seasons only significantly changed for females, with increased afternoon activity and reduced early morning activity during the rainy season (06:00 to 09:00 hrs). The dry season presented more significant changes in activity directionality among age classes (Figure 6). Watson tests indicated significant directionality differences between males and females, and males and juveniles, with males showing a strong activity peak in the late afternoon (16:00 to 17:00 hrs) and juveniles



Figure 3. Directionality of daily activity of different sexes and age classes in order females, males, juveniles, and fawns. The red arrows show the average daily activity, and the black arrows between groups indicate significant differences in directionality. The x-axis scale, represented as a circle, shows hours in 24 hours, while the y-axis is shown outside the circle and represents the frequency of activity.



Figure 4. Daily activity density curves and overlap between different sexes and age classes of white-tailed deer in the Arenillas Ecological Reserve. The gray color represents the area of overlap of daily activity.

in the early morning (5:00 hrs; Figure 3). No significant differences were observed among age classes during the rainy season.

The season did not significantly affect the overlap for any age class, with activity patterns remaining consistent throughout the year. A significant difference in overlap between juveniles and fawns was noted in both seasons (Figure 7).

Discussion

White-tailed deer exhibit a broad distribution across various ecosystems along an extensive latitudinal range. While it is known that the species' daily activity pattern responds to changes in abiotic conditions and varies with age, these responses have predominantly been studied in temperate ecosystems. Despite the species' high occupancy in SDTF (<u>Haro-Carrión *et al.* 2021</u>), their daily activity patterns have not been thoroughly evaluated (but see <u>Sánchez-Rojas *et al.* 1997</u>).

This study found that the daily activity patterns of whitetailed deer in SDTF show predominantly crepuscular peaks, varying by age class, sex, and seasonality. Adult individuals follow the widely reported pattern for the species (<u>Cornicelli et al. 1996</u>; <u>Webb et al. 2010</u>; <u>Gallina and Bello Gutierrez 2014</u>), with higher activity peaks during twilight, while fawns are most active in the early morning hours. Adult activity is distributed over 24 hours, though males tend to be more active at night than females. According to <u>Bowyer</u>



Figure 5. Activity index (a, c) and proportion of diurnal activity (b, d) during the dry and the rainy seasons for different sex, and age classes of white-tailed deer. The pairwise test with a Bonferroni correction is shown in letters and with brown color bar.

(2004) temporal segregation between sexes may reduce competition for resources when there are no differences in their diets. Furthermore, adult males, being larger, may be less susceptible to predation (Main *et al.* 1996; Fulbright and Ortega-Santos 2013; Lashley *et al.* 2014), allowing them to access resources at night.

Females showed no differences in their activity pattern compared to fawns. Although overlap with juveniles is less, the activity period of the latter falls within the females' activity period. This similarity in activity patterns between females, juveniles, and fawns is expected due to the lactation process and females being more tolerant of these groups in terms of access to food resources (Hawkins and Klimstra 1970). It has even been reported that juveniles may increase their feeding activity when adult females are present (Stone et al. 2017). On the other hand, juveniles tend to be most active at times different from higher-ranking adult males to avoid antagonistic encounters (Townsend and Bailey 1981; Donohue et al. 2013), which could explain the differences in activity patterns between these two groups. According to Cherry et al. (2015), many juveniles do not reach sexual maturity, so their activity budget would be primarily allocated to body growth rather than reproduction.

As expected, the daily activity pattern of white-tailed deer changed between seasons. During the rainy season, there was a general decrease in daily activity (Figure 5a). This could be because greater resource availability during this season reduces the need for exploratory behavior for adequate forage (Crimmins *et al.* 2015). Results also showed greater overlap and the same directionality in the activity of different groups during rainy season. This overlap could be explained by the mating and breeding season

coinciding with the rainy season (Mandujano and Gallina 1996; Gallina-Tessaro 2019). According to Mandujano y Gallina (1996), in SDTF, white-tailed deer breading occurs at the beginning of the rainy season, while mating happens towards the end. In other ecosystems, some studies suggest that during the mating season, activity patterns between females and males coincide (Holzenbein and Schwede 1989; Fuller *et al.* 2020), whereas during the rearing, fawns and juvenile females typically forage together with their mothers (Hawkins and Klimstra 1970; Mandujano and Gallina 1996). These results suggest that, during the rainy season, reproductive and post-reproductive behavior was more important than intraspecific competition.

The strong climatic seasonality characteristic of SDTF limits resource availability during the dry season, especially foliar biomass (Mandujano et al. 2004; Arceo et al. 2005; Gallina-Tessaro 2019) and increases stress for water and solar radiation (Jara-Guerrero et al. 2021). According to our hypotheses, the balance between intraspecific competition and environmental stress should define the overlap or temporal partitioning of the daily activity of white-tailed deer. Resource scarcity implies an increase in intraspecific competition for food and a possible temporal partitioning of daily activity. Conversely, increased environmental stress should lead to greater overlap among groups as all would avoid peak stress hours to reduce energy expenditure (Mandujano and Gallina 1995; Sánchez-Rojas et al. 1997; Gallina and Bello 2010). Our results show a general increase in activity and daily activity periods during the dry season, supporting the idea of decreased resources. Additionally, we found a partition in daily activity periods among groups (Figure 6), which could be explained by reduced resource access and increased intraspecific competition.



Figure 6. Seasonal changes in the directionality of daily activity of different sex, and age classes, in order females, males, juveniles, and fawns. The red arrows show the average daily activity, and the black arrows between groups indicate significant differences in directionality. The x-axis scale, represented as a circle, shows hours in 24 hours, while the y-axis is shown outside the circle and represents the frequency of activity.

Anthropogenic activities have been shown to impact large mammal populations, such as the white-tailed deer, altering their behavioral patterns and daily activity (Root et al. 1988; Osterhaus and Jensen 2019). Due to the narrow shape of the REA, ranging between nine and six km at the study site, human activities could be influencing deer activity patterns (Espinosa et al. 2016). In SDTF, human pressure mainly involves extensive use of the forest for activities such as livestock farming with free-range cattle, firewood and timber extraction, and hunting (Antongiovanni et al. 2020; Jara-Guerrero et al. 2019, 2021; Singh 1998). Throughout nearly three years of study, we found no records of domestic animals or livestock within the study site, except for three records of Canis lupus familiaris. These data, along with the substantially higher number of deer records compared to other similar reserves like Pacoche and Machalilla (Espinosa et al. 2016; Lizcano et al. 2016), suggest that the REA is subject to relatively low anthropogenic pressure.

Hunting is another pressure that could modify the activity patterns of white-tailed deer within the study area, although this effect would depend on the intensity of hunting pressure (Osterhaus and Jensen 2019). Osterhaus and Jensen (2019) found no effect of hunting on the activity pattern of white-tailed deer in Kansas, United States, attributing the lack of effect in their study to low levels of daily hunting activity (0.065 hours/ha) compared to levels reported by Root *et al.* (1988; 0.45 hours/ha per day) in a similar ecosystem in Missouri, United States, where an effect of hunting on deer activity patterns was observed. In our study area, based on human records from camera traps, we estimate that human daily activity was 0.03 hours/ha, a reduced pressure compared to other studies. These results allow us to propose that the activity patterns reported in this work are those expected under natural conditions. However, it is necessary to conduct studies to analyze deer behavior under different levels of hunting pressure in conditions with different anthropogenic pressures to better understand the impact of human activities on the daily activity of this important mammal.

In conclusion, white-tailed deer in dry forests exhibit a daily activity pattern slightly different from that observed in other ecosystems, with activity patterns more extended throughout the day. The overall analysis of activity showed different activity patterns for each age group; however, this difference changes when we analyze the activity pattern in each season. The results suggest that reproductive and post-reproductive behavior during the rainy season determine the overlap in activity among different age classes. Whereas, during the dry season, food availability and the consequent increase in competition lead to a partition in daily activity among the different groups.





Figure 7. Seasonal change in the daily activity density curves and overlap between different age classes of white-tailed deer in the Arenillas Ecological Reserve. The gray color represents the area of overlap of daily activity.

Acknowledgments

We thank the Ministerio del Ambiente del Ecuador for the assistance in providing logistical facilities during our project, especially the staff of the Arenillas Ecological Reserve. This project was conducted under permit No. MAE-DNB-CM-2015-0016, and funded by the Universidad Técnica Particular de Loja through the PROY_CCNN_1054.

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Associated editor: Rafael Reyna Submitted: July 27, 2023; Reviewed: August 21, 2023 Accepted: April 1, 2024; Published on line: May 30, 2024 WHITE-TAILED DEER'S RHYTHMS

Corrigendum

Montero-Bagatella, S. H., F. A. Cervantes, and A. González-Romero. 2023. Population parameters of the Phillips kangaroo rat (*Dipodomys phillipsii*). Therya 14:351-359.

Population parameters of the Phillips kangaroo rat (Dipodomys phillipsii)

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Change in:

Figure 3. Average sex and age breakdowns of captured Phillips' kangaroo rat (*Dipodomys phillipsii*) by season for 2012-2016, from Perote, Veracruz.



CORRIGENDUM