Therya

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La portada
Grupo familiar de vampiro espectral (*Vampyrum spectrum*) perchando dentro de una cueva del Departamento de Rivas, Nicaragua. Marzo del 2017. Fotografía de José G. Martínez-Fonseca.

Issue cover
Family group of spectral bats (*Vampyrum spectrum*) roosting inside a cave in the Department of Rivas, Nicaragua. March 2017. Photograph by José G. Martínez-Fonseca.

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. El signo decimoprimero en la cosmogonía mexica, "Ozomatli", es una representación pictórica del mono araña (*Ateles geoffroyi*). La especie de primate de más amplia distribución en México". Es habitante de los bosques, sobre todo de los que están por donde sale el sol en Anáhuac. Tiene el dorso pequeño, es barrigudo y su cola, que a veces se enrosca, es larga. Sus manos y sus pies parecen de hombre; también sus uñas. Los Ozomatin gritan y silban y hacen viajes a la gente, arrojan piedras y palos. Su cara es casi como la de una persona, pero tienen mucho pelo."

Contenido

Therya

Volumen 16, número 3 Septiembre 2025

EDITORIAL

Murciélagos y Zoonosis: retos para la conservación y salud pública 323

Celia Isela Selem-Salas

ARTICLES

Seasonal Composition of the Diet of Free-Range Equines in Northern Coahuila, Mexico 325

José Javier Ochoa-Espinoza, José Isidro Uvalle-Sauceda, César Martín Cantú-Ayala, Fernando Noel González-Saldívar, Eloy Alejandro Lozano-Cavazos, José Antonio Hernández-Herrera, Fernando Isaac Gastelum-Mendoza, and Michelle Ivonne Ramos-Robles

Distribution, ecology, and natural history of the Spectral bat (*Vampyrum spectrum*) in Colombia 335

Tatiana Velásquez-Roa, Christian Cabrera-Ojeda, Andrea Bernal-Rivera, Cristian Calvache-Sánchez, Pamela Carv ajal-Nieto, Sara Medina-Benavides, and Jonathan Granobles-Cardona

Phylogenetic relationships between Monotremata and Monotremaformes: parallelism with appendages and habits of aquatic moles 351

Edson Patrick de Oliveira Quintes, and Leila Maria Pessôa

Postnatal growth and age estimation in the broad-tailed bat (*Nyctinomops laticaudatus*) 363

Eduardo Sánchez-Garibay, and Jorge Ortega

Changes in the activity pattern of white-tailed deer in the Tehuacán-Cuicatlán Biosphere Reserve, Mexico 371

Eva López-Tello, Salvador Mandujano, and Sonia Gallina

Medium and large terrestrial mammals in an area voluntarily designated for conservation in the northern Yucatan Peninsula, Mexico 383

Sol de Mayo A. Mejenes-López, Guadalupe de los Ángeles Cab-Paat, Jorge A. Vargas-Contreras, Griselda Escalona-Segura, Orlando Pelaez-Cruz, and Oscar G. Retana-Guiascón

Neotropical insectivorous bats adjust echolocation calls in response to vegetation structure 397

María Camila Salazar-Pérez, and Sergio Estrada-Villegas

Trophic ecology of marine mammals in the Mexican Pacific Ocean: Prey diversity, network structure, and overlap with fisheries 409

Luis Medrano-González, Tania Benavidez-Gómez, Vicente N. Vargas-Navidad, and Nelly Zepeda-López

Fisiología del epitelio vaginal en hembras de *Anoura latidens* (Chiroptera: Phyllostomidae), anotaciones sobre la estructura social de la colonia en refugios artificiales, Calarcá, Quindío, Colombia 427

Andrés Felipe Vargas Arboleda, y Hugo Mantilla Meluk

fascículo 48 https://mastozoologiamexicana.com/therya/index.php/THERYA/issue/view/48
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El objetivo y la intención de THERYA es ser una revista científica para la publicación de artículos sobre los mamíferos. Estudios de investigación original, editoriales y artículos de revisión son bienvenidos.

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Editorial

Murciélagos y Zoonosis: retos para la conservación y salud pública

Los murciélagos constituyen el segundo grupo más diverso de mamíferos, con cerca de 1,500 especies descritas. Su relevancia ecológica es indiscutible: cumplen funciones clave como la polinización, la dispersión de semillas, la regeneración de selvas y el control natural de plagas. Estos servicios ecosistémicos favorecen la estabilidad ambiental y, de forma indirecta, aportan beneficios económicos a sectores agrícolas y forestales, siendo inclusive de importancia económica para el ser humano.

Sin embargo, su relación con la salud humana ha generado fuertes controversias. La emergencia del SARS-CoV-2 intensificó el estigma hacia todos los murciélagos, lo que provocó ataques directos, destrucción de refugios y medidas de control poco fundamentadas. Esta reacción, motivada más por miedo y desinformación que por evidencias científicas, amenaza la conservación de este grupo de mamíferos y pone en peligro la funcionalidad de los hábitats naturales.

Los murciélagos son reservorios de agentes infecciosos, incluyendo virus, bacterias, hongos y parásitos. Las zoonosis o enfermedades infecciosas asociadas a murciélagos incluyen la rabia, ébola y coronavirus, entre muchas otras. No obstante, la sola presencia de estos patógenos no implica una transmisión directa a humanos, ya que en la mayoría de los casos los datos concretos sobre su impacto real en la salud pública son limitados y poco documentados científicamente. Enfermedades como la histoplasmosis, por ejemplo, se asocian erróneamente con la transmisión directa por murciélagos, cuando en realidad es causada por el hongo *Histoplasma capsulatum* que se desarrolla en sus heces, pero no depende de los murciélagos como vectores.

Una de las zoonosis más importantes en Latinoamérica es la rabia, transmitida principalmente por murciélagos hematófagos conocidos como vampiros, que afecta principalmente al humano y a los animales domésticos. La expansión de las actividades ganaderas ha contribuido al crecimiento de las poblaciones de estos murciélagos y a la incidencia de esta enfermedad. Actualmente, numerosas campañas de control de los vampiros se han llevado a cabo

Figura.1. Murciélago vampiro, *Desmodus rotundus*, especie hematógafa de murciélagos. Foto: Frederico Sonntang.

Figura 2. Emergencia de murciélagos insectívoros de la familia Mormoopidae, los cuales se alimentan de toneladas de insectos cada noche. Foto: Emmanuel Solís.

como respuesta a los brotes de rabia, incluyendo acciones de vigilancia, control de los vampiros, vacunación del ganado y tratamientos profilácticos post-exposición a humanos.

Tradicionalmente, los mecanismos de control han consistido en la eliminación masiva de colonias de murciélagos hematófagos mediante vampiricidas. Sin embargo, este método puede resultar contraproducente, ya que la eliminación de adultos inmunes facilita la propagación de juveniles susceptibles y fomenta la recolonización por individuos externos, aumentando la incidencia de la rabia en las poblaciones y acentuando la prevalencia del virus. La alternativa más eficaz y sostenible es la vacunación del ganado y de las personas en riesgo, además de la mejora en la infraestructura de viviendas rurales para prevenir mordeduras en humanos, así como la modificación de conductas de pastoreo del ganado para disminuir los puntos de contacto con los murciélagos.

Un problema de fondo radica en que muchas instituciones gubernamentales mantienen estrategias obsoletas, sin suficiente actualización científica, con poco personal capacitado y acceso limitado a la vacunación preventiva. Asimismo, la falta de coordinación entre autoridades, ganaderos, académicos y comunidades rurales perpetúa prácticas ineficaces y dañinas.

Actualmente, ante la emergencia de la miasis del gusano barrenador del ganado, la falta de información, capacitación y liderazgo del gobierno ha generado acciones de manejo y control ineficaces, enfocadas a la matanza de los murciélagos sin evidenciar la correcta identificación de las especies hematófagas, sin contar con evidencia sólida de la participación de éstos en el ciclo de transmisión del gusano barrenador, revelando la vulnerabilidad del sector agropecuario frente a enfermedades que podrían prevenirse con mejores prácticas de manejo y control.

El crecimiento de la población humana, la pérdida del hábitat y el cambio climático son factores que incrementan el riesgo de los brotes zoonóticos al aumentar el contacto del humano con la fauna silvestre, por lo que los murciélagos no deben concebirse solo como amenazas sanitarias, sino como actores fundamentales en el equilibrio de los ecosistemas, al cumplir con roles ecológicos clave en los ecosistemas. La demonización injustificada obstaculiza tanto su conservación como la implementación de estrategias de control basadas en la ciencia. Es imperativo replantear la gestión de zoonosis a partir de un enfoque integral y multidisciplinario, que combine salud pública, ganadería y conservación de la biodiversidad. Solo así será posible garantizar un equilibrio entre las necesidades humanas y la preservación de estos mamíferos esenciales.

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Seasonal Composition of the Diet of Free-Range Equines in Northern Coahuila, Mexico

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Donkeys and horses are large non-ruminant herbivores distributed throughout the world. They are part of the cultural heritage of many countries and consume natural resources when they graze freely. In Mexico, the potential or current impacts of these animals on natural habitats shared with wildlife and other domestic animals have been scarcely documented. The objectives of this study were to evaluate the ecological variables diversity, richness, and diet similarity, and to identify the most commonly consumed plant species in spring and summer. The study was conducted in northern Coahuila. The dietary content of equine fecal samples was analyzed using microhistology. A total of 43 plant species were identified in the diet, with biodiversity values ranging from 2.12 to 2.34. Plant composition varied between seasons, showing less than 60% similarity. The most frequent species included *Opuntia macrocentra*, grasses such as *Cynodon dactylon*, *Erioneuron pulchellum*, *Bouteloua gracilis*, and *B. curtipendula*, and *Aloysia wrightii* among the herbaceous plants. Grasses made up most of the diet in spring, while herbaceous plants were dominant in summer. This information serves as a reference for grazing management in northern Coahuila, where cattle farming is becoming increasingly challenging. Diversifying land use and balancing it with the habitat needs of wildlife should be considered.

Keywords: donkeys, feeding preferences, horses, microhistology.

Los burros y caballos son grandes herbívoros no rumiantes presentes en todo el mundo formando parte de la cultura de muchos países y que también hacen uso de los recursos naturales cuando están en libre pastoreo. En México se han documentado escasamente los impactos potenciales o reales que estos animales causan en los hábitats naturales que comparten con fauna silvestre y otros animales domésticos. El objetivo fue evaluar las variables ecológicas de diversidad, riqueza y similitud de la dieta, e identificar las especies de plantas más consumidas en primavera y verano. El estudio se llevó a cabo en el norte de Coahuila. Se analizó el contenido de la dieta en excretas de equinos usando microhistología. La riqueza de plantas que componen la dieta fue de 43 especies, el valor de biodiversidad fluctuó entre 2.12 y 2.34 y la composición botánica no fue muy similar (<60%) entre estaciones. Se identificaron especies con mayor ocurrencia, como *Opuntia macrocentra*, especies de gramíneas como *Cynodon dactylon, Erioneuron pulchellum, Bouteloua gracilis* y *B. curtipendula*, además de *A. wrightii* en el grupo de herbáceas. En primavera las gramíneas constituyen la mayor parte de la dieta, mientras que en verano lo fueron las herbáceas. Esta información marca una referencia a considerar para el manejo de agostaderos en el norte de Coahuila, donde la cría de ganado bovino es cada vez más un reto, su diversificación y el balance con las necesidades de hábitat de la fauna silvestre deben ser consideradas.

Palabras clave: burros, caballos, microhistología, preferencias alimenticias.

Equines were first introduced to North America by the Spaniards round 1530 (Lugo and Alatriste-Contreras 2020). Donkeys were used as one of the main labor forces and expanded into the United States during the gold rush, when their use in mining activities increased due to their hardiness (Seegmiller and Ohmart 1981; Wolfe 1983). For their part, horses have played a key role in various cultures, as a component of war strategies and facilitating new communication modalities. In Mexico, horses have gained a prominent place within the national culture, especially in the context of the sport known as *Charrería* (Palomar 2004; Robledo-Reyes *et al.* 2024).

The original habitat of donkeys is predominantly desert (Seegmiller and Ohmart 1981; Wang *et al.* 2022), while horses evolved in open areas dominated by grasses and herbaceous plants, with little visual obstruction (Mihlbachler *et al.* 2011; Secord *et al.* 2012). Despite their cultural and economic importance, there is limited information available on equine populations in Mexico. In 2007, approximately 72 800 equine heads (horses, mules, and donkeys) were reported in the state of Coahuila, particularly in the municipalities of Cuatro Ciénegas, Saltillo, and Ocampo (INEGI 2013). The National Agricultural Survey (Encuesta Nacional Agropecuaria; INEGI 2020) estimated the existence of 1.5 million horses, mares, and their offspring, as well as approximately one million donkeys in the country. However, there is no specific inventory of equines for Coahuila, nor is there updated data on free-range equines. This situation is even more critical for feral populations, mainly donkeys, which live with little or no human contact in the strip bordering Coahuila and the United States of America (Stangl *et al.* 2007). These animals exhibit behaviors similar to those of native wildlife, which pose additional challenges in understanding their food ecology and impact on native vegetation.

In northern Coahuila, rural communities depend on economic activities such as the extraction of *candelilla* wax from *Euphorbia antisyphilitica* Zucc. and extensive livestock farming. In this context, donkeys play a central role as pack animals, facilitating access to remote mountainous areas during *candelilla* harvest (Ochoa *et al.* 2017; Jurado-Guerra *et al.* 2021). Similarly, cow-calf production is the main cattle-farming activity in the region, supplemented by breeding goats for cabrito and horses for labor (De los Reyes 2018). Historically, extensive livestock farming in Mexico has primarily focused on cattle, goats, and sheep, which has influenced land-use planning and carrying-capacity estimates, leaving horses in the background (Beck *et al.* 2020; Wells *et al.* 2022). Although equines have market value, they are rarely integrated into livestock calculations, unless the productive entities are dedicated solely to equine breeding (Fleurance *et al.* 2016).

There is scarce knowledge about the diet of equines and the plant resources consumed by them under wild or semi-wild conditions. Equines are known to consume a wide range of plant species, including grasses, herbs, and shrubs (Scasta *et al.* 2016; Gordon and Prins 2023). However, most studies have been conducted in temperate or arid regions of the United States and Europe, with a particular focus on grassland management, competition with other domestic species, or habitat conservation (Menard *et al.* 2002; Launchbaugh and Howery 2005; Harris *et al.* 2017). This geographic bias limits the understanding of equine food ecology in tropical, subtropical, or non-pastoral landscapes, where their interaction with vegetation could be significantly different (Harris *et al.* 2017). At the country level, studies on equine diet are virtually non-existent, which limits our understanding of their ecological role, especially in semiarid regions of northern Mexico where their presence is prevalent but scarcely documented.

Several studies have investigated the diet composition of free-range equines in various regions of the world using direct observation, microhistological analysis, DNA barcode identification in fecal samples, and near-infrared spectroscopy (NIRS) (Duncan 2012; Kartzinel *et al.* 2015; Harris *et al.* 2017; Castellaro *et al.* 2021). Horses exhibit a preference for grasses and pastures, although they can adjust their diet according to seasonal food availability (Scasta *et al.* 2016; Castellaro *et al.* 2021). Likewise, a review of the diet of wild donkeys in arid areas indicates that this species consumes around 175 plant species, with a marked preference for grasses and herbaceous plants (Abella 2008). Menard *et al.* (2002) suggest that although feral equines can exploit niches supplementary to those of domestic ruminants and, therefore, coexist with them under certain management conditions, this coexistence largely depends on the season of the year, since the trophic overlap between both groups may increase in certain periods. This background information suggests that the diet of equines varies not only according to the type of ecosystem but also in response to seasonal, physiological, and social factors, underscoring the need for regional studies that consider these variables.

The lack of regional studies on equine food ecology hinders the design of sustainable management strategies. The seasonal variability of their diet, the selection of plant species, and the potential impact on vegetation structure have not yet been systematically evaluated. Therefore, the objectives of this study were to: (1) evaluate the richness, diversity, and similarity of the equine diet during the wet and dry seasons; (2) describe the composition of the diet and identify the plant species that are consumed most frequently. This information will expand our knowledge about the ecological role of equines in semiarid ecosystems, enabling the implementation of sustainable management strategies that benefit both the conservation of natural resources and the optimization of equine livestock in natural grasslands of northern Mexico. These findings will set the foundation for future adaptive management strategies in landscapes where traditional human activities coexist with populations of domesticated or feral equines.

Materials and methods

Study area. - The study was conducted in the north of the Ocampo municipality, state of Coahuila, where rosetophyllous and microphyllous desert shrublands predominate. The most representative shrub species include the creosote bush (*Larrea tridentata* (DC.) Coville), Joshua tree (*Yucca* spp.), mesquite (*Neltuma glandulosa* Torr.), and prickly pear cactus (*Opuntia macrocentra* Engelm.). The most common native grasses belong to the genera *Bouteloua* spp. and *Aristida* spp., while the dominant species in the herbaceous stratum belong to the families Asteraceae, Fabaceae, and Brassicaceae (Powell 1998; Ochoa *et al.* 2017). The predominant soil type is Calcaric Regosol, mainly in lowlands or flat areas (INEGI 2021).

According to the climate classification of Peel *et al.* (2007), the region has an arid hot-desert climate with cool winters (BWh). The nearest meteorological station is San Miguel (National Meteorological Service code 5013), with records dating back to 1960. The mean annual precipitation is 244.2 mm, with 2010 being the rainiest of the past 15 years, with 592 mm; July is the month with the highest mean precipitation, while March is the driest month. The mean annual temperature is 20.9 °C, with maximum and minimum records of 41.1 °C and -4 °C, respectively. The study area is characterized by rugged relief with prominent mountain ranges such as *El Terminal*, *San Vicente*, *Carrasco*, and *La Harina*. The maximum altitude is 1700 m a.s.l. in the Sierra de San Vicente, while the minimum altitude is 550 m on

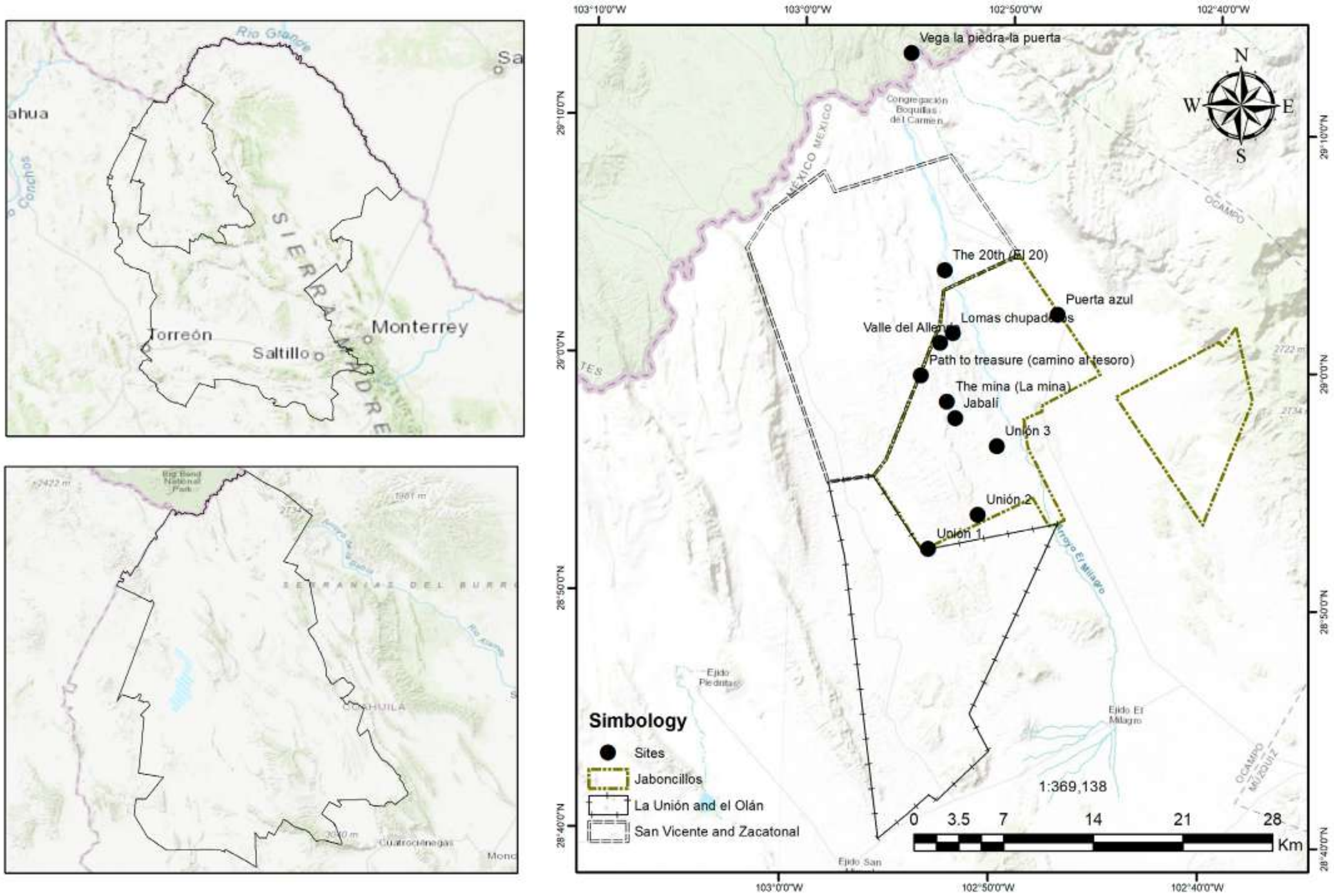

Figure 1. Study area and equine excreta collection sites in the ejidos *San Vicente y Zacatonal*, *Jaboncillos Grande*, and *La Unión y El Olán*, north of the Ocampo municipality, state of Coahuila, Mexico.

the bank of the Rio Grande. This study covered three ejidos, Jaboncillos Grande, La Unión y El Olán, and San Vicente y Zacatonal, all in the municipality of Ocampo, Coahuila (Figure 1). The study area extends over 68,000 hectares in which cattle move freely, since the ejidos are not bordered by metal fences that may restrict their movement. Land use in the region is dominated by extensive livestock farming, mainly cattle and goats, under a continuous grazing regime.

Fieldwork. – During April (referred to as spring) and July (referred to as summer) 2012, we traveled the dirt road network in the study area, collecting feces samples randomly in areas where the local inhabitants reported the presence of feral or free-range equines. At each sampling site, the presence and use of the area by equines was verified by observing signs such as resting areas, browsing traces, paths, tracks, or physical presence of animals. Between three and five fecal samples were collected per site, within a radius of 10 to 20 meters from the point where the most recent feces (excreta) were found. Excreta were considered fresh if they were soft, without signs of degradation by insects, and not completely dry after prolonged sun exposure. The fecal samples were stored in paper bags and labeled with the date, coordinates, and site code.

In total, 18 fecal samples were collected from five sites during spring and 20 samples from six sites during summer (Figure 1). The sample size was defined based on the number of excreta collected in spring; then, the same number of samples was collected in summer, following a non-probabilistic sampling principle (Makwana *et al.* 2023). Given the broad extension of the study area and the unique occupation of equines (Bakaloudis *et al.* 2024), the number of sampling sites adequately represents the use of space by these animals. In the sampling area, no horses were observed in the wild, and occupation mainly involved feral donkeys. However, since horse, donkey, and mule excreta are morphologically indistinguishable in the field, all collected samples are referred to herein as equine excreta.

Laboratory testing. – We employed the microhistological identification technique described by Holecheck *et al.* (1982), which is based on the comparison of plant cells in excreta with those of potentially consumed plant species. To this end, at each site, we collected samples from the edible parts of plants potentially consumed, as well as those that showed evidence of consumption, to create a reference catalog.

Sample processing began with a sun-drying phase followed by oven-drying at 75 °C for at least 48 hours to prevent fungus growth. Subsequently, the excreta and plant samples were ground, sieved to a particle size of no larger than 1.70 mm, and mounted on slides. For identification of cell structures, images were captured using a camera coupled to a stereo microscope using 20x

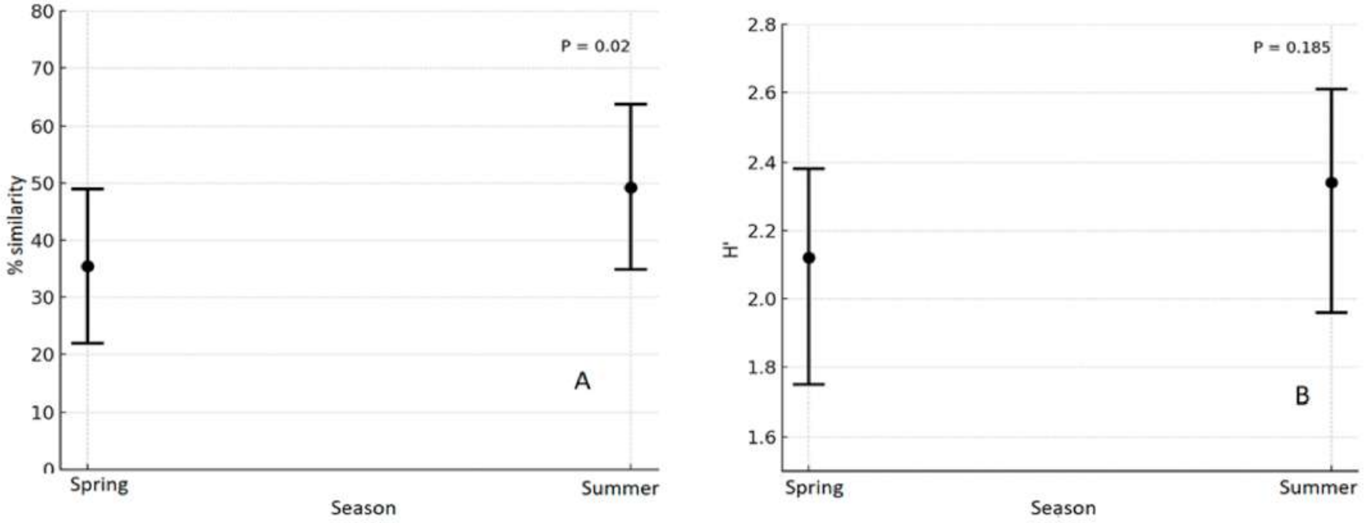

Figure 2. Bray-Curtis similarity index (A), mean and standard deviation; and Shannon-Wiener diversity index (B), with 95 % CI, of the diet of free-range equines during spring and summer in northern Coahuila.

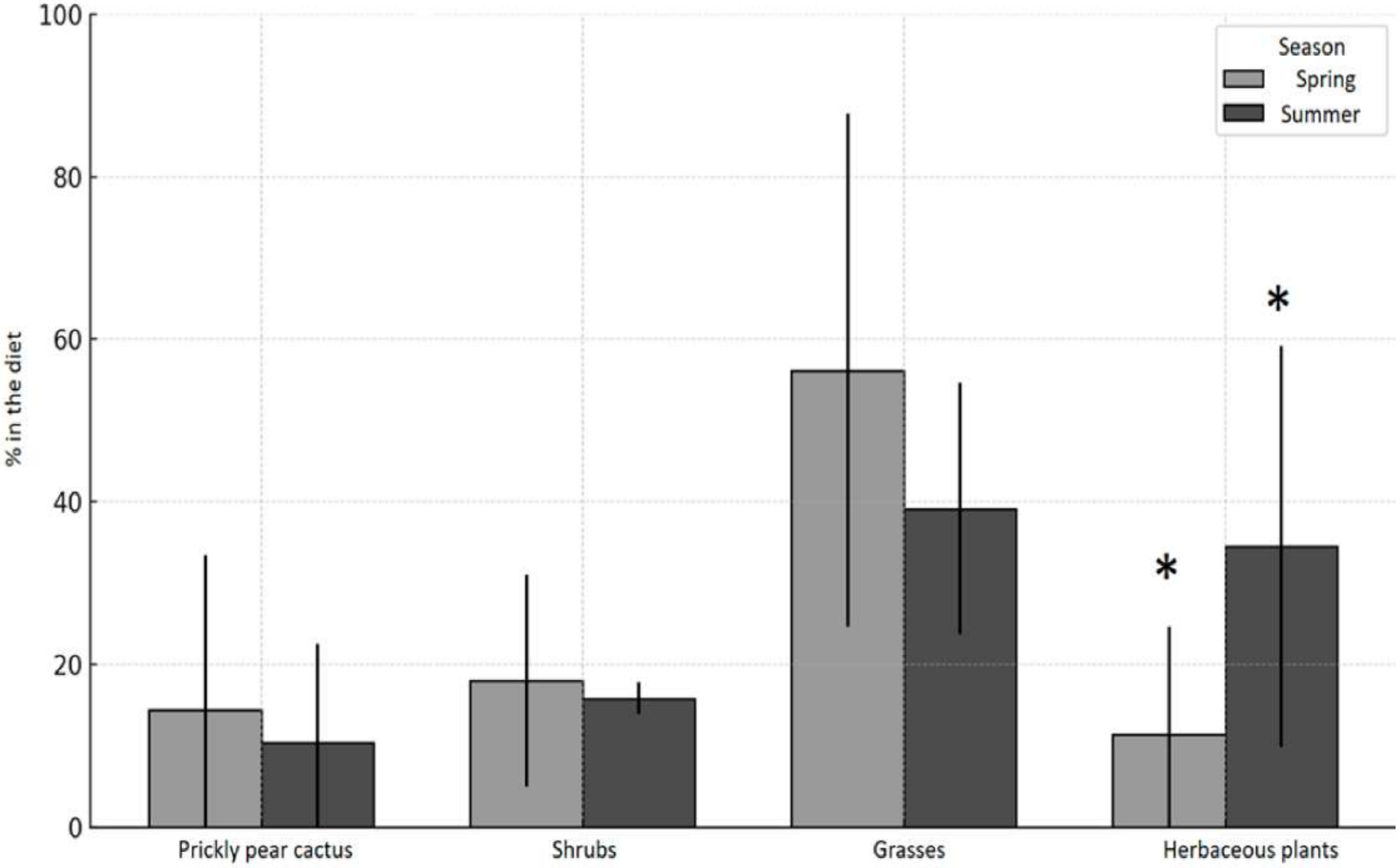

Figure 3. Composition of the diet of free-range horses by biological form, highlighting the participation of prickly pear cactus (*Opuntia macrocentra*), during spring and summer in northern Coahuila. Different letters indicate a significant difference ($P < 0.05$).

and 40x magnifications. These images were integrated into the reference catalog used in the microhistological identification process.

Microscopic analysis. - For each study site, a composite sample was prepared by thoroughly mixing the collected feces to ensure a homogeneous mixture. Five slides of each composite sample were mounted, each with 20 observation fields, for a total of 100 objective fields per site. Overall, 600 fields were examined for spring and 500 for summer. Each sample was examined under the microscope to determine the seasonal plant composition of the diet based on the relative frequency of cell traces of each identified species (Fracker and Brischle 1944; Holecheck *et al.* 1982; Garnick *et al.* 2018).

A database was created that included the identification of the species consumed in each season and their classification within functional groups: grasses, herbaceous plants, and shrub species. However, given the high percentage of prickly pear cactus (*O. macrocentra*) fibers in the diet, this species was analyzed separately to evaluate possible food preferences, keeping it within the shrub species group for functional classification purposes.

Data analysis. – The Shannon-Wiener index (H'), species richness (S), and Bray-Curtis similarity index were calculated; the latter was selected for its ability to integrate data on the relative abundance of species, its ease of interpretation, and its applicability in quantitative ecology studies (Ricotta and Podani 2017). The analyses were performed using the PAST program, version 3.19 (Hammer 2024). Seasonal differences in dietary diversity were evaluated using the Student's t-test ($\alpha \leq 0.05$). To identify similarity patterns between dietary components, a cluster analysis was applied using the averaging method (Ter Braak, 1987). Dissimilarity was measured using the Euclidean distance, which quantifies the proximity between cases in a multidimensional space. Finally, the reliability of the hierarchies projected in the dendrogram generated in PAST was determined by calculating the cophenetic correlation coefficient, which measures the degree of agreement between the original distances and the distances in the dendrogram.

Results

Ecological variables. - Regarding species richness, we identified a total of 43 plant species in the diet of equines in the study area, 36 in spring (83.7 % of the total) and 37 in summer (86.0 %), with 30 species common to both seasons. The mean number of taxa per site was slightly higher in spring (20.33 ± 4.17) than in summer (19.80 ± 2.38), with no significant difference between seasons ($P > 0.05$).

Only one cell structure did not match the species recorded in the reference catalog; therefore, it was classified as an "unidentified plant". For the functional group of shrubs, the following species were detected at trace levels in spring: *Atriplex canescens* (Pursh) Nutt., *Ephedra antisyphilitica* Berland., *Karwinskia humboldtiana* (Schult.) Zucc. and *Berberis trifolilata* Moric. In the case of grasses, *Muhlenbergia porteri* Scribn. accounted for less than 1 % of the diet.

During summer, also at the trace level, five shrub and herbaceous species were identified: *Buddleja marrubiifolia* Benth., *Celtis pallida* Torr., *Viguiera stenoloba* S.F. Blake, *Dyssodia setifolia* (Lag.), and *Senecio flaccidus* (Less.).

The dietary diversity, assessed by the Shannon-Wiener index, was higher in summer compared to spring (2.34 vs. 2.12, respectively), although the difference was not statistically significant ($P > 0.05$) (Figure 2). On the other hand, the similarity in the composition of the diet, based

Table 1. Percentage composition of the diet of free-range horses by plant species and biological form in two seasons (spring and summer) in northern Coahuila.

Species	Spring	Summer	Species	Spring	Summer
Shrubs (%)					
Acacia farnesiana	0.51	3.06	*Krameria erecta*	1.16	0.81
Acacia rigidula	2.08	5.49	*Larrea tridentata*	2.27	1.70
Atriplex canescens	0.18		*Leucophyllum frutescens*	5.26	0.79
Buddleja marrubifolia		0.63	*Berberis trifoliolata*	0.18	
Celtis pallida		0.22	*Parthenium incanum*	2.40	2.12
Dalea bicolor	0.35	0.40	*Neltuma glandulosa*	1.92	0.47
Ephedra antisyphilitica	0.56		*Tiquilia canescens*	11.46	0.48
Euphorbia antisyphilitica	0.67	0.25	*Viguiera stenoloba*		0.16
Guaiacum angustifolium	0.76	0.46			
Karwinskia humboldtiana	0.34		*Opuntia macrocentra*	13.19	8.10
Grasses (%)					
Aristida spp.	1.08	3.47	*Cynodon dactylon*	11.44	7.75
Arundo donax	2.04	1.02	*Erioneuron pulchellum*	13.72	11.57
Bouteloua curtipendula	6.35	3.79	*Heteropogon contortus*	0.43	2.25
Bouteloua gracilis	6.82	4.24	*Muhlenbergia porteri*	0.53	
Bouteloua ramosa	2.18	0.17	*Setaria leucopila*	0.48	0.22
Herbaceous plants (%)					
Abutilon wrigthii	2.86	17.88	*Parthenium confertum*	0.35	1.90
Allionia incarnata	0.54	0.57	*Selaginella spp*	0.53	1.01
Cologonia spp.	1.00	0.35	*Senecio flaccidus*		1.07
Croton dioicus	2.36	6.52	*Sida abutifolia*	1.01	0.53
Dyssodia setifolia		3.06	*Solanum elaeagnifolium*	1.76	1.50
Evolvulus alsinoides	0.50	0.19	Herbaceous plant not identified	0.75	1.63
Lesquerella fendleri		4.18			

on the relative abundance of plant traces in the samples, ranged between 64.5 % and 14.9 % in spring, with a mean of 35.4 ± 13.5 %, and between 72.5 % and 29.5 % in summer, with a mean of 49.2 ± 14.3 %; in this case, the difference between seasons was statistically significant ($P = 0.023$) (Figure 2).

Composition and consumption. The plant composition of the diet in spring was dominated by grasses (45.1 %), followed by shrubs (30.0 %), excluding the mountain prickly pear cactus (*O. macrocentra*), which accounted for 13.2 % and represented as much as 40 % of the diet at some sites. Herbaceous plants recorded the lowest percentage of the diet in spring (11.6 %).

In contrast, the most representative plants in the diet in summer were herbs (40.4 %), followed by grasses (34.5 %). In this same season, the proportion of shrubs, including prickly pear cactus, represented approximately 25 % of the diet (Figure 3).

The most frequent plant species in the equine diet in spring were the woody crinklemat (*Tiquilia canescens* [A. DC.] A.T. Richardson, 11.46 %) and the cenizo (*Leucophyllum frutescens* [Berland.] I.M. Jhonst., 5.26 %). Both species have soft, tomentose leaves that are particularly palatable among the species available this season. However, their consumption was significantly lower in summer ($P < 0.05$), likely because most species were highly dehydrated at that time.

In the case of grasses, spring and summer recorded a high consumption of fluffgrass (*Erioneuron pulchellum* [Kunth] Tateoka) and Bermuda grass (*Cynodon dactylon* [L.] Pers.) (Table 1). The former is abundant in rosette-shaped shrubland areas, while the latter is abundant on the banks of the Rio Grande, suggesting that equines probably move to the riverbanks in search of water, hence taking advantage of the local availability of this species. Other species also abundant in spring include the blue grama (*Bouteloua gracilis* [Kunth] Lag. ex Griffiths) and the sideoats grama (*B. curtipendula* [Michx] Torr.), both of which were abundant in the diet.

Regarding herbaceous plants, high consumption percentages of *Abutilon wrightii* A. Gray (17.9 %) and *Croton dioicus* Cav. (6.5 %) were observed in summer. Coinciding with the preferred shrubs in spring, these species have soft leaves in summer. The rest of the species in this group were recorded only at trace levels.

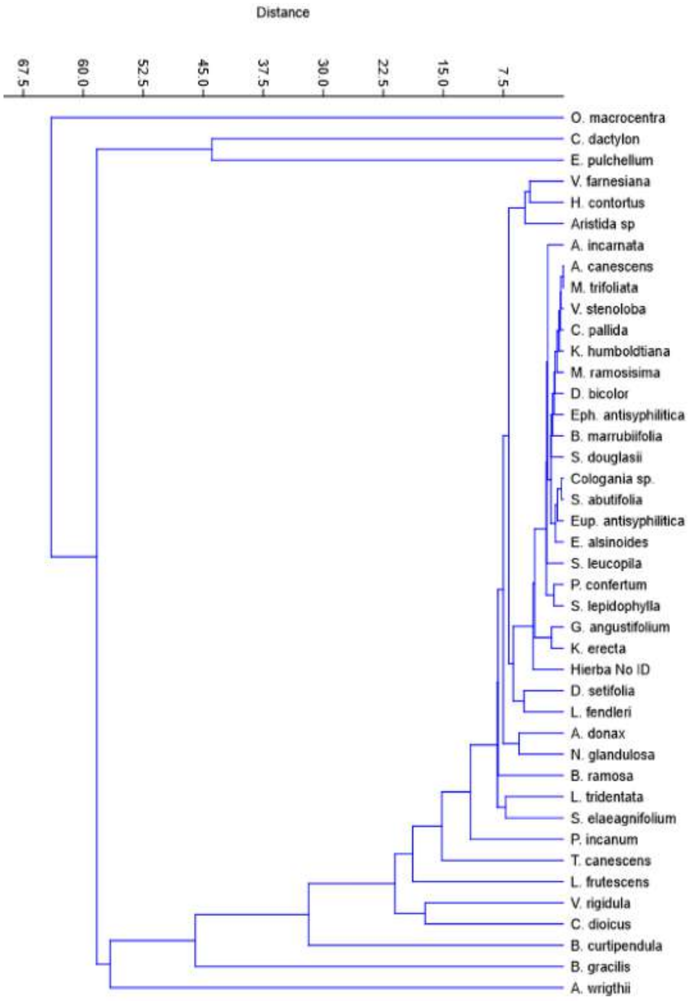

Figure 4. Hierarchical canonical correspondence analysis of the species that make up the diet of free-range equines during spring and summer in northern Coahuila.

The cluster analysis between sites, seasons, and species yielded a cophenetic correlation value of 0.95, indicating that the dendrogram adequately represents the data clustering (Figure 4). Four well-defined groups were identified. The first included mountain prickly pear cactus, whose presence in the diet differed markedly from that of all other species, with a frequency of 65 % in both seasons. The second group consisted of *A. wrightii* and four species of grasses: *C. dactylon, E. pulchellum, B. curtipendula*, and *B. gracilis,* with Euclidean distances corresponding to relative frequencies in the diet ranging from 35 % to 55 %. The third group (between 20 % and 17 % Euclidean distance) comprised blackbrush (*Vachellia rigidula* [Benth.]) and cenizo (*Leucophyllum. frutescens*), both shrub species, and *Croton dioicus*, a small semi-woody herbaceous species. These species are particularly important dietary components in the summer, after the rains. The fourth group comprised approximately 30 species, with a low contribution to the diet, all of which had Euclidean distances less than 15 %, indicating a low relative frequency and a limited contribution to the equine diet.

Discussion

The results of this study indicate that the composition of the diet of free-range equines varied between seasons, with a higher consumption of herbaceous plants in summer, clearly associated with higher humidity and, therefore, more abundant vegetation available for food. In contrast, grass consumption was significantly higher in spring, when it was dry and there were no fresh forage resources in the herbaceous stratum. The trend to increased forage availability after the summer rainy season was reported by Ochoa *et al.* (2023), who documented increased vegetation indices (NDVI) concurrent with higher precipitation levels in rainy years in the Chihuahuan desert. Peters *et al.* (2021)

also reported that heavy rainfall events led to a higher richness of plant species during summer in the same desert biome.

The above results coincide with previous studies that have described seasonal fluctuations in the diet of equines. It has been reported that the diet of donkeys during summer in the Sonoran and Mojave deserts accounted for between 33 % and 61 %, while in spring it fluctuated between 3 % and 30 % (Esmaeili *et al.* 2023). On the other hand, King and Schoenecker (2019), working in an equine herd management area in Colorado, United States, reported higher grass consumption in the horse diet in spring (68.7–78.5 %), similar to the numbers reported in the present study. A coincidence was reported in the proportion of shrub species consumed in spring and grass species in summer, both contributing approximately 30 % of the diet. This pattern is like the findings of Mikicic *et al.* (2023), who reported the preference of equines for open areas and a selective preference for herbaceous plants, with a consumption percentage of up to 80 %.

Regarding the ecological variable of species richness, 43 plant species were identified in the equine diet. This number is lower than the one reported by Esmaeili *et al.* (2023), who recorded 65 species, but similar to the number reported by Pansu *et al.* (2022), that is, 31 taxa (17–40 species) in the diet of large herbivores in southeast Africa.

These differences can be attributed to methodological factors. In particular, the microhistological analysis used in this study tends to underestimate the proportion of herbaceous species in the diet (43 species) compared to molecular methods such as those based on DNA markers (King and Schoenecker 2019). Additionally, the environmental context of this study, characterized by common land affected by severe drought, could limit the availability of plant resources, influencing the species richness observed in the diet.

In terms of dietary diversity, the Shannon-Wiener index calculated in this study was higher than that reported by Esmaeili *et al.* (2023), who documented H' values ranging from 1.40 to 1.94, and was close to the H' values documented by Pansu *et al.* (2022), which ranged from 1.58 to 2.57. These results suggest a moderate level of dietary diversity for equines in both seasons. However, contrary to our expectation, according to the hypothesis, no significant differences in dietary diversity were found between seasons. In addition to the diversity value, it is relevant to analyze the similarity in the composition of the diet, either between species, as addressed in most studies (MacCracken and Hansen 1981; Marshal *et al.* 2012; Gómez and Núñez 2016), or between seasons of the year, as in the present work. In the first case, the similarity analysis enables the identification of possible overlaps in the use of resources between species or vegetation strata, which can evidence ecological competition. This study did not compare competition with other herbivore species. However, given their body size, equines are potential competitors of domestic cattle and wild ungulates, such as bighorn sheep and mule deer, which thrive in the study area (Espinosa and Contreras 2010), although the latter two species exhibit foraging habits that mainly involve browsing (Hosten *et al.* 2007). It is worth noting that shrubs comprise between 15 % and 30 % of the equine diet, so active competition for this vegetation stratum is likely to occur to some extent.

Regarding competition with other animal species, the number of equines, both feral and domestic, has not been documented in detail anywhere in Mexico. However, estimating it is essential to prevent future conflicts with wildlife regarding competition not only for forage resources, but also for other components of the habitat. This is the case documented in the western United States, where the increase in the number of feral horses led to a decrease in the abundance of greater sage-grouse (*Centrocercus urophaisanus*) to approximately 2.5 % (Coates *et al.* 2021). The impact on the use of available space by this bird and the pronghorn (*Antilocapra americana*) has also been reported, with which equines compete not only for food, but also for displacement routes and watering troughs (Henning *et al.* 2022).

The similarity in diet between seasons of the year can help identify patterns of non-random site and food selection, likely determined by the availability of certain plant species. A study by Ochoa *et al.* (2017) in the same study region identified the plant species showing the highest ecological importance value, i.e., those best represented in terms of frequency, coverage, and density. Mesquite was the most important species, while prickly pear cactus was the most consumed species in the equine diet, being among the 10 species with the highest ecological importance value. Among grasses, blue grama produced the highest importance value (Ochoa *et al.* 2017), but fluffgrass was the species most consumed in spring and summer (13.7 % and 11.5 %, respectively). These three species were found in the equine diet, with prickly pear cactus and blue grama being the main components, at approximately 12 % and 6 %, respectively, implying that they are consumed in proportion to their abundance. In contrast, mesquite, despite its high importance value (71/100), only represented 1.9 % of the diet in spring, when it renews its leaves, suggesting non-random consumption.

The cluster analysis revealed two well-defined groups of plant food resources. The first included three of the five sites evaluated in summer and one in spring, which shared a similar diet composition, indicating that foraging sites could be selected based on the composition of plant species, namely *A. wrigthii* and four grass species (*B. gracilis, B. curtipendula, C. dactylon*, and *E. pulchellum*). The second cluster comprised five of the six sites evaluated in spring, showing a more diverse composition. The overall similarity of the diet between seasons was significantly different, with greater homogeneity in summer (50 %) than in spring. This similarity suggests a regularity in food selection in summer. Previous studies have reported a dietary overlap of up to 60 % between equines and mule deer (*Odocoileus hemionus*) in periods of high forage production (Marshal *et al.* 2012), as well as 50 % in the wet season and up to 70 % in the dry season between guanacos (*Lama guanicoe*) and feral donkeys in arid ecosystems (Gómez and Núñez 2016). Since equines are large herbivores, their intake rate and bite size can increase the likelihood of competition for forage resources with other species (MacCracken and Hansen 1981).

Finally, this study recorded a higher consumption of grass species in both seasons of the year, in terms of both the percentage in the diet composition and the relative frequency of consumption of species. This selection pattern is consistent with data reported in previous studies, where grass consumption by donkeys and zebras can exceed 50 % and even be the sole component in their diet under certain conditions (King and Schoenecker 2019; Pansu *et al.* 2022).

Conclusions

The consumption patterns observed in equines in the present study show seasonal variations in diet composition, with a higher consumption of herbaceous plants in summer and grasses in spring, possibly related to the increased availability of plants after the rainy season. Although these results differ from those reported in some previous studies, consistency was found in the consumption of shrubs and grasses, which account for approximately 30 % of the diet. These findings are consistent with observations suggesting that equines prefer open areas covered by herbaceous vegetation.

A total of 43 plant species were identified in the equine diet, which recorded a lower richness compared to some previous studies, likely due to differences in the methodology used and the environmental conditions at the study site, affected by drought and low availability of plant resources. Although prickly pear cactus (*O. macrocentra*) was identified as a major food source, grasses were the main functional group in the diet, highlighting their central role in the diet of these herbivores.

The results of this study provide relevant information on the diet of free-range equines in northern Mexico and underline the importance of considering these animals in the planning and management of grazing lands. Currently, this is practically not done, neither from an academic perspective nor for determining the productive parameters of grazing land (e.g., grazing coefficient, animal load, animal inventory) and is scarcely and poorly documented in decision-making in production units. Some of the regulatory initiatives could include the specific classification of grazing land for use by this type of livestock, reviewing ejido regulations to adjust the number of equine heads using grazing land at a given space and time, or implementing ethical measures for the control of feral populations that disrupt habitats and cause damage to other species.

Given the size and feeding habits of equines and their competition or overlap with other domestic and wild herbivorous species, this interaction should be considered in future studies, especially when evaluating the feasibility of conservation strategies or reintroduction of wildlife species, since their coexistence with equines could reduce the availability of resources or even jeopardize the survival of other species (Beck *et al.* 2024).

Unlike other countries such as the United States, where feral equine management is regulated and population estimates are conducted to assess their impact on ecosystems (Scasta *et al.* 2016), in Mexico, there is scarce information and a mixed social perception about donkeys, horses, and mules. Therefore, it is essential to improve our understanding of their ecology and develop appropriate management strategies to mitigate the potential adverse effects on natural ecosystems.

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Distribution, ecology, and natural history of the Spectral bat (*Vampyrum spectrum*) in Colombia

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Species distribution and ecology are heavily influenced by ecosystem structure and dynamics, where shifts in land use and habitat loss jeopardize the survival of populations. This is especially important for top predators, such as Spectral bat **(***Vampyrum spectrum),* which is thought to depend on conserved areas with large resource availability. It is the largest bat in the Americas. Here, we report the second record of the Spectral bat for Valle del Cauca, Colombia, and the first record for the region's Tropical Dry Forest. We also report key morphological and ecological information associated to a couple individuals from Colombia's Pacific region. Further, we analyzed the association of the Spectral bat's presence in Colombia with biome, ecosystem, and vegetation cover type. Historical distribution analysis shows a decrease in stable forest areas ranging from 1990 to 2017. Most *V. spectrum* records are from the Andean region, with significant associations of biomes and vegetation cover. Surprisingly, the areas where the species has been recorded in the last years correspond to a mix of forests, urban areas and agricultural lands. The diet of the carnivore Spectral bat includes 12 vertebrate species, predominantly rodents and birds, while sugar assimilation seems to be low and slow, as expected for its protein rich meal consumption with low sugar content. This study contributes to the understanding of *V. spectrum's* ecological needs and highlights critical areas for future research and conservation efforts to ensure the species' survival in Colombia.

Key words: Bats, diet, forest and non-forest, habitat, top predator.

La distribución y la ecología de las especies están influidas por la estructura y la dinámica de los ecosistemas, donde los cambios en el uso del suelo y la pérdida de hábitat ponen en peligro la supervivencia de las poblaciones. Esto es especialmente importante para los depredadores tope, como el murciélago espectral (*Vampyrum spectrum*), que se cree que depende de zonas conservadas con gran disponibilidad de recursos. El murciélago espectral es el murciélago más grande que se encuentra en el continente americano. Aquí, reportamos el segundo registro del murciélago espectral para el Valle del Cauca, Colombia, y el primer registro para el Bosque Seco Tropical de la región. También reportamos información morfológica y ecológica clave asociada a un par de individuos de la región Pacífica de Colombia. Además, analizamos la asociación de la presencia del murciélago espectral con biomas, ecosistemas y tipo de cobertura vegetal presentes en Colombia. El análisis de la distribución histórica muestra una disminución en las áreas de bosque estable entre 1990 y 2017. La mayoría de los registros de *V. spectrum* proceden de la región andina, con asociaciones significativas a biomas y cobertura vegetal. Sorprendentemente, las zonas donde se ha registrado la especie en los últimos años corresponden a una mezcla de bosques, zonas urbanas y tierras agrícolas. La dieta del murciélago espectral carnívoro incluye 12 especies de vertebrados, predominantemente roedores y aves, mientras que la asimilación de azúcares parece ser baja y lenta, como era de esperar por su consumo de comidas ricas en proteínas con bajo contenido en azúcares. Este estudio contribuye a la comprensión de las necesidades ecológicas de *V. spectrum* y destaca áreas críticas para futuras investigaciones y esfuerzos de conservación para asegurar la supervivencia de la especie en Colombia.

Palabras clave: Bosque/no bosque, dieta, hábitat, murciélagos, depredador tope.

The spatial distribution of species is strongly influenced by the structure and dynamics of the ecosystems in which they inhabit (Gaston 2003). Bats are highly sensitive to changes in land cover and ecosystem transformation (Meyer *et al.* 2016). The Spectral bat (*Vampyrum spectrum*) is recognized as the largest carnivore bat species present in the New World (Altringham 2011). It is currently classified as Near Threatened (NT) by the International Union for Conservation of Nature (Solari 2018). It is associated with a variety of habitats that provide shelter and food resources, from tropical rainforests to transitional ecosystems (Emmons and Feer 1997). However, changes in land-use patterns and loss of natural ecosystems can significantly affect the distribution and survival of this species (Simmons and Voss 1998).

The Spectral bat, which ranges from southern Mexico to Bolivia and across the Amazon Basin, is considered rare due to low abundance and limited records (Greenhall 1968; Sousa *et al.* 2016; Acosta *et al.* 2019; Pacheco-Figueroa *et al.* 2022). It is thought to prefer conserved forested areas, where it functions as a top predator, preying on birds, rodents, and other bats (Vehrencamp *et al.* 1977; Gardner 2008; Solari 2018). Despite its ecological significance, aspects of its ecology and population dynamics remain largely unknown. In Colombia, it inhabits various ecosystems across all biogeographic regions (Esquivel *et al.* 2020), but the few records hinder understanding of its population status and local distribution. Existing reports indicate a declining population trend, reflecting its rarity despite widespread range (Reid 2009; Solari 2018).

Because of its poorly documented history in Colombia, it has not been included in any risk assessments in the past (Rodríguez-Mahecha *et al.* 2006) nor in the most recent assessment of wildlife endangered species of Colombia (Resolución 0126 de 2024 by MADS 2024). Many aspects of natural history, population ecology and current distribution of *Vampyrum spectrum* are still unknown in the country, having just 38 valid records in a frame of approximately 80 years (1942 to 2023) (Rivas-Pava *et al.* 2007; Pinto-Orozco *et al.* 2023) out of 344 records on its distributional range (GBIF 2023) which limits the assessment of its conservation status. Overall, studies have been focused in understanding *V. spectrum*'s diet (Gardner 1977; Navarro and Wilson 1982; Gardner 2008), others have provided information about its movement and echolocation (Martínez-Fonseca *et al.* 2022), home range (Vehrencamp *et al.* 1977, Sousa *et al.* 2016), its local distribution (Esquivel and Rodríguez-Bolaños 2018; Pinto-Orozco *et al.* 2023), and biogeographic affinity (Esquivel *et al.* 2020), being the latter the most comprehensive approximation for Colombia. In Valle del Cauca, a department located in the Pacific region of Colombia, the first record of the species was recent; it was reported in a small fragment of premontane humid forest in the Bosque de Yotoco Natural Reserve (Pinto-Orozco *et al.* 2023), confirming its presence in this region in the southwestern part of the country.

However, none of the mentioned studies have tested the habitat affinity of the Spectral bat, a key aspect to understand and predict the species response to habitat changes (López-Bosch *et al.* 2022). The relationship between changes in land cover and biome structure provides a comprehensive perspective on habitat requirements of *Vampyrum spectrum*, especially in humid tropical and mountain biomes, as well as in transitional ecosystems between primary and secondary forests (Sierra *et al.* 2007). Studying the distribution of the species in relation to changes in cover and biome structure can lead to the identification of habitat displacement trends and the impact of fragmentation (Laurance *et al.* 2014), which provides valuable information for conservation strategies at the national level. Hence, our aim was to determine, for the first time, the association between *Vampyrum spectrum* records and: 1) biomes, 2) ecosystems, and 3) vegetation covers in Colombia. We hypothesized that *V. spectrum* would show a stronger association with vegetation cover, and therefore a higher number of records in well-conserved areas. Furthermore, we present ecological and morphological data and report the first record of the species within the highly fragmented Tropical Dry Forest of Valle del Cauca, Colombia. Additionally, our Bayesian model indicates that the variation in the number of *V. spectrum* records is best explained by the nested structure of Biome, Ecosystem, and Cover; suggesting that ecological context at multiple spatial scales plays a significant role in shaping distribution patterns. This information is essential for species conservation, particularly considering the rapid transformation of forested areas in the Neotropics.

Materials and methods

Our study area included 10 remnants of Tropical Dry Forest (TDF) of Valle del Cauca, Colombia from 2019 to 2021 (Velásquez-Roa and Calvache-Sánchez 2021). TDF is characterized to present a bimodal regimen climate, with rainy seasons between April – May and October – November, which represents 70% of annual precipitation, and two dry seasons between January – February and July – August (Armbrecht and Ulloa-Chacón 1999). The average altitude of the places visited was 1050 m above sea level. These remnants are characterized by a high intervention rate, logging, and the presence of *Chusquea* spp. The new record in Colombia for Spectral bat was captured at Hacienda La Venta, which is a private area in the municipality of Bugalagrande, Valle del Cauca. It is part of the basin of Paila river and Cauca's River hydrographic region (Figure 1). This area is dominated by grass used for livestock and 120 ha of TDF. The individual was captured under the collect permit 100 No. 1122 of October 23, 2018, given to the project "Contribución a la conservación del Bosque Seco Tropical del Valle del Cauca a través del fortalecimiento Valle del Cauca occidente" and deposited at the Colección Zoológica de referencia científica, INCIVA (IMCN 165). Seven dominant plant species at the Hacienda La Venta were *Croton schiedeanus, Aniba perutilis, Zanthoxylum rhoifolium, Miconia spicellata, Machaerium capote, Clarisia biflora* and *Nectandra* sp., all of them with thin stem and height between three m to 20 m, which is indication of a highly modified environment.

Historical distribution and ecological affinity. To evaluate habitat changes in areas where *Vampyrum spectrum* has been recorded in Colombia, we compiled occurrence data from Esquivel *et al.* (2020) and Pinto-Orozco *et al.* (2023) along with the new record reported in this study, totaling 39 occurrences (N=39) and we compiled a forest and non-forest map from IDEAM (2019) between 1990 and 2017

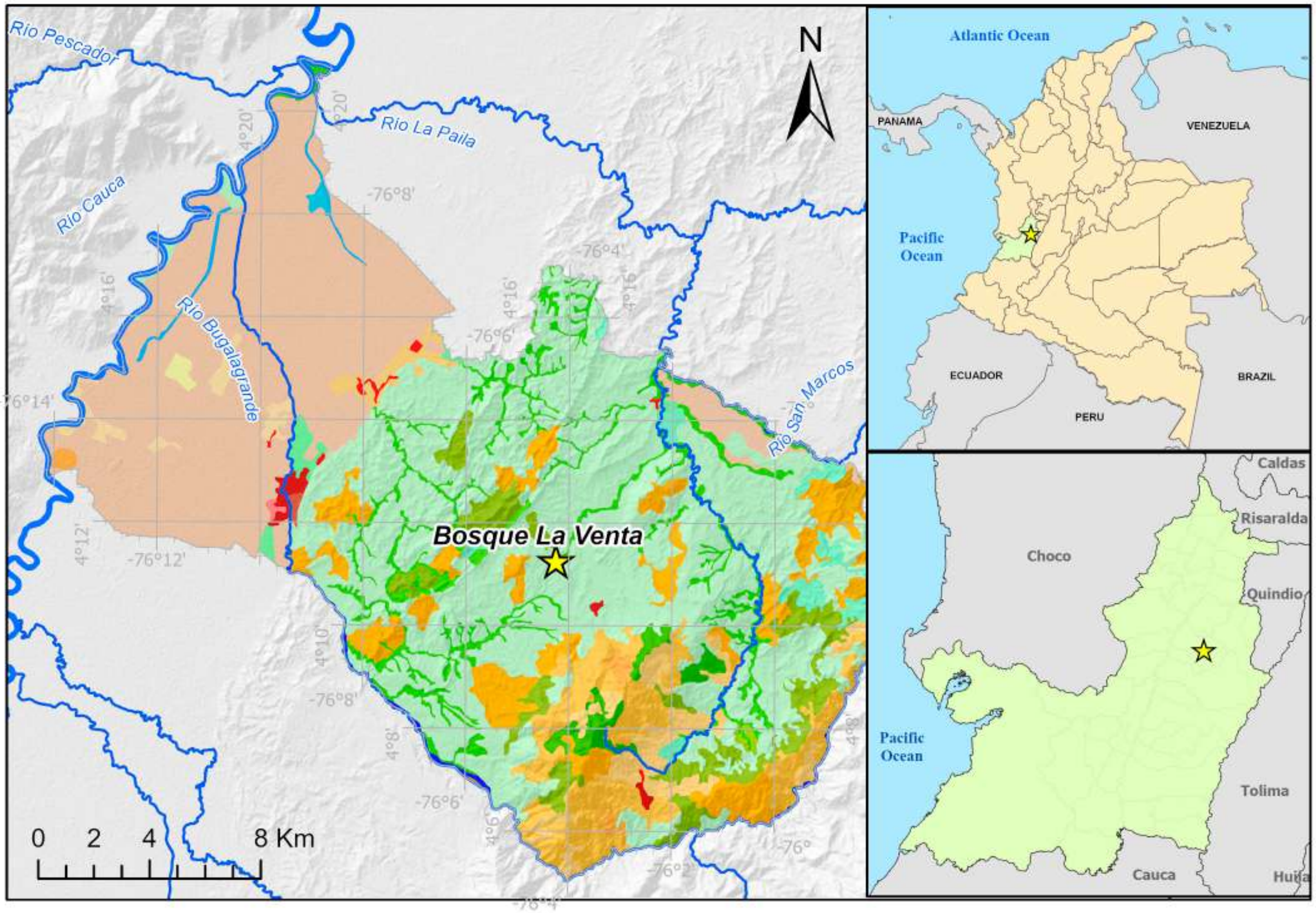

Figure 1. Vegetation covers of the habitat where *Vampyrum spectrum* was captured during the current study; Hacienda La Venta, Bugalagrande, Valle del Cauca, Colombia (★). Sources: IDEAM: Coverages 2020, CVC: Basic cartographic 2021, IGAC: Basic cartographic 2019. Light green areas: secondary forest.

(~30 m). IDEAM classifies habitat changes into five classes: stable forest, non-stable forest, deforestation, regeneration, and areas with no available information. Furthermore, we incorporated the terrestrial, marine, and coastal ecosystem map and the biome classification by the Instituto Humboldt (1:100.000, IDEAM *et al.* 2017), which classifies Colombian ecosystems based on ecological and structural attributes. These resources are essential for assessing deforestation, regeneration, and forest stability, providing key insights into the spatial dynamics of the species. Furthermore, we quantified the extent of area loss by comparing the forest and non-forest raster data from 2017 with that from 1990, calculating the difference to identify areas of change over time. To categorize the magnitude of vegetation loss, we applied a threshold-based classification using the following criteria: significant loss (^{3} 30% decrease), moderate loss (10.1% to 29.9% decrease) and minor loss (0.1% to 10% decrease). These thresholds were implemented using conditional logic in the Raster Calculator tool in ArcGIS Pro 3.4.0 (ESRI 2024). The resulting classified raster was then used to calculate the total area of loss per category by multiplying the number of pixels in each class by the area of a single pixel (900 m^{2}). The results were expressed in square kilometers and as percentages of the total affected area.

To examine associations between biome, ecosystem, and vegetation cover in relation to Spectral bat records, we applied a randomization test to account for the small sample size, followed by a Chi-square test at a significance level of 0.05, as an exploratory analysis. Additionally, due to the small sample size, we employed a Bayesian approach, fitting a hierarchical model with nested random intercepts to evaluate whether the nested structure of Biome, Ecosystem, and Cover explained the variation in bat records (counts) in Colombia. We fitted a Poisson bayes model using the brm function from the brms package (Bürkner 2021) with 3 chains, 30000 iterations, 10000 warmup and thin equal 1. We defined our priors as normal (0, 1) for the intercept (assumes the log of expected counts is centered around 0 with moderate uncertainty) and exponential (1) for random effect standard deviations (encourages smaller group-level variation unless strongly supported by the data). The analyses were conducted in R software version 2024.12.0+467 (R Core Team 2024).

Morphology. We took external and craniodental measurements of the specimen captured at Hacienda La Venta. Additionally, to increase the available morphological

Figure 2. Cranioventral, lateral and dorsal views of the *Vampyrum spectrum* specimen's cranium and dorsal view of specimens' skin (IMCN 165) collected in Hacienda La Venta, Bugalagrande, Valle del Cauca, Colombia.

information of the species from the Pacific region of Colombia we also visited two mammal collections. We report measurements of a specimen deposited in the Colección Zoológica at Universidad de Nariño (PSO-Z MUN 663 and its neonate MUN 663B) and two specimens deposited in the Colección de Mamíferos at Universidad del Valle (UV-13281, UV-13282) that have not been previously published (Figure 2). Following Simmons and Voss (1998), standard external (n = 3) and craniodental (n = 13) measurements were taken using digital calipers of 0.01 mm accuracy. For all the individuals, the external measures were hind foot length (HF), ear length (E) and forearm length (FA), while the craniodental measures were: Greatest length of the skull (GLS), Condyloincisive length (CIL), Condylocanine length (CCL), Zygomatic breadth (ZB), Maxillary toothrow length (MTRL), Mandibular toothrow length (MANDL), Postorbital breadth (PB), Braincase breadth (BB), Braincase height (BH) without the sagittal crest, Palatal length (PL), Canine-Canine breadth (C-C), Superior Canine length (SCL) and Inferior Canine length (ICL) both considered in teeth without wear.

Diet. Diet information from the individual caught at Hacienda La Venta was not possible to identify (feathers); however, we compiled diet information from an individual captured in El Algodonal village, municipality of Taminango, department of Nariño by one of this study's authors (October 2013). The specimen was caught in a shallow cavern of 30 m (5x7) above the south bank of the Mayo River. The cave was inhabited by a group of four adult Spectral bats, one of which was a female with a neonate, collected and deposited at Colección Zoológica Universidad de Nariño (PSO-Z) (MUN 663 – MUN 663B). Approximately 1 kg of biological material

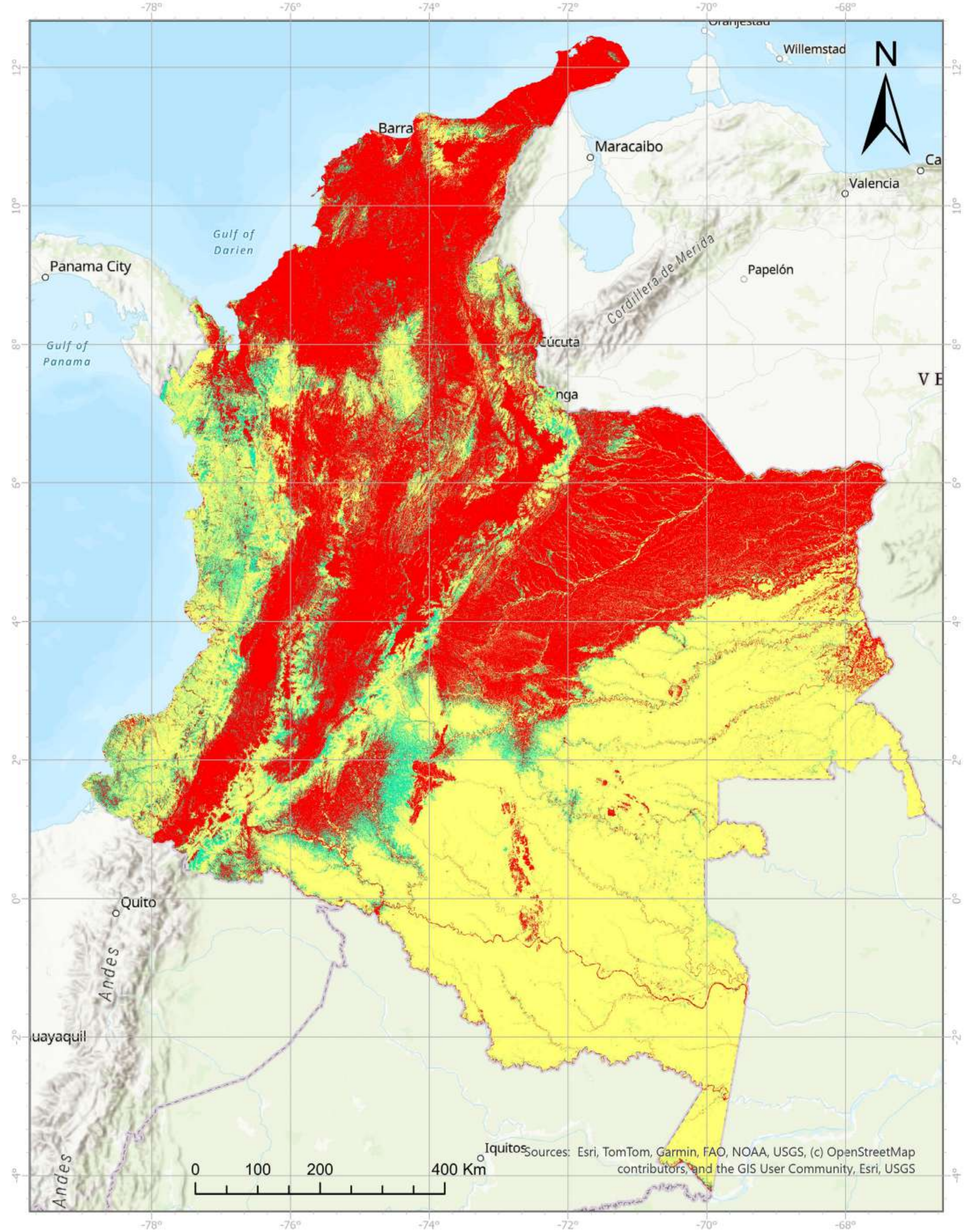

Figure 3. Categorization of vegetation loss in Colombia (1990–2017) based on a 30% reduction threshold derived from forest and non-forest cover data (IDEAM 2019). The map indicates areas of high vegetation loss (red), moderate vegetation loss (green), and minor vegetation loss (yellow) in Colombia.

Table 1. Standard external and craniodental measurements in millimeters (mm) for four adult specimens and a neonate of *Vampyrum spectrum. The mean ($\bar{x}$), standard deviation (sd) and sex are provided only for adult specimens.* These specimens are housed in the Colección Zoológica de Referencia INCIVA (IMCN), the Colección de Mamíferos at Universidad del Valle (UV), and the Colección Zoológica at Universidad de Nariño (PSO-Z), respectively. *Cranial measurements are not included.

	INCIVA (This study) IMCN	**Universidad del Valle**		**Universidad de Nariño**		**$\bar{x}$ ± sd**
		UV		**PSO-Z**		**Adult specimens**
Measurements	IMCN-165	UV-13281	UV-13282	MUN-663	MUN-663B	
(mm)	♀	♀	♂	♀	Neonate*	
E	43.49	37.37	39.03	38.80	19.40	39.67 ± 2.65
HF	27.67	19.16	25.78	28.40	25.65	25.25± 4.21
FA	109.09	96.98	103.55	106.40	41.15	104.01 ± 5.20
TL	146.56	138.73	113.74	147.71	58.48	136.69 ± 15.81
GLS	53.26	50.43	51.00	52.49		51.80 ± 1.31
CIL	44.37	42.24	43.73	43.82		43.54 ± 0.91
CCL	44.51	42.10	43.22	43.34		43.29 ± 0.99
ZB	24.45	22.75	24.25	23.52		23.74 ± 0.77
MTRL	21.14	19.78	20.85	21.28		20.76 ± 0.68
MANDL	22.95	21.96	23.05	23.32		22.82 ± 0.59
PB	8.31	7.48	8.15	8.14		8.02 ± 0.37
BB	16.58	15.32	16.4	15.99		16.07 ± 0.56
BH	18.01	15.51	18.32	17.99		17.46 ± 1.31
PL	20.18	19.70	21.41	21.29		20.65 ± 0.84
C-C	8.37	8.17	9.38	9.22		8.79 ± 0.60
SCL	8.03	7.19	8.06	7.49		7.69 ± 0.43
ICL	8.08	6.71	8.72	7.8		7.83 ± 0.84
Weight	158	120	-	>250		

Conventions

External: E: ear length, HF: Hind foot length, FA: Forearm, TL: Total length

Cranial: Greatest length of the skull (GLS), Condyloincisive length (CIL), Condylocanine length (CCL), Zygomatic breadth (ZB), Maxillary toothrow length (MTRL), Mandibular toothrow length (MANDL), Postorbital breadth (PB), Braincase breadth (BB), Braincase height (BH) without the sagittal crest, Palatal length (PL), Canine-Canine breadth (C-C), Superior Canine length (SCL) and Inferior Canine length (ICL) both considered in teeth without wear.

found at the bottom of the roosting site was collected. The material was separated into identifiable/non-identifiable components and separated into items by morphological similarity. The birds' feathers and skulls recovered were compared with specimens housed at the PSO-Z and the Colección de Mamíferos of Universidad del Valle (UV) to determine the lowest taxonomic level possible for the preys. This information was simultaneously contrasted with information on the species list of the sampled locality.

Sugar assimilation. We measured glucose assimilation in the individual caught at Hacienda La Venta, Bugalagrande, Valle del Cauca (2020). After a 10-hour fasting period, we measured blood glucose levels using a glucometer GlucoQuick G30a (Diabetrics®). We extracted a blood drop from the forearm using a lancet and the measurement was directly done through the meter strips. Subsequently, we fed the bat with a 20% glucose solution 5.4 g/kg body weight based on Kelm *et al.* (2011). Then we measured blood glucose levels 10, 30 and 60 minutes after the glucose ingestion to build a glucose tolerance curve. The essays were conducted ensuring the welfare of the animal during sample collection according to the guidelines of the American Society of Mammalogists for the use of wild mammals in research and education (Sikes and ACUC 2016), and the physiological study was conducted under the permit of the National Authority of Environmental licenses and the Ministry of Environment and Sustainable Development of Colombia, Resolution 1070, 28 August 2015.

Results

During the fieldwork at Hacienda La Venta, an adult post-lactating female *V. spectrum* was captured using ground mist nets in August 2020. The exact location where the individual was caught was an edge between a secondary forest and a grassland (Figure 1). This represents the second confirmed record for Valle del Cauca and the first for the very fragmented and diminished Tropical dry forest in the department. Morphometrics for our specimen (IMCN -165) are presented in Table 1, along with previously unpublished data for specimens housed at the Colección de Mamíferos

Table 2. Summary of results from the hierarchical Bayesian model with nested random effects. The model includes random intercepts for Biome, Ecosystem (nested within Biome), and Cover (nested within Ecosystem and Biome). Estimates are presented alongside their lower (l-95% CI) and upper (u-95% CI) 95% credible intervals, standard deviation (sd), standard error (Est.error), convergence diagnostics (Rhat) and effective sample sizes (Bulk_ESS and Tail_ESS). Parameters whose credible intervals do not include zero are considered to have a statistically significant effect (bold numbers).

Multilevel	**Hyperparameters:**						
~Biome	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
sd(Intercept)	0.37	0.31	**0.01**	**1.14**	1	22265	24533
~Biome:Ecosystem							
sd(Intercept)	0.3	0.22	**0.01**	**0.8**	1	21533	24274
~Biome:Ecosystem:Cover							
sd(Intercept)	0.29	0.22	**0.01**	**0.8**	1	21887	24872
Regression	**Coefficients:**						
Intercept	0.56	0.3	-0.09	1.1	1	35548	32093

Draws were sampled using sampling (NUTS). For each parameter, Bulk_ESS and Tail_ESS are effective sample size measures, and Rhat is the potential scale reduction factor on split chains (at convergence, Rhat = 1).

of Universidad del Valle (UV-13281, UV-13282) and Universidad de Nariño (MUN-663, MUN-663B).

Historical distribution and ecological affinity. To assess changes in the forest cover and geographical distribution of *V. spectrum* in Colombia (in forest and non-forest areas), we analyzed habitat transformations over time. Between 1990 – 2017, stable forest areas showed a marked decline, with notable losses in the Pacific, Andean and Amazon regions. Similarly, areas classified as "no information", were predominant between 1990 and 2010. Deforestation was consistently present from 1990 to 2012. The category of regeneration, in contrast, was minimally represented due to its localized nature, making it difficult to visualize at a broader scale. Non-stable forests, which dominate most of the national territory, are associated with regions experiencing higher levels of anthropogenic activity. Most *V. spectrum* records have been reported in the Andean region, followed by the Pacific, Orinoquia, Amazon and, to a lesser extent, the Caribbean region. To further quantify these changes, we applied a 30% decrease threshold to classify the severity of loss. This analysis revealed that approximately 468,168 km^2 experienced a significant loss (43%), 100,130 km2 underwent a moderate loss (9%) and 533.514 km2 experienced a minor loss (48%) (Figure 3, Table 3).

Table 3. Area and proportion of vegetation loss categories in Colombia (1990–2017) based on 30% decreased threshold. Area values were calculated by multiplying the pixel count by 0.0009 km^2, due to spatial resolution of 30 meters per pixel.

Category	Pixel count	Area (Km^2)	Percentage total loss
Significant loss (^{3} 30%)	520205048	468,168.54	42.49%
Moderate loss (10.1% to 29.9%)	111255970	100,130.37	9.09%
Minor loss (0.1% to 10%)	592793639	533,514.28	48.42%

Statistical analysis revealed a significant association between *V. spectrum* records and biome type (X2 = 18.8, p value = 0.001) as well as vegetation cover (X2 = 13.6, p value = 0.04) but not significant association with ecosystem type (X2 = 7.3, p value = 0.21) (Figure 4A). Many records were located within the Tropical Humid Zonobiome (ZHT) and were associated with the grasslands (PA) and mosaics vegetation cover types (MO) (Figure 4B, 4C). On the other hand, the posterior estimates obtained through the Bayesian model revealed that the variation in bat counts is meaningfully structured across all three spatial levels (Table 2). The standard deviations of the random intercepts were Biome: 0.37 (95% CI: 0.01–1.14), Ecosystem within Biome: 0.30 (95% CI: 0.01–0.80) and Cover within Ecosystem and Biome: 0.29 (95% CI: 0.01–0.80) (Figure 5). These estimates suggest that each level of the ecological hierarchy contributes to explaining the variability in *V. spectrum* records, with no single level dominating the structure. All model diagnostics indicated good convergence ($\hat{R}$ = 1.00) and high effective sample sizes, supporting the reliability of the posterior estimates (Table 2). These results underscore the importance of accounting for multi-scale ecological structure when modeling species distribution data, especially in complex landscapes like those found in Colombia. Analysis of present-day vegetation cover (IDEAM *et al.* 2017) in areas where *Vampyrum spectrum* has been recorded indicates that the species primarily occupies highly heterogeneous landscapes. Notably, only five out of 39 records correspond to dense forest areas, while nearly half are in proximity to urban developments or agricultural zones, suggesting a degree of adaptability to modified environments.

Diet and sugar assimilation. We recorded 12 vertebrate species in the diet of the Spectral bat, where four species were mammals, and eight were birds. In the case of mammals, we found a total of 41 skulls of rodents (Cricetidae) where 63.4% corresponded to *Zygodontomys brunneus* (26)

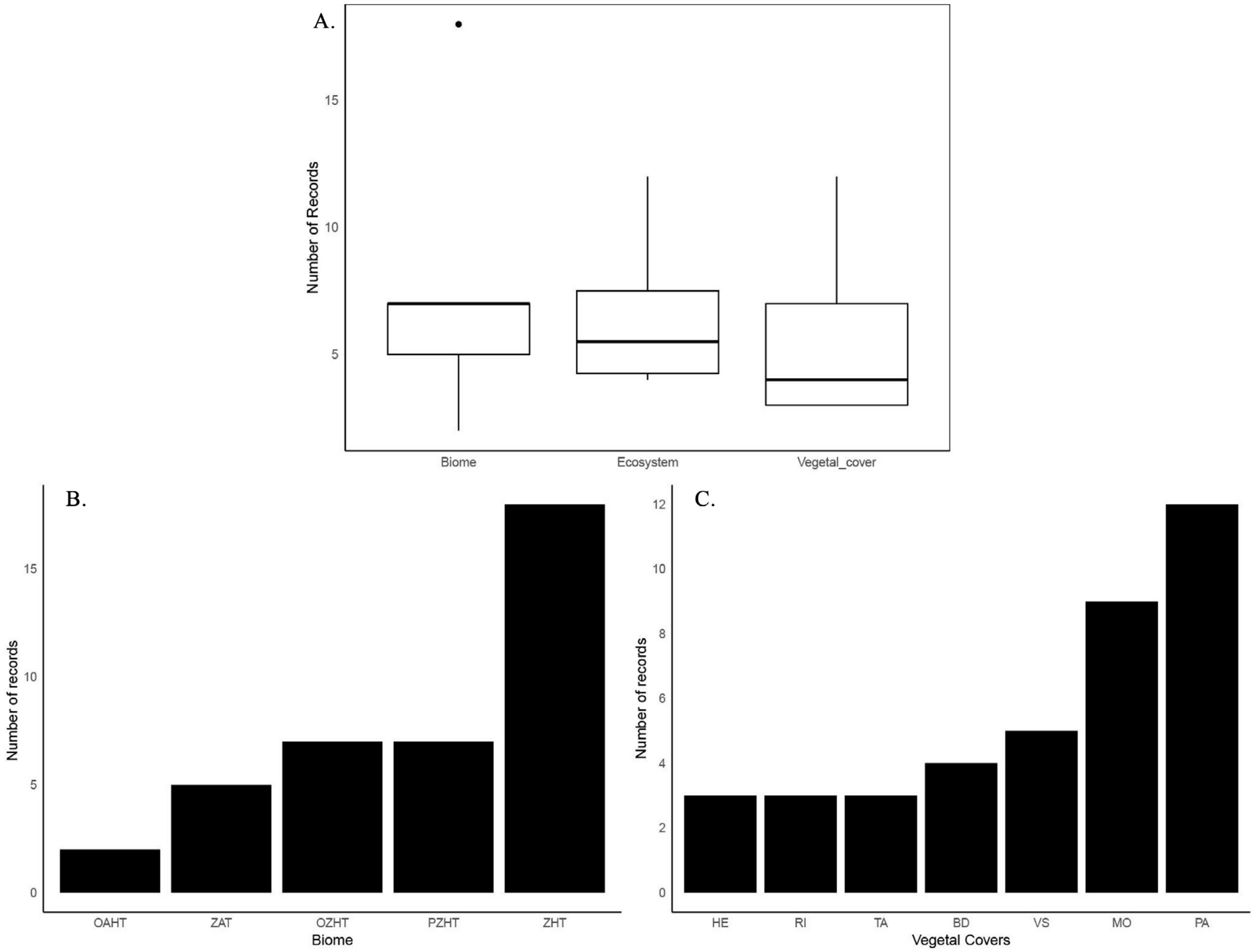

Figure 4. Visual representation– Boxplot – of the distribution of the number of records of *Vampyrum spectrum* per biome, ecosystem and vegetal cover (A). Comparison of number of records of *V. spectrum* by biome (B) and by vegetal covers (C). For acronyms see IDEAM et al. (2017).

and 21.95% corresponded to *Reithrodontomys mexicanus* (9). Additionally, we found two bat species, *Phyllostomus discolor* (n=2; Phyllostomidae) and *Tadarida brasiliensis* (n=4; Molossidae). Regarding birds, we recorded four orders: Columbiformes, Psittaciformes, Passeriformes and Piciformes, 14 feather samples could not be identified. The prey species consumed exhibit a mass range between 13 and 160 g with an average of 46.5 g. On the other hand, the fasting blood glucose level was recorded for the bat captured at Hacienda La Venta as 99 mg/dL, while the glucose assimilation was slow, since during the one hour experiment the blood glucose levels just increased. The bat exhibited an absorption peak after 60 minutes of glucose ingestion with glucose levels of 225 mg/dL (Figure 6; Camacho *et al.* 2024).

Discussion

Morphology. The morphometric data reported in this study (Table 1) does not differ noticeable from the previous published morphometric data of *V. spectrum* (Esquivel *et al.* 2020), indicating little variability among the Colombian populations. However, as far as we know our study is the first providing external morphometric measurements for a neonate. Esquivel *et al.* (2020) pointed out that differences observed in the ear length and forearm could be explained by the way the collectors historically took this measurement in the former and, in the latter, with clinal variation also observed in other bat species (Kelly *et al.* 2018); aspects that we did not address in this study. Our specimens exhibit a standard fur coloration (reddish brown, Figure 2); however, it has been reported that specimens of *V. spectrum* could show variation in external characteristics such as fur coloration, ranging from grayish to reddish brown (Navarro and Wilson 1982). This data is important for comparing morphological measurements with information obtained in the past and captures from different altitudes or ecosystems, which must be explored to evaluate whether the differences are associated with clinal variation or environmental conditions.

Historical distribution and ecological affinity. The distribution of *V. spectrum* is generally associated with well-conserved habitats, with forest cover as a key factor

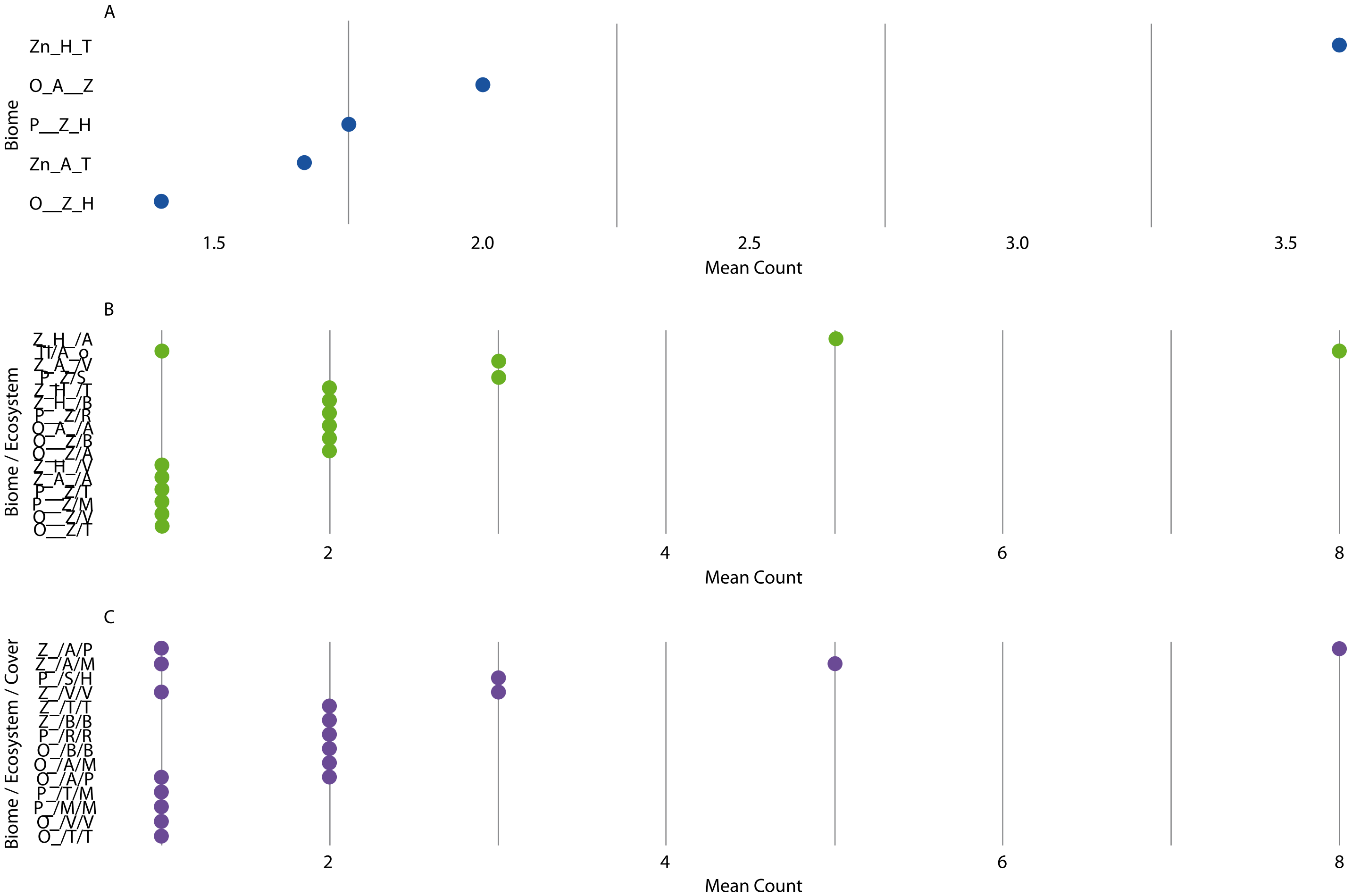

Figure 5. Mean *Vampyrum spectrum* counts across hierarchical ecological levels in Colombia. The figure displays the average number of *V. spectrum* records at three nested spatial scales: (A) Biome, (B) Ecosystem nested within Biome, and (C) Cover nested within Ecosystem and Biome. Each point represents the mean count for a given category, with labels abbreviated for clarity. The visualization highlights the structured variation in counts across ecological contexts, supporting the conclusion that habitat use by *V. spectrum* is influenced by multi-scale environmental factors rather than vegetation cover alone. For acronyms see IDEAM et al. (2017).

influencing its occurrence (Solari 2018). However, our historical and current vegetation cover analyses indicate that the species is undergoing significant environmental changes, 43% (Figure 3). The earliest recorded observation of *V. spectrum* in Colombia dates to 1942 (Rivas-Pava *et al.* 2007). Unfortunately, the oldest available data on forest and non-forest cover in the country is from 1990 (IDEAM 2019), leaving a 48-year gap in our understanding of habitat availability and preferences for the species.

Although we initially hypothesized that *V. spectrum* would exhibit a strong dependence on vegetation cover, with higher occurrence in well-conserved areas, our results did not support this assumption. Instead, the Bayesian hierarchical model revealed that its distribution is better explained by a multi-scale ecological structure encompassing Biome, Ecosystem, and Cover. Visualizations of the mean counts by Biome, Ecosystem, and Cover (Figure 5) illustrate clear differences in *V. spectrum* records across these nested spatial scales. The model's random intercepts captured meaningful variability at each level, with posterior estimates indicating non-negligible standard deviations for Biome (0.37), Ecosystem within Biome (0.30), and Cover within Ecosystem and Biome (0.29).

These findings suggest that *V. spectrum*'s habitat preferences are not solely determined by vegetation integrity, but rather by a multifactorial set of ecological drivers acting across different spatial and temporal scales. This interpretation aligns with the results of Aguiar *et al.* (2015), who demonstrated that the distribution of Tonatia saurophila is likely shaped by a complex interplay of factors including climate, topography, habitat structure, and potential evolutionary divergence. Such parallels underscore the importance of considering hierarchical environmental and a broader ecological and evolutionary contexts when modeling species distributions, particularly for wide-ranging species like *V. spectrum*. The apparent absence of a simple vegetation-cover dependency underscores the complexity of habitat selection in heterogeneous landscapes such as those found in Colombia and calls for more integrative approaches that account for spatial structure and ecological interactions.

Armenteras *et al.* (2013) estimated that between 1990 and 2005, Colombia experienced an annual deforestation rate of 0.62%, equivalent to approximately 341,071 hectares (ha) of forest lost per year. The habitat alterations observed in the forest and non-forest cover map for the period 1990–2017 (Figure 3), underscore the extensive habitat loss that has occurred over time. This pattern suggests that Spectral bat's environments are likely to continue undergoing transformation. Habitat loss and shift in land use in Colombia has been primarily driven by agricultural expansion and livestock farming, which are closely linked to deforestation (González *et al.* 2011; Negret *et al.* 2019). Notably, the Andean region, where a significant proportion of *V. spectrum*'s records are concentrated, has experienced some of the highest deforestation rates in the country (Etter *et al.* 2006).

National-scale updates on forest cover changes since 2017 are still pending. However, recent reports indicate that the deforestation rates have declined in recent years after peaking in 2017, when 425,000 ha of forest were lost. Notably, in 2023 primary forest loss decreased 49% compared to 2022, when 266,000 ha were deforested, according to Global Forest Watch (World Resources Institute 2024). The status of the Tropical Dry Forest (TDF) remains critical, ranking among the most threatened ecosystems globally. In Colombia, over 92% of its original vegetation cover has been lost *(*Pizano and García 2014). This decline is primarily driven by human activities, such as livestock grazing, agricultural expansion, mining, and timber harvesting (Álvarez-Álvarez *et al.* 2018; Scolozzi *et al.* 2012; Ruiz *et al.* 2013). In Valle del Cauca, most of the remaining TDF fragments are scarce and are currently under protection due to their valuable vegetation cover and unique plant formations (Alvarado-Solano and Otero-Ospina 2015; Salazar *et al.* 2002). However, knowledge about smaller TDF fragments and their associated species remains limited.

This study documents the second record of the species in the Valle del Cauca, and the first within the department's TDF. Statistical analysis revealed that the distribution of *V. spectrum* is better explained by a multi-scale ecological structure encompassing Biome, Ecosystem, and Cover (Figure 5), suggesting that the species' habitat preferences are not solely driven by vegetation integrity, but rather by a combination of ecological factors operating at different spatial scales. In Colombia, information is scarce about the species' association with specific biomes, or vegetation cover types occupied by *V. spectrum*. Esquivel *et al.* (2020) identified that *V. spectrum* mainly inhabits primary and secondary forests (Cabrera 2011; Esquivel and Rodríguez-Bolaños 2018), tropical dry forests (Maria 2004), and sub-Andean forests, particularly in areas with high precipitation. The presence of *V. spectrum* primarily across diverse habitats with varying degrees of human disturbance suggests a potential tolerance to habitat transformation. This finding aligns with our results, which show an association between the species and the tropical humid zonobiome and higher occurrences in grasslands and agricultural mosaics (Figure 3). One possibility is the species' adaptability to habitat changes. Given its extensive home range (Vehrencamp *et al.* 1977; Solari 2018), *V. spectrum* likely moves between roosting sites to water and food resources, navigating through fragmented, degraded areas. Additionally, grasslands may provide abundant and easily accessible prey in simplified environments with fewer obstacles and reduced prey refuges.

Roost availability is a critical factor for the persistence of *V. spectrum*, as the species relies on large, mature trees with natural cavities and caves (Altringham 2011). Our results suggest that the species has been recorded in highly transformed landscapes, raising questions about the quality of available roosting sites. An analysis of the forest structure in 10 tropical dry forest fragments in central-northern Valle del Cauca, including La Venta (the site where our specimen of *V. spectrum* was captured) reveals that most trees belong to the smallest diameter classes, with fewer individuals in intermediate and larger size categories (Saldaña and Moncaleano 2021). However, La Venta exhibits a more balanced diameter class distribution, indicating a higher presence of large trees compared to other remnants assessed (Saldaña and Moncaleano 2021).

These findings suggest that although *V. spectrum* can occupy fragmented landscapes, the scarcity of large-diameter trees in many tropical dry forest remnants may limit its long-term persistence. Previous studies have found that large-bodied bat species strongly depend on trees with natural cavities or artificial structures when natural roost availability is low (Dinets 2016). Given the advanced transformation of Colombia's tropical dry forest, the conservation of large trees and the restoration of forest fragments with potential for cavity development could be key strategies for protecting the species.

Diet and sugar assimilation. There are some reports on the diet of Spectral bat across its distribution, most of them conducted in Central America. However, it is difficult to determine the identity of the consumed species from stomach contents due to gastric destruction of the samples (Casebeer 1963; Petterson and Kirmse 1969). Therefore, finding a roost like the one reported here is important to document the identity of the Spectral bat's preys. This is the first diet report for the species in Colombia and in a sub-xerophytic forest ecosystem (Esquivel *et al.* 2020). The area where the diet information comes from is characterized by shrub and sub-arboreal deciduous or semi-deciduous strata typical of the families Fabaceae, Euphorbiaceae, Cactaceae, Bignoniaceae, and Rutaceae of dry forest. The sub-xerophytic forest of the Patía Valley has a high degree of anthropogenic intervention and differs in structure from other dry forests in Colombia, having some of the smallest patches in the country (Cabrera-Ojeda *et al.* 2016), which poses significant threats to the Spectral bat in this ecosystem.

Through the analysis of remains in refuges, up to 18 bird species have been recorded (Vehrencamp *et al.* 1977), while Martínez-Fonseca *et al.* (2022) presented a list of 27 species detected through barcoding from feces, of which *Leptotila verreauxi* and *Phyllostomus discolor* are shared with this study. Some new contributions to the diet of

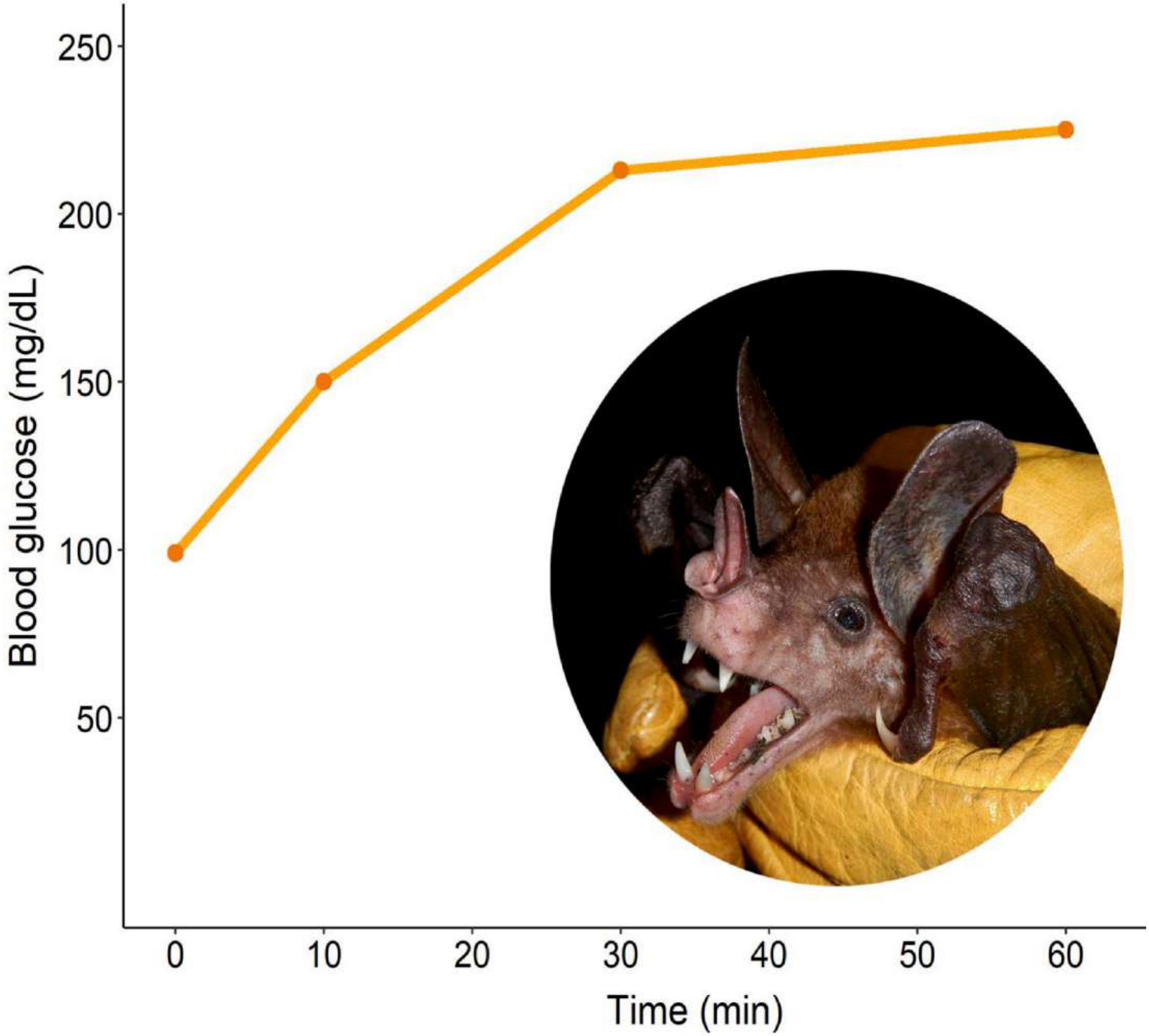

Figure 6. Sugar assimilation curve for the individual of *Vampyrum spectrum* captured in Hacienda La Venta, Bugalagrande, Valle del Cauca, Colombia. Adapted from Camacho et al. (2024).

the *V. spectrum* are the birds *Zenaida auriculata*, *Pheuticus aureoventris*, *Pyrocephalus rubinus*, *Colaptes puntigula*, *Forpus conspicillatus*, and *Thamnophilus multristriatus*. These species range in weight from 14 to 125 g, like those reported in other studies (Vehrencamp *et al.* 1977; Martínez-Fonseca *et al.* 2022). The reported diet does not seem to show a specific preference for any prey; however, the presence of non-passerine birds stands out. It has been suggested that the olfactory sense of the Spectral bat may play an important role in hunting these types of birds, which can have a strong odor and occupy gregarious perches (Vehrencamp *et al.* 1977). The new mammal records in this diet are the cricetid rodents *Zygodontomys brunneus*, *Reithrodontomys mexicanus*, and the molossid bat *Tadarida brasiliensis*. *R. mexicanus* and *T. brasiliensis* are the first confirmed records for TDF in the Nariño department, which highlight the importance of predator refugees' analysis for the documentation of poorly known species. It has been reported that the foraging of Spectral bats is limited to the mid and upper strata of the forest, catching birds and mammals that rest at a certain height, limiting their ability to forage at lower heights (Vehrencamp *et al.* 1977). However, it seems that some foraging strategies include low flights (Pacheco-Figueroa *et al.* 2022). The rodents recorded in *V. spectrum*'s diet in this study are typically on the ground, especially *Z. brunneus*, an open grassland and shrub mouse (Voss 1991), suggesting that the Spectral bat actively hunts over lower forest strata. Likely, olfaction is also an important factor in the search for mammals, as the recorded species, like bats, are also characterized by strong body odors (Nielsen *et al.* 2006; Kwiecinski 2006).

In terms of the Spectral bat physiology, we observed what was expected for an organism with a low-sugar diet, a slow glucose assimilation (Figure 6, Camacho *et al.* 2024*)*. During the one-hour period of the experiment the process of glucose incorporation into the tissues was never higher than the previous intestinal glucose absorption process. This demonstrates a low sugar assimilation capability compared to bats with rich-sugar diets, in which blood glucose levels decrease after 10 and 30 minutes of glucose ingestion and reach much higher blood glucose levels (Kelm *et al.* 2011: Peng *et al.* 2017; Camacho *et al.* 2024). The glucose tolerance was like the one observed in insectivorous bats (Peng *et al.* 2019), which is very likely due to its carnivory preferences. The species' nutrition is mostly based on proteins and very few glucoses, mainly coming from the prey's blood (birds and small mammals), so glucose absorption does not compare to the speed at which nectarivore or frugivore bat species absorb glucose (Camacho *et al.* 2024).

Conservation strategies and landscape management for V. spectrum. The conservation of the Spectral bat requires an integrated approach focused on enhancing habitat connectivity, promoting sustainable land-use practices, and involving local communities. Key strategies include creating ecological corridors (Hilty *et al.* 2021), restoring roosting trees like *Ficus* spp., *Anacardium excelsum*, and *Brosimum alicastrum* (Ramos-Perez and Silverstone-Sopkin 2018), adopting agroforestry systems (Arroyo-Rodríguez *et al.* 2019), and incentivizing landowners through ecosystem service payments (Ardila-Vargas and Estrada 2021). Long-term monitoring and collaboration with communities and conservation groups are essential for effective, lasting protection.

This study highlights urgent deforestation and habitat loss in Colombia, emphasizing the need for focused conservation efforts on protecting and restoring critical habitats. The likely *V. spectrum*'s resilience to habitat changes suggests conservation should also include the already transformed landscapes surrounding protected forests. Human activities, particularly agriculture and livestock farming, significantly contribute to habitat degradation. Therefore, conservation strategies must promote sustainable land use and mitigate anthropogenic impacts. Our study offers valuable ecological insights that are essential for future conservation planning. It highlights the importance of maintaining ongoing monitoring of habitat changes and species distributions to effectively adapt strategies. Additionally, it is critical to raise awareness of *V. spectrum*'s ecological significance to support these initiatives. Ultimately, although more research is needed to fully understand the species' habitat needs, it is crucial to advocate for policies that protect fragmented ecosystems, enhance connectivity, promote sustainable practices, and conserve preys' diversity for the long-term survival of the species. Further studies should focus on understanding how the species deals with landscape transformation in terms of physiological stress and ecological adaptability. Integrative studies are needed to assess what the optimal and suboptimal habitat characteristics for the species are, to evaluate if the species distribution range or home range is expanding or contracting, and how large the populations remaining in extremely disturbed and fragmented ecosystems like the Tropical Dry Forest.

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Phylogenetic relationships between Monotremata and Monotremaformes: parallelism with appendages and habits of aquatic moles

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For a long time, Monotremata included Mesozoic taxa such as *Steropodon* and *Teinolophos*, as well as Cenozoic species. However, fossil discoveries from the Cretaceous have revealed significant differences between Cenozoic monotremes and their ancestors, with Cenozoic monotremes being more closely related to each other than to earlier forms, thereby forming the crown group of Monotremata. Among extant mammals, moles exhibit several morphofunctional parallels with monotremes. These similarities may reflect comparable evolutionary trajectories. Here, we investigate evolutionary parallels between monotremes and Talpidae, aiming to determine whether their resemblances could indicate a similar evolutionary pathway and what implications this would have for our understanding of Monotremata's evolutionary history. Our objectives were to identify additional morphological characters to differentiate Monotremata from their ancestors and to outline possible evolutionary parallels between monotremes and Talpidae. To this end, specimens of monotremes and talpids housed in the collection of the Museu Nacional-UFRJ were analyzed, along with a literature review on the evolution, morphology, and fossil record of these groups. Among the non-dental morphological characteristics identified, we noted the presence of a lateral projection on the anterior portion of the mandible extending beyond the mandibular symphysis, forming the bony support of the "beak" in present-day monotremes, and the presence of a reinforced pectoral girdle with several hypertrophied bones and joints not observed in other tetrapods. The swimming method of platypuses, which involves a series of alternating strokes of the forelimbs, is highly similar to the digging or swimming motions observed in moles. Thus, it is possible that, like semi-aquatic moles, monotremes may have descended from an ancestor with a specialized fossorial lifestyle.

Key Words: Cretaceous, Eimer's organs, monotremes, Talpidae, *Teinolophos*.

Durante mucho tiempo, los Monotremata incluyeron taxones mesozoicos como *Steropodon* y *Teinolophos*, además de especies del cenozoico. Sin embargo, los descubrimientos fósiles del cretácico revelaron una diferencia importante entre los monotremas del cenozoico y sus ancestros, ya que los monotremas del cenozoico estaban más estrechamente relacionados entre sí que con formas anteriores, formando así el grupo corona de Monotremata. Entre los mamíferos vivos, los topos tienen una serie de paralelos morfofuncionales con los monotremas. Estos paralelos pueden reflejar trayectorias evolutivas similares. Nuestros objetivos fueron: encontrar caracteres morfológicos adicionales para diferenciar a los Monotremata de sus ancestros y rastrear posibles paralelos evolutivos entre ellos y Talpidae. Para ello, se evaluaron especímenes de monotremas y tálpidos almacenados en la colección del Museu Nacional-UFRJ, así como la investigación bibliográfica sobre la evolución, morfología y registro fósil de estos grupos. Entre las características morfológicas no dentales identificadas aquí están la presencia de una proyección lateral en la porción anterior de la mandíbula que se extiende más allá de la sínfisis mandibular, formando el soporte óseo del "pico" en los monotremas actuales y la presencia de una cintura escapular reforzada, con varios huesos y articulaciones hipertrofiadas no vistas en otros tetrápodos. El método de natación de los ornitorrincos, que implica una serie de golpes alternados de las extremidades delanteras, es muy similar al de los topos cuando excavan o nadan. Así, es posible que, al igual que los topos semiacuáticos, los monotremas desciendan de un ancestro con un estilo de vida fosorial especializado.

Palabras clave: Cretácico, monotremos, Talpidae, *Teinolophos*, órganos de Eimer.

Monotremes, the egg-laying mammals, are currently the only remaining members of the Yinotheria mammal lineage (Luo and Yuan 2007). Among living mammals, they are by far one of the most distinctive groups due to both their peculiar appearance and retention of several characteristics present in many Mesozoic mammals or even in previous synapsids such as egg laying, the presence of potentially venomous spurs on the hind legs, and a spread-out posture of the limbs (Hurum *et al.* 2006; Gambaryan and Kuznetsov 2013).

Due to the unique distinction of monotremes, for a long time represented by a scarce fossil record that was composed only of Cenozoic taxa closely related to living species, a strict definition of them in relation to the other groups of mammals was not possible. However, older taxa of the Monotremata lineage were eventually discovered (Archer *et al.* 1985; Flannery *et al.* 1995; Rich *et al.* 1999). These new fossil species were readily classified as members of the order Monotremata but given the great morphological differences between some of these taxa and their modern relatives, such as the possession of teeth in the anterior region of the mandible, including incisors and canines, the presence of a Meckel's groove in the posterior part of the mandible, and the probable absence of a "beak" (Archer *et al.* 1985; Musser 2003; Rich *et al.* 2016), it was necessary to revise the definitions of Monotremata.

Initially, the apomorphies used to differentiate monotremes from other Yinotheria were the presence of a partially enlarged mandibular canal, the marked separation in size between the last premolar and the first molar, the absence of a paraconid cusp on the lower first molar, and the presence of large and wide talonids (Pian *et al.* 2016). Following this definition, the order Monotremata included genera such as *Kollikodon,* a large Cretaceous mammal with bunodont teeth (Flannery *et al.* 1995), *Steropodon*, a peculiar animal with teeth similar to tribosphenic teeth and which was initially and erroneously interpreted as basal platypus (Archer *et al.* 1985), and *Teinolophos,* the oldest known member of this lineage, around 123 million years old, and all species of Cenozoic monotremes (Rich *et al.* 1999).

The new fossil discoveries have revealed significant differences between Cenozoic monotremes and their Cretaceous ancestors (Rich *et al.* 2016). Cenozoic monotremes are much more closely related to each other than they were to any of the Cretaceous ones, forming the crown group Monotremata Chimento *et al.* (2023). The broader group containing the Monotremata and older forms such as *Teinolophos*, *Kollikodon*, and *Steropodon* was named Monotremaformes. The analysis that based this arrangement was exclusively based on dental morphology, disregarding any non-dental character.

Chimento *et al.* (2023)'s proposal was made considering the possession of low dilambdodont molar teeth, expanded mesodistally, with the anterior lobe equivalent to the trigonid positioned lower than the posterior lobe, which would correspond to the talonid. The talonid is composed of two transverse lophs instead of one and lacks a labial cingulid. Thus, Chimento *et al.* (2023) did not include a broader analysis that encompassed non-dental characters of this group. While tooth morphology is an essential trait for the taxonomy of many mammalian groups, other types of characters—such as the mandible, middle ear, and scapular girdle—are also crucial, particularly in groups with highly specialized morphologies like monotremes.

Among living placental mammals, some representatives of the family Talpidae (Laurasiatheria, Eulipotyphla) (true moles, shrew moles, and desmans) appear to exhibit a series of morphological and functional similarities with living monotremes, such as a modified rostrum containing specialized mechanosensory organs, which are essential for spatial orientation (Catania 2000), and a type of swimming driven by alternating movements of the forelimbs (Hickman 1984; Fish *et al.* 1997). Inasmuch as these similarities reflect convergent evolutionary trajectories, understanding the evolutionary history of moles could help understand the still obscure evolutionary history of monotremes. Therefore, we herein carry out a bibliographical review on the evolution and morphology of monotremes and their relatives in search of non-dental characters to be analyzed in the light of the phylogenetic proposal of Chimento *et al.* (2023).

In addition, we will also draw possible evolutionary parallels between monotremes and talpids, to determine whether their similarities could in fact represent a similar evolutionary trajectory, and what implications this would have for our understanding of the evolutionary history of the order Monotremata.

Materials and methods

Regarding monotremes and talpids, we based our character survey on those documented in the literature (detailed below) and in two skins and a skeleton of *Ornithorhynchus anatinus*, as well as two specimens of *Tachyglossus aculeatus*, and a skin and skull of *Desmana moschata*; four Myrmecophagidae and Manidae, variation in the shape of the mandibular symphysis was assessed from five articulated dentaries of *Tamandua tetradactyla* and two dentaries of *Manis sp*. All specimens are housed in the collection of the Museu Nacional (MN), Universidade Federal do Rio de Janeiro. The specimens were first analyzed macroscopically and then photographed using an Olympus OM-D E-M5 Mark II camera. Specimens deposited in the collection of the National Museum-UFRJ receive a numbering starting with the letters MN.

Regarding the monotremes and talpids, we observe morphological traits documented in the literature. For Myrmecophagidae and Manidae, variation in the shape of the mandibular symphysis was assessed from specimens in Museum to comparative purposes with that of echidnas.

Specimen provenances: the *Desmana moschata* specimen (MN 93184) originated from present-day Kazakhstan, formerly part of the USSR, with a collection label dated November 1931. The articulated mandibles of *Tamandua tetradactyla* evaluated in this study belong to the following specimens: MN 5883 from Santa Teresa, Espírito Santo; MN 9677 from Ilhéus, Bahia; MN 63499 from São Raimundo Nonato, Piauí; MN 68357 from Telêmaco Borba, Paraná; and MN 79449 from Duque de Caxias, Rio de Janeiro. The Monotremata specimens (MN 1 to MN 5) and the Pholidota specimens (MN 46 and MN 47) were purchased in Europe in the early XX century, and their exact geographical origins are unknown.

A comprehensive search of Google Scholar was conducted for data published between 1925 and 2025, using various combinations of keywords such as: Montramata + fossil; Monotremata + Shoulder Girdle; Monotremes + Evolution; Monotremata + middle ear; Monotremata + Eletroreception; Aquatic mole + Locomotion; Platypus + Locomotion; Echidna + Evolution. Priority was given to articles focusing on evolution, morphology (including jaw structure, shoulder girdle, and sensory organs), the fossil record of monotremes, other monotreme-like species, and semi-aquatic moles. Additionally, the bibliographic references of selected articles and books were thoroughly reviewed, with no restrictions on publication dates.

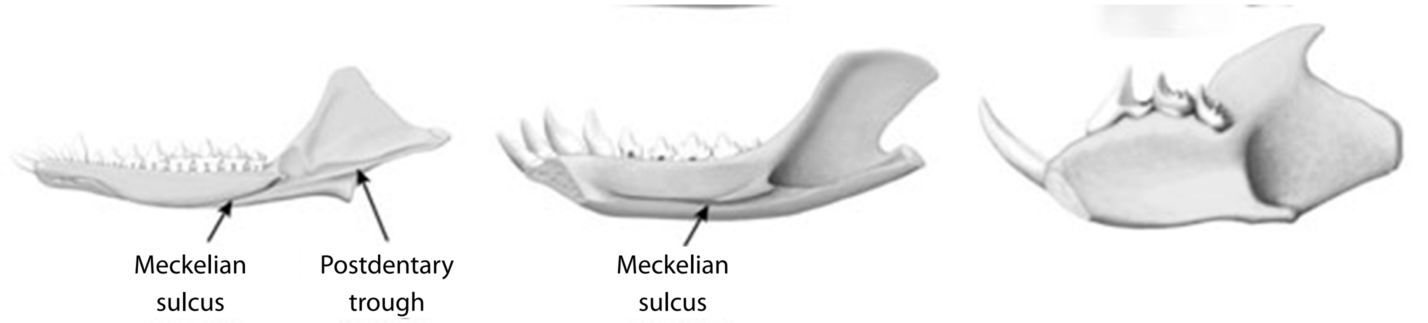

Figure 1. The three patterns of middle ear existing in mammals: (Right) *Morganucodon*, a postdentary attached ear: the jaw presents a Meckelian groove and a post-dental trough where the Meckelian cartilage and the post-dental bones would be inserted, respectively; (Center) *Lyaconodon*, a Meckelian-attached ear: a prominent Meckelian sulcus highlighting the possession of a Meckelian cartilage that may or may not be ossified and a lack of a post-dental trough. (Left) *Vilevolodon*, a detached ear, characterized by the complete absence of Meckelian groove or postdentary trough in the jaw. This stage represents the complete separation between the jaw and the bones of the mammalian ear (Modified from Wang *et al.* 2021)

Results

As a result of the bibliographic research, a total of 49 references on evolution, Monotreme morphology, and semi-aquatic moles were gathered and reviewed. These sources are divided into 40 articles and two notes published in journals, as well as seven book chapters. All these works found are cited and referenced below.

Middle Ear Patterns. Mammals can present up to three different types of middle ear patterns, which are defined by the level of connection between the dentary and the middle ear bones (Ramírez-Chaves *et al.* 2016; Wang *et al.* 2021). The mandibular middle ear of cynodonts (MMEC), in which the postdentary is attached in the mandible (Luo 2011) is the most primitive pattern, preceeding true mammals and being found in derived cynodonts. Additionally, in some primitive mammals such as *Henosferus molus* from the Lower Jurassic of Argentina (Rougier *et al.* 2007), the ear bones are fully fixed in the dentary, rendering them as a joint between the skull and the jaw. The bones are evidenced in incomplete fossils by the presence of a post-dental trough in the back of the jaw of these animals.

The second pattern is the partial mammalian middle ear (PMME), also known as "ear with Meckelian attachment" (Meng *et al.* 2011). In this pattern, the middle ear bones are still connected to the mandible by a Meckel's cartilage, which can be completely cartilaginous or ossified. The PMME can be inferred by a pronounced Meckel's groove in the dentary of incomplete fossils.

Finally, the definitive mammal's middle ear (DMME) or "detached ear", has no direct connection between the ear bones and dentary since they completely migrated to the middle ear. The presence of this pattern is attested by the absence of both the postdentary trough and of Meckel's groove in the dentary (Figure 1); this is the condition found in all living mammals (Allin and Hopson 1992).

Both *Teinolophos* and *Steropodon* were originally interpreted as having post-dentary-attached (Luo *et al.* 2002; Rich *et al.* 2005). However, with the discovery of more complete materials of *Teinolophos*, it lacks a post-dental trough in its jaw and presents a very well-developed Meckel's groove in its dentary, indicating the presence of an ossified Meckel's cartilage, and a Meckelian-attached ear (Rich *et al.* 2016). Regarding *Steropodon*, a more in-depth analysis of the holotype revealed that its supposed postdentary trough was a pre-depositional break that the fossil had suffered. On the other hand, the mandible of *Steropodon* also presented a Meckelian groove, indicating an ear with a Meckelian-attached. The validity of this interpretation has been questioned by Ramírez-Chaves *et al.* (2016), but two additional jaw fragments of *Steropodon* described by Flannery *et al.* (2024) preserved a more posterior region of the jaw than that of the holotype. These additional specimens have a slight depression on the lingual surface of the dentary, which may be a remnant of a rudimentary Meckelian groove.

A possible additional taxon of Steropodontidae, the *Parvopalus clytiei,* probably had an ear detached, as it does not have a postdentary trough or even a Meckelian groove on its dentary (Flannery *et al.* 2024), so if its classification as a Steropodontidae alongside *Steropodon* is correct, it means that a detached ear may have arisen independently in different lineages of Monotremaformes.

The type specimen of the fossil taxon *Opalios splendens* has a possible Meckel's groove in its jaw; however, this is so reduced that it is likely that it was absent (Flannery *et al.* 2024).

All known taxa, living or extinct, of Ornithorhynchidae and Tachyglossidae have a detached ear, meaning their jaws do not show any trace of a postdentary trough or a Meckel's groove (Pascual 1992; Musser and Archer 1998; Rich *et al.* 2005; Chimento *et al.* 2023; Flannery *et al.* 2024). *Teinolophos* and *Opalios* are the closest taxa to the crown group of Monotremata and possibly have an ear Meckelian-attached, (Chimento *et al.* 2023; Flannery *et al.* 2024), while members of the crown group themselves have a detached ear. This suggests that this characteristic arose independently in this lineage in relation to the others (Figure 2).

The acquisition of a detached ear can be interpreted as a synapomorphy of the crown group of Monotremata among the Monotremaformes. Alternatively, if *O. splendens* does not possess a Meckelian groove, the presence of a detached ear could qualify as a synapomorphy of the lineage that includes both the crown group of Monotremata and its sister taxon *O. splendens*.

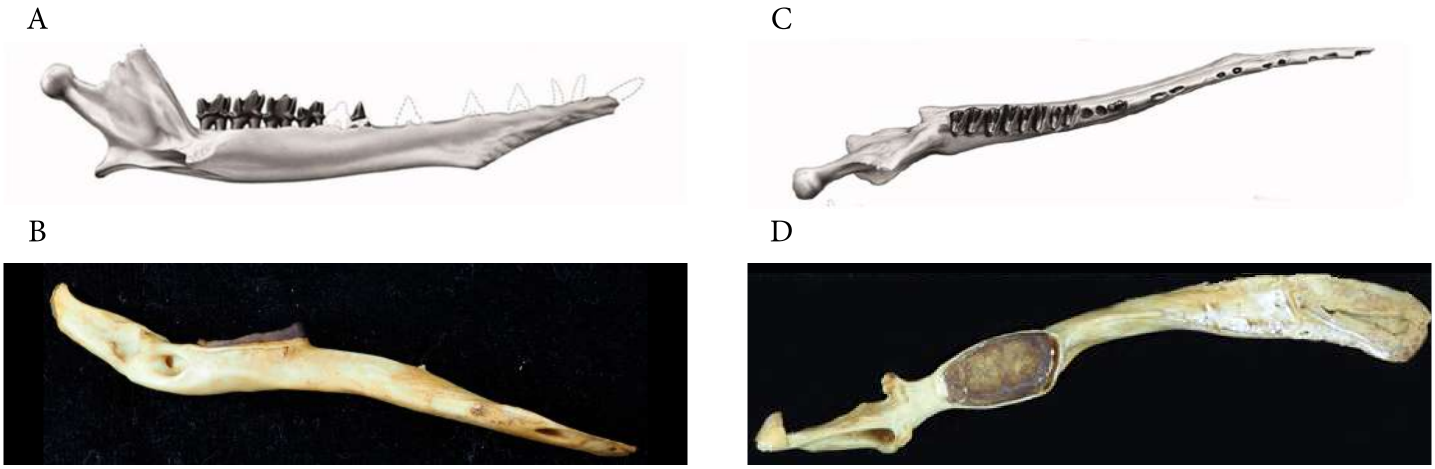

Figure 2. Lateral view of the jaws of a *Teinolophos trusleri* (A) and a platypus (*Ornithorhynchus anatinus*) (B). *T. trusleri* has a prominent Meckel's groove in the jaw, indicative of an ossified Meckel's cartilage. In contrast, the platypus does not have any trace of this structure (the middle ear is separate from jaw). (C) a superior view of the jaws of *T. trusleri* (Modified from Rich *et al*. 2016) and a platypus (D). The distal end of the jaws of *T. trusleri* projects inward to form the mandibular symphysis. In contrast, in the platypus, the distal end of the jaw curves outward, extending beyond the mandibular symphysis. Bar = 5 mm

Differentiation of the number of molars between Ornithorhynchidae and their predecessors. Cenozoic toothed monotremes such as *Monotrematum sudamericanum*, the species of the genus *Obdurodon,* and juveniles of the modern *Ornithorhynchus anatinus* have three pairs of molar teeth in their jaws, with a much-reduced M3, with only one tooth root holding it in the jaw. This same dental arrangement can be found in the fossil *Dharragarra aurora*, which is one of the characters contributing to its identification as an Ornithorhynchidae (Flannery *et al.* 2024), and as the oldest known member of the crown group of Monotremata.

Meanwhile, other Monotremaformes, such as *Teinolophos trusleri* and *Opalios splendens*, possess five pairs of molar teeth in their jaws with two tooth roots in all molars (Rich *et al.* 2016; Flannery *et al.* 2024). As these are the closest taxa to the crown group, one can assume that the reduction in the number of molars in the mandible, together with the significant decrease in the size of M3, may be synapomorphies of Monotremata.

Other Monotremaformes such as *Steropodon* and *Parvopalus* also have three molars in their jaws (Archer *et al.* 1985; Flannery *et al.* 2024); however, *Steropodon* has at least a very different dental morphology from that found in *T. trusleri* or in species of the crown group of Monotremata (Flannery *et al.* 2022; Chimento *et al.* 2023). Regarding *P. clytiei*, no other materials are known besides the holotype. As it only consists of a toothless jaw, its dental morphology is unknown, and its number of molar teeth is only known due to the dental alveoli present in the holotype (Flannery *et al.* 2024).

Kollikodontidae taxa, such as *Kollikodon ritchiei* has four molar teeth per quarter of the jaw, its M4 being significantly reduced, with only one tooth root (Pian *et al.* 2016; Flannery *et al.* 2022). This condition is not very different from the M3 of the Ornithorhynchidae; however, its tooth morphology is markedly different from that seen in other Monotremaformes or even in other mammals (Flannery *et al.* 1995; Pian *et al.* 2016). Therefore, the reduction in the number of molars probably occurred in separate ways in different lineages of Monotremaformes.

Rostrum Commonly Referred to as "Beak" or "Bill". One of the most striking characteristics of living monotremes is the possession of a rostrum that is generally referred to as a leathery "beak" full of electroreceptors and mechanoreceptors, which these animals use to locate their prey (Pettigrew 1999). To transmit environmental information from the "beak" to the brain, these animals increased the size of their mandibular nerves, resulting in the formation of an enlarged mandibular canal in their jaws (Musser and Archer 1998; Asahara *et al.* 2016; Rich *et al.* 2016). Given this functional correlation between the presence of an enlarged mandibular canal and the possession of a "beak", the presence of the former has been used as an indication of the presence of the latter (Figure 3).

Thus, all Mesozoic members of the monotreme lineage that had an enlarged mandibular canal, such as *Steropodon*, *Teinolophos*, and *Kollikodon*, were automatically interpreted as having a "beak", similarly to their living relatives (Musser and Archer 1998; Rowe *et al.* 2008). This line of thought was predominant for a long time, as the fossils of these Mesozoic taxa were very fragmentary, with only incomplete jaw remains that consisted only of the posterior part of the jaw (Flannery *et al.* 2022). This such an association was questioned after the discovery of a complete jaw of *Teinolophos trusleri* by Rich *et al.* (2016), who reported that despite the presence of an enlarged mandibular canal in the dentary *T. trusleri* did not present the characteristic "beak" of living monotremes. Its mandibular symphysis extends to the tip of their mandible, as in most other mammals, as opposed to modern platypuses whose anterior portion of the mandible extends beyond the symphysis and projects laterally outward, forming the bony support for its "beak". This indicates that *T. trusleri* did not have a "beak" (Figure 2). In both *Teinolophos* and *Obdurodon*), therefore, the presence

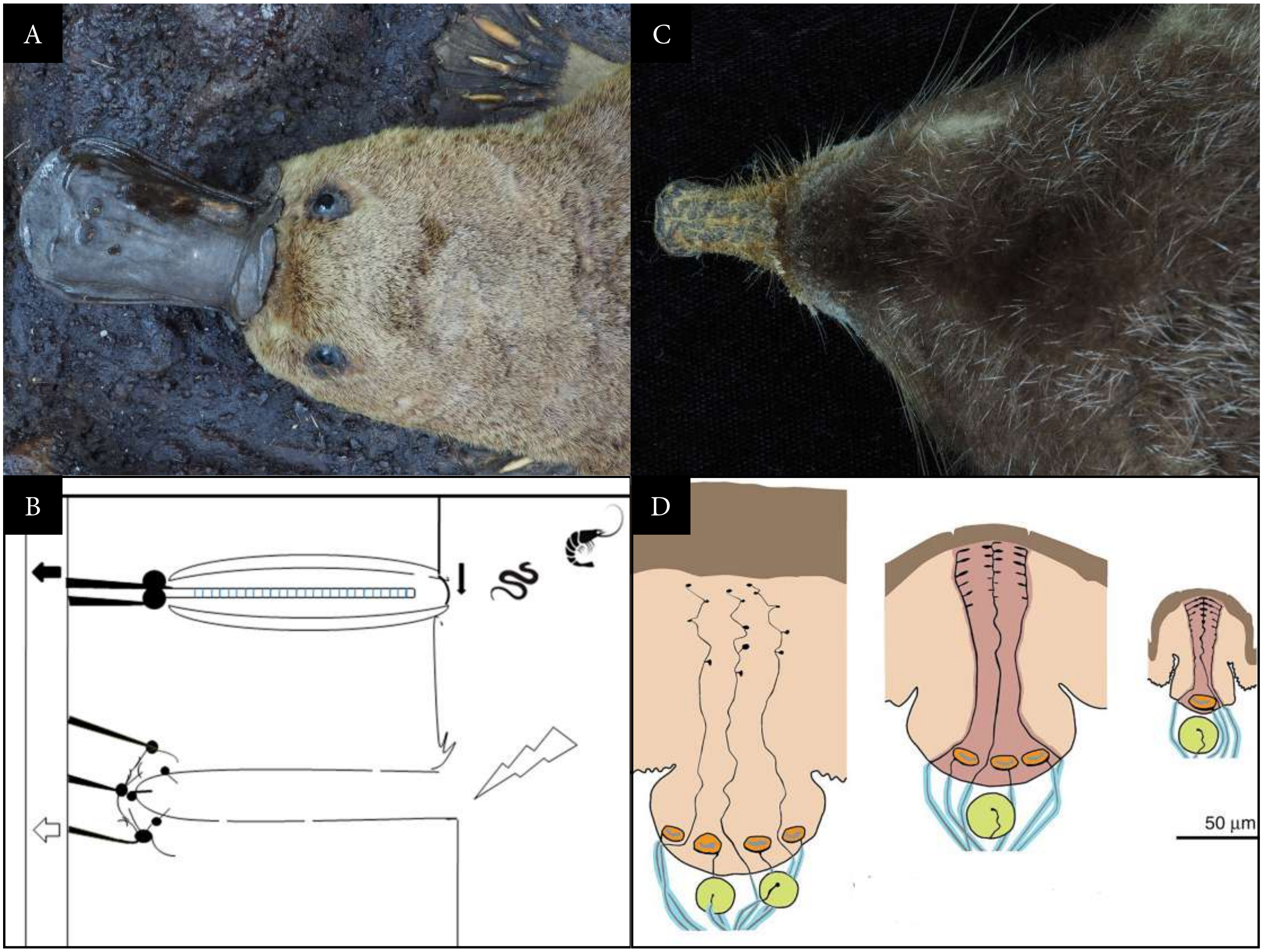

Figure 3. Upper: Top view of the rostrums of a platypus (*Ornithorhynchus anatinus*) (A-MN 2) and a Russian desman (*Desmana moschata*) (C-MN 93184). Both animals have a laterally wide rostrum covered by naked and highly sensitive skin. Lower: representations of the specialized sensory structures in the rostrums of both animals (B), (Modified by Fish *et al*. 1997) and D (Modified by Catania *et al*. 2020), with emphasis on the mechanoreceptor structures that are very similar to each other.

of an enlarged mandibular canal should not be interpreted as unequivocal evidence of the possession of this structure.

In the same way, *Opalios splendens* does not have projections on the mandible extending beyond the mandibular symphysis. However, *O. splendens* has some mandibular characteristics seen in platypuses, such as a torsion of the horizontal ramus of the dentary, the presence of a deep hemispherical pit for enlarged masseter and the possession of a very evident horizontal flattening in the anterior portion of the mandible (Flannery *et al.* 2024). This may indicate that *O. splendens* perhaps had some intermediate structure between a basic mammalian snout and a "beak" derived from monotremes. This is one of the reasons why it is classified as the closest taxon to the Ornithorhynchidae (Flannery *et al.* 2024), and by extension, the crown group of Monotremata.

Among the known Monotremaformes, the closest taxa to the crown group of Monotremata (*Teinolophos* and *Opalios*), do not have a true "beak", which strongly suggests that this feature was not present in other Monotremaformes from the "middle Cretaceous". Therefore, this structure must only be present in members of the crown group of Monotremata and could consequently be a synapomorphy of this clade.

Both *Patagorhynchus* and *Monotrematum* do not have known complete jaw remains and, therefore, it is not known whether they had a lateral projection that extends beyond the mandibular symphysis. However, both taxa have teeth which are morphologically almost identical to the teeth of *Obdurodon*, are found in sediments of ancient bodies of freshwater (Chimento *et al.* 2023). In addition, *Monotrematum* presents postcranial remains that show adaptations for swimming (Forasiepi and Martinelli 2013). These traits suggest that they present a typical general morphology of a platypus and, by extension, a lateral projection of the mandible that extends beyond the mandibular symphysis.

It should also be noted that echidnas (Tachyglossidae) do not have a lateral projection of the mandible that extends beyond the mandibular symphysis like platypuses, but they still have a "beak" (Pettigrew 1999; Augee *et al.* 2006). However, despite the absence of concrete fossil

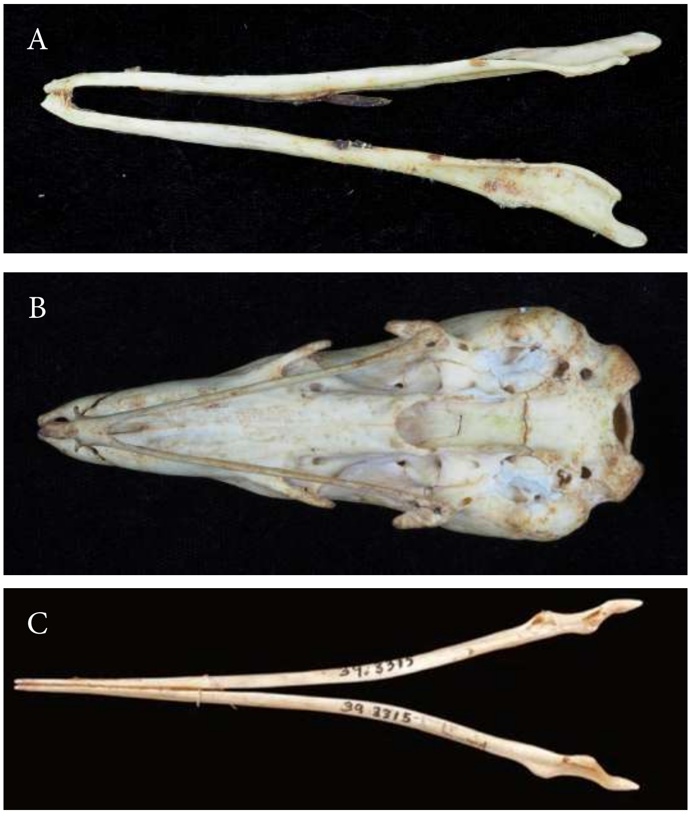

Figure 4. (A) Ventral view of the jaws of a southern tamandua (*Tamandua tetradactyla* MN 68359), (B) an Asian pangolin. (*Manis sp.* MN 47), and a long-beaked echidna C. (*Zaglossus brujinii*) modified from Helgen *et al.* 2012. The distal ends of the echidna jaws do not have an inward curvature to form the mandibular symphysis as in other myrmecophagous; instead, the jaws extend parallel to their distal end forming a long mandibular symphysis also seen in platypuses.

evidence (Camens 2010), echidnas evolved from ancestors very similar to platypuses (Phillips *et al.* 2009; Flannery *et al.* 2022) and, therefore, both share morphological characteristics.

Some characteristics present in echidnas may suggest that their ancestors had jaws like those of platypuses. The possession of a lateral twist of the jaws greater than that of other myrmecophagous mammals, such as in anteaters and pangolins, is not very different from the condition in platypuses. The two sides of the echidna's jaws do not contort inward to form the mandibular symphysis but instead run parallel to the tip of the mandible, in a very similar way to the mandibular symphysis of platypuses, but without the post-symphysis projections (Figure 4). Thus, the absence of these projections in modern echidnas is due to their derived facial morphology and does not affect the possession of a "beak" as a synapomorphy of Monotremata.

Shoulder girdle. Mammals are divided into two major groups: Yinotheria and Theriimorpha. These groups can be distinguished based on the structure of the pectoral girdle. Yinotheria possess a rigid and reinforced pectoral girdle that retains all the bones found in earlier therapsids, whereas the Theriimorpha have a mobile pectoral girdle with a lighter and more simplified suspension, exhibiting a significant reduction in the number of bones (Augee *et al.* 2006; Luo and Yuan 2007). Within Yinotheria, monotremes have the most reinforced pectoral girdle, featuring a hypertrophied interclavicle whose upper portion almost entirely overlaps the extent of the clavicles. Additionally, they possess a hypertrophied (meta)coracoid that extends to the manubrium-interclavicle joint, forming an articulation with these respective bones (Luo and Yuan 2007; Luo 2015) (Figure 5).

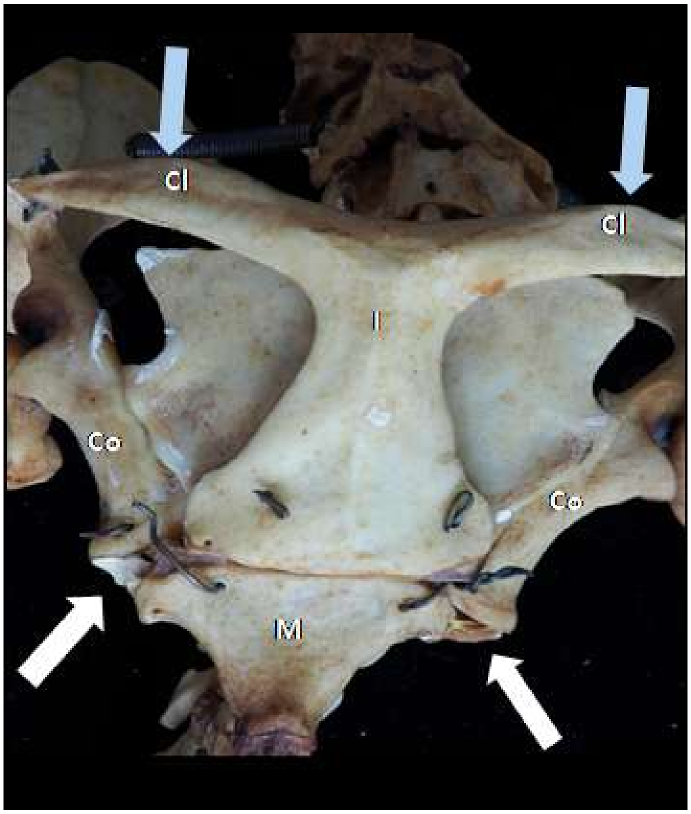

Figure 5. Ventral view of the shoulder girdle of the modern platypus (*Ornithorhynchus anatinus*, MN 3). Gray arrows point to clavicles parallel to the interclavicle, white arrows point to the articulation between the coracoid, manubrium and interclavicle. Captions: Cl = Clavicle; I = Interclavicle; Co = Coracoid; M = Manubrium. Photo: João Alves de Oliveira.

The reinforced arrangement of the shoulder girdle of monotremes provides additional support for the powerful shoulders and forelimbs of these animals. In general, the reason for the existence of such a structure is attributed to the digging habits of these mammals, which were secondarily co-opted to carry out the swimming movements of semi-aquatic platypuses. To date, there are no known remains of the shoulder bones of any of the pre-Cenozoic fossil monotremaformes, and as the only other Yinotheria whose shoulder girdle is known are the Shuotheriidae from the Middle Jurassic of Asia. There's a gap of more than 100 million years between a primitive Yinotheria shoulder girdle and the Monotremata shoulder girdle (Luo and Yuan 2007; Luo 2015; Flannery *et al.* 2022).

Because all fossil monotremes from the Upper Cretaceous to early Cenozoic were semi-aquatic (Phillips *et al.* 2009; Chimento *et al.* 2023), it is possible that the reinforced shoulder structure of these animals evolved to allow the execution of their unique swimming movements (Fish *et al.* 1997). Therefore, it could be possible to infer that the reinforced shoulders of modern monotremes may have evolved along with a semi-aquatic lifestyle, as among the Monotremaformes only monotremes have semi-aquatic forms (Flannery *et al.* 2022; Chimento *et al.* 2023). The reinforced shoulder could qualify as a synapomorphy of this clade, however, given the large gap in the fossil record of these animals, this proposal must be taken with caution, as new discoveries could completely change this reconstruction of the evolutionary history of these animals.

Sensitive snouts and unusual swimming. One of the most striking characteristics, which can be found in both talpids and monotremes, is the possession of a highly sensitive snout full of mechanosensory organs. Monotremes have a highly specialized snout, superficially similar to the beak of a bird, that is full of mechanoreceptors and electroreceptor (see Figure 3), while moles have a procumbent snout, similar to a proboscis, filled with a set of specialized mechanoreceptive organs known as Eimer's organs. Desmans (a group of aquatic moles) have an extensive network of Eimer's organs aligned in a complex configuration of rosettes, with a micro-vibrissa located within each rosette (Catania 2000; Catania 2020).

The mechanoreceptive organs of monotremes and the Eimer's organs of talpids are remarkably similar both morphologically and functionally. In addition, aquatic talpids (*Desmana*, *Galemys* and *Condylura*), have an even more modified snout than their terrestrial and fossorial relatives, with star-nosed moles having a series of nasal tentacles, while desmans have an elongated snout like a proboscis that has a notable morphological similarity to the "beak" of platypuses (Catania 2000; Catania 2020).

Among extant aquatic mammals, platypuses exhibit a unique swimming motion characterized by a series of alternating strokes of their forelimbs (Fish *et al.* 1997). These swimming movements closely resemble the motions performed by moles and other fossorial mammals while digging. Notably, semi-aquatic moles, such as the star-nosed mole (*Condylura cristata*), display a swimming pattern highly similar to that of platypuses, using a series of predominantly alternating strokes with their forelimbs to propel themselves through water. These movements closely resemble those used by these and other moles for soil excavation; however, unlike platypuses, star-nosed moles also employ their hindlimbs, to a lesser extent, for additional underwater propulsion (Hickman 1984).

Despite their vaguely beaver-like form, platypus tails do not play an active role in locomotion. Instead, they serve other functions, such as transporting nesting materials and storing fat (Guiler 1983; Fish *et al.* 1997; Thomas *et al.* 2017). The fat-rich tails of platypuses were highly valued by Indigenous Australian peoples, particularly during winter (Robinson and Plomley 1966; University of New South Wales, Sydney [Accessed May 18th, 2025]).

Desmans also swim using a series of alternating limb movements; however, unlike platypuses and star-nosed moles, desmans utilize their hindlimbs for aquatic propulsion, in addition to their long, laterally flattened tails (Ivlev *et al.* 2010). Given that the few mammals that move through water via alternating limb strokes originate from fossorial species and possess a relatively well-documented evolutionary history, this may suggest that other mammals with similar swimming styles have followed comparable evolutionary trajectories. Although predominantly terrestrial, echidnas are nonetheless adept swimmers, capable of crossing rivers and occasionally even swimming in the ocean (Augee *et al.* 2005; Phillip *et al.* 2009). They can dive underwater, which aids their survival during sudden flooding events. When caught in such situations, an echidna reduces its heart rate from 60 beats per minute to just 12 beats per minute, conserving oxygen for its heart and brain—the organs most sensitive to oxygen deprivation. Laboratory data have revealed that the cardiovascular system of an echidna resembles that of a seal (Augee *et al.* 2005), further supporting the hypothesis that this trait is inherited from ancestors, similarly to the platypus. When swimming, echidnas, like platypuses, engage in alternating strokes of their forelimbs. However, their swimming rhythm is slower, and their hindlimbs also contribute to additional propulsion.

Discussion

Non-dental synapomorphies of Monotremata. In addition to the dental characters described by Chimento *et al.* (2023), the crown group of Monotremata can be distinguished from the other Monotremaformes by a suite of characters: (1) the absence of a Meckel groove in the posterior part of the dentary, a lateral projection in the anterior portion of the mandible that is extends beyond the mandibular symphysis (Rich *et al.* 2016; Flannery *et al.* 2024); (2) a reduction in the number of molars in the mandible, with a marked simplification of M3, which has only one tooth root (Flannery *et al.* 2024), and (3) the possession of a reinforced shoulder girdle with expanded interclavicles that completely overlap the clavicles and a hypertrophied (meta) coracoid that extends to the joint between the manubrium and interclavicles (Augee *et al.* 2006; Luo and Yuan 2007; Luo 2015).

Based on dental morphology, Chimento *et al.* (2023) recovered *Teinolophos trusleri* as the closest relative to the crown group Monotremata, but the subsequent discovery of *Opalios splendens*, changed this view. In addition to sharing several unique characteristics with *T. trusleri*, such as the possession of five molars in the jaw, *O. splendens* also shares some derived characters present in monotremes, such as a flattened snout and perhaps a prominent ear (Flannery *et al.* 2024). Thus, it probably represents an intermediate form between the Teinolophidae and the Monotremata, being a sister taxon of this last. It is worth highlighting that *O. splendens* may not be the ancestor of the crown group, as it was contemporary with the platypus *Dharragarra aurora,* the oldest known member of the crown group of Monotremata (Flannery *et al.* 2024).

Therefore, the old synapomorphies used to define monotremes cited in Pian *et al.* (2016) should not be discarded, but rather repositioned, as although they are not enough to define what Monotremata is, they can still be used to define what Monotremaformes are.

Definitive Middle Ear and Chewing. To date, few studies have been conducted on why different lineages of mammals independently evolved a detached ear. Grossnickle (2017) in his work proposed that in Cladotheria mammals (placentals, marsupials, and Meridiolestida), the evolution of a detached ear would be associated with the predominant transverse chewing movement present in this group, as the ossified Meckel's cartilage present in several lineages of earlier mammals would considerably restrict the jaws from performing these mediolateral movements. Thus, the first Cladotheria significantly reduced Meckel's cartilage, so that it did not limit the ability to move their jaws, allowing them to move their jaws without limitation.

In addition to the Cladotheria, another very well-known group of mammals that developed a detached ear were the Allotheria represented by Euharamyida, Multituberculata, and Gondwanatheria (Kielan-Jaworowska 1997; Krause *et al.* 2020; Mao 2023). These rodent-like mammals were among the most abundant and diverse mammals of their time (Krause *et al.* 2021), some of them like *Vilevolodon diplomylos* had ear ossicles very similar to those of living monotremes (Wang *et al.* 2021). However, unlike Cladotheria, Allotheria did not have transverse chewing movement but rather a palinal chewing movement (Lazzari *et al.* 2010; Zheng *et al.* 2013; Schultz *et al.* 2014), similar to that of many contemporary herbivorous dinosaurs such as ceratopsids and hadrosaurids (Varriale 2016). Therefore, the transverse chewing movement itself cannot be considered the only event by which mammals could have developed a detached middle ear.

Basal Monotremaformes such as *Teinolophos* and *Steropodon* had a chewing movement that was substantially ortal rather than transverse due to the accessory complements of the mandible (ossified Meckel's cartilage) and the molar teeth deeply rooted in the mandible. On the other hand, the Monotremata have a predominantly transverse chewing movement that can be evidenced by the extensive transverse wear on the molars of species of the genus *Obdurodon* (Ashwell 2013). Monotremata and Cladotheria possess a prominent angular process, positioned at the posterior part of the mandible (Rich *et al.* 2016; Grossnickle 2017). The presence and location of this structure were also noted by Grossnickle (2017) as being correlated with the evolution of transverse mastication and, by extension, with the distinct middle ear. Multituberculates and other Allotheria lack an angular process in their mandibles (Kielan-Jaworowska 1997). Based on this evidence, we propose that, as in Cladotheria, the reduction and subsequent loss of ossified Meckel's cartilage in Monotremaformes is probably associated with a predominantly transverse chewing movement performed by the jaws of these animals.

Additional evidence for the platypus-like ancestry of echidnas. As previously stated, today we know that echidnas are derived from terrestrial platypuses, with several aspects of their biology referring to their aquatic ancestry (Phillips *et al.* 2009; Flannery *et al.* 2022). We propose two additional morphological characteristics that corroborate the proposal that echidnas diverged from platypuses (Figure 4): (1) the presence of a well-marked lateral curvature in their jaws, not recorded in other mammals with similar facial morphology, such as pangolins and anteaters, and (2) the shape of the mandibular symphysis. In most mammals, including the myrmecophagous forms already mentioned, the distal end of the jaws curves inwards, forming the mandibular symphysis; however, in echidnas, this pattern is very similar to that seen in platypuses. Based on this evidence, we propose that, despite extensive cranial specializations for myrmecophagy, echidnas still possess the same base jaw shape as their platypus-like ancestors.

The "Bill" of the Platypus and the Proboscis of the Mole. It has been proposed that the electroreceptive and mechanoreceptive senses of the Monotremaformes evolved in the polar forests of southern Gondwana during the Early Cretaceous (Flannery *et al.* 2022). These conditions enabled the highly sensitive snouts to locate the small invertebrates on which they fed. As the senses of electroreception and mechanoreception are more efficient in an aqueous environment than on dry land, it is not surprising that, over time, some of these Monotremaformes adopted a semiaquatic lifestyle.

In the water, these animals were able to make even more use of their electrical and tactile senses using their typical mammalian snouts on extensive platforms full of electroreceptors and mechanoreceptors, thus forming the characteristic "beak" of members of the order Monotremata (Pettigrew 1999; Asahara *et al.* 2016). A similar process occurred in a later lineage, the mole family Talpidae.

Moles in general have a unique characteristic, the Eimer's organs (Figure 3); these are a set of tactile organs located on the surface of the nasal epidermis, which give the animal an extraordinary sense of touch. In terms of structure and function, Eimer's organs are very similar to the mechanoreceptor organs of monotremes and their ancestors (Catania 2000), thus being an example of convergent evolution. Given the morphological and functional similarity between both structures, it is possible to draw certain parallels between the evolutionary history of Monotremaformes and that of talpids.

The first moles were small animals that lived above ground and foraged in the leaf litter of the ancient forests of Asia during the Paleogene, in a similar way to early Monotremaformes of the lower Cretaceous like *Teinolophos trusleri*. Like these, when foraging in the dense forest environment and in low light, the moles were able to make use of the sharp mechanosensory system, to locate their prey. A few basal talpids still maintain this ancestral lifestyle.

The shrew moles of the subfamily Uropsilinae are the most primitive living talpids, forming a sister lineage to all other moles. These animals lack the typical adaptations for the fossorial lifestyle of their relatives, still retain external auricles, and have the most rudimentary Eimer's organs of their family. Over time, most moles became fossorial and began to use their Eimer's organs to locate earthworms and other invertebrates underground. However, at least two mole lineages, the Condylurini and the Desmanini, have a semiaquatic lifestyle and can therefore utilize even more complex sensory structures like monotremes (Shinohara *et al.* 2003; Wan *et al.* 2013; Bannikova *et al.* 2015).

The star-nosed mole *(Condylura cristata)* is the only semi-aquatic mole native to North America, as well as the only living representative of the tribe Condilurini (Wan *et al.* 2013; Bannikova *et al.* 2015). It lives mainly in regions with humid soil, such as in swamps and on the banks of rivers and lakes.

In general, star-nosed moles spend most of their time digging, but they also forage in water, particularly in the winter. To pick up signals from their prey in their moist environments, these moles use a series of nasal appendages that increase the surface area of their snouts, giving them one of the most developed mechanosensory systems of any other mole (Hamilton 1931; Hickman 1984).

The "desmans", two species native to northern Eurasia members of the Desmanini tribe (Wan *et al.* 2013; Bannikova *et al.* 2015), are the most morphologically modified talpids for a semiaquatic lifestyle. Similarly to the star-nosed mole, desmans further developed their mechanosensory systems in response to their aquatic lifestyle; they utilized their snout into a true proboscis that is wide laterally and compressed vertically (Richard and Vallette Viallard 1969), which makes it visually very similar to the "beak" of aquatic monotremes such as the platypuses. Although thinner, this proboscis has an extensive network of Eimer's organs aligned in a complex configuration of rosettes, with a micro-vibrissa located inside each rosette, which gives the animal not only the ability to find its prey underneath water, but also to detect and avoid submerged obstacles (Catania 2000), in the same way as the "beaks" of platypuses.

A Reinforced Shoulder for Swimming and Digging. Due to the significant scarcity of postcranial remains of Mesozoic Yinotherian mammals, the precise timing of the emergence of the reinforced shoulder structure found in modern monotremes remains unknown. However, the foundational arrangement of this structure was already present in basal Yinotheria, such as *Pseudotribos robustus* (Luo and Yuan 2007), a Middle Jurassic Shuotheriidae from Asia. *P. robustus* is known from a partial skeleton, including pectoral girdle elements such as a large and robust interclavicle, whose anterior portion extensively overlaps the clavicles. This pectoral girdle arrangement has been associated with a fossorial lifestyle.

Kryoryctes cadburyi is the only known representative of Mesozoic Monotremaformes with postcranial remains, specifically a single humerus. This humerus exhibits significant morphological similarity to that of echidnas, to the extent that *Kryoryctes* was initially interpreted as a basal Tachyglossidae (Pridmore *et al.* 2005). Consequently, it was proposed that *K. cadburyi* had a specialized fossorial lifestyle.

To test the hypothesis that *Kryoryctes* was indeed fossorial, Hand *et al.* (2025) analyzed the bone microstructure of the only known specimen of this taxon. Their results indicated that, despite its external morphology closely resembling fossorial species, the humerus of *K. cadburyi* exhibits a bone microstructure highly similar to that of extant aquatic mammals, featuring a particularly thick cortex and a significantly reduced medullary cavity. Based on these findings, the authors proposed a semi-aquatic burrowing lifestyle for this taxon. As a semi-aquatic burrower, *Kryoryctes* would benefit significantly from the reinforced shoulder structure found in Monotremata.

Therefore, it would not be unreasonable to suggest that this structure evolved to facilitate more efficient swimming. Since within Monotremaformes only Monotremata includes semi-aquatic species, the reinforced shoulder structure could be considered a synapomorphy for the clade. The only postcranial fossil evidence of aquatic locomotion in Cenozoic monotremes is a humerus from *Monotrematum sudamericanum*, which exhibits morphology nearly identical to that of modern platypuses and likely shared a similar lifestyle (Forasiepi and Martinelli 2013).

Among extant aquatic mammals, platypuses—and by extension early Monotremata—are characterized by a unique swimming motion involving a series of alternating strokes of the forelimbs (Fish *et al.* 1997). Among all living mammals, only one species is known to employ a similar movement for underwater propulsion: the star-nosed mole. This small fossorial and semi-aquatic mammal uses a series of predominantly alternating strokes of its forelimbs to move through water. These movements closely resemble those used by moles for digging; however, unlike platypuses, star-nosed moles also utilize their hindlimbs to a lesser extent for additional underwater propulsion (Hickman1984).

Thus, the reinforced shoulder structure has played a fundamental role throughout the evolutionary history of these groups, influencing their ability to occupy various habitats and often contributing to their survival in transitional and dynamic environments.

In conclusion, we describe four morphological characteristics, in addition to dental morphology, that can be identified as synapomorphies of the crown group of Monotremata, namely: the absence of a Meckel groove in the posterior part of the dentary, the presence of a lateral projection in the anterior portion of the mandible that extends beyond the mandibular symphysis, the reduction in the number of molars in the mandible, with marked simplification of the M3, and probably due to the possession of a reinforced shoulder girdle with expanded interclavicles that overlap entirely with the clavicles, and a hypertrophied (meta) coracoid which extends to the joint between the manubrium and the interclavicles.

We propose that as in Cladotheria mammals, the evolution of a prominent ear in Monotremata is related to a transverse chewing movement. We also suggest that the shape of the echidna's jaw remains remarkably like that of its ancestors, despite the evident morphological changes associated with myrmecophagy. Finally, we observed that the evolutionary history of monotremes in fact has several parallels with the evolutionary history of aquatic moles. Based on this, we propose that, like the Desmanini and Condylurini, monotremes are descendants of ancestors with a specialized fossorial lifestyle.

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Postnatal growth and age estimation in the broad-tailed bat (*Nyctinomops laticaudatus*)

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Postnatal growth in bats has been studied in various species, but its documentation often depends on access to roosts, the ability to handle pups, and reliable marking systems for recapture. We examined the postnatal growth and development of the Broad-eared Free-tailed Bat (*Nyctinomops laticaudatus*) in a colony at the Governor's Palace, Uxmal Archaeological Zone, Yucatán, Mexico. Marked pups were measured from birth until the onset of flight for forearm length, body mass, and fourth digit length. Growth curves were generated, from which growth rates and age-prediction equations were derived. At birth, forearm length, fourth digit length, and body mass were 45%, 29%, and 31% of postpartum female values, respectively. These traits grew linearly up to 40 days, with average rates of 0.44 mm/day (forearm), 0.54 mm/day (fourth digit), and 0.14 g/day (body mass). Growth rates slowed after the onset of flight. Forearm length provided a reliable predictor of age for pups between 1 and 40 days old when measurements were ≥37 mm. Flight trials indicated that pups began flapping and short horizontal movements at 15 days and achieved sustained flight by day 40. The growth rates observed in *N. laticaudatus* exceeded those reported for many other tropical insectivorous bats. Documenting postnatal development in molossids enhances our understanding of this critical stage in bat life history, and comparative studies across tropical species will provide valuable insights into the evolution of bat growth strategies.

Key words: age-estimation, development, Molossidae, *Nyctinomops laticaudatus*, postnatal growth.

El crecimiento postnatal en murciélagos se ha estudiado en varias especies, pero su documentación a menudo depende del acceso a los refugios, la posibilidad de manipular a las crías y la disponibilidad de sistemas de marcaje confiables para su recaptura. Examinamos el crecimiento y desarrollo postnatal del murciélago de cola suelta ancha (*Nyctinomops laticaudatus*) en una colonia ubicada en el Palacio del Gobernador, Zona Arqueológica de Uxmal, Yucatán, México. Las crías marcadas fueron medidas desde el nacimiento hasta el inicio del vuelo en longitud del antebrazo, masa corporal y longitud del cuarto dedo. Se generaron curvas de crecimiento, a partir de las cuales se derivaron tasas de crecimiento y ecuaciones para la predicción de la edad. Al nacer, la longitud del antebrazo, la longitud del cuarto dedo y la masa corporal correspondían al 45%, 29% y 31% de los valores registrados en hembras posparto, respectivamente. Estos rasgos crecieron de manera lineal hasta los 40 días, con tasas promedio de 0.44 mm/día (antebrazo), 0.54 mm/día (cuarto dedo) y 0.14 g/día (masa corporal). Las tasas de crecimiento disminuyeron después del inicio del vuelo. La longitud del antebrazo resultó un predictor confiable de la edad para crías de entre 1 y 40 días, cuando las mediciones fueron ≥37 mm. Las pruebas de vuelo indicaron que las crías comenzaron a aletear y realizar movimientos horizontales cortos a los 15 días, y alcanzaron el vuelo sostenido alrededor del día 40. Las tasas de crecimiento observadas en *N. laticaudatus* superaron las reportadas para muchos otros murciélagos insectívoros tropicales. Documentar el desarrollo postnatal en molósidos amplía nuestra comprensión de esta etapa crítica en la historia de vida de los murciélagos, y los estudios comparativos entre especies tropicales proporcionarán información valiosa sobre la evolución de las estrategias de crecimiento en estos mamíferos.

Palabras clave: crecimiento postnatal, desarrollo, estimación de edad, Molossidae, *Nyctinomops laticaudatus*.

An organism's life history comprises those events or traits in its life cycle that influence its fitness. In this context, life history theory explains variations in survival and reproductive success—both of which play a fundamental role, as they allow inferences about evolutionary processes and help assess population viability (Stearns 1992). The main characteristics that comprise an organism's life history are gestation length, birth size, postnatal growth, growth rate, age and size at maturity, offspring size and number, senescence related to longevity, and mortality rates (Stearns 2000; Roff *et al.* 2006; Fabian and Flatt 2012).

The postnatal growth period is a time when young mammals develop appropriate sensorial and locomotor skills, necessary to become independent of the mother (Baptista *et al.* 2000). Many researchers have studied this period with respect to changes in behavior, physiology, and ecology to investigate life history traits (Kunz *et al.* 2009; Eastick *et al.* 2022). Postnatal growth studies are essential for understanding how life history traits are influenced by extrinsic and intrinsic factors (*e.g.*, local and regional climates, food supply, latitude, seasonal variations, roosting environments, litter sizes, maternal conditions, gender, birth timing; Kunz *et al.* 2009; Eghbali and Sharifi 2023).

Growth and development of bats were studied during the postnatal period (Orr 1970; Tuttle and Stevenson 1982), under both natural (Stern and Kunz 1998) and captive conditions (Hughes *et al.* 1995). Studies from captive conditions do not reflect the compromises and constraints imposed on animals in the wild, as these conditions did not reflect their natural environments (Kunz and Robson 1995; Mclean and Speakman 2000). Studies conducted in the field, based on pups marked at birth and recaptured

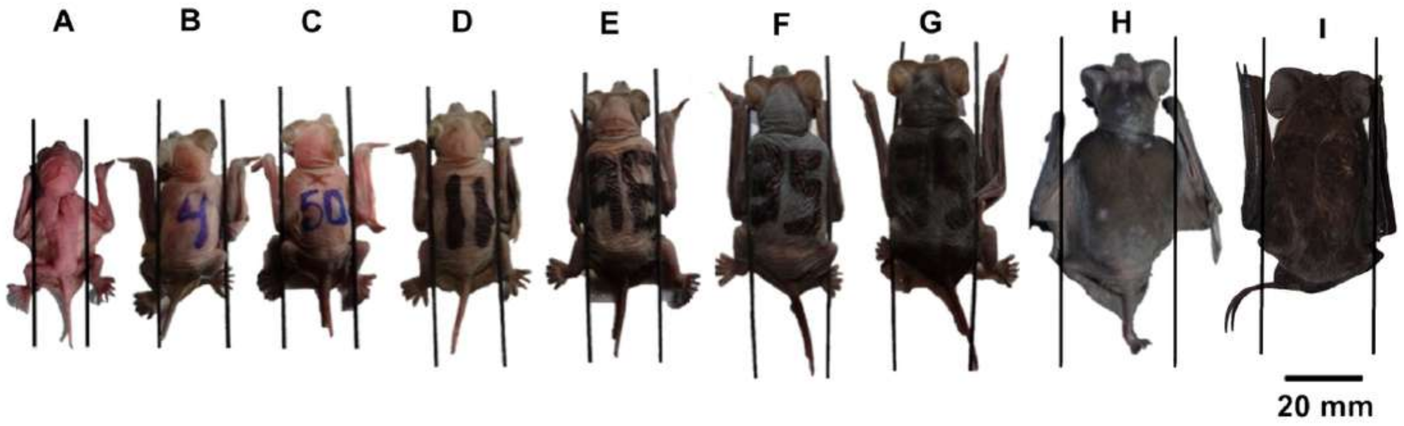

Figure 1. Development of *N. laticaudatus*: A.- Newborn (1 day); B.- juvenile five days old; C.- Juvenile 10 days old; D.- Juvenile 15 days old; E.- Juvenile18 days old; F.- Juvenile 21 days old; G.- Juvenile 27 days old; H. Juvenile 35 days old; I.- Adult (45 days).

at regular intervals, should yield the most ecologically meaningful results on postnatal growth in bats. Previous studies have shown that measurements of the length of the forearm, the length of the fourth finger, and body mass are helpful to estimate the age of bats during the early postnatal period (Kunz and Anthony 1982; Kunz and Robson 1995; Stern and Kunz 1998). Forearm length is the most accurate and reliable character for estimating age during the early linear growth period of bats.

Most studies on postnatal growth in bats were conduct on vespertilionids (Jones 1967; O'Farrell and Studier 1973; Isaac and Marimuthu 1996; Hoying and Kunz 1998; Baptista *et al.* 2000; Koehler and Barclay 2000); with only a few efforts on the Molossidae (Short 1961; Pagels and Jones 1974; Kunz and Robson 1995; Allen *et al.* 2010); which is widespread in the tropics and subtropics of the world.

The Broad-eared Free-tailed Bat *Nyctinomops laticaudatus* (Chiroptera: Molossidae) is rare or uncommon throughout most of its geographic range, as confirmed by the few records from several countries (Barquez *et al.* 1999). However, it is relatively common in some areas, especially within the Yucatan Peninsula (Jones *et al.* 1973; Bowles *et al.* 1990). This species is listed as a least concern by the IUCN (Barquez *et al.* 2015). However, nothing about the external characters at birth or postnatal growth has been reported, and little information is known concerning the life history of *N. laticaudatus* (Avila-Flores *et al.* 2002). This paper aimed to provide such background data on the pattern of postnatal growth for *N. laticaudatus* from México and to derive age-specific growth equations based on a quantitative analysis of forearm, fourth finger. and body mass.

Materials and Methods

Study area and collecting data. The study was conducted between June and August 2015 in Governor's Palace at the Archeological Zone of Uxmal, Yucatán, México (20° 21´N, 89° 46´W). This Mayan ruin housed a colony of approximately 5,000 *N. laticaudatus* (Málaga and Villa 1956; Ortega *et al.* 2010). The Governor's Palace was constructed on a platform 12 meters high and 100 meters long and is composed of three independent blocks, with nine interior chambers and 11 exteriors. There are cavities in the corner of every chamber with particular dimensions of approximately 30 X 30 centimeters, and an average depth of 70 centimeters. The nursery roost of this species is located inside the cavities of the chambers (Ortega *et al.* 2010).

Marking and recapture. Prior investigation indicated that the females of *N. laticaudatus* in this area give birth from late June to early July (Ortega *et al.* 2010). Therefore, we checked the cave daily from about 10 days before birthing. When newborn pups were observed, we began collecting them by hand. Once neonates were found, we would gently hand-capture them during the morning (usually at 09:00 h). Young bats were immediately placed in warm cloth bags and taken into a temporary laboratory near the roost. Neonates with an attached umbilical cord were assumed to be one day old (Kunz and Robson 1995). Following determination of the sex of pups, a unique number was placed on the body (temporal marker, *Sharpie*®) of each bat for individual identification (Figure 1). Forearm length and fourth finger were measured to the nearest 0.01 mm with a digital caliper (Digimatic Cd-6 B 500-133, *Mitutoyo*®). Body mass was recorded to the nearest 0.01 g using an electronic balance (Electronic balance # 501A-*LG*®). To minimize errors in using calipers, individual measurements were repeated three times, and the means were used in the analysis.

To limit disturbance and possible abandonment, the bats were returned to the sites of capture after observations and measurements had been obtained. We visited the roost every day for one month, and bats were collected and recorded daily. Fieldwork procedures followed the guidelines approved by the American Society of Mammalogists (Sikes *et al.* 2011). All permits needed for the capture and handling of bats were obtained from Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT—SGPA/DGVS/12598/15) and Instituto Nacional de Antropología e Historia (INAH).

Statistical analysis. A chi-square with Yate's correction test was applied to evaluate whether sex ratio at birth was different from chamber (Sokal and Rohlf 1979). We used a multivariate analysis of variance (*Manova*) test to compare length of forearm, length fourth finger and body mass of males and females at birth. The proportions of size to the birth of the pups with regard to that of adult mothers was obtain across the comparison of the variables, with 20 nursing females gathered during the period of study (Kunz and Robson 1995). The growth rates (mm/day or g/day) were calculated using the equation of McOwat and Andrews (1995): where "R" is the growth rate of postnatal growth, "L1" and "L2" are the length or mass for the captures one and two, and "t1" and "t2" is the time in the captures one and two. By using the rates of growth of the recaptured newborn, the precision of the equation will be improved. We used a linear regression analysis, with age as the dependent variable, to derive age predictive equations based on the data of linear changes of the forearm length, length of the fourth finger, and body mass.

Results

We sampled 31 crevices from the nursery roost from June to August 2015. Inside cavity, neonates with umbilical cords were captured and marked as *N. laticaudatus*, of which 48 were females (49%) and 50 males (51%). The sex ratio (1.04♂: 0.96♀) did not differ significantly from unity (X2 Yates= 0.01, d.f.= 1, P= 0.92034). No significant difference was found between the length of the forearm, fourth finger, and body mass of male and female pups at birth (Manova: λ-Wilk: 0.9741, F= 0.8636, P= 0.4787; Table 1).

Table 1. Descriptive statistics of the variables used to evidence sexual dimorphism in pups of *N. laticaudatus* in Uxmal, Yucatán. n= number of marked bats; SD= standard deviation; CV= coefficient of variation.

	Forearm (mm)		
Sex	mean ± SD	min-max	CV (%)
Male (n= 50)	19.4824 ± 1.0892	15.84 - 22.23	5.59
Female (n= 48)	19.2250 ± 1.0608	17.04 - 22.64	5.51
	Forth finger (mm)		
Sex	mean ± SD	min - max	CV (%)
Male (n= 50)	16.6762 ± 0.8021	14.92 - 18.65	4.81
Female (n= 48)	16.6441 ± 0.9156	14.44 - 19.59	5.50
	Body mass (g)		
Sex	mean ± SD	min - max	CV (%)
Male (n= 50)	3.642 ± 0.5140	2.04 - 6.08	14.11
Female (n= 48)	3.559 ± 0.4221	2.77 - 4.79	11.86

The size of the pups at birth in relation to the adult (20 nursing females) presented the following proportions: 45% of the forearm, 29% of the fourth finger, and 31% of the body mass (Table 2).

The results obtained by the equation McOwat and Andrews (1995), from 385 recaptures, indicated that the average growth rate of the forearm (mean ± SD) is 0.44010 ± 0.1391 mm/day, the fourth finger is 0.5389 ± 0.2003 mm/day, and body mass is 0.093 ± 0.1415 g/day. These rates remained constant during the pre-flight young.

Table 2. Size at birth of *N. laticaudatus* and its proportion with regard to the size of the adult, SD= standard deviation.

	Pups at birth (n= 98) mean ± SD	Adult (n= 20♀) mean ± SD	% of the adult
Forearm	19.35 ± 1.07 mm	43.05 ± 1.62 mm	45%
Fourth finger	16.66 ± 0.85 mm	58.34 ± 2.08 mm	29%
Body mass	3.60 ± 0.47 g	11.52 ± 0.62 gr	31%

The linear regression analysis of the variables of growth with respect to age showed high determination coefficients for the variables: forearm (R2= 0.8807; Figure 2a), fourth finger (R2= 0.875; Figure 2b), and body mass (R2= 0.8446; Figure. 2c), which indicates a high degree of relationship between age and growth variables. The coefficient of determination for the variables no forearm (1-R2= 0.1193; 11.9%), fourth finger (1-R2= 0.1250; 12.5%) and weight (1-R2= 0.1554; 15.5%) lower privileges, as among 11.9% and 15.5% of the variation in the variables was not explained by the regression model with respect to age.

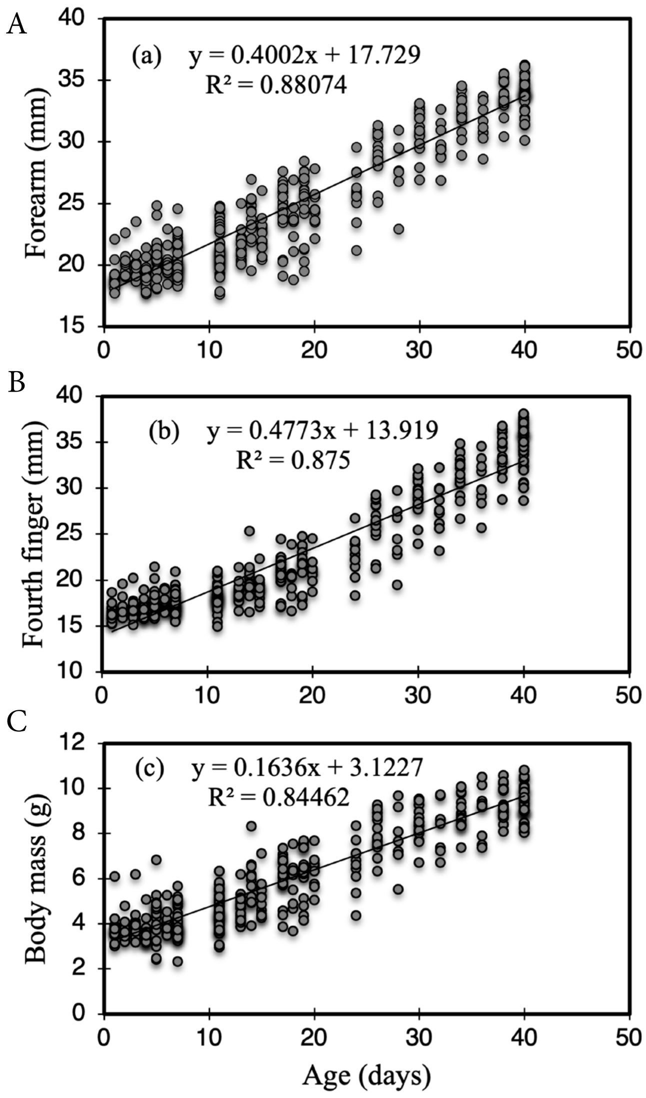

Figure 2. Age-related changes in (a) forearm, (b) fourth finger, (c) body mass during the postnatal growth of *N. laticaudatus*. Growth trajectories were fit using unary linear regression equations.

Age was estimated quantitatively based on linear changes observed in the length of the forearm (1 - 40 days). A linear regression equation allowed age estimates according to forearm length values ranging from 17.64 to 36.12 mm. High values of the correlation coefficient (R2= 0.880) indicated that the length of the forearm was a reliable character for estimating the age of young *N. laticaudatus* in the first 40 days of age (Figure 3).

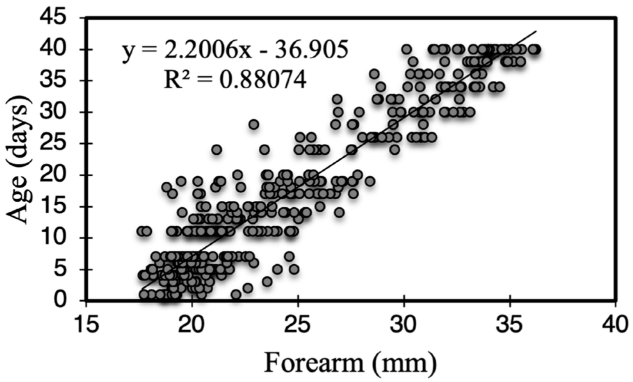

Figure 3. Age-predictive equations for *N. laticaudatus* young based on length of forearm; the independent variable (age) was placed on the Y axis to facilitate prediction of age.

All females collected with young had single offspring with closed eyes at birth. The eyes of the pups opened during the second day after birth. The ears are folded toward the head at birth and take their stand normal to second day. Fur was visible predominantly on the back and ventral side of the body from the third week. However, the development of adult fur is visible up to 43 days. The young were able to flutter and glide when dropped from their hands at 20 days of age. At 30 days, pups were able to fly freely inside the cave. At 45 days, it was not possible to catch young of *N. laticaudatus* at the nursery site.

Discussion

The sex ratio in pups was 1: 1 (1.04♂: 0.96♀; *P*= 0.92034) in the area, likewise this ratio remained constant to the stage preflight. For this species in Uxmal, two previous records in 2006 and 2013 (Ortega *et al.* 2010) and in this study showed no sex ratio deviations in pups. Also, in previous studies, the sex ratio 1: 1 have been report for other of insectivorous bats: *Myotis austroriparius* (Rice 1957), *M. lucifugus* (Smith 1957), *Eptesicus fuscus* (Mills *et al.* 1975), *Myotis yumanensis* (Milligan and Brigham 1993), *Tadarida brasiliensis* (Kunz and Robson 1995), *Pipistrellus subflavus* (Hoying and Kunz 1998), *Myotis velifer* (Loucks and Caire 2007), and *Hipposideros terasensis* (Cheng and Lee 2002).

The size of newborns, as well as their growth and development, are affected by factors such as sex, litter size, geographical location of the population, the temperature and relative humidity of the shelter. The influence of sex is remarkable in species where there is sexual dimorphism, since the rate of growth is faster in the sex of larger size, such as in the males of *Phyllostomus hastatus*, or females *Lasiurus cinereus* (Stern and Kunz 1998; Koehler and Barclay 2000).

Neonates are large compared with those of other small mammals; they come to represent between 12 and 43 % of postpartum maternal weight (Kunz *et al.* 2009). The size of the forearm of *N. laticaudatus* pups at birth is 45% relative to the size of the forearm of the mother, the fourth finger is 29 % the size of the mother's, and their weight is 31% of the mother's. Comparing these proportions with other tropical bats, it shows that the size of the forearm corresponds to half the size of the mother's forearm, similar in all species (Kunz *et al.* 2009). The exception is *Phyllostomus hastatus*, probably because this species is considerably larger than the other phyllostomids, where there is an inverse relationship between the size of the adult and litter size at birth (Tuttle and Stevenson 1982). The mass of pups observed was within the range, but they were slightly heavier than most insectivorous pups (Kunz *et al.* 2009).

The average growth rates of the forearm (0.44 ± 0.13 mm/day) and weight (0.14 ± 0.09 g/day) were low compared to those reported for the family Vespertilionidae (1.2 ± 0.6 mm/day, and 0.9 ± 0.4 g/day), Phyllostomidae (0.9 ± 0.8 mm/day and 0.3 ± 0.09 g/day) and sister species of family Molossidae (0.8 ± 0.2 mm/day and 0.4 ± 0.2 g/day; Short 1961; Pagels and Jones 1974; Kunz and Robson 1995; Allen *et al.* 2010). It is known that changes in temperature and humidity influence growth rates of bats, so that a decrease in the temperature of the shelter retards the rate of growth and development of the fetus or the cost of thermoregulation increases (Tuttle and Stevenson 1982). However, the temperature increase accelerates the metabolism and consequently the growth rate (Hoying and Kunz 1998). Therefore, it is possible that the constant ambient conditions (temperature and humidity) in the Palace of Governor during the present study do not directly influence an increase or decrease in growth rates; instead, this species may be seeking a thermostable site condition for the hanger nursery. Although in this study we could not compare two colonies of *N. laticaudatus*, for two roosting sites of *Tadarida brasiliensis* found significant differences in growth rates associated with changes in the temperature (Allen *et al.* 2010). These results contrast with a report for *Phyllostomus hastatus* (Stern and Kunz 1998), where the authors could not link any variable to differences in the size of infants from different years. It is possible that this variation is a result of not evaluated intrinsic factors such as sex, food availability, maternal care and some social factors (Klima and Gaisler 1968; Gould 1975; Tuttle and Stevenson 1982; Hoying and Kunz 1998; Porter and Wilkinson 2001).

The analysis of growth models allows comparison of the parameters derived with other studies regardless of the size and analyzed growth period body (Kunz and Robson 1995). The adjustment of variables forearm, fourth finger, and body mass of *N. laticaudatus* were higher for the linear model for the pre-flight, contrary to other bat species where the logistic model is the most common or the only one is tested (*Tadariada brasiliensis*, *Noctilio albiventris*, and most species of Vespertilionidae and Phyllostomidae family (Kunz *et al.* 2009).

It is challenging to compare growth parameters in these studies and that most of them do not realize the division as a turning point in the stages of pre-flight and post-flight during the development of the young, because several authors agree that growth models should be tested in two stages and each of these have physiological, ecological and behavioral characteristics different from each other (Lin *et al.* 2011). There are some articles where high coefficients of determination for the linear model for the pre-flight phase are reported, as in the case of *Myotis myotis* (Paz 1986), *M. lucifugus* (Baptista *et al.* 2000), *M. emarginatus* (Eghbali and Sharifi 2018), *M. capaccinii* (Mehdizadeh *et al.* 2018), *Tadarida brasiliensis* (Allen *et al.* 2010), *Hipposideros pomona* (Lin *et al.* 2011), *Pteropus giganteus* (Sudhakaran *et al.* 2013), *Natalus mexicanus* (Martínez-Coronel *et al.* 2021), and *Leptonycteris yerbabuenae* (Martínez-Coronel *et al.* 2014).

Nyctinomops laticaudatus pups are altricial, as they are born hairless and with their eyes closed. Their tendency to cluster into nursery groups suggests that they have poor thermoregulatory abilities, are incapable of self-feeding, and possess an underdeveloped echolocation system, as well as immature wing muscles and bones.

The results of this study provide valuable insight into the postnatal growth patterns of *Nyctinomops laticaudatus*, revealing an initial phase of linear growth in key morphological traits, followed by a slowdown as the flight stage approaches. These findings not only enhance our understanding of ontogenetic development in this species but also allow for the establishment of functional parameters for age estimation in pups—an essential tool for studies on population dynamics, management, and conservation.

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Changes in the activity pattern of white-tailed deer in the Tehuacán-Cuicatlán Biosphere Reserve, Mexico

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The activity patterns of white-tailed deer are intricately linked to their fundamental requirements for nutrition, rest, and reproduction. These patterns naturally fluctuate in response to seasonal variations and environmental conditions. This study analyzed the daily activity patterns of the white-tailed deer, *Odocoileus virginianus*, inhabiting the scrubland and tropical dry ecosystem within the Tehuacán-Cuicatlán Biosphere Reserve (TCBR), Mexico. Our investigation specifically focused on comparing these activity patterns across different reproductive periods (rut, gestation, and fawning) considering potential variations based on the individual's sex and geographical location. We used 100 camera trap stations across four localities from February 2012 to February 2016. We analyzed white-tailed deer activity with circular statistics (Watson tests), kernel density estimates, Watson tests and activity range core. We obtained a total sampling effort of 22,809 days-trap, resulting in 1,656 independent records. Our findings revealed that the white-tailed deer presented a bimodal activity pattern during the day, with activity peaks between 06:00-12:00 h and 17:00-19:00 h. We found significant differences in the activity peaks between the rut and fawning periods with the gestation period, between females and males, and between localities in the TCBR. White-tailed deer show remarkable adaptability to habitats with varying environmental and ecological conditions, as temperate, semi-arid, and tropical regions. Generally, it is considered crepuscular because it tends to present greater activity during dawn and dusk. However, our results diverge from this classification, as the white-tailed deer's activity pattern was mainly diurnal, and their activity peaks changed depending on the physiological period, sex, and local habitat conditions. These variations could be attributed to habitat characteristics because extreme temperatures are not present in this tropical dry forest, as in arid and semi-arid zones. Moreover, the vegetation cover may protect against heat during the rainy season, and some plant species offer water sources for deer during the dry season. These findings contribute valuable insights into the biology and behavior of this species inhabiting the tropical dry forests of Mexico.

Key words: fawning; gestation; Oaxaca; *Odocoileus virginianus*; photo trapping; rut.

Los patrones de actividad de los venados cola blanca están relacionados con la necesidad de cubrir sus requerimientos nutricionales, de descanso y reproducción, por lo que varían dependiendo de la estación y las condiciones ambientales. En este trabajo analizamos el patrón de actividad del venado cola blanca *Odocoileus virginianus* del bosque tropical seco en la Reserva de la Biosfera Tehuacán-Cuicatlán (RBTC), México. Nuestra investigación se enfocó en comparar el patrón de actividad entre épocas reproductivas (celo, gestación y crianza), sexos y localidades. Colocamos 100 estaciones de muestreo con cámaras trampa en cuatro localidades, entre febrero de 2012 y febrero de 2016. Analizamos la actividad del venado cola blanca con estadística circular (prueba Watson), estimaciones de densidad kernel e intervalos de actividad núcleo. Obtuvimos un esfuerzo de muestreo de 22,809 días trampa y 1,656 registros independientes. El venado cola blanca mostró un patrón de actividad bimodal en el día con picos de actividad de 06:00 a 12:00 h y 17:00 a 19:00 h. Encontramos diferencias significativas en los picos de actividad entre el periodo de apareamiento y crianza con el de la gestación, entre hembras y machos, y entre localidades en la RBTC. El venado cola blanca muestra marcada adaptabilidad a hábitats con diferentes condiciones ambientales y ecológicas, como regiones templadas, semi-áridas y tropicales. Generalmente es considerada crepuscular porque presenta mayor actividad en el amanecer y atardecer. Sin embargo, nuestros resultados divergieron de esta clasificación, ya que su patrón de actividad fue principalmente diurno y sus picos cambiaron dependiendo del periodo fisiológico, el sexo y las condiciones locales del hábitat. Estas variaciones pueden deberse a las características del hábitat, ya que en este bosque tropical seco no se presentan temperaturas extremas como en las zonas áridas y semi-áridas. Además, la cobertura vegetal protege al venado del calor durante la época de lluvias y algunas especies de plantas les proveen recursos hídricos en la época seca. Nuestros resultados aportan nueva información sobre la biología y el comportamiento de esta especie en los bosques tropicales secos de México.

Palabras clave: Apareamiento; crianza; fototrampeo; gestación; Oaxaca; *Odocoileus virginianus*.

Activity patterns are a crucial element contributing to the optimization of energy balance in animals (Cid *et al.* 2020). These patterns in mammals are linked to the basic needs of nutrition, rest, and reproduction (McFarland 1999). It is known that activity patterns can be regulated by intrinsic factors such as age, sex, and physiological status, alongside extrinsic variables including diurnal-nocturnal transitions, temperature, precipitation, lunar phases, food availability, and interspecies interactions (Oliveira-Santos *et al.* 2009; Harmsen *et al.* 2010; Foster *et al.* 2013; Monterroso *et al.* 2013). Some studies have demonstrated that the presence of domestic species can also influence the activity patterns of wild species, thereby reducing encounters and consequent competition or predation risks (Carter *et al.* 2015; Zapata-Ríos and Branch 2016; Díaz-Ruiz *et al.* 2016). However, studying mammal activity through direct observation is especially difficult when the species of interest have shy behavior or if it inhabits spatially complex environments, which hinders the robust acquisition of ecological data.

Camera traps are a benchmark field method for acquiring data about activity patterns since they can obtain a large amount of data without disrupting the behavior of the target species and can be settled across diverse field conditions (O'Connell *et al.* 2011; Rovero and Zimmermann 2016). This technique is employed to address specific research on ecological patterns and the response of mammal populations to management actions. As such, camera trapping research provide information about the impact of accessibility to hunters on activity patterns (Espinosa and Salvador 2017), the correlation between frugivorous species activity and fruiting tree phenology (Mendoza *et al.* 2019; Mandujano and López-Tello 2022), predator-prey dynamics (Caravaggi et al. 2018), resource partitioning through activity patterns (Xue *et al.* 2018), as well as ecological research and management of megaherbivores species (Thapa *et al.* 2019), arboreal mammals (Astiazarán *et al.* 2020), among others. Consequently, camera traps appear as indispensable tools for investigating the activity patterns of various ungulate species, including the white-tailed deer (*Odocoileus virginianus*) (e.g., Tobler *et al.* 2009, Chen *et al.* 2019; Ikeda *et al.* 2019; Rahman and Mardiastuti 2021).

The white-tailed deer (*Odocoileus virginianus*, hereafter deer) is a species with a wide geographical distribution in the Americas and an adaptive capacity that allows it to inhabit distinct types of habitats with different environmental conditions (Gallina *et al.* 2019; Ortega-Santos *et al.* 2011). Also, it is considered crepuscular because it is most active at dawn and dusk (Holzenbein and Schwede 1989, Webb *et al.* 2010, Massé and Côté 2013). In places with marked seasonality, such as the tropical dry forest, the deer adjusts its activity depending on the availability and quality of food. In the season with a greater abundance of food resources, the deer spends more time moving and foraging, while in the season with lower abundance, it dedicates more time to digestion to avoid energy loss (Holzenbein and Schwede 1989, Webb *et al.* 2010, Massé and Côté 2013). Moreover, some studies suggest that the activity of females and males is related to their metabolic demands, with the former requiring more time to forage to have a better nutrition than males (Beier and McCullough 1990; Gallina *et al.* 2005). Another factor that can influence deer activity is human activities, such as hunting (Kilgo *et al.* 1998) and livestock (Cooper *et al.* 2008; Kukielka *et al.* 2013). Some studies report that deer decrease their activity in places where livestock is present (Cooper *et al.* 2008; Kukielka *et al.* 2013), and that they are more active at night during the hunting season (Kilgo *et al.* 1998). Deer can also modulate their activity to avoid natural predators such as coyotes, especially during the fawning season, with nursery groups being more diurnal compared to nocturnal activity by coyotes (Crawford *et al.* 2021).

In the central Mexican region encompassing the Tehuacán-Cuicatlán Biosphere Reserve (TCBR), a diverse assemblage of wild ungulate species can be found, such as white-tailed deer, collared peccary (*Pecari tajacu*), and red brocket deer (*Mazama temama*), including domestic ungulates such as goats (*Capra hircus*), cattle (*Bos taurus*), horses (*Equus caballus*), and donkeys (*Equus asinus*) (Mandujano *et al.* 2019; Ortíz-García *et al.* 2012). White-tailed deer hold significant importance for local human communities within TCBR (Mandujano *et al.* 2016a). Subsistence hunting of white-tailed deer has long been a customary practice in the region, with trophy hunting emerging as a contemporary addition (López-Téllez *et al.* 2016). This species has been subject to extensive ecological and behavioral research as part of a comprehensive, long-term monitoring initiative (Barrera-Salazar *et al.*, 2015; Ramos-Robles *et al.*, 2013; Yañez-Arenas and Mandujano, 2015; Yañez-Arenas *et al.* 2012).

In this study we describe the activity pattern of white-tailed deer in four localities within the La Cañada region of TCBR. We hypothesized that the physiological seasons, sex and anthropogenic pressure regulate the activity pattern of deer. Therefore, we expected that 1) activity would be greater at twilight during the gestation period to avoid predation, 2) males present greater nocturnal activity than females, and 3) activity will vary between localities as a function of differences in habitat structure and anthropogenic pressures (livestock, free-roaming dogs and poaching). This study presents a comprehensive investigation into the activity patterns of white-tailed deer, shedding light on the complex interplay of factors governing their behavior within the unique ecological context of the TCBR.

Methods

Study are. The TCBR encompasses the northwestern sector of the Meseta of Oaxaca, situated within the La Cañada region, as a constituent of the Sierra Madre del Sur. Geographically, it spans the extreme southeastern region of the state of Puebla and northeastern Oaxaca, encompassing coordinates ranging from 17°39' to 18°53' N latitude and 96°55' to 97°44' W longitude (Figure 1). The reserve covers 490,187 hectares and has an elevation gradient extending from 600 to 2,950 meters above sea level. Climatically, the region experiences an average annual temperature range of 18° to 24.5°C (Mandujano *et al.* 2016a). Precipitation patterns within the valley region display an annual average range of 250 - 500 millimeters, primarily concentrated between May to October, with peak precipitation occurring from June to September (CONANP 2013).

For our study, we selected four localities within the state of Oaxaca, namely San Gabriel Casa Blanca (CBL), San Juan Los Cues (LCU), San Juan Bautista Cuicatlán (CUI), and San Pedro Chicozapotes (CHI) (Figure 1). The dominant vegetation types in CBL and LCU comprise crassicaule thicket and dry tropical forest (Scrub-TDF), characterized by a prevalence of cacti from the *Neobuxbamia* genus. In

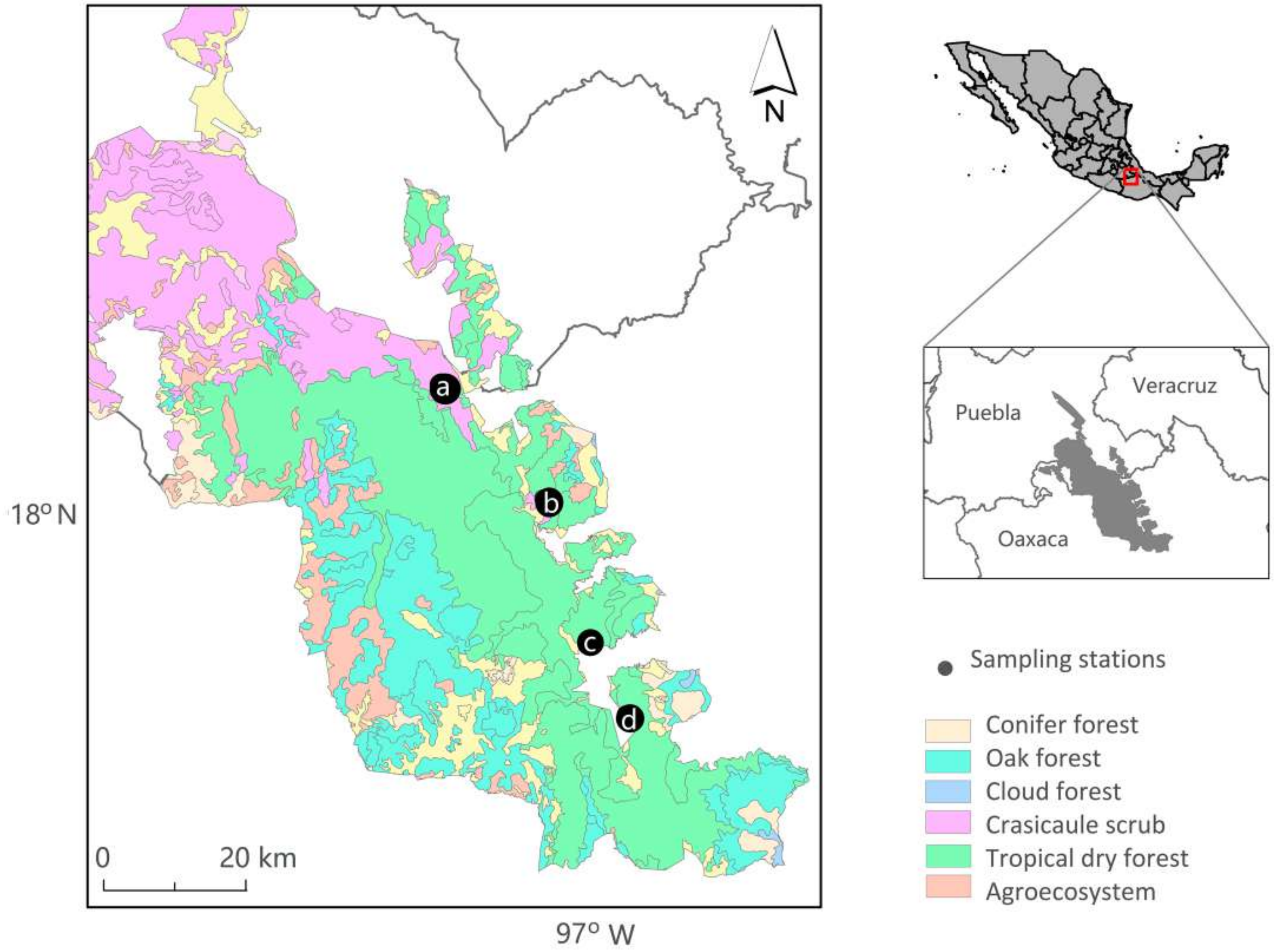

Figure 1. Geographical localization of the Tehuacán-Cuicatlán Biosphere Reserve, the main types of vegetation, and the location of four localities Casa Blanca (A), Los Cues (B), Cuicatlán (C), and Chicozapotes (D) in the region of La Cañada, Oaxaca.

contrast, CUI and CHI are predominantly characterized by tropical dry forests (TDF), featuring a denser and taller tree canopy, and areas with steeper topography (Barrera-Salazar *et al*. 2015). CBL is a site with rough terrain between canyons, hills, and mountains such as Nahualtepec, Cihualtepec, and Petlanco; LCU is located within a plateau in the Cañada region; CUI has a very rugged orography and has large hills; and CHI has a topography made up of alluvial plain, colluvial slope, high hills, and steep slopes and peaks (INAFED 2010). Across all four localities, the primary economic activities consist of agriculture and goat farming, semi-extensive cattle ranching is prevalent in LCU, CUI, and CHI (INEGI 2005).

Data collection. To document the activity patterns of the white-tailed deer, we employed digital camera traps equipped with motion sensors (Cousins Truth Cam 35® and Moultrie Game Spy D-55IR® models). These camera traps were placed in trees, maintaining a consistent height of 20 to 30 cm above ground. To ensure spatial independence, we adhered to a minimum linear distance of 500 meters between each camera. In total, our study encompassed 100 sampling sites, which were distributed across the following periods and localities: 65 sites in CBL, spanning from February 2012 to February 2016; 13 sites in CHI, covering the period from February 2012 to April 2013; 14 sites in CUI, spanning from July 2012 to July 2014; and eight sites in LCU, extending from August 2012 to April 2013. The number of camera sites in CBL and CUI varied across years, maintaining an annual average of 16 and 11 cameras, respectively. All camera traps were programmed to capture a sequence of 3 photographs at intervals of 10 to 15 seconds, remaining operational for a continuous 24-hour cycle. Monthly maintenance checks were conducted to replace storage cards and verify the proper functioning of the camera traps. Subsequently, the collected images were organized and classified using the *camtrapR* package (Niedballa *et al.* 2016). An independent record was considered as a sequence of photographs separated by a minimum time interval of one hour or when distinct individuals were discernible within a shorter period, based on distinctive features such as scars or sexual dimorphism (Peral *et al.* 2022; Suárez López 2023).

Data analysis. Independent records of the white-tailed deer were subjected to kernel density transformation and graphically visualized using the *overlap* package (Ridout and Linkie 2009), and we estimate the deer activity range core (50%) for season, sex, and locality (Oliveira-Santos *et al.* 2013) with the *circular* package (Agostinelli and Lund 2013). We were interested in modeling the activity range core, or the 50% kernel isopleth, since it represents the peaks of activity (Oliveira-Santos *et al.* 2013). Within this analysis, we derived estimations for both mean circular, standard deviation circular parameters and von Mises confidences intervals (90%) with 1000 resamples. We explored potential fluctuations in white-tailed deer behavior corresponding to the species' physiological needs by comparing activity patterns across various dimensions. First, we examined activity patterns during different physiological periods, specifically the rut (November-February), is when the females enter in estrus and the males seek them out to mate; gestation (March-June), is when the males shed their antlers and the females are pregnant; and fawning (July-October), is when the females are lactating and the males begin to grow their antlers (Bello *et al.* 2001). These periods were established by observing the photos before running analysis. We also consider activity as diurnal from 7:00 to 18:59 h, nocturnal from 20:00 to 05:59 h, and crepuscular from 06:00-06:59 and from 19:00-19:59 h. Additionally, we conducted a comparative analysis of activity patterns between male and female deer. Finally, we compared deer activity patterns by locality to determine whether difference in habitat characteristics and anthropogenic pressure influences their activity. For this we obtained the frequency of capture (FC) of livestock, hunters, and free-roaming dogs (*Canis familiaris*) and considered that localities with higher FC for these species have more anthropogenic pressures, while localities with lower FC have less anthropogenic pressures.

For estimate the FC, we use the follow formula:

$$FC = \frac{N\ tot}{Day\ tot} X\ 100\ night\ traps$$

Where:
FC = Frequency of capture
N tot = Number of independent records
Day tot = sampling effort (number of cameras * number of active days of the camera)

The multifaceted comparisons were executed with the Watson's two-sample test (Jammalamadaka and SendGupta 2001) and overlap coefficient in activity range core (Oliveira-Santos *et al.* 2013) from the *circular* package (Agostinelli and Lund 2013), facilitating robust statistical evaluation. All statistical analyses were performed within the R 3.4.1 statistical language (R Core Team 2021).

Results

The total sampling effort was 22,809 trap days, and we obtained a total of 1,656 independent records of white-tailed deer. These records included 736 females, 513 males, and 407 instances where sex could not be identified (Table 1). Across all four study localities, white-tailed deer exhibited predominantly diurnal activity patterns characterized by bimodal peaks occurring from 06:00 to 14:30 h and 16:00 to 19:00 h.

Table 1. Sampling effort, independent records, and frequency of capture, in parentheses, to white-tailed deer, livestock, and illegal hunters.

	Locality				**Total**
	Casa Blanca	**Los Cues**	**Cuicatlán**	**Chicoza-potes**	
Sampling effort	14,649	1,372	3,573	3,215	22,809
Number of camera traps	65	8	14	13	100
Deer records	1,201 (8.20)	238 (17.35)	145 (4.06)	72 (2.24)	1,656 (7.26)
Females	565 (3.86)	126 (9.18)	25 (0.70)	20 (0.62)	736 (3.22)
Males	347 (2.36)	74 (5.39)	58 (1.62)	34 (1.06)	513 (2.25)
Unidentified	289 (1.97)	38 (2.77)	62 (1.74)	18 (0.56)	407 (1.78)
Cattle records	0 (0)	170 (12.39)	447 (12.51)	15 (0.47)	632 (2.77)
Donkey records	0 (0)	0 (0)	193 (5.40)	0 (0)	193 (0.85)
Free domestic dog records	9 (0.06)	0 (0)	55 (1.54)	2 (0.06)	66 (0.29)
Poaching records	0 (0)	1 (0.07)	5 (0.14)	0 (0)	6 (0.03)

During the rut season, deer showed two activity range core (50%) from 06:58 to 12:45 h and from 15:57 to 18:52; in gestation was from 06:00 to 11:19 and from 17:32 to 20:57; while in fawning was from 06:10 to 13:03 and from 15:52 to 17:49 (Figure 2). We found significant differences in the activity hour mean between the rut (11:56 ± 1.73, CI = 11:09-12:49) and gestation (07:47 ± 2.20, CI = 04:12-10:36; Table 2) periods with overlap coefficient of 0.64 in activity range core (50%). Additionally, we found a significant difference in the activity hour mean between the gestation and fawning (11:16 ± 1.67, CI = 10:37-11:57; Table 2) periods with overlap coefficient of 0.63 in activity range core (50%). During gestation, deer showed less activity between 11:20 and 17:00 h and increased nocturnal activity, whereas during the fawning season, deer were more active during the daytime (Figure 2). We did not find significant differences in the activity hour mean between the rut and fawning season and, overlap coefficients in activity range core (50%) was 0.86.

Table 2. Comparison of activity pattern of white-tailed deer by locality, sex, and season. Watson test (U^2).

Season / Sex / Locality	**U^2**	**p**
Rut - Gestation	0.70	0.001*
Rut - Fawning	0.12	0.10
Gestation - Fawning	0.67	0.001*
Female - Male	0.20	<0.05*
Casa Blanca - Los Cues	0.22	<0.05*
Casa Blanca - Cuicatlán	0.08	0.10
Casa Blanca - Chicozapotes	0.06	0.10
Los Cues - Cuicatlán	0.22	<0.05*
Los Cues - Chicozapotes	0.09	0.10
Cuicatlán - Chicozapotes	0.09	0.10

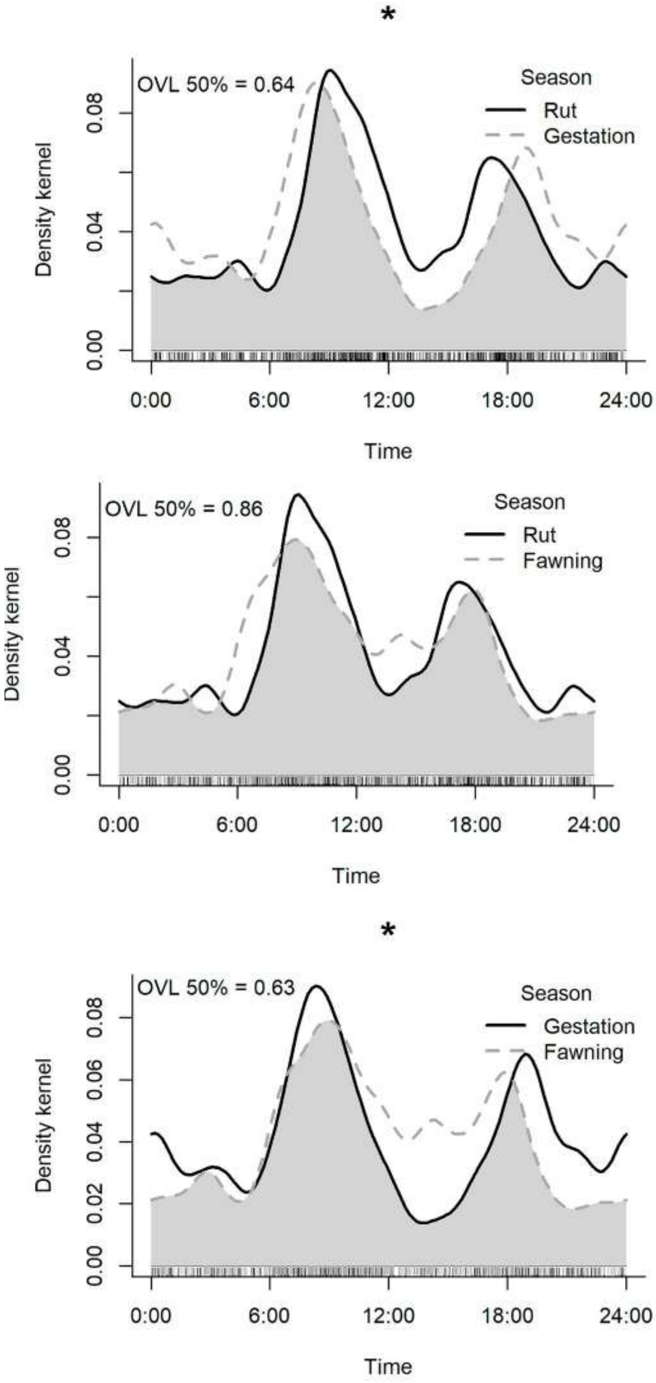

Figure 2. White-tailed deer activity pattern for physiological season in TCBR. The * indicates statistically significant differences between activity hours means and OVL 50% is overlap activity range core (50%).

In terms of sex-based comparisons, both sexes showed two activity range core (50%): females one activity range core from 06:37 to 12:22 h and the other from 16:07 to 18:48 h, while males one activity range core from 06:00 to 13:09 h and the other from 16:16 to 17:57 h (Figure 3). Our analysis indicated that activity hour means of females (12:00 ± 1.65, CI = 11:09-12:31) were statistically different when compared to males (10:20 ± 1.78, CI = 09:28-11:18; Table 2) and overlap coefficients in activity range core (50%) was 0.85.

In three localities (CBL, LCU, and CUI), deer also showed two activity range core (50%), one in the morning and another in the afternoon, while they were unimodal in CHI. Specifically, deer was active from 06:31 to 12:22 h and from 16:01 to 19:07 h in CBL; from 06:00 to 12:16 h and from 16:55 to 17:37 h in LCU; from 06:02 to 13:06 h and from 17:50 to 20:20 h in CUI; and from 06:00 to 14:34 h in CHI (Figure 4). Furthermore, we found significant statistical differences in activity hour mean between CBL (11:47 ± 1.81, CI = 11:09-12:29) and LCU (10:02 ± 1.57, CI = 09:05-11:09) with overlap coefficient of 0.75 in activity range core (50%), as well as between CUI (11:10 ± 1.91, CI = 09:01-13:52) and LCU with overlap coefficient of 0.62 in activity range core (50%) (Table 2). We did no find significant statistical differences were observed with CHI (10:03 ± 1.76, CI = 07:33-12:45) and overlap coefficients in activity range core (50%) were 0.67 (CBL), 0.73 (LCU) and 0.78 (CUI).

Discussion

White-tailed deer show remarkable adaptability to habitats with varying environmental and ecological conditions, as temperate, semi-arid, and tropical regions (Ortega-Santos *et al.* 2011; Gallina *et al.* 2019). Generally, deer are regarded as crepuscular because they tend to exhibit greater activity during dawn and dusk, consistent with a behavioral strategy aimed at conserving energy and water during extreme temperature hours (Beier and McCullough 1990; Kammermeyer and Marchinton 1977). In regions characterized by marked seasonality, deer adjust their activity patterns based on the availability and quality of food resources. During periods of abundant food, they allocate more time to movement and foraging, whereas with of food scarcity, they increase hours of digestion to conserve energy (Holzenbein and Schwede 1989; Webb *et al.* 2010; Massé and Côté 2013; Gallina and Bello 2014).

Our results show that white-tailed deer is mainly diurnal in TCBR, an activity pattern that contrasts with the results of the studies previously mentioned. These differences could be related to habitat characteristics, such as type of vegetation, temperature, and anthropogenic pressure. For example, our study area does not present such marked changes in temperature unlike the other studies (North of country, United States, and Canada); the predominant vegetation is dry tropical forest, while that other studies are closed forest, open forest, swamp, or scrub (Holzenbein and Schwede 1989; Webb *et al.* 2010; Massé and Côté 2013; Gallina and Bello 2014). These factors possibly benefit diurnal activity because the vegetation cover may protect against heat during the rainy season, and some plants species offer water sources for deer during the dry season, when water availability decreases (Arceo *et al.* 2005; Ramos-Robles *et al.* 2013). Another important characteristic of our study area, which could have influenced deer activity, was the presence of poachers, cattle, and free-roaming dogs, of the four localities, only one (CBL) had few records of this kind. Moreover, camera trapping studies have also reported that this specie presented greater diurnal activity in tropical dry forest and other types of habitats (Hernández-Saintmartín *et al.* 2013; Soria-Díaz and Monroy-Vilchis 2015;

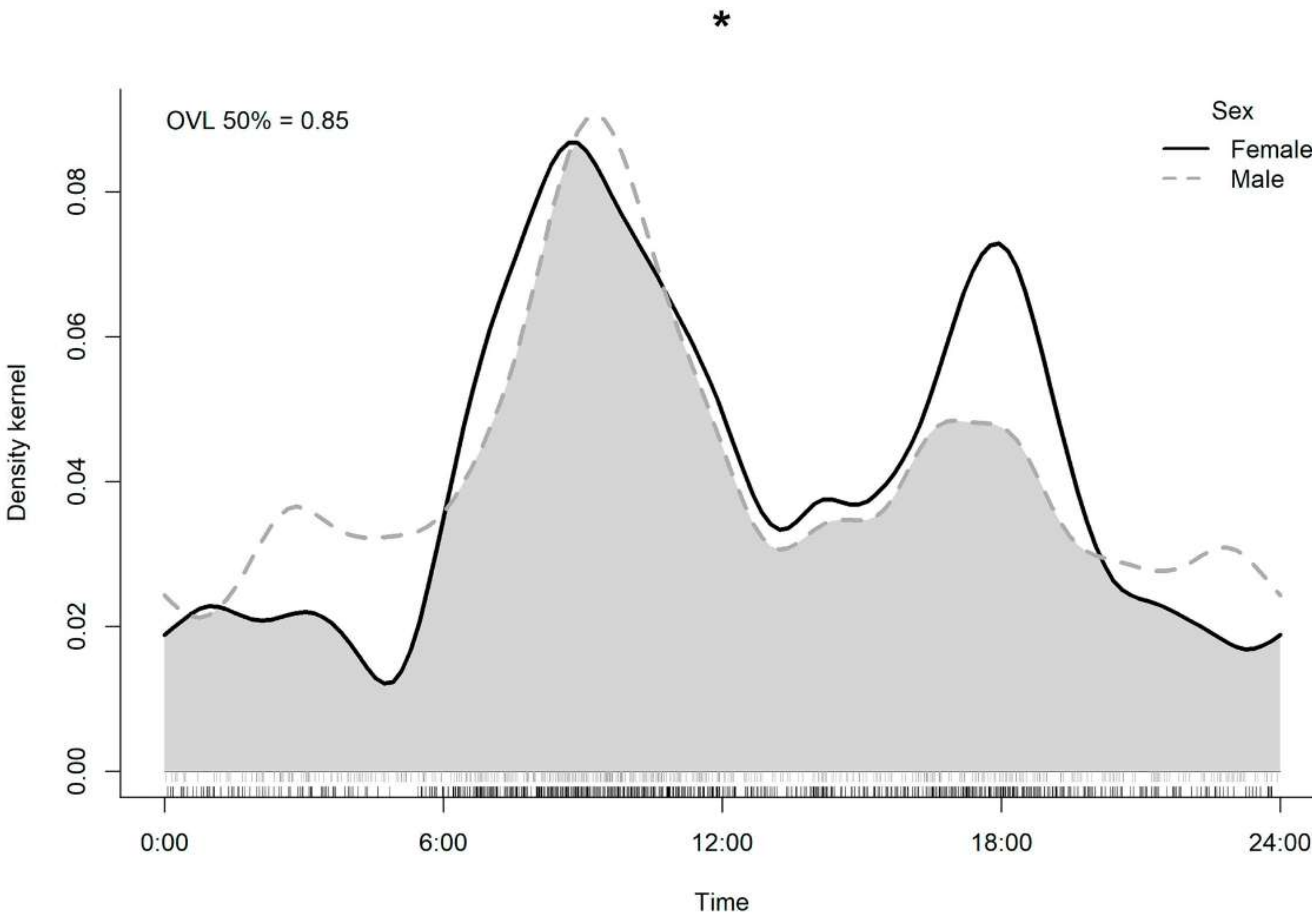

Figure 3. Females and males white-tailed deer activity pattern in the TCBR. The * indicates statistically significant differences between activity hours means and OVL 50% is overlap activity range core (50%).

Higdon *et al.* 2019), including our preliminary study in the TCBR (López-Tello *et al.* 2015).

Across all three physiological periods (rut, gestation, and fawning), the white-tailed deer consistently displayed diurnal activity patterns, with varying expressions of their activity peaks. Moreover, during gestation, deer showed greater crepuscular activity supporting our prediction that deer would be more active during this period to avoid predation, because it is crucial in this life stage. In addition, we found statistically significant differences and low overlap coefficient in activity range core between the rut and gestation periods, as well as between fawning and gestation. During the gestation period, deer decreased their activity range core in the morning, whereas was greater in rut and fawning, also showed less activity range core in the afternoon during fawning. These findings agree with prior studies that suggest variations in deer activity across physiological periods (Kammermeyer and Marchinton 1977; Massé and Côté 2013). Demands to search for mates (in the case of males) or raise offspring (in the case of females) can be influenced mainly by changes in vegetation and predator activity (Cueva-Hurtado et al. 2024). For instance, female deer decrease their activity and movement during gestation, while raising their young to minimize predation risk (Holzenbein and Schwede 1989; Beier and McCullough 1990). In contrast, males increased their activity during the mating season, as they actively seek potential mates (Holzenbein and Schwede 1989; Beier and McCullough 1990).

In the TCBR, the primary predator of white-tailed deer is the puma (*Puma concolor*), a species classified as cathemeral, showing greater activity during nighttime hours (Monroy-Vilchis *et al.* 2009; Hernández-Saintmartín *et al.* 2013; Ávila-Nájera *et al.* 2016). While the coyote can prey on the fawn during nighttime hours, especially in the fawning season (Crawford *et al.* 2021). We did not obtain sufficient puma and coyote records to compare their activity peaks with those of white-tailed deer. However, we do not discard that puma and coyote activity likely influence deer behavior. To mitigate predation risk, deer may show more activity in the morning, when its main predators are less active.

Our results showed that activity patterns of female and male deer, in general, were similar and overlap coefficient in activity range core was high. However, we found significant differences between their mean activity hour and von Mises confidence intervals were narrow. The activity range core of males was greater in the morning than that of females and presented less activity during the sunset, while females

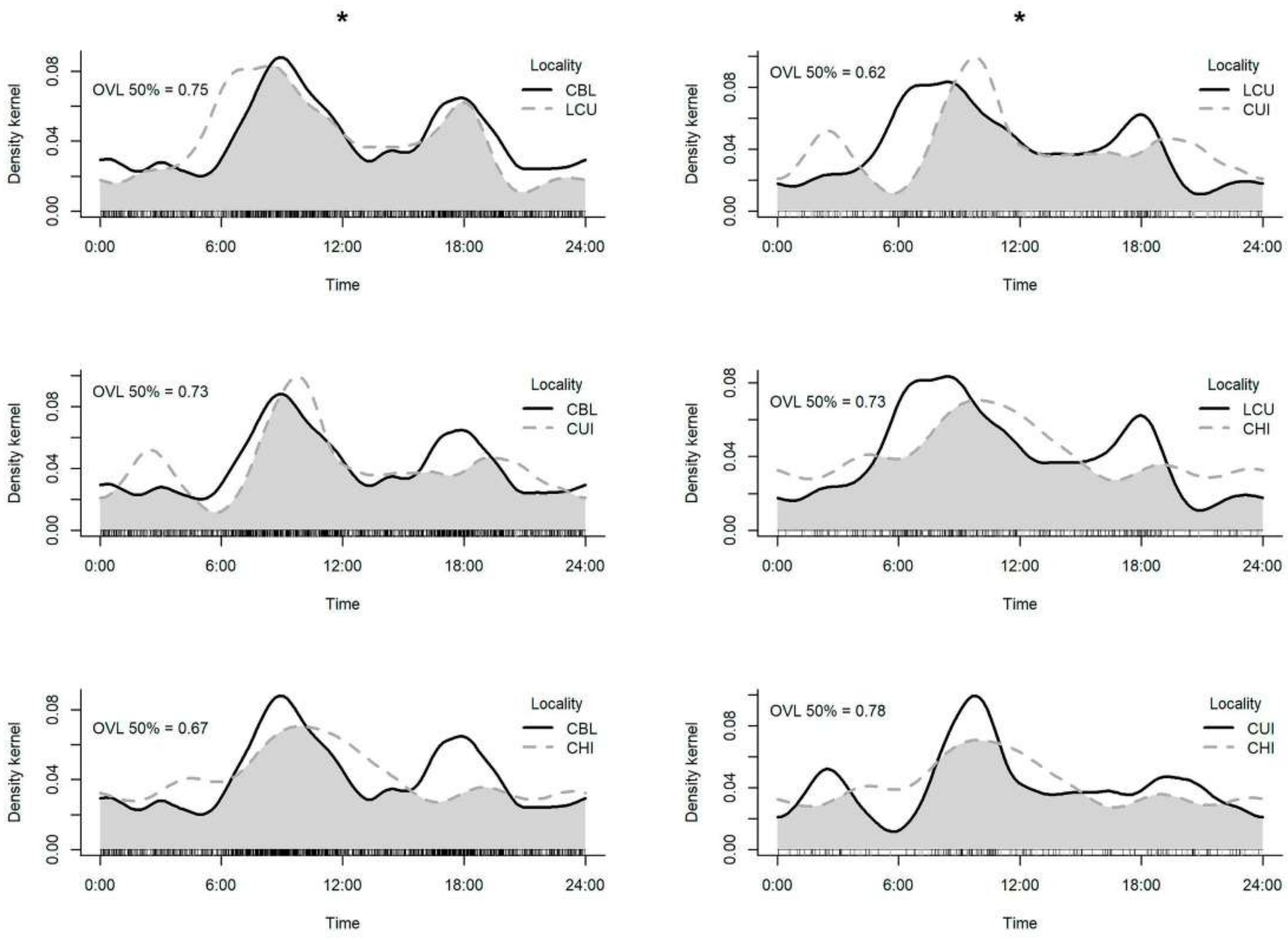

Figure 4. White-tailed deer activity pattern for locality in TCBR. Abbreviations: Casa Blanca (CBL), Los Cues (LCU), Cuicatlán (CUI) and Chicozapotes (CHI). The * indicates statistically significant differences between activity hours means and OVL 50% is overlap activity range core (50%).

displaying peak activity at noon. These results do not agree with our prediction since males had less nocturnal activity, but it suggest that sex-specific physiological requirements play a crucial role. This phenomenon agrees with studies emphasizing sexual dimorphism and metabolic demands, as females necessitate higher-quality food resources and, consequently, allocate more time to foraging (Beier and McCullough 1990; Gallina and Bello 2014). However, some studies have reported similar activity patterns between male and female deer, with distinctions emerging in specific seasons (Beier and McCullough 1990; Gallina *et al.* 2005; Webb *et al.* 2010; Cueva-Hurtado et al. 2024). We did not analyze sex-specific differences within each physiological season due to limited identification in some independent records. However, our results suggest that differences in activity between sexes in the TCBR could be linked to their metabolic requirements and physiological seasons. Females may maintain higher daytime activity during the mating and fawning seasons, coinciding with increased rainfall and vegetation cover, while reducing daytime activity during gestation to avoid exposure to high temperatures when vegetation cover decreases (Beier and McCullough 1990; Webb *et al.* 2010). In addition, other studies have shown that females decrease their activity and movements before parturition due to the decrease in food availability and quality, as well as to avoid predation (Sánchez-Rojas *et al.* 1997; Gallina and Bello 2014). Meanwhile, males increased their movements during rut in search of receptive females (Webb *et al.* 2010), maybe this could explain their activity during the morning, as well as to avoid predators and illegal hunters.

Our results indicate that the white-tailed deer's activity pattern in the TCBR is mainly diurnal across all four study localities, being bimodal in three localities (CBL, LCU, CUI), and unimodal in CHI. However, we found significant differences in deer activity hour means between LCU and CBL, as well as between LCU and CUI. In LCU, the deer activity range core was more restricted in general and in the afternoon compared to CBL and CUI. Moreover, in CUI, the locality with more anthropogenic pressure (measured by greater frequency of captures by domestic species and poachers), deer presented more activity in sunset and night hours. Although the von Mises confidence intervals in CUI were wide and overlap with confidence intervals of LCU, we suggest that the difference between localities should are related to habitat characteristics and anthropogenic pressure, because LCU y CUI presented more open vegetation, higher shrubs, and greater proximity to main roads and agroecosystems unlike CBL (López-Tello 2014). In CBL primarily has goat farming and poaching has decreased due to the establishment of a legal white-tailed deer hunting management unit (UMA by its acronym in Spanish) in 2012 (Mandujano et al. 2016b). Our results are consistent with our prediction and other studies that have been demonstrated that these anthropogenic factors can alter the activity patterns of wildlife species, as they tend to reduce their activity during hours with more human or domestic animal activity to avoid encounters (Kilgo *et al.* 1998; Wang *et al.* 2015).

In conclusion, our study suggests that white-tailed deer in the TCBR exhibit diurnal activity patterns, and their activity peaks are related mainly to sex and physiological periods since their metabolic demands change during different periods of the year, therefore, validating our hypotheses. Also, we found that the activity can be influenced by anthropogenic factors, such as poaching and the presence of domestic species, since deer activity was more nocturnal and crepuscular in CUI, the most disturbed area. However, we suggest that it is important to increase the sampling effort in CHI, CUI and LCU to understand how these variables influence the deer activity pattern. It would also be valuable to obtain a greater number of records of the puma, coyote, domestic species (free-roaming dogs and livestock) and humans to compare their activity patterns against those of deer. Overall, our research provides new insights into the biology and behavior of white-tailed deer in the tropical dry forest of the Tehuacán-Cuicatlán Biosphere Reserve.

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Medium and large terrestrial mammals in an area voluntarily designated for conservation in the northern Yucatan Peninsula, Mexico

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Conservation areas designated voluntarily are part of a government strategy to protect biodiversity in Mexico and are important for the conservation of medium-sized and large mammals. The objective of this study was to contribute to the knowledge of the diversity of medium- and large-sized mammals in the Voluntarily Designated Conservation Area in the state of Yucatán, Komchén de los Pájaros. Activity patterns and species under some risk category were determined, as well as estimates of species richness, diversity, the relative abundance index of each species. Systematic sampling was conducted from December 2019 to December 2021, selecting four permanent sites and simultaneously carrying out non-systematic sampling The relative abundance index and sampling effort were calculated with non-parametric estimators, Chao 1 and Chao 2, using the EstimateS program. The species accumulation curve was constructed, and the activity patterns were established. Histograms with hours of activity were obtained using the Oriana Version 4.0 software. Fifteen mammal species were identified with a sampling effort of 4256 trap nights, 33 % of which were protected. The independent records and relative abundance index prioritized *Nasua narica, Odocoileus virginianus*, and *Urocyon cinereoargenteus*. Fourteen species were recorded in the northern season, six during rainfall, and six were permanent. The pattern of activity was obtained, being diurnal in *N. narica*, nocturnal in *Didelphis virginiana*, *Dasypus novemcinctus*, *Sylvilagus yucatanicus*, *Canis latrans,* and *Procyon lotor,* and cathemeral in *U. cinereoargenteus* and *O. virginianus*. Photo trapping not only allowed us to know mammal diversity, but also to document their behavior and determine their activity patterns (diurnal, nocturnal, and cathemeral) and degree of sociability (group or solitary). Preserving this habitat is essential to protect regional diversity, particularly for mammals currently threatened by various anthropogenic activities.

Key words: activity patterns, conservation island, diversity, photo records, relative abundance, risk categories, Yucatan Peninsula.

Las áreas destinadas voluntariamente a la conservación forman parte de una estrategia gubernamental para proteger la biodiversidad en México y son importantes para la conservación de mamíferos de talla mediana y grande. El objetivo de este estudio fue contribuir al conocimiento de la diversidad de mamíferos medianos y grandes en el área destinada voluntariamente a la conservación en el estado de Yucatán. Se determinaron los patrones de actividad y las especies en alguna categoría de riesgo, así como estimar la riqueza, diversidad y el índice de abundancia relativa de cada especie. El muestreo sistemático se llevó a cabo de diciembre de 2019 a diciembre de 2021, seleccionando cuatro sitios permanentes y realizando simultáneamente muestreos no sistemáticos. El índice de abundancia relativa y el esfuerzo de muestreo se calcularon con los estimadores no paramétricos Chao 1 y Chao 2, utilizando el programa EstimateS. Se construyó la curva de acumulación de especies y se establecieron los patrones de actividad. Se obtuvieron histogramas con horarios de actividad empleando el software Oriana versión 4.0. Se identificaron 15 especies de mamíferos con un esfuerzo de muestreo de 4256 días-trampa, de las cuales 33 % estuvieron bajo alguna categoría de protección. Los registros independientes y el índice de abundancia relativa destacaron a *Nasua narica, Odocoileus virginianus* y *Urocyon cinereoargenteus*. Se registraron 14 especies en la temporada de nortes, seis durante lluvias y seis fueron permanentes. Se obtuvieron los patrones de actividad, siendo diurno en *N. narica*; nocturnos en *Didelphis virginiana*, *Dasypus novemcinctus*, *Sylvilagus yucatanicus*, *Canis latrans,* and *Procyon lotor,*; y catemerales en *U. cinereoargenteus* y *O. virginianus*. El fototrampeo no solo permitió conocer la diversidad de mamíferos, sino también documentar su comportamiento y determinar sus patrones de actividad (diurnos, nocturnos y catemerales) y su grado de sociabilidad (gregarios o solitarios). Conservar este hábitat es fundamental para proteger la diversidad regional, particularmente de los mamíferos amenazados actualmente por diversas actividades antropogénicas.

Palabras clave: categorías de riesgo, diversidad, isla de conservación, fotoregistros, abundancia relativa, patrones de actividad, Península de Yucatán.

In the Yucatán Peninsula, the presence and distribution of 152 mammal species have been documented; of these, 123 species are terrestrial mammals, belonging to 89 genera, 29 families, and 11 orders (Sosa-Escalante *et al.* 2013), representing approximately 26 % and 2 % of the terrestrial mammal fauna recognized for Mexico and the world, respectively (Ramírez-Pulido *et al.* 2005; Wilson and Reeder 2005). The state of Yucatan has 128 species of mammals, of which 99 are terrestrial, grouped into 78 genera, 29 families, and 11 orders (Sosa-Escalante *et al.* 2014); 36 are of medium size, and five are considered large. Of the total species in this area, 22 are at risk (three subject to special protection, 10 threatened, and nine endangered) according to the Mexican standard NOM-059-SEMARNAT-2010 (SEMARNAT 2010, 2019); the Convention on International Trade in Endangered Species (CITES 2025), lists 12 species (seven in Appendix I and five in Appendix II; https://cites.org/sites/default/files/esp/app/2022/S-Appendices-2022-06-22.pdf) and the International Union for Conservation of Nature (IUCN 2025) lists 96 species (three threatened, two vulnerable, three endangered, one extinct, 87 least concern).

There is a marked variation in mammalian body size and morphology (Arita and Figueroa 1999), traits considered for the various classifications of mammals (Rumiz *et al.* 1998; Garmendia *et al.* 2013; Hernández-Pérez *et al.* 2015; Coronel-Arellano *et al.* 2018; Hernández *et al.* 2018; Pérez-Solano *et al.* 2018; Ruiz-Gutiérrez *et al.* 2020); particularly, body size is of interest in this work to classify mammals into medium-sized (1–30 kg) and large (>30 kg; Pineda-Muñoz *et al.* 2016).

A central aspect in the study of mammals is the direct observation of individuals under natural conditions; however, many species are difficult to observe due to their behavior patterns, low densities, and elusiveness (Chávez *et al.* 2013). For this reason, one of the techniques most widely used worldwide for studying large and medium-sized mammals is photo trapping, which consists of the use of camera traps that are automatically activated when they detect movement or temperature changes, recording the species distributed in the area of interest (Chávez *et al.* 2013; Hernández-Pérez *et al.* 2015).

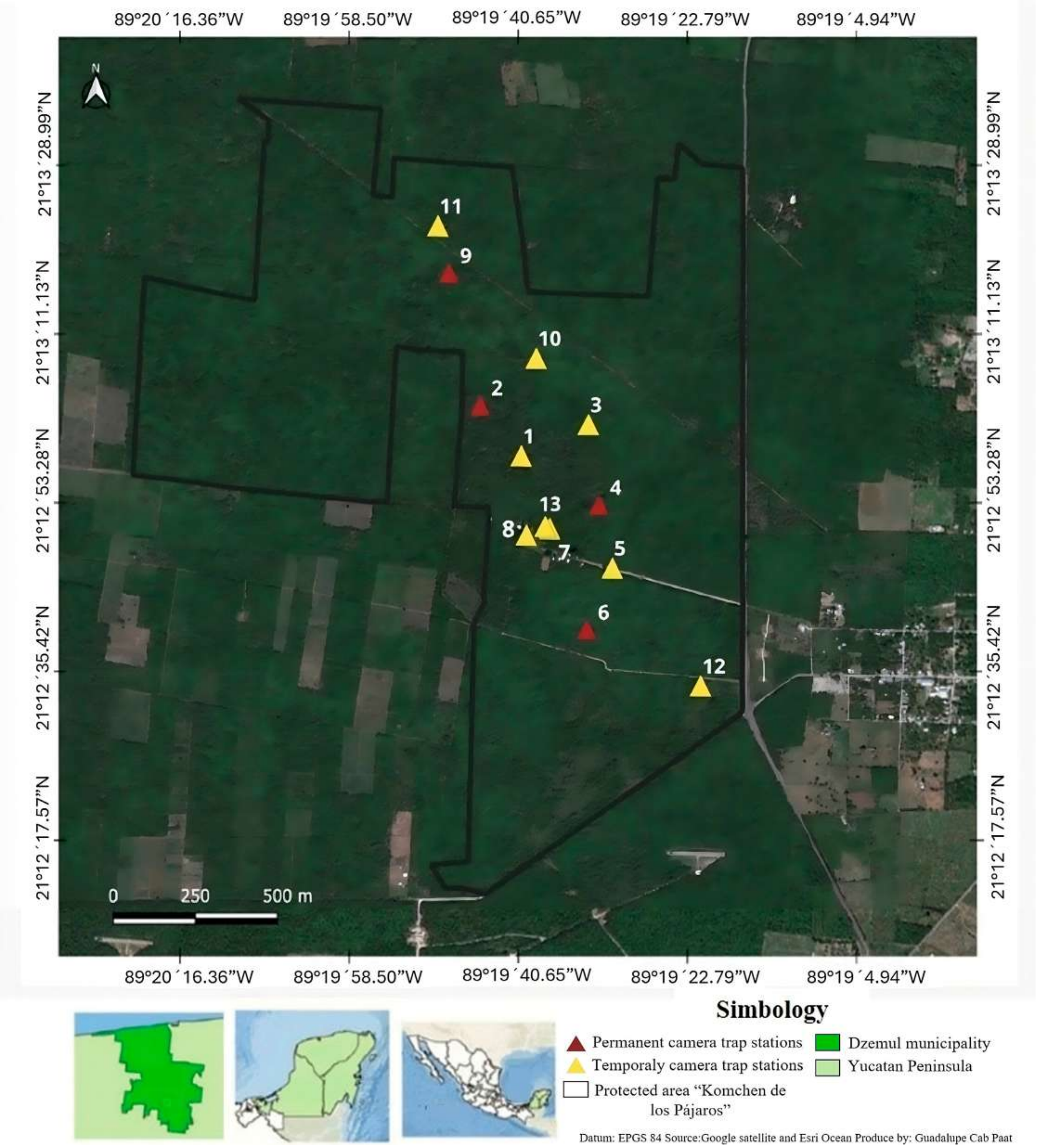
After Mandujano (2019), which summarizes the use of camera traps in Mexico, at least 19 formal publications have emerged that employed photo trapping as a primary or supplementary method for obtaining data or records of the presence of medium-sized and large mammal and bird species in Mexico. This study reports 34 species of mammals and six species of birds in the title of 188 documents as of 2017. For mammals, the most studied species were *Panthera onca, Leopardus pardalis, Puma concolor, Tapirus bairdii, Tayassu pecari, Odocoileus virginianus,* and *Cuniculus paca*; for birds, *Penelope purpurascens* was the species most frequently studied. Additionally, it mentions that the topics covered include the expansion of the geographic range, species diversity, estimates of relative abundance and population density using capture-recapture, activity patterns between species, and ecological interactions.

Inventories carried out with the photo-trapping technique for studies focused on diversity (Hernández-Pérez *et al.* 2015; Coronel-Arellano *et al.* 2018), population ecology and activity patterns (Monroy-Vilchis *et al.* 2009; Hernández-Hernández *et al.* 2018) for large and mid-sized mammals represent a reliable and non-invasive tool (Silveira *et al.* 2003; Monroy-Vilchis *et al.* 2009); when these are combined with the use of attractants (artificial drinking fountains and olfactory stimuli), they are highly efficient to detect both diurnal and nocturnal animals, and even cryptic, rare and evasive species that are hard to detect with other techniques.

Other works that demonstrate the effectiveness of photo trapping include those of Hidalgo-Mihart *et al.* (2017). These authors used camera traps to inventory medium-sized and large mammals in the Laguna de Términos and Pantanos de Centla wetlands, in southeastern Mexico, which are highly complex and hard-to-access habitats, recording 30 native species, including *Cuniculus paca*, five feline species, *Lontra longicaudis, Eira barbara,* and an introduced species, *Sus scrofa*.

Over the years, several studies on terrestrial mammals have been carried out within the peninsula and the state of Yucatan. A recent work at the peninsular scale using photo trapping is that of Hernández-Pérez *et al.* (2015). At the local level in the state of Yucatán, several studies have also employed photo trapping (Hernández-Betancourt *et al.* 1996; Faller-Méndez *et al.* 2005; Mejenes-López *et al.* 2021; Peláez-Cruz *et al.* 2022). Additionally, recent studies have employed other techniques for recording diversity (Balam-Ballote *et al.* 2020; Cimé-Pool *et al.* 2020). The results of these studies provide information required to confirm the presence of mammal species in unexplored areas; additionally, there are projects aimed at identifying suitable zones for maintaining viable populations in the long term (Hernández-Pérez *et al.* 2015). Recent contributions focusing on confirming mammal diversity at local and state levels enrich the information on this group for the state of Yucatan; the latest update of the taxonomic list was contributed by Sosa-Escalante *et al.* (2014). However, the great extension and diversity of the state of Yucatan require focusing research efforts on the diversity of mammals in Protected Natural Areas (private, municipal, state, and federal) and on the evaluation of priority areas for ecological restoration (Sosa-Escalante *et al.* 2013). These would provide solid scientific ground for the design of strategies, since mammals should not be a focal group only because of their market or use value but should be considered marker groups for assessing the health status of habitats (Sosa-Escalante *et al.* 2014), with particular emphasis on the species listed in a risk category.

According to their activity patterns, mammals are classified into diurnal, nocturnal, crepuscular, and catameral. Diurnal species are active mainly during daylight hours; nocturnal species are active mainly at night; crepuscular species show peak activity during sunrise and sunset; and catameral species distribute their activity evenly



Datum: EPGS 84 Source:Google satellite and Esri Ocean Produce by: Guadalupe Cab Paat

Figure 1. Photo trapping stations and geographical location of the conservation area designated voluntarily Komchén de los Pájaros, Dzemul, Yucatán, Mexico.

throughout the 24-h cycle or at peaks of activity, particularly regarding feeding or displacements, in both daytime and at night (Tattersall 2006). These patterns are useful for understanding how species utilize their local environment, predicting the times and seasons with the highest impact due to hunting, and contributing to management elements for the conservation of the area.

For the above, this work aims to contribute to the knowledge of the local diversity of medium-sized and large mammals in a conservation area designated voluntarily (AVDC, in Spanish) in the state of Yucatán to highlight it as a wildlife refuge; particularly, our objectives are to estimate the diversity and relative abundance index (RAI) for each species, species richness using Chao 1 and 2,

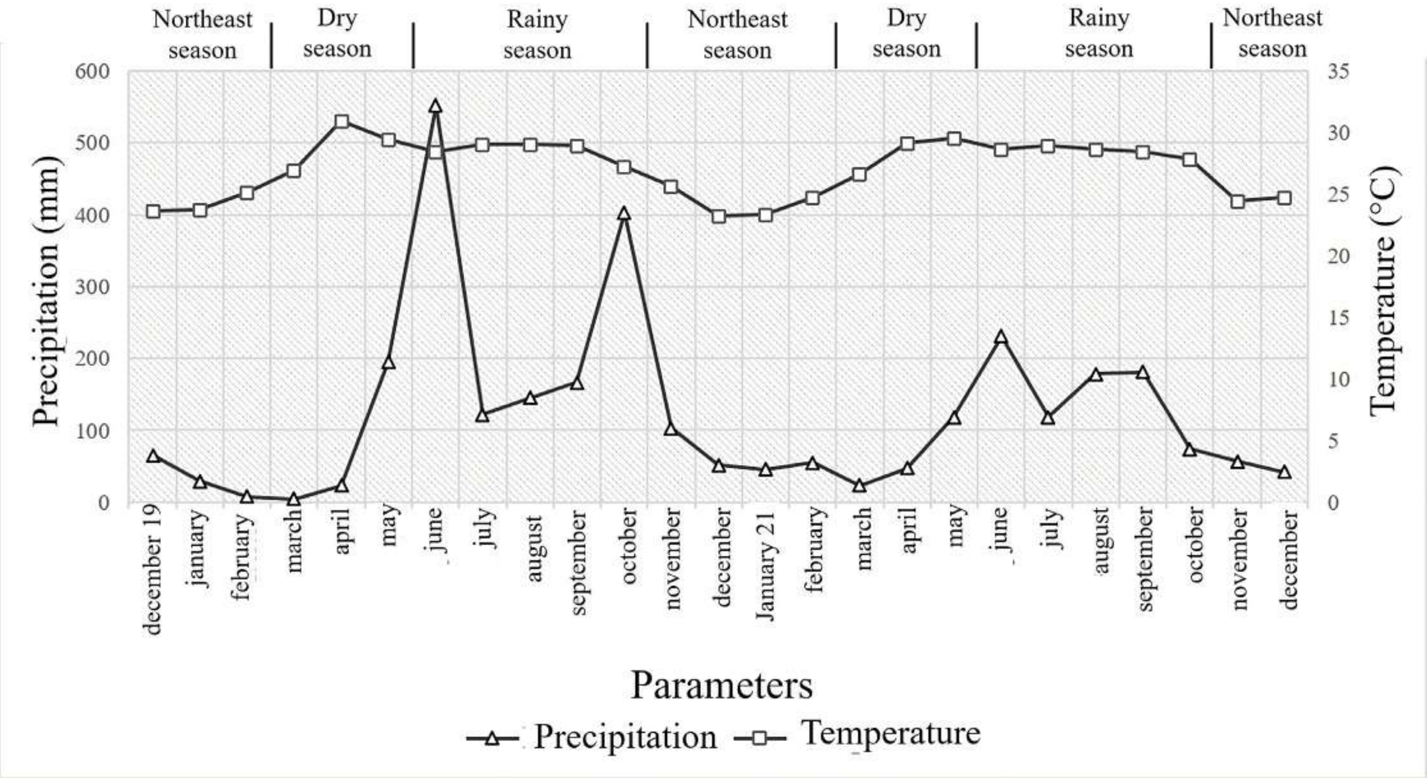


Figure 2. Climate chart with monthly mean temperature and precipitation from 2020 to 2021 in the conservation area designated voluntarily Komchén de los Pájaros, Dzemul, Yucatán, Mexico.

and determine the species in risk categories, and activity patterns using the photo trapping technique.

Materials and methods

Study area. The voluntary designated for conservation area (AVDC) named Komchén de los Pájaros (KdlP) is located at kilometer 1.5 of the Dzemul-Xtampú road pass, south of the municipality of Dzemul, in the north of the state of Yucatán (Figure 1); it has an area of 300 ha within an altitude range of 0 to 15 m (Rzedowski 1978; INEGI 2005; Morrone 2005). The soil type is Leptosol with outcrops of limestone rocks and slabs, well drained, and with little moisture retention (Flores and Espejel 1994; Olmstead *et al.* 1995; INEGI 2009). The local vegetation is a low deciduous tropical forest, characterized by trees less than 15 m in height with non-thorny trunks and that shed their leaves in the dry season; the dominant species belong to the family Fabaceae (Miranda and Hernández-X. 1963), with the presence of columnar cacti (*Pterocereus gaumeri* and *Nopalea gaumeri*; Flores and Espejel 1994). The regional climate is warm and semi-dry, with a mean annual temperature ranging from 18 °C to 24 °C, and a mean annual precipitation between 700 mm and 1200 mm (Miranda and Hernández-X. 1963; Holdridge *et al.*1971). The climate seasons for the study area were defined more accurately by reviewing the annual temperature and precipitation summaries for the sampling period (2020, 2021) based on data recorded by the Telchac Puerto weather station obtained from CONAGUA (2020, 2021). In those years, the mean annual temperature was 27.3 °C and 27.1 °C, and the mean annual precipitation was 1802.2 mm and 1172.6 mm, respectively. These characteristics confirm the temporal pattern reported by Herrera-Silveira (1994) for the Yucatán peninsula, where the dry season occurs from March to May, in which temperature is high and precipitation is extremely low; it is followed by the rainy season from June to October, with high precipitation and lower temperatures; finally, the "*nortes*" season occurs from November to February, recording the lowest temperatures and precipitation (Figure 2).

Fieldwork. Sampling was carried out from December 2019 to December 2021, using eight camera traps, one Moultrie (model M-4000), one Primo (model 63053), three Bushnell HD (model 119876), and three CuddeBack (model h-1453). The sites were selected based on accessibility (trails) and water availability (artificial water fountains and cenotes) to capture the highest species diversity (O'Brien *et al.* 2011). As a systematic sampling, we selected four permanent sites (positions 2, 4, 6, and 9; Figure 1); at the same time, we performed random sampling by installing four temporary cameras for variable periods of time at each site (positions 1, 3, 5, 7, 8, 10, 11, 12, and 13; Figure 1), selected based on the previous finding of indirect records of mammals (stools/feces or footprints). Permanent sites 2 and 6 functioned as dual stations for six months (January–July 2021). Camera traps were installed on live tree trunks approximately 0.5 m above the ground, each along trails near water sources. Each camera trap was set to produce three consecutive shots when activated and operated for 24 h, until the batteries were depleted. Camera traps were reviewed once a week, and the cards were replaced each month. Camera traps

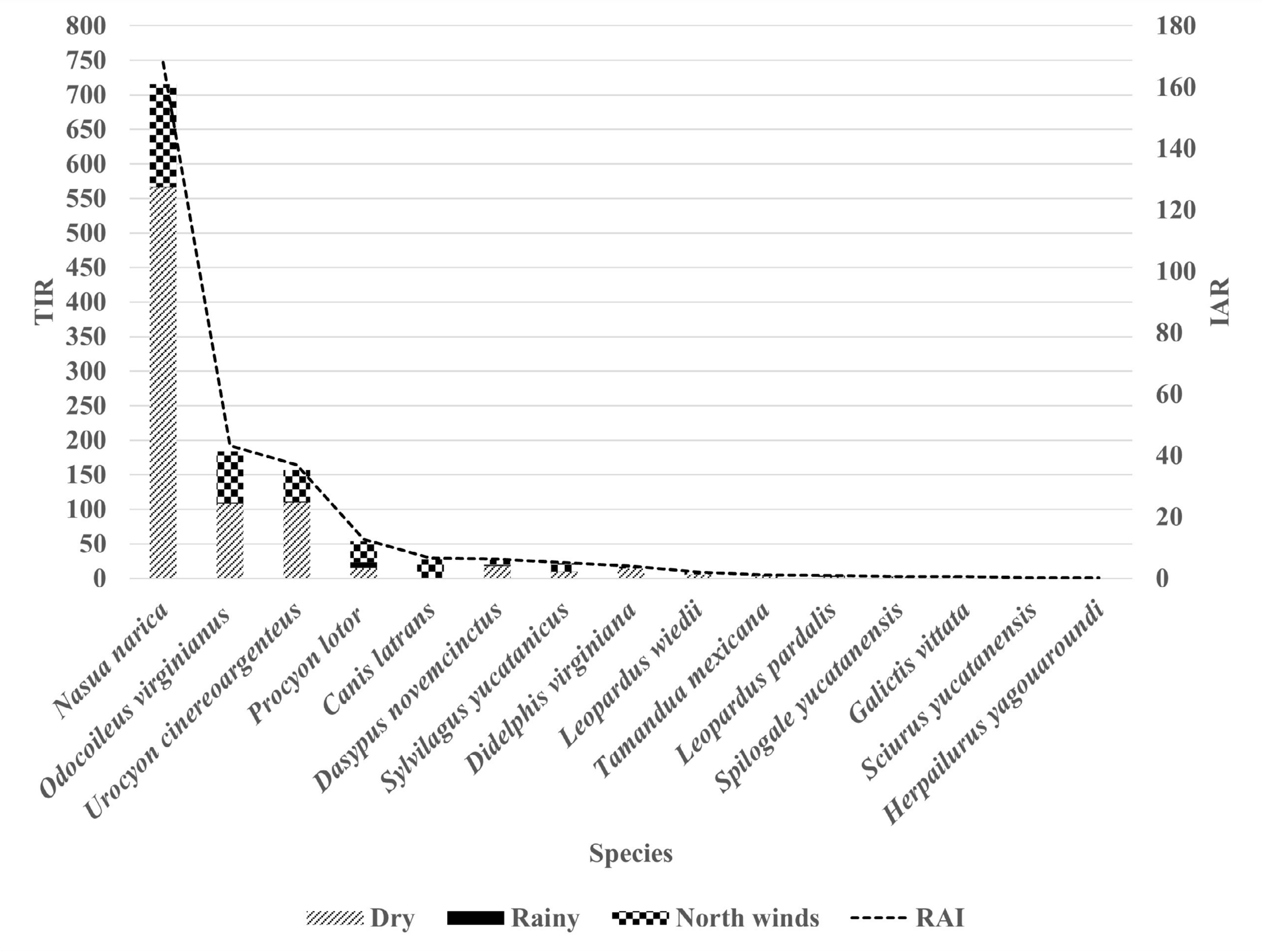


Figure 3. Total independent records (TIR) and Relative Abundance Index (RAI) for each species recorded in the conservation area designated voluntarily Komchén de los Pájaros, Dzemul, Yucatán, Mexico.

were in operation for 89 weeks. We divided the sampling into three periods due to the flooding of the study area in early May, leading to the following periods: first period, from December 2019 to May 2020, with seven camera traps and covering a total of 20 effective weeks; second period, from June to December 2020, with only four camera traps and covering a total of 20 effective weeks; and third and last period, from January to December 2021, with eight camera traps operating for 48 effective sampling weeks.

The sampling effort was calculated using a formula that considers the number of camera traps multiplied by the number of sampling days (Medellín *et al.* 2006; Lira-Torres and Briones-Salas 2012). This formula was adapted to meet the project needs, as the sampling time was divided into periods. We first calculated a partial sampling effort per period, and then the three results were added to obtain the total effort (SE_T): $SE_T = SE_{P1} + SE_{P2} + SE_{P3}$. The relative abundance index (RAI; Medellín *et al.* 2006; Lira-Torres and Briones-Salas 2012) was calculated with the formula $RAI = C/SE^{*}$ 1000 trap nights, where C = number of captures or independent photographed events, SE = sampling effort (number of camera traps multiplied by number of monitoring days), and 1000 trap nights = (standard unit).

Office work. In order to estimate the total number of independent records (TIR), to avoid counting the same individual several times, only the following cases were considered independent photo captures: 1) consecutive photographs of different individuals; 2) consecutive photographs of individuals of the same species separated by more than 24 h; this criterion was applied when it was not clear whether a series of photographs corresponded to the same individual; 3) non-consecutive photographs of individuals of the same species (Medellín *et al.* 2006; Monroy-Vilchis *et al.* 2011; Lira-Torres and Briones-Salas 2012). Photo-recorded species were determined by comparison with several guides (Reid 1997; Ceballos and Oliva 2005; Aranda 2012), and scientific names were standardized according to Ramírez-Pulido *et al.* (2014). An Excel data matrix was constructed (Microsoft Windows 10) entering the data of records selected as independent captures (scientific name, common name, number of individuals, year, season (rainy, dry, or "*nortes*"), photo-capture date and time, coordinates

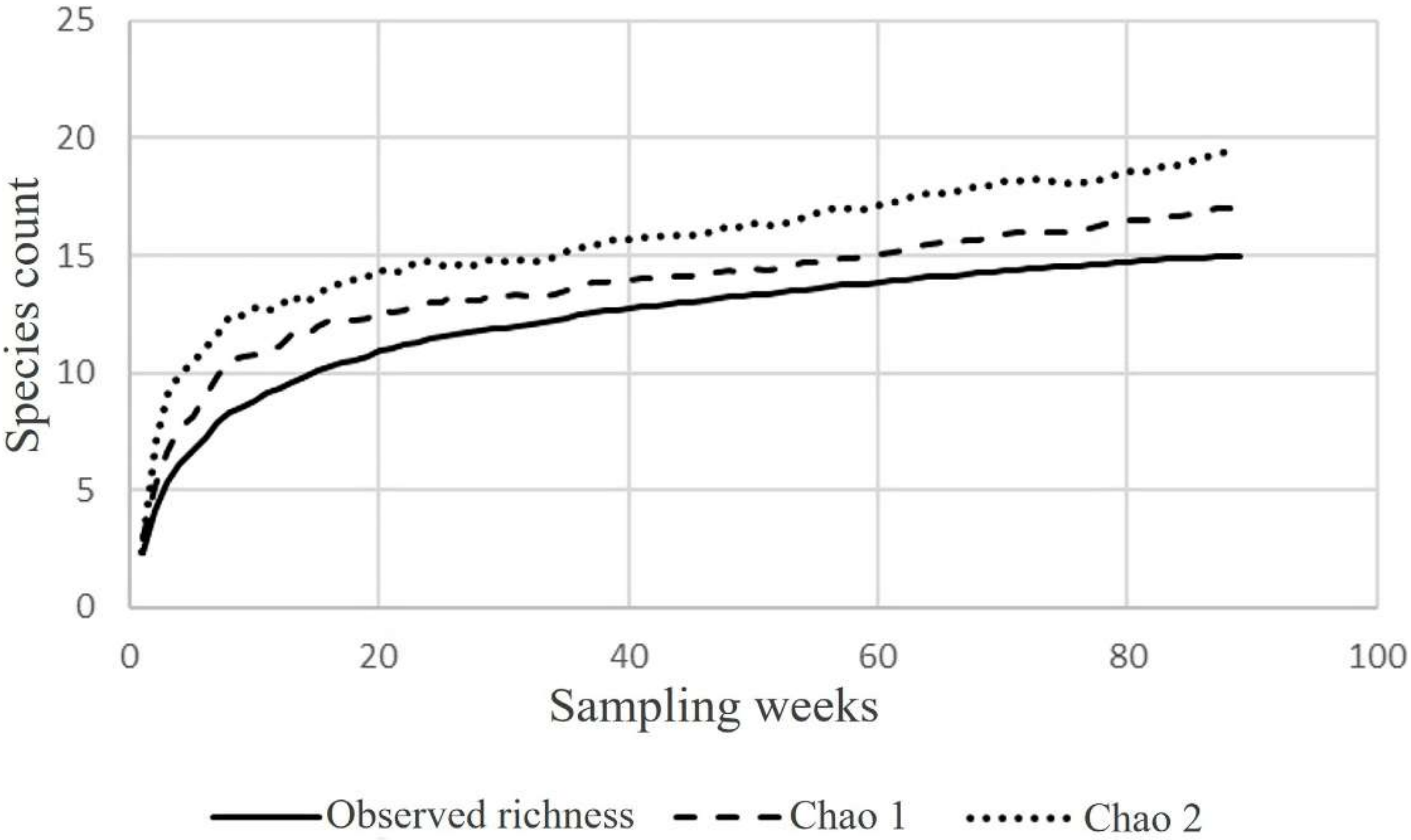


Figure 4. Accumulation curve of observed species and values of species richness estimators in the conservation area designated voluntarily Komchén de los Pájaros, Dzemul, Yucatán, Mexico.

(latitude and longitude, in degrees, minutes, and seconds), sex and age of the specimen: female (F), male (M), young offspring (Y), juvenile (J), or adult (A), and remarks.

We reviewed the Mexican Official Standard NOM-059-SEMARNAT-2010 (SEMARNAT 2010, 2019) and the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN 2025; https://www.iucnredlist.org/) to identify the species listed in a risk category or as threatened.

All the records captured were organized into data matrices and statistically analyzed with two non-parametric estimators (Villarreal *et al.* 2004) using the EstimateS program (version 9; Colwell 2013). The estimator based on record abundance was Chao 1: $S = S_{obs} + F2/2G$, where S_{obs} = number of observed species in a sample, F = number of singletons, G = number of doubletons; additionally, the estimator used for presence-absence data was Chao 2: $S = S_{obs} + (L2/2M)$, where S_{obs} = number of observed species in a sample, L = number of species recorded in only one sample (unique species), and M = number of species recorded in exactly two samples (duplicated species). The values of species richness were used to construct the species accumulation curve in Excel (Microsoft Windows 10).

To establish activity patterns, we considered species with at least 11 independent records, including the time of photo capture (Maffei *et al.* 2002, Monroy-Vilchis *et al.* 2009). Afterwards, the records for each time interval were counted and classified into four patterns based on the time of peak activity: diurnal, peak activity from 08:01 h to 17:59 h; nocturnal, from 20:01 h to 05:59 h; crepuscular, from 06:00 h to 08:00 h and from 18:00 h to 20:00 h), and catameral (species that are active all day and night; Monrroy-Vilchis *et al.* 2011). With this information, histograms of activity periods were constructed using the Oriana version 4.0 software (Kovach Computing Services, 2011).

Results

A sampling effort of 4256 trap nights resulted in a total of 1230 independent photo captures, identifying 14 species of medium-sized mammals (93 %) and one large mammal (7 %), grouped into 11 families and six orders (Table 1). We found that 33 % (5) of these species are listed in a risk category established in the Mexican Official Standard NOM-059-SEMARNAT-2010 (SEMARNAT 2010, 2019), while 14 species are listed as Least Concern (LC) and one as Near Threatened (NT) by the IUCN (2025; Table 1).

The TIR and RAI of each species prioritized *Nasua narica* (*n* = 715, RAI = 168.00), followed by *Odocoileus virginianus* (*n* = 184, RAI = 43.23), *Urocyon cinereoargenteus* (*n* = 157, RAI = 36.89), in contrast to those that yielded lower values, such as *Sciurus yucatanensis* and *Herpailurus yagouaroundi* (each with TIR = 1, RAI = 0.23; Figure 3). The highest species richness was recorded during the *nortes* season (December to February), with 14 species, in contrast to the rainy season, which recorded six species. On the other hand, the species recorded in the three seasons were *Dasypus novemcinctus*, *Sylvilagus yucatanicus*, *U. cinereoargenteus*, *N. narica*, *Procyon lotor*, and *O. virginianus* (Table 1).

Table 1. Taxonomic list of medium and large mammals associated with ecological data in "Komchén de los Pájaros", Dzemul, Yucatan according to Ramírez-Pulido *et al.* (2014). Conservation status according to NOM-059-SEMARNAT-2010 (P = endangered and A = threatened) and IUCN (LC = least concern and NT = near threatened). Abbreviations: TIR (Total independent records), RAI (Relative abundance index of each species), seasonality (D = dry season, R = rainy season, N = north winds season), and body size (M = medium and L = large). Feeding habits are based on Mayani-Parás *et al.* (2023). Activity: blank data are not recorded, while superscript 1 was taken from Mejenes-López *et al.* (2021).

	Feeding habits/ Activity	Conservation status		TIR	RAI	Number of records per season			Size M / L
		NOM-059/UICN				D	R	N	
CLASS MAMMALIA Linnaeus, 1758									
ORDER DIDELPHIMORPHIA									
FAMILY DIDELPHIDAE									
Didelphis virginiana Kerr,1792	Omnivore/ Nocturnal		LC	17	3.99	14	-	3	M
ORDER CINGULATA									
FAMILY DASYPODIDAE									
Dasypus novemcinctus Linneo, 1758	Invertivore/ Nocturnal		LC	27	6.34	18	1	8	M
ORDER PILOSA									
FAMILY MYRMECOPHAGIDAE									
Tamandua mexicana (de Saussure. 1860)	Invertivore/	P	LC	5	1.17	3	-	2	M
ORDER LAGOMORPHA									
FAMILY LEPORIDAE									
Sylvilagus yucatanicus (Allen, 1890)	Herbivore/ Nocturnal		LC	22	5.17	9	1	12	M
FAMILY SCIURIDAE									
Sciurus yucatanensis Allen, 1877	Frugivore/		LC	1	0.23	-	-	1	M
ORDER CARNIVORA									
FAMILY FELIDAE									
Herpailurus yagouaroundi (Hilaire, 1803)	Carnivore/	A	LC	1	0.23	-	-	1	M
Leopardus pardalis (Linneo, 1758)	Carnivore/	P	LC	4	0.94	2	-	2	M
Leopardus wiedii (Schinz, 1821)	Carnivore/	P	NT	9	2.11	6	-	3	M
FAMILY CANIDAE									
Canis latrans (Say, 1822)	Omnivore/ Nocturnal		LC	28	6.58	1	-	27	M
Urocyon cinereoargenteus (Schreber, 1775)	Omnivore/ Cathemeral[1]		LC	157	36.89	110	1	46	M
FAMILY MEPHITIDAE									
Spilogale yucatanensis (Howell, 1902)	Invertivore/ Nocturnal[1]		LC	3	0.70	3	-	-	M
FAMILY MUSTELIDAE									
Galictis vittata (Schreber, 1776)	Carnivore/	A	LC	3	0.70	-	-	3	M
FAMILY PROCYONIDAE									
Nasua narica (Linneo, 1766)	Omnivore/ Diurnal		LC	715	168.00	566	1	148	M
Procyon lotor (Linneo, 1758)	Omnivore/ Diurnal		LC	54	12.69	15	9	30	M
ORDER ARTIODACTYLA									
FAMILY CERVIDAE									
Odocoileus virginianus (Zimmermann, 1780)	Herbivore/ Cathemeral		LC	184	43.23	108	2	74	L

The two recorded canid species, *Canis latrans* and *U. cinereoargenteus*, differed in their relative abundances (RAI = 6.58 and 36.89, respectively). However, it should be mentioned that the coyote was always recorded in groups of two or three individuals and in the *nortes* season (n = 27), while fox specimens were virtually always captured as single individuals (97 %), mostly in the dry season (n = 110) and the *nortes* season (n = 46). We expected to record high abundances of both species, since they are generalists commonly found in the study region. *Dasypus novemcinctus, Sylvilangus yucatanicus, U. cinereoargenteus*, *N. narica*, *P. lotor*, and *O. virginianus* were recorded in all seasons. The three felid species differed in the season of capture: *Leopardus weidii* and *Leopardus pardalis* were recorded only in the dry

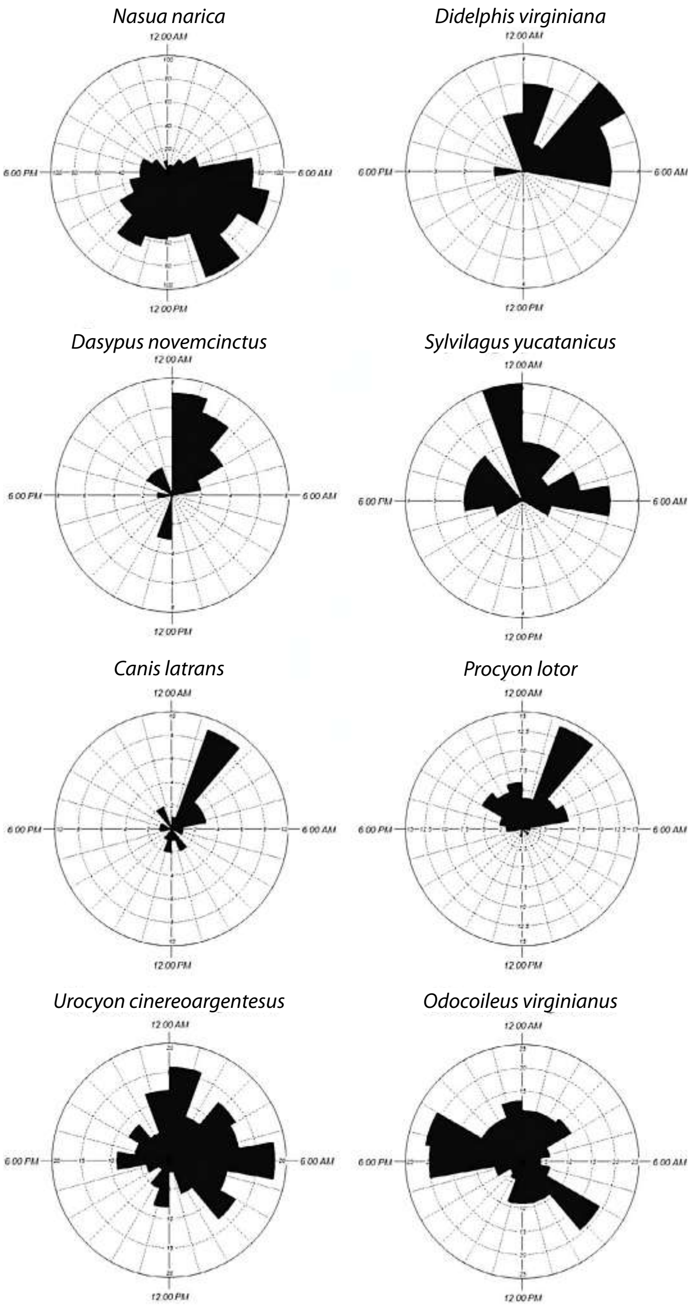

Nasua narica — *Didelphis virginiana* — *Dasypus novemcinctus* — *Sylvilagus yucatanicus* — *Canis latrans* — *Procyon lotor* — *Urocyon cinereoargentesus* — *Odocoileus virginianus*

Figure 5. Activity patterns of medium-sized and large mammals with more than 10 independent records in the conservation area designated voluntarily Komchén de los Pájaros, Dzemul, Yucatán, Mexico.

season (*n* = 2; *n* = 6, respectively) and the *nortes* season (*n* = 2, *n* = 6), while *H. yagouaroundi* was active only during the *nortes* season (Table 1). The accumulation curve shows a gradual increase that did not stabilize after the 89 weeks of sampling, suggesting the possibility of adding new records. This was confirmed with estimators, with Chao 1 being the most accurate and least biased, as it is closer to the observed richness, suggesting the potential presence of two additional species (*n* = 17); for its part, Chao 2 estimates a higher number of species (*n* = 19.45; Figure 4).

The activity pattern was obtained only for eight of the 15 species documented in this study. The records reflected that mammals are active throughout the day, but each showed a particular preference (based on the highest number of records): diurnal, *N. narica*; nocturnal, *D. virginiana, D. novemcinctus*, *S. yucatanicus*, *C. latrans*, and *P. lotor*; whereas *U. cinereoargenteus* and *O. virginianus* were recorded evenly throughout the 24 hours, so they are considered catameral species (Figure 5).

Discussion

This study reports for the first time the diversity of medium-sized and large mammals inhabiting KdlP AVDC, where low deciduous tropical forests predominate, in the municipality of Dzemul, Yucatán. This work confirms the presence of 15 species (37 %) recorded for the state of Yucatán (Sosa-Escalante *et al.* 2014). Given this, the KdlP AVDC, a 300-ha fragment of typical vegetation (low deciduous tropical forest), is important as it maintains a diverse functional group of mammals, including omnivores, carnivores, invertivores, herbivores, and frugivores (Mayani-Parás *et al.* 2023).

The species richness recorded in this study was intermediate compared to other studies carried out in the state. This is the case of the Dzilam State Reserve, with a land area of 42 555 ha, where six species of medium-sized mammals were recorded, 67 % of which are shared with those recorded in the present study (*Didelphis marsupialis, Tamandua mexicana, U. cinereoargenteus*, and *N. narica*; Hernández-Betancourt *et al.* 1996); in El Zapotal Private Reserve in the municipality of Tizimín, with an area of 2300 ha, 21 species were recorded, 43 % of which are shared (*D. marsupialis, T. mexicana, Sciurus yucatanensis, L. pardalis, L. wiedii, U. cinereoargenteus, N. narica, P. lotor*, and *O. virginianus* (Faller-Méndez *et al.* 2005); in the ejido Progresito, municipality of Peto, only 67 % of 12 documented species are shared (*D. novemcinctus, T. mexicana, Sciurus yucatanensis, U. cinereoargenteus, Spilogale yucatanensis, N. narica, P. lotor*, and *O. virginianus* (Cimé-Pool *et al.* 2020); and in the ejido of Xcan, municipality of Chemax, with an area of 24 516 ha, 35 % of the 17 species recorded are shared (*Sylvilangus yucatanicus, Sciurus yucatanensis, Spilogale yucatanensis, N. narica, P. lotor*, and *O. virginianus* (Balam-Ballote *et al.* 2020). *Nasua narica* is present at the five sites, displaying a wide distribution. Considering the extension of all the areas just mentioned, KdlP AVDC stands out due to its intermediate-to-high richness of medium-sized and large mammals despite its smaller size. This work is considered relevant because its intensive sampling effort provides a close approximation to the species richness of medium-sized and large mammal species in the area. Additionally, we believe that including non-accessible areas and increasing the sampling effort could increase the number of species, as suggested by the estimators (Chao 1 and 2; Figure 4).

The participation of local communities and other landowners is crucial for raising awareness and promoting the responsible use and conservation of wildlife, with photo trapping as its primary tool. For example, Duque-Moreno *et al.* (2024), in their study on potential ecotourism based on the observation of wild mammals in the AVDC

Centauro del Norte in Calakmul, Campeche, Mexico (9722.75 ha), recorded 14 species of medium-sized and large mammals of importance for ecotourism with a sampling effort of 549 trap nights. On the other hand, Medina-Torres *et al.* (2015) documented participatory sampling with a sampling effort of 426 trap nights in the ejido San Ignacio, municipality of Morelos, Chihuahua. This AVDC has an area of 7662.2 ha where the presence of nine species of medium-sized and large mammals was reported in different types of vegetation.

In other private conservation areas, the sampling effort and size undoubtedly determine the differences between them. For example, only considering photo-trap sampling, Cruz-Bazán *et al.* (2017), with a sampling effort of 42 trap nights, reported eight mammal species for the Talhpan private conservation area in Papantla, Veracruz, with an extension of 24 ha that includes different habitats; most species recorded in the medium semi-evergreen tropical forest. In contrast, Galindo-Aguilar *et al.* (2024), in a study involving community monitoring in 18 AVDCs in Oaxaca along an altitudinal gradient of 50 to 3000 m asl that included a mosaic of conditions, with the predominance of tropical forests, captured 26 species of medium-sized and large mammals representing 49 % of the 53 species reported for the state, with a sampling effort of 4384 trap nights. It is worth mentioning that these 18 AVDCs are immersed in a gradient of conditions over areas of differentiated sizes, the smallest of 600 ha and the largest of 9670 ha (Miguel A. Briones Salas, pers. comm.), and are a public policy conservation strategy where the communities are involved (Galindo-Aguilar *et al.* 2024).

In the Ceratozamia Protection and Development Area located in Ixhuatlán del Sureste, in the south of the State of Veracruz, Mexico, covering 100 ha and managed under a private regime, Pelayo-Martínez *et al.* (2023) investigated the patterns of daily activity of arboreal mammals and their degree of overlap with a sampling effort of 996 trap nights in 50 ha of forest area, a remnant of evergreen tropical forest. These authors documented the presence of seven species of medium-sized mammals (*Potos flavus, Caluromys derbianus, Coendou mexicanus, D. marsupialis, T. mexicana, P. lotor*, and *Philander opossum*).

Comparing the areas under the community and private management regimes mentioned above with our study area data in terms of surface area (24–24 516 ha) and species richness of medium-sized and large mammals (7–26), we found that KdlP AVDC is of intermediate size (300 ha) and has an intermediate richness of medium-sized and large mammals (15 species) relative to the data reported to date, despite being under anthropic pressure due to changes in land use and hunting. Therefore, we maintain that the AVDC studied is a wildlife protection site that should be included in monitoring and conservation programs. Additionally, according to Padilla *et al.* (2025), it can also be considered a "conservation island", as the surrounding matrix is being altered and is adjacent to a human community.

We agree with Briones-Salas *et al.* (2023) in highlighting that records of mammals in AVDCs by community monitors reflect the central role of indigenous or local communities in the knowledge and conservation of biodiversity; some of these mammal species are listed in a risk category. For example, according to NOM-059-SEMARNAT-2010 (DOF 2019), of the species listed for the state of Yucatán, KdlP AVDC reports 33 % of the species classified as endangered of extinction and 20 % as threatened, which together represent 22.7 % of the protected terrestrial mammal species (Sosa-Escalante *et al.* 2014). This data highlights the importance of KdlP AVDC for mammalian conservation. However, this mammal fauna is under pressure, as evidenced by roadkill incidents and hunting activities observed during the study period.

The coati (*N. narica*) was the species with the highest number of records in the sampling area, likely due to the availability of food and water; this species is reported to be common in low deciduous tropical forests of Mexico (Alfaro-Espinosa *et al.* 2006; Pérez-Irineo and Santos-Moreno 2012).

The largest number of mammal records in this study corresponds to nocturnal mammals (20:01 to 05:59), probably because most Neotropical mammals are nocturnal (Srbek-Araujo and Chiarello 2005). Among these, *P. lotor* displayed a peak of activity at midnight, an observation consistent with the preferred time of activity recorded by Valenzuela (2005). *Leopardus wiedii* also showed activity during the night, coinciding with the report of Valenzuela (2005). In the case of *Sylvilagus yucatanicus*, *D. virginiana*, and *D. novemcinctus*, Monrroy-Vilchis *et al.* (2011) mention that their preference for nocturnal/crepuscular habits is related to the avoidance of predation, as their body mass is less than 10 kg; this behavior was confirmed in the present study, recording the peak of activity of these species at night: *Sylvilagus yucatanicus*, from 23:00 h to 00:00 h; *D. virginiana*, from 03:00 h to 04:00 h; and *D. novemcinctus*, from 00:00 h to 01:00 h. In the case of cathemeral species, *U. cinereoargenteus* has been reported as a mainly nocturnal species (Reid 1997; Mejenes-López *et al.* 2021); however, this work showed diurnal and crepuscular activity, probably due to lower food availability in some seasons, which requires more time and effort to meet its needs (González-Pérez *et al.* 1992; Pelaez-Cruz *et al.* 2022). *Odocoileus virginianus* was recorded by Monrroy-Vilchis *et al.* (2011) to be active throughout the day, a finding that aligns with the present study, without showing a preference for any particular time of day.

The work geographically closest to our study area is that of Hernández-Pérez *et al.* (2015). These authors provided a list of 16 species of medium-sized and large mammals that inhabit the northwest and north coasts of the Yucatán Peninsula, of which seven are shared with KdlP AVDC. Of these, three species are nocturnal (*D. novemcinctus*, *L. pardalis*, and *L. weidii*) and another three are cathemeral (*N. narica, P. lotor*, and *O. virginianus*), which is consistent with our records in the present study; the only exception is *U. cinereoargenteus*, reported as diurnal by Hernández-

Pérez *et al.* (2015), with three records, and as cathemeral in the present study, with 157 records, of which 66 are diurnal. Therefore, we consider that a larger number of independent records helps represent and characterize the activity patterns of mammals more accurately.

When sociability is encouraged, animals can form groups ranging from pairs to large congregations. In mammals, sociability can be beneficial because it provides greater protection from predators, improves the success in locating or maintaining access to resources, creates mating opportunities, reduces vulnerability to infanticide, or facilitates mate selection. However, sociability can have drawbacks for individuals because it exposes them to infections, can increase their visibility to predators, and intensifies competition for access to resources and mating opportunities. Animals living in larger groups may attempt to expel other residents, disperse, or attempt to exclude immigrants (Silk 2007). These are possible explanations for the finding that 11 out of 15 species recorded in the tropical forests of the northern Yucatan Peninsula are solitary.

Kappeler *et al.* (2013) discuss the social organization of adult mammals that can lead a solitary life (e.g., in the KdlP AVDC, *U. cinereoargenteus, D. novemcinctus, Sylvilagus yucatanicus, P. lotor, O. virginianus, L. weidii, L. pardalis, H. yagouaroundi, D. virginianus, T. mexicana, Spilogale yucatanensis*, and *Galictis vittata*), coordinate their activities with a member of the opposite sex forming pairs (not predominant in the study area), or associate and coordinate their activities with two or more conspecifics forming groups (*P. lotor, C. latrans*, and *N. narica*). However, this author does not mention interspecific groupings, such as the case of *Spilogale yucatanensis* and *U. cinereoargenteus, which were* recorded in the AVDC studied (Mejenes-López *et al.* 2021).

The group behavior of some mammals was recorded by photo recording. For example, *N. narica* was recorded forming herds of 13 to 17 individuals in the dry season (April 2020 and March to April 2021). For its part, *O. virginianus* was observed in groups, one with one female and two juveniles in the dry season (April to May 2020, March to June 2021) and in the *nortes* season (November and December 2021); these records contrast with studies carried out in temperate forests of Oaxaca, Morelos, and the State of Mexico, where the largest population was found in October (Ortiz-Martínez *et al.* 2005) and in winter (Flores-Armillas *et al.* 2011; Beltrán and Díaz de la Vega 2017), and coincides with the study of Mandujano and Gallina (1993) in tropical areas with deciduous and semi-evergreen vegetation, which recorded deer between February and June, with a lower record between November and January. Additionally, *P. lotor* was recorded in pairs in the dry season (March 2021) and in groups of three in the rainy season (June 2021) and the *nortes* season (December 2021). The data recorded in this study on the size of *N. narica* herds are similar to those reported by various authors (Burger and Gochfeld 1992; Valenzuela 2005; Di Blanco and Hirsch 2006).

Solitary behavior has been scarcely documented and is exhibited by more than 80 % of species in the order Carnivora (Gittleman 1989). Sandell (1989) noted that solitary individuals have little social interaction, except in the mating season. Meanwhile, Bekoff *et al.* (1984) mentioned that they communicate with each other through olfactory, auditory, and even visual signals, thus avoiding contact and maintaining distribution areas or territories that do not overlap; to obtain food, these individuals depend on being stealthier and more cryptic to catch prey dispersed in complex habitats. In the present study, *O. virginianus* was recorded not only in groups but also individually; for example, solitary females in the dry season (March 2021), the rainy season (June 2021), and the *nortes* season (from November to December 2021), and solitary males in the dry season (June 2021) and the *nortes* season (from November to December 2021). Additionally, one adult of *G. vitata* was recorded in the dry season (May 18, 2021, at 15:25 h) and in two rainy seasons (June 4, 2021, at 07:38 h and June 5, 2021, at 15:25 h); this same species was also photo recorded in a group of three individuals outside the sampling period, on February 4, 2023 (Asis Alcocer García, pers. comm.). Based on these observations, a mid- to long-term study is recommended to define patterns of group or individual behavior.

Finally, the photo trapping technique facilitated better recording of mammal species that are difficult to detect (e.g., *H. yagouaroundi, Spilogale yucatanensis*, and *G. vittata*). Furthermore, we confirmed that it is a reliable non-invasive method that provides physical evidence of the presence of individuals, in addition to contributing data regarding the natural history of the species mentioned here. Compared to other conservation areas in the region, KdlP AVDC is a small area of 300 ha of low deciduous forest that is home to various functional groups of mammals, including omnivores, herbivores, carnivores, and invertivores, and functions as a wildlife refuge or conservation island. In addition, it protects one-fifth of the mammal species of Yucatan according to NOM-SEMARNAT-2010, which are being threatened by strong anthropogenic stressors, including urban growth, hunting, and deforestation, all of which jeopardize the persistence of the habitat and its natural communities. The results obtained here should promote environmental education and long-term monitoring.

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Neotropical insectivorous bats adjust echolocation calls in response to vegetation structure

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Echolocation is a crucial process in bat ecology, but it is influenced by different biotic and abiotic factors. The structural complexity of the vegetation can filter which species can be part of an assemblage because bats have to modify the spectral and temporal parameters of their echolocation calls. We evaluated how insectivorous bats modulate their echolocation calls among sites that vary in vegetation structural complexity in the Llanos of Colombia in three habitats. We used canonical correspondence analysis (CCA) to correlate vegetation variables per plot with the spectral and temporal variables of the echolocation signals from bats using those habitats. We found that delta frequency and delta time had the greatest variability in relation to the percentage of tree cover and diameter at breast height. Our results indicate that pulses, in terms of frequency and time, tend to fluctuate more in cluttered habitats. We then selected the two species with the highest number of recordings (*Saccopteryx bilineata* and *Neoeptesicus* cf. *furinalis*) and compared spectral and temporal variables among sites. For *Neoeptesicus* cf. *furinalis* showed a predictable behavior: pulses were shorter, and more frequency modulated in highly cluttered habitats. However, and contrary to our expectations, the pulses of *Saccopteryx bilineata* tended to be longer and with a smaller frequency modulated component in highly cluttered habitats. These results demonstrate that bats modulate their echolocation calls in different ways according to the structure of vegetation. Modulation of echolocation has important implications for sensory ecology and bat composition in tropical landscapes.

Key words: Audiomoth, functional diversity, habitat filtering, plant structural complexity, savannas.

La ecolocalización es un proceso crucial en la ecología de los murciélagos, pero está influenciada por diferentes factores bióticos y abióticos. La complejidad estructural de la vegetación puede determinar qué especies pueden formar parte de un ensamblaje, ya que los murciélagos deben modificar los parámetros espectrales y temporales de sus llamadas de ecolocalización. Evaluamos cómo los murciélagos insectívoros modulan sus llamadas de ecolocalización en lugares con diferentes niveles de complejidad estructural de la vegetación en los Llanos de Colombia en tres hábitats. Aplicamos un análisis de correspondencia canónica (CCA) para correlacionar las variables de vegetación por parcela con las variables espectrales y temporales de las señales de ecolocalización de los murciélagos que habitan esos lugares. Descubrimos que la delta de frecuencia y la delta de tiempo presentaron una mayor variabilidad en relación con el porcentaje de cobertura arbórea y el diámetro a la altura del pecho. Nuestros resultados indican que los pulsos tienden a fluctuar más en términos de frecuencia y tiempo en hábitats densamente estructurados. Posteriormente, seleccionamos las dos especies con mayor número de grabaciones (*Saccopteryx bilineata* y *Neoeptesicus* cf. *furinalis*) y comparamos sus variables espectrales y temporales en los distintos lugares. En *Neoeptesicus* cf. *furinalis* mostró un comportamiento predecible: los pulsos eran más cortos y presentaban una mayor modulación de frecuencia en hábitats muy densos. Sin embargo, y contrario a nuestras expectativas, los pulsos de *Saccopteryx bilineata* tendieron a ser más largos y con un componente de modulación de frecuencia menor en hábitats muy densos. Estos resultados demuestran que los murciélagos modulan sus llamadas de ecolocalización de diferentes maneras de acuerdo con la estructura de la vegetación. La modulación de la ecolocalización tiene implicaciones importantes para la ecología sensorial y la composición de murciélagos en paisajes tropicales.

Palabras clave: Audiomoth, complejidad estructural de la vegetación, diversidad funcional, hábitat como un filtro, sabanas.

Functional diversity plays a crucial role in regulating community structure, determining ecosystem functioning (Tilman *et al.* 1997; Naeem and Wright 2003), and influencing niche partitioning, guild membership and habitat selection in animals (García-Morales *et al.* 2016; Pigot *et al.* 2020). Functional diversity refers to the range of morphological or physiological traits among species, with functional traits being the key attributes that enable organisms to cope successfully with their environment (Hooper *et al.* 2005; Villéger *et al.* 2010). Therefore, functional diversity helps us understand how habitats can filter which species are found in a given area (Petchey and Gaston 2006). For insectivorous bats, the functional diversity of echolocation signals can help determine how vegetation structure at small spatial scales may filter species based on their ability to discriminate prey echoes from background clutter or emit signals that optimize detection in open areas (Schnitzler and Kalko 2001).

Echolocation is a sensory system that operates along a continuum, rather than being strictly divided into open-space and clutter-adapted signals. The spectral and temporal characteristics of echolocation calls allow bats to detect, classify and locate prey in different levels of vegetation clutter (Schnitzler and Kalko 2001). In densely vegetated environments, bats require broadband, short-duration pulses to navigate efficiently around obstacles

(Broders *et al.* 2004; Barataud *et al.* 2013). In contrast, in open areas, bats emit echolocation calls with narrower bandwidths and longer durations, which enhance long-range prey detection (Kalko and Schnitzler 1993). However, some species show a high degree of plasticity, modulating their echolocation parameters to different vegetation structures and foraging conditions (Neuweiler 2003).

Bats can also modify the timing and rate of their echolocation pulses depending on environmental constraints (Fenton *et al.* 2016). For instance, during critical hunting moments, bats decouple their call emissions from wingbeats to enhance sensory flow, rapidly increasing their call rate when they are close to their prey (Stidsholt *et al.* 2021). However, this behavior comes at an energetic cost, so when not actively hunting, bats synchronize their calls with wingbeats to optimize energy efficiency (Stidsholt *et al.* 2021). Similarly, when navigating through highly cluttered environments, bats tend to increase their call emission rates to improve spatial resolution (Falk *et al.*, 2014). Yet, in the presence of conspecifics, they reduce emission rates and even omit pulses to minimize interference, which can ultimately impact their navigation and collision avoidance (Adams *et al.* 2017).

Vegetation complexity, which serves as a proxy for clutter, can act as a strong selective force, shaping echolocation structures and filtering community composition (Yovel *et al.* 2009). Greater habitat heterogeneity provides more ecological niches, which promotes species diversity (Langridge *et al.* 2019). Conversely, a reduction in structural complexity negatively affects bat diversity (Meyer *et al.* 2016) and poses a threat to several taxonomic groups (Meyer and Kalko 2008; Jones *et al.* 2009; Estrada-Villegas *et al.* 2010; Cruz *et al.* 2016). For example, Díaz-B *et al.* (2023) demonstrated that environmental conditions in Colombian dry forests significantly influence functional traits of bats, including echolocation pulse structure, diet, vertical foraging behavior and trophic level. However, despite increasing research on the drivers of functional diversity in Neotropical bats, few studies have examined how vegetation complexity modulates the functional diversity of echolocation signals in insectivorous bat ensembles (Cisneros *et al.* 2015; Núñez *et al.* 2019). This knowledge gap is particularly relevant in naturally heterogeneous landscapes such as savannas. In the Colombian Llanos, savannas are often flanked by gallery forests, and sometimes there are soft forest edges with sparce tree cover in the transition between gallery forests and savannas (Romero-Ruíz *et al.* 2004).

To better understand how vegetation structure influences echolocation in Neotropical bats, we assessed the effect of vegetation complexity on the functional diversity of echolocation pulses in an ensemble of insectivorous bats in the savannas of Colombia. Based on Denzinger *et al.* (2016), we predicted that bats in cluttered habitats would echolocate at higher frequencies and shorter durations, whereas bats in open areas would emit lower frequency, longer duration calls. We also expected the acoustic parameters of the bat ensemble to vary with habitat complexity. Specifically, we predicted that species should modulate their vocalizations to the structural characteristics of their environment.

Previous studies have shown that species such as *Saccopteryx bilineata* and members of the genus *Eptesicus* modify their echolocation signals according to vegetation complexity: in cluttered habitats they emit highly modulated frequency pulses, whereas in open areas they reduce modulation (Ratcliffe *et al.* 2011; Jakobsen *et al.* 2012). In this study, we first describe the acoustic parameters of the insectivorous bat ensemble and then test our predictions by correlating vegetation clutter with the variability of spectral and temporal parameters of echolocation calls for all species in the ensemble, as well as for *Saccopteryx bilineata* and *Neoeptesicus* cf. *furinalis* separately.

Materials and Methods

Study area and vegetation coverage. We conducted our study in El Caduceo Natural Reserve, municipality of San Martin, department of Meta, Colombia (3.6711° N, -73.6585° W, Elevation: 377.8 MASL). The reserve is located at the piedmont of the Eastern Cordillera and the onset of the Llanos (Basto-González 2009) (Supplementary material, Figure 1). The Colombian Llanos are mainly composed of natural savannas and gallery forests (Lasso *et al.* 2011). Savannas are mostly covered with native grasses and are mainly used for cattle grazing, and the forest boundaries between gallery forests and savannas have not shifted due to stable land use patterns and the lack of significant encroachment or deforestation (Romero-Ruíz *et al.* 2004).

In El Caduceo, 70 hectares are covered with gallery forests while the remaining 103 hectares are savannas used for cattle farming and agriculture in small parcels (Casallas-Pabón *et al.* 2017). The area experiences a monomodal rainfall regime, with a dry season from December to March (Correa *et al.* 2006). We selected three habitats: open (S), cluttered (BT), and highly cluttered (BM) environments. Open areas (S) consisted mainly of pastures used for cattle grazing and agricultural crops, such as pineapples. Cluttered sites (BT) were in the transitional area between open areas and highly cluttered environments, and were composed of scattered, thin, and tall pioneer trees characteristic of early successional stages, interspersed with open savanna patches. Highly cluttered sites (BM) were located within gallery forests, which exhibited greater structural complexity, including a diverse assemblage of tall trees, palms, dense understory vegetation, as well as numerous saplings.

We measured diameter at breast height (DBH), canopy width and percent cover in 18 plots, six per habitat. We also measured the percentage of vegetation coverage with a concave spherical densiometer (Rivera-Gallego and Pinzón-Florián 2022) in each plot. Vegetation structure data were collected on the same sites where we conducted

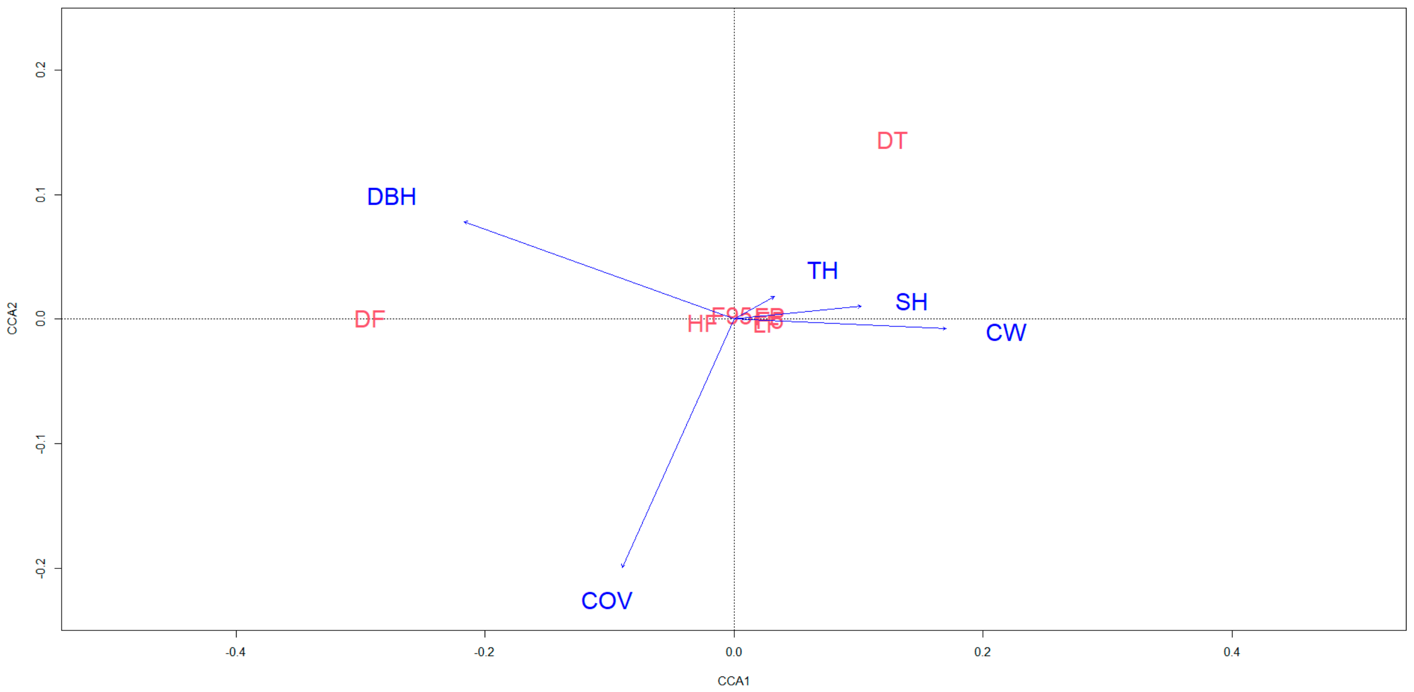

Figure 1. Canonical correspondence analysis (CCA) showing that spectral and temporal parameters vary according to the vegetation variables. The parameters showing the most significant changes were Δ Frequency (DF) and Δ Time (DT). The eigenvalues for the first and second axes were 0.3790 and 0.0474, respectively. Spectral and temporal parameters in red. LF: Low frequency, High frequency: HF, Δ Time: DT, Frequency 5%: F5, Frequency 95%: F95, Δ Frequency: DF, Peak frequency: FP. Vegetation variables in blue. Total height: TH, Stem height: SH, Canopy width: CW, DBH, % Coverage: COV.

acoustic monitoring to ensure comparability between environmental variables and acoustic parameters across sites (please see below). Since vegetation structure remains stable over short time periods (months or years), data collection of vegetation variables did not require the same sampling effort as bat monitoring (Willcox *et al.*, 2017).

Bat capture and reference calls. We captured and recorded bats from February 11th to March 9th, 2023. Four 6 × 2.5 m nets were used on each habitat and deployed from 17:30 to 23:30 for 18 nights. We relocated nets every night. Captured bats were placed in cloth bags, measured, and sexed (Supplementary material, Figure S2). We followed the Guidelines of the American Society of Mammalogists for proper handling protocols (Sikes *et al.* 2011).

We used taxonomic keys to identify all the captured bats (Díaz *et al.* 2016 ; Díaz *et al.* 2021). All bats from the family Phyllostomidae bats were released immediately. Although some phyllostomid bats are insectivorous, we did not include any species from this family because their echolocation calls are unidirectional (all other families are omnidirectional) and are not adequately sampled with our sound recorders (Jakobsen *et al.* 2013). For reference calls, we used a Pettersson M-500 USB microphone, with Pettersson-BatMicRecorder software at a sampling rate of 386 kHz and a resolution of 16 bits. We started recording when the individual was released until it was no longer in sight. The recording methodology involved releasing bats at the site where they were found on the night of capture. Our study is in compliance with Permit No. 2467 (25 October 2023) issued by the Colombian Environmental Licensing Authority for collecting and manipulating wildlife for non-commercial scientific research purposes.

Echolocation calls were recorded and analyzed using Raven Pro 1.6. (Cornell Lab of Ornithology, Cornell Univeristy, USA). Both reference and passive acoustic recordings (see next section) were processed in Raven Pro 1.6. Spectrograms were generated using a Hamming window with a 512-point FFT, 100% frame size, and 80% overlap. A sonotype can be defined as a distinct acoustic category that groups echolocation calls sharing similar structural characteristics, regardless of their signal frequency (Roemer *et al.* 2021). Based on this definition, we labeled each call and measured the following spectral-temporal parameters: start time (s); end time (s); low frequency (kHz); high frequency (kHz); Δ Time delta (s); 5% frequency (kHz); 95% frequency (kHz); Δ Frequency delta (kHz); peak frequency (kHz). We also labeled each recording with sonotype; taxon; sex; recording type; and quality. The audio quality was evaluated using the signal-to-noise ratio (SNR), which quantifies the quality of a signal in the presence of noise and is expressed in decibels (dB). The selection of these parameters was based on Martínez-Medina *et al.* (2021).

Passive acoustic sampling. We used nine Audiomoth recorders using the following parameters: 250 kHz sampling rate, medium gain, 1740 seconds of inactive time, and 60 seconds of active time. We sampled from 17:30 to 5:30 daily for three weeks. In total, we had nine recording sites, three in each habitat. Each recorder was placed four meters above ground level in all three habitats and at least 150 meters apart to avoid pseudoreplication (Pryde and Greene

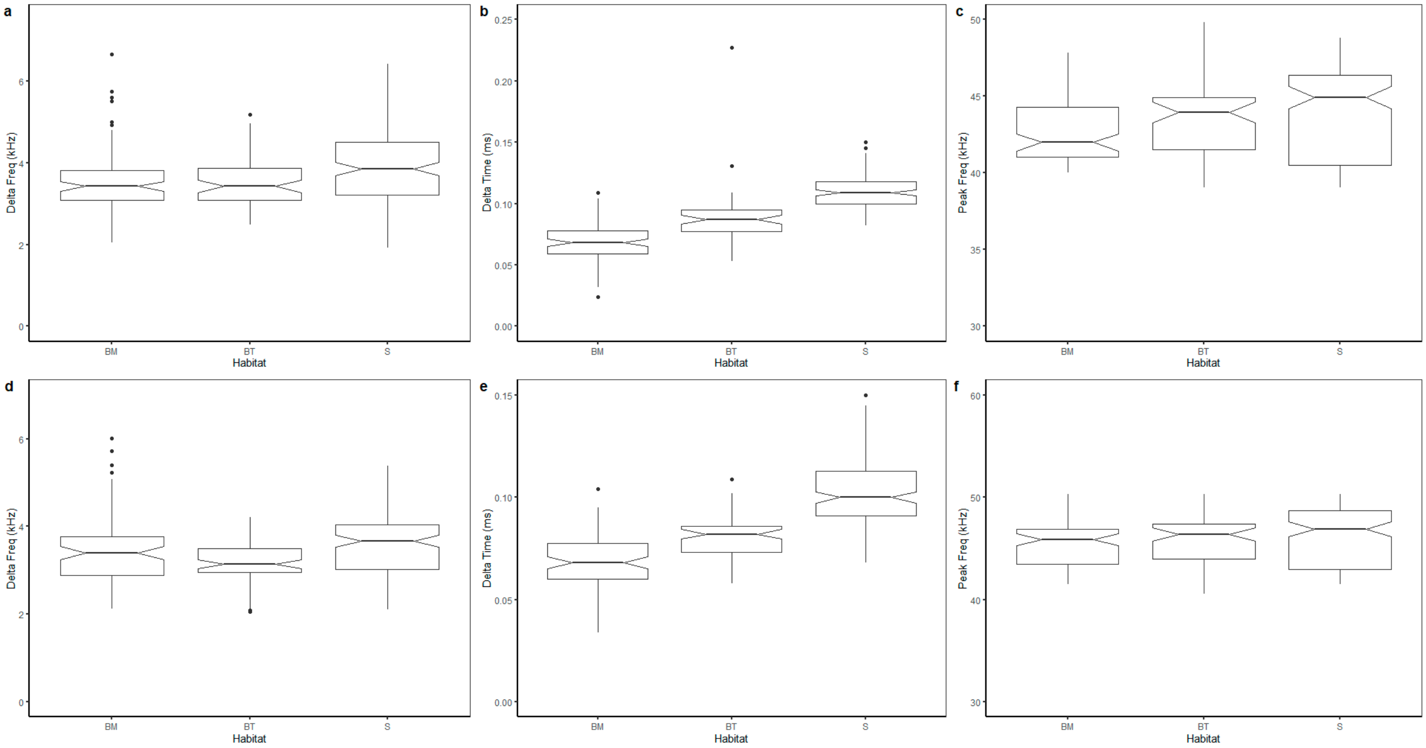

Figure 2. Spectral and temporal parameters of the two *Saccopteryx bilineata* pulses vary between the different sites, Open (S), Clutter (BT) and High Clutter (BD), as indicated by the lack of overlap in the notches of the box plots. **a.** Δ Frequency of the low pulse **b.** Δ Time of the low pulse **c.** Peak frequency of the low pulse **d.** Δ Frequency of the high pulse **e.** Δ Time of the high pulse **f.** Peak frequency of the high pulse.

2016). Recording sites were relocated weekly to avoid nights without recordings. Spectral-temporal parameters from search calls were analyzed and labeled in Raven Pro1.6. (Cornell Lab of Ornithology, Cornell University, USA), including start and end times, low and high frequencies, Δ Time delta, bats presence, sonotype, taxon, habitat classification, feeding behavior, and recording quality (Martínez-Medina *et al.* 2021). Sonotype identifications were informed by previous studies (Jung *et al.* 2007; Jung *et al.* 2014; Arias-Aguilar *et al.* 2018).

Data Analysis. We used a Principal Component Analysis (PCA) to identify patterns in vegetation structure, using the following R libraries: FactoMineR (Lê *et al.* 2008), factoextra (Kassambara and Mundt 2020), REdaS (Maier 2022) and ggplot2 (Wickham 2016). Then we used canonical correspondence analysis (CCA) to determine whether the spectral and temporal parameters of the insectivorous bat ensemble were modulated given the complexity of the vegetation. To do this, we first averaged the data obtained for each vegetation variable and each spectral and temporal variable per plot across the three habitats (18 plots in total). Then we created two matrices with the same number of rows, one with the vegetation data per plot, and another with the spectral and temporal variables per plot. Then, we used the function "scale" to normalize the data because all variables were in different units and then calculated the CCA using the library Vegan (Oksanen *et al.* 2022).

To determine differences in spectral and temporal parameters of echolocation signals among habitats, we selected species recorded at least 10 times in each habitat to ensure sample representativeness. Only two species met our criteria: *Saccopteryx bilineata* (Number of recordings per habitat: BT= 33, BM= 28, S= 49) and *Neoeptesicus* cf. *furinalis* (Number of recordings per habitat: BT= 15, BM= 10, S= 49).

We calculated boxplots for the following parameters: Bandwidth (Δ Frequency, kHz), Pulse Duration (Δ Time, s), and Peak Frequency, as these parameters best characterize echolocation pulses (Jung *et al.* 2007). Subsequently, we used a Kruskal-Wallis test to compare the means of each parameter across habitats, and used a Dunn-Bonferroni test for multiple comparisons (Supplementary material, Table S1, S2). For the aforementioned tests, we used the following R libraries: PMCMRplus (Pohlert 2021), ggplot2 (Wickham 2016), and ggpubr (Kassambara and Mundt 2020), o and the FSA library (Ogle *et al.* 2025). Given that vegetation variables were not used in these analyzes, our comparisons between habitats for *Saccopteryx bilineata* and *Neoeptesicus* cf. *furinalis* are based on our whole sampling (three weeks).

Results

We found 19 species, being *Saccopteryx bilineata* and *Neoeptesicus* cf. *furinalis* the most relatively abundant across all three habitats (Supplementary Material, Table S3 and Figure S4 to S6). The first PCA axis of vegetation variables explained 38.3% of the data, whereas the second axis explained 19.9%. The variables that explained most of the variance in Axis 1 and 2 were DBH, and Total Height, respectively (Supplementary material, Table S4). The PCA plot indicated that BM and BT are clustered together. Since there were no vegetation variables in habitat S, all plots from S are aggregated into a single point (Supplementary material, Figure S3).

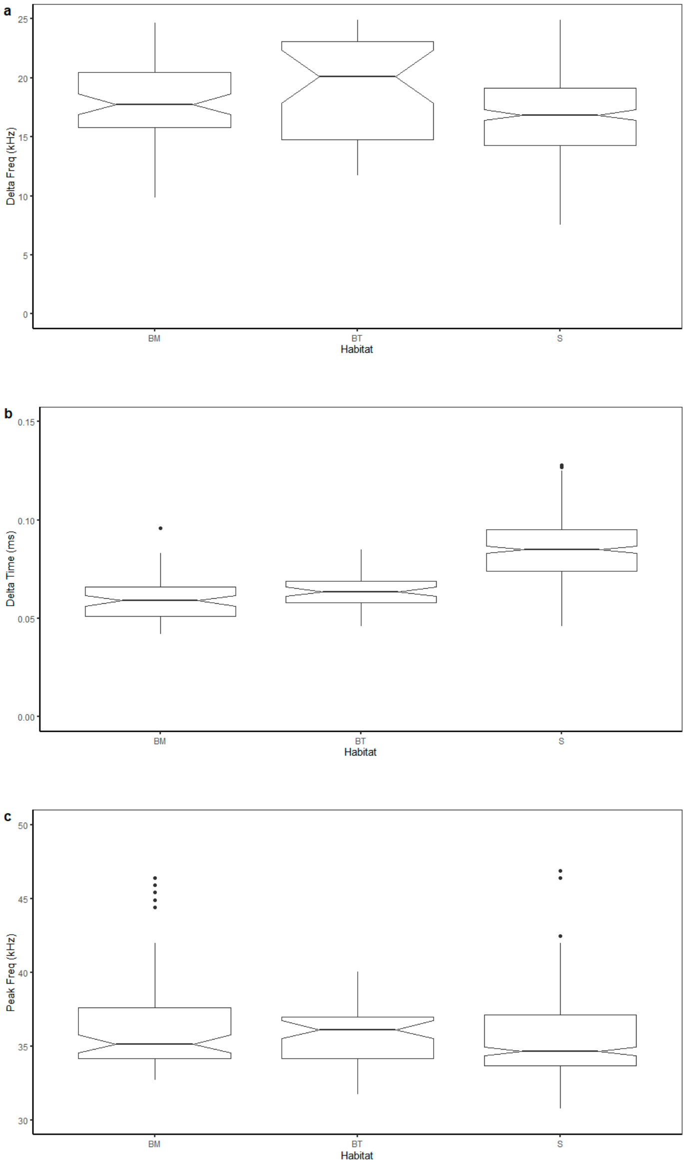

Figure 3. Spectral and temporal parameters of the *Neoeptesicus* cf. *furinalis* pulses vary between the different sites, Open (S), Clutter (BT) and High Clutter (BD), as indicated by the lack of overlap in the notches of the box plots. **a.** Δ Frequency (kHz) **b.** Δ Time (s) **c**. Peak frequency (kHz).

Relationship between vegetation variables and acoustic parameters. The first axis of the CCA indicates that DBH had the strongest influence on the spectral and temporal parameters (-0.67), while percentage cover (COV) was the most influential variable on the second axis (-0.62). Among the spectral-temporal variables, Δ Frequency (kHz) was most influenced by the first axis (-0.29), while Δ Time (s) was mainly influenced by the second axis (0.14) (Figure 1).

Spectral and temporal differences in two species present in the three habitats. We found that the Δ Frequency (kHz) (*P*= 1.987e-05), Δ Time (ms) (*P*= 2.2e-16), and peak frequency (kHz) (*P*= 4.89e-12) of the low pulse of *Saccopteryx bilineata* (Figure 2) were significantly different among habitats. Multiple comparisons for peak frequency only showed a significant difference between BM-S (*P*= 0.002). For Δ Frequency (kHz), we observed significant differences between BM-S (*P*= 0.002) and BT-S (*P*= 0.004). For Δ Time (ms), we found significant differences between BM-BT (*P*= 1.32e-04), between BM-S (*P*= 9.34e-40), and between BT-S (*P*= 3.23e-13).

Moreover, we also found significant differences among habitats for the high pulse of *Saccopteryx bilineata* in Δ Frequency (kHz) (*P*= 0.0001), Δ Time (ms) (*P*= 1.72e-05), and peak frequency (kHz) (*P*= 4.89e-12). The multiple comparisons tests only showed a significant difference between BM-S (*P*= 0.031). For Δ Frequency (kHz), we only found significant difference between BT-S (*P*= 0.0001). However, for the Δ Time (s), there is a significant difference between BM-BT (*P*= 1.40e-03), between BM-S (*P*= 2.3e-34), and between BT-S (*P*= 1.84e-13). These results indicate that environmental factors significantly influence the echolocation calls of *Saccopteryx bilineata* (Supplementary material, Figure S7). The significant differences in Δ Frequency and peak frequency between habitats suggest that this species is modifying their vocalizations, probably to optimize foraging efficiency and sound detection in response to the specific characteristics of their environment.

For *Neoeptesicus* cf. *furinalis* (Figure 3), there were significant differences among habitats in the Δ Frequency (kHz) (*P*= 0.004), Δ Time (s) (*P*= 2.20e-16), and peak frequency (kHz) (*P*= 1.39e-05). Regarding Δ Frequency (kHz), there was a significant difference among all three habitats: BT-S (*P*= 2.44e-06), BM-S (*P*= 5.99e-02), and BM-BT (*P*= 4.70e-02). For the Δ Time (s), there is a significant difference between BM-S (*P*= 1.13e-30), between BT-S (*P*= 4.15e-17), and finally between BT-BM (*P*= 6.81e-01) (Supplementary material, Figure S8).

Discussion

Our results confirm that bats can modify their echolocation calls according to the vegetation structure of their surroundings. These results suggest that vegetation structure, particularly tree size (*i.e.*, DBH), plays a key role in the variation of bat call frequency, whereas canopy cover affects the temporal aspects of calls, such as pulse duration. Our results indicate that different aspects of habitat complexity influence different acoustic parameters, reflecting how bats modify their echolocation to navigate and forage in environments with varying levels of clutter.

The most variable parameters were Δ Frequency (kHz) and Δ Time (s) for the whole ensemble. For *Saccopteryx bilineata*, we found rather unusual results that contradicted our predictions (Fenton *et al.* 2016). Initially, we assumed that in a more cluttered environment, pulses would be shorter and with a wider Δ Frequency delta to better avoid environmental obstacles (Moss *et al.* 2011). However, our results suggest that *S. bilineata* does the opposite: we recorded a shift to larger frequencies and a shorter in open spaces compared to cluttered environments. In comparison, and as we expected, *Neoeptesicus* cf. *furinalis* had longer duration calls with narrower Δ Frequency delta in open spaces, and short pulses with a higher Δ Frequency delta in highly cluttered areas (Moss *et al.* 2011).

Bats adjust their spectral and temporal parameters according to vegetation structure. To understand how the temporal and spectral parameters of echolocation pulses vary in bats, it is key to first determine how vegetation structure varies. The cluttered area (BT) is a transitional forest that has been restored for about 15 years. In this area, tall trees predominate, but with a lower DBH and with a smaller canopy width. In comparison, the highly cluttered space (BM) is a mature forest with very little disturbance in the last 50 years, according to landowners. Trees present in the highly cluttered area have different heights and diameters, with an average height of 25 m, and an average DBH higher than BT. Therefore, the highly cluttered space has a thick intertwined canopy whereas the cluttered space did not.

Bats adjusted the spectral and temporal parameters of their echolocation calls according to the complexity of vegetation structure. The type of acoustic environment that bats need to detect, classify and avoid is directly related to structural variables such as tree height, canopy density, and DBH because these parameters determine the number and type of obstacles and need to be sorted out (Schnitzler and Kalko 2001). For instance, Δ Frequency increased in sites with higher DBH, while Δ Time increased in areas with lower percentage cover. These results indicate that echolocation pulses become more modulated in more cluttered environments, supporting our predictions.

Larger trees generally contribute to greater clutter because of their sheer size and because they promote a denser and taller understory (Chazdon *et al.* 2010). Moreover, pulse interval became longer in less cluttered spaces, also supporting our prediction. Sites with lower percentage of canopy coverage, by definition, are much more open and less obstructed. In these types of conditions, bats are not forced to increase their rate of sound emission to update their perception of their environment (Fenton *et al.* 2016). We do not exclude other factors that may influence how bats modulate their echolocation signals. For instance, bat size (López-Cuamatzi *et al.* 2020) and feeding behavior (Fenton *et al.* 2016) also play a role in structuring echolocation calls. Yet, our results support our prediction that bats will echolocate at higher frequencies for shorter durations in cluttered areas (Denzinger *et al.* 2016).

Differences in spectral-temporal parameters in two species across three habitats. Saccopteryx bilineata is known for its vocal learning abilities (Knörnschild *et al.* 2006) and exhibits complex acoustic social interactions (Davidson and Wilkinson 2004). As shown by Ratcliffe *et al.* (2011), we observed that *S. bilineata* can alter its echolocation signals as needed, especially in relation to the type of habitat where it is foraging. For instance, Jakobsen *et al.* (2012) showed that *S. bilineata* significantly reduced the intensity and duration of its calls when flying in a flight cage, a proxy for a closed space environment. This aligns with our findings; Δ Time was higher in open environments (S) compared to sites with some obstacles (BT) or to highly cluttered environments (BM). In other words, pulses were longer in open spaces than in cluttered spaces. Our results support the idea that bats reduce the duration of their echolocation calls in cluttered environments. In cluttered habitats, the speed of receiving and processing environmental information is crucial, and this modification in call duration could facilitate such processing (Denzinger *et al.* 2016).

On the other hand, Δ Frequency and peak frequency showed a surprising behavior. We expected that Δ Frequency should be highest in cluttered spaces as bats increase their frequency modulation so they can effectively differentiate prey from vegetation (Denzinger *et al.* 2016). However, and contrary to our predictions, we observed that Δ Frequency and peak frequency were significantly higher in open spaces compared to cluttered spaces. Both results contradict existing literature. We think there are three different, not mutually exclusive hypotheses that can help us explain these unforeseen results.

First, call structure could change due to prey size (Aldridge and Rautenbach 1987). It is possible that smaller insects are found in greater numbers in the savanna (open spaces), so increasing both peak frequency and the range of frequency modulation of the echolocation signal would be advantageous and facilitate the detection of smaller prey at closed distances. Further studies assessing prey size across habitats would help us evaluate this hypothesis.

Second, *S. bilineata* might be increasing the frequency of their calls in open spaces to increase the directionality of their sonar beam. Jakobsen *et al.* (2012) found that *S. billineata* emits at higher frequencies to achieve more directional sonar beams, which increases the resolution and level of detail in their acoustic field of view. Seems contradictory to increase frequency and frequency modulation in open spaces because a reduction in call intensity also reduces the chances that a bat will detect an echo from a distant prey (Jakobsen *et al.* 2012). However, a narrower sound beam produces more intense sounds at the center of the beam, increasing the range at which bats can receive echoes, thus increasing the chances of detecting prey (Jakobsen *et al.* 2013). It is commonly assumed that bats use more energy when emitting echolocation calls at higher frequencies.

Initial studies by Speakman *et al.* (1989) showed that bats expend more energy at rest as vocal frequency increases. However, later studies showed the energy demand of echolocation was negligible compared to the high energy demand of flight (Speakman and Racey 1991; Voigt and Lewanzik 2012). Currie *et al.* (2020) found that increased signal intensity, but not frequency, increased energy expenditure even during flight. Therefore, more detailed studies in echolocation physiology will help us determine whether *S. billineata*, and maybe other species, increase their frequency range of emission to produce narrower beams in open spaces.

A third option as to why *S. bilineata* increased its frequency and decreased its pulse duration in open areas could be to avoid acoustic interference. *Saccopteryx leptura*, *Peropteryx macrotis*, and *Molossops temminckii* forage in similar areas as *S. bilineata*, and there could be an overlap with the echolocation calls of these species and *S. bilineata*. These three other species produce calls in similar frequency ranges (Supplementary material, Table 5), which could lead to acoustic interference.

Acoustic interference is related to acoustic niche partitioning, where coexisting species adjust their acoustic signals to minimize masking and enhance foraging efficiency (Siemers and Schnitzler 2004; Ey and Fischer 2009; Roemer *et al.* 2019). However, we believe this hypothesis seems unlikely because the abundance of these three species in the sampled habitats was low, reducing the likelihood of significant acoustic interference with *S. bilineata*.

Alternatively, the shift in *S. bilineata*'s call structure might not be solely related to acoustic overlap but could also be a strategy to reduce direct competition with other insectivorous bats in cluttered environments (Beilke *et al.* 2021). In cluttered habitats, where prey availability and species overlap may be higher, *S. bilineata* might exploit open spaces to reduce competition with species that hunt similar prey sizes. In these cluttered environments, where dense vegetation makes prey capture more difficult (Rainho *et al.* 2010), *S. bilineata* often shows a proportional aggregative response to increased prey abundance, whereas clutter-adapted species may remain unaffected by such variations (Müller *et al.* 2012). These findings support the hypothesis that *S. bilineata* may shift to open spaces as a strategy to access prey more efficiently and reduce competitive interactions.

Identifying the specific Molossidae species present in each habitat would help clarify whether this behavioral shift is driven by competition avoidance or by other adaptive factors. In sum, our results suggest that *S. bilineata* may have greater plasticity in its echolocation structure than previously thought. Further studies on prey size, sonar beam adjustments, and call overlap avoidance would be needed to better understand the potential costs of this plasticity.

Contrary to our results for *S. bilineata*, the spectral and temporal parameters of *N.* cf. *furinalis*, were consistent with theory (Barataud *et al.* 2013). Δ Frequency was higher in cluttered spaces compared to open spaces, suggesting that this species indeed benefits from having a broader frequency range in cluttered environments to efficiently navigate obstacles. Moreover, *N.* cf. *furinalis* showed shorter pulses in cluttered environments and longer pulses with lower frequency modulation in open spaces. The peak frequency for this species did not show significant differences between habitats.

Limitations. This study had two major limitations. First, our sampling period is short. We only sampled for one month and during the peak of the dry season. We do not know whether our results would be different in other times of the year where insect population abundance would be different across habitats, or if tree deciduousness would alter the acoustic space between habitats that bats must sort. It would be advisable that other studies integrate different climatic seasons to get a more complete picture of how vegetation complexity affects the echolocation structure of the insectivorous bat ensemble. The second limitation was the lack of data on insect size present in the different habitats, and the prey size that *S. bilineata* prefers among habitats. More information on the foraging ecology of this species would help us understand the unusual behavior we found.

Conclusions

Vegetation complexity significantly influences temporal and spectral parameters of bat echolocation calls in an insectivorous bat ensemble in the Llanos of Colombia. The structure of echolocation calls were significantly affected by diameter at breast height (DBH) and percentage of vegetation coverage (COV). We found unexpected call behavior in *Saccopteryx bilineata*, potentially influenced by various factors such a s prey sizes, sonar beam adjustments, and overlap avoidance, including direct competition with other species of similar size, even in the absence of acoustic overlap. Sorting among these hypotheses would help elucidate the potential costs of echolocation plasticity. Contrary to *S. bilineata*, the echolocation behavior of *N.* cf. *furinalis* followed the expected pattern according to theory. Understanding how bats change their spectral and temporal parameters according to different levels of vegetation complexity can provide insights into how species manage, or fail to manage, naturally open areas, such as the Colombian Llanos, or open areas resulting from anthropogenic activities.

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Trophic ecology of marine mammals in the Mexican Pacific Ocean: Prey diversity, network structure, and overlap with fisheries

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During the 20th century, interaction between marine mammals and fisheries pervaded all Mexican seas and fishing activities, with severe impacts for both fishermen and mammals, generating the need to investigate these animals' trophic ecology to better manage the country's fisheries. Aimed to characterize the trophic ecology of marine mammal fauna in the Mexican Pacific Ocean and their interaction with fisheries, here we built a network for the diet similarity of marine mammals and examined its modularity to identify trophic guilds and analyze its relationship with trophic level, prey diversity, and trophic overlap with fisheries. We reviewed literature and data in our group to identify and validate for comparison, 380 prey species of 40 marine mammal species from the Mexican Pacific Ocean. We determined a similarity matrix between marine mammal diets that depended on the diversity and ingested amount of 8 prey types. From this matrix, we built a non-directional and weighted network, with mammal species being the nodes, and diet similarities the edges. We examined modularity and other network traits in relation to mammals' trophic level, prey richness, and overlap with fisheries. We identified 5 network modules of marine mammals that we defined as trophic guilds, being I) planktophagic, II) ichthyophagic, III) teuthophagic of low trophic level, IV) teuthophagic of high trophic level, and V) sarcophagic. We observed a wide variation among mammals for their weighted degrees (added pairwise similarities), prey richness, and trophic levels that combine differentially known diets and diets with different prey diversities. Inverse relationships between prey richness and weighted degree at the species level, and between trophic level and weighted degree at the guild level, indicate that Mexican Pacific marine mammals belong to two trophic systems –surface and deep waters– mainly structured by competitive exclusion, which is stronger at higher trophic levels. Marine mammals with greater trophic overlap with fisheries in the Mexican Pacific Ocean occur in guilds I and II, principally *Phocoena sinus*, *Zalophus californianus*, *Tursiops truncatus*, and *Delphinus bairdii*.

Key words: Competitive exclusion; Functional group; Network modularity; Trophic guild; Trophic system

Durante el siglo XX, la interacción entre mamíferos marinos y pesquerías se extendió a todos los mares mexicanos y actividades pesqueras con impactos severos para ambos, pescadores y mamíferos, generando la necesidad de investigar la ecología trófica de estos animales para desarrollar una mejor gestión de los recursos pesqueros del país. Para caracterizar la ecología trófica de la mastofauna marina del Pacífico Mexicano y su interacción con las pesquerías, aquí construimos una red de similitud en la dieta de mamíferos marinos, examinando su modularidad para identificar gremios tróficos y analizar su relación con el nivel trófico, la diversidad de presas y el traslape trófico con las pesquerías. Revisamos literatura y datos de nuestro grupo para identificar y validar para comparación, 380 especies presa de 40 especies de mamíferos marinos del Pacífico Mexicano. Determinamos una matriz de similitud entre las dietas de los mamíferos marinos, dependiente de la diversidad y la cantidad ingerida de 8 tipos de presas, con la que construimos una red no direccional con pesos, siendo las especies de mamíferos los nodos y sus similitudes las aristas. Examinamos la modularidad y otros atributos de la red en relación con el nivel trófico de los mamíferos, la riqueza de sus presas y su traslape con pesquerías. Identificamos 5 módulos en la red de mamíferos marinos que definimos como gremios tróficos, siendo éstos: I) planctófagos, II) ictiófagos, III) teutófagos de bajo nivel trófico, IV) teutófagos de alto nivel trófico y V) sarcófagos. Observamos una amplia variación entre los mamíferos en sus grados ponderados (la suma de sus similitudes pareadas), riquezas de presas y niveles tróficos, lo cual combina dietas diferencialmente conocidas y dietas que en realidad tienen diferentes diversidades de presas. Relaciones inversas entre la riqueza de presas y el grado ponderado a nivel de especies, así como entre el nivel trófico y el grado ponderado a nivel de gremio, indican que los mamíferos marinos del Pacífico Mexicano pertenecen a dos sistemas tróficos, –aguas superficiales y profundas–, mayormente estructurados por exclusión competitiva que es más fuerte en altos niveles tróficos. Los mamíferos marinos de mayor traslape trófico con las pesquerías en el Pacífico Mexicano son de los gremios I y II, principalmente *Phocoena sinus*, *Zalophus californianus*, *Tursiops truncatus* y *Delphinus bairdii*.

Palabras clave: Exclusión competitiva; Gremio trófico; Grupo funcional; Modularidad de redes; Sistema trófico

Most marine mammals are highly vulnerable to the ongoing environmental deterioration as they inhabit broad geographic distributions in which they travel long distances and are impacted by different human activities with complex effects on their health, foraging, reproduction, and dispersion (Katona and Whitehead 1988; Harwood 2001; Acevedo-Whitehouse and Duffus 2009; Smith *et al.* 2009). Marine mammal ecology is sensitive to shifts in the oceans, making their foraging and reproduction reflect changes in marine food webs, and their movements also reflect changes in large-scale biophysical signals. For these reasons, research on marine mammals focuses on inferring changes in the structure and function of marine ecosystems from them, under the concept of sentinel species (Ross 2000; Aguirre and Tabor 2004; Moore 2008; Bossart 2011; Moore and Kuletz 2019).

Marine mammals have medium to high trophic levels and consume large amounts of food, which importantly contribute to the flows of matter and energy in the oceans. They transport nutrients from deep to shallow waters, and their long-distance movements are relevant to horizontally spread fertilizing materials across the oceans, all of which stimulates primary productivity (Gaskin 1982; Katona and Whitehead 1988; Papale and Giacoma 2025). Especially large cetaceans have recently been recognized as being of great importance to the global dynamics of carbon (Roman *et al.* 2014; Pearson *et al.* 2023). However, the high trophic levels and high metabolic rates of marine mammals also make them assimilate large amounts of pollutants, which are involved in severe physiological failures, especially immunological and reproductive, indicating that such pollutants also affect other species in the marine trophic webs (*e.g.*, Aguilar *et al.* 1987, 1999; Reijnders 1988; Jefferson *et al.* 2006). Another important ecological trait of marine mammals is their high trophic plasticity by which these animals can adapt to diverse environmental changes, but by which they also negatively interact with the varied and intensive human activities at sea nowadays, especially fisheries (Nemoto 1970; Gaskin 1982; Northridge 1985, 1991, 2009a, b; Northridge and Hoffman 1999; Fertl, 2009; Plagányi and Butterworth 2009). Interactions between marine mammals and fisheries have occurred for centuries. Still, since the mid-20th century, fisheries drastically intensified and underwent technological revolutions, leading to profound changes in marine ecosystems, including high mortalities of marine mammals and other amniotes worldwide. The interaction between marine mammals and fisheries is a complex and dynamic problem that results in the annual death of several hundred thousand individuals and inflicts dreadful wounds on many thousands more. This problem has worsened during the 21st century, with gillnets accounting for 84% and 98% of fisheries-caused mortality of odontocetes and pinnipeds, respectively (Anderson 2001; DeMaster *et al.* 2001; Read *et al.* 2006; Northridge 2009a, b; Reeves *et al.* 2013; Sonne *et al.* 2024). Gillnets are now the greatest risk for the imminent extinction of the vaquita (*Phocoena sinus*) and another 12 units in critical risk of 5 small cetacean species (D'Agrosa *et al.* 2000; Rojas-Bracho *et al.* 2006; Jaramillo-Legorreta *et al.* 2017; Brownell Jr. *et al.* 2019).

Since the 19th century, several marine mammal conservation issues in Mexico have become important nationally and internationally, involving management, economy, politics, and social welfare. Such issues currently include interaction with fisheries, physical, chemical, and biological pollution, collisions with ships, interaction with touristic activities, habitat deterioration, and the synergisms of these impacts with climate change (Aurioles 1993; Arellano-Peralta and Medrano-González 2013, 2015; Heckel *et al.* 2020). Between 46 and 50 marine mammal species inhabit the Mexican Pacific Ocean, some of which have been economically, politically, or socially important throughout history. The Mexican Pacific Ocean encloses the oceanographic and biogeographic transition between the Eastern Tropical Pacific and the Northeastern Pacific, and thus exhibits species with different biogeographic and environmental affinities (Ballance *et al.* 2006; Medrano González *et al.* 2008; Medrano González and Urbán Ramírez 2019; Heckel *et al.* 2020), as well as one of the very few endemic marine mammals in the world: the vaquita.

Knowledge on marine mammal foraging accelerated throughout the 1980s when the collapse of several fisheries favored culling campaigns against diverse marine mammals, urging the need to assess interactions between fisheries and marine mammals and their impacts on both (Northridge 1985, 1991; Kaschner and Pauly 2005). Data on marine mammals feeding in Mexico have been obtained through direct observation (*e. g.*, Gendron and Urban 1993; Sánchez-Pacheco *et al.* 2001), examination of feces (*e. g.*, Bautista Vega 2000, 2002; Porras Peters 2004) and stomach contents (*e. g.*, Pérez-Cortés Moreno *et al.* 1996), analysis of fatty acids content (*e. g.*, Nolasco Soto 2003; López Montalvo 2005, 2012; Rueda Flores 2007; Traconis Corres 2010) and stable isotope proportions for different elements (*e. g.*, Gendron *et al.* 2001; Jaume Schinkel 2004; Porras-Peters *et al.* 2008; Elorriaga-Verplancken *et al.* 2013; Busquets-Vass *et al.* 2021), molecular scatology (Jiménez Pinedo 2010; Guerrero de la Rosa 2014), and metagenomics (Brassea-Pérez *et al.* 2019). These and other studies have examined the relationship of marine mammals with the abundance of their prey in the context of environmental variation (*e. g.*, Gendron Laniel 1990; Gendron and Urban 1993; García Rodríguez 1999; Jaquet and Gendron 2002; Porras-Peters *et al.* 2008). Nonetheless, not much research in Mexico has treated marine mammal trophic ecology at the community level, or on a non-local scale, *i. e.*, mesoscale.

In this work, we compile and compare the prey diversity of 40 marine mammal species from the Mexican Pacific Ocean. We built a network, with nodes being mammal species and edges their pairwise diet-similarities, to examine its modularity, weighted degree of connections, trophic level, and prey diversity, to identify trophic guilds and their relationships. We also examine diet similarities to examine the species' propensity for competition and interaction with fisheries.

Materials and methods

Diet data. We thoroughly reviewed literature and data from our group not reported in literature (Sánchez Arias 1992; Unpublished observations of author LMG), to identify 380 prey species taken by 8 mysticetes, 27 odontocetes, and 5 pinnipeds from the Mexican Pacific Ocean (Table 1; Figure S1; Supplementary Excel file). Prey compilation and validation are updated until September 2021. Since diet diversity is highly underestimated (Pauly *et al.* 1998; Trites 2019), we assumed that species or genera listed as prey in other regions are also taken in the Mexican Pacific Ocean if they are present there. This decision could cause a few false positives which surely

Table 1. Prey diversity (richness; PR), trophic level (TLMP), weighted degree (WD), and prey overlap with fisheries (%; POF) in the diet network of 40 marine mammal species from the Mexican Pacific Ocean.

Species	Acronym	Guild	PR	TLMP	WD	POF
Eubalaena japonica	Ejap	I	6	3.20	24.2	0.0
Balaenoptera musculus	Bmus	I	8	3.21	24.2	0.0
Eschrichtius robustus	Erob	I	3	3.28	24.6	0.0
Megaptera novaeangliae	Mnov	I	9	3.61	25.6	22.2
Balaenoptera borealis	Bbor	I	12	3.29	25.0	33.3
Balaenoptera physalus	Bphy	I	12	3.34	25.1	41.7
Balaenoptera edeni	Bede	I	7	3.37	26.2	42.9
Balaenoptera acutorostrata	Bacu	I	3	3.41	26.0	66.7
Steno bredanensis	Sbre	II	7	4.13	27.0	14.3
Delphinus delphis	Ddel	II	56	4.25	20.6	17.9
Phocoenoides dalli	Pdal	II	28	4.16	25.3	21.4
Phoca vitulina richardii	Pvit	II	27	3.98	22.8	22.2
Aethalodelphis obliquidens	Lobl	II	38	4.08	23.3	23.7
Arctocephalus townsendi	Aobl	II	35	3.86	23.4	25.7
Delphinus bairdii	Dbai	II	27	4.19	24.2	37.0
Arctocephalus galapagoensis	Agal	II	8	4.08	27.8	37.5
Tursiops truncatus	Ttru	II	56	4.20	18.6	39.3
Zalophus californianus	Zcal	II	102	4.05	10.1	42.2
Mesoplodon carlhubbsi	Mcar	III	8	4.20	26.7	0.0
Mesoplodon stejnegeri	Mste	III	2	4.20	27.2	0.0
Mesoplodon peruvianus	Mper	III	1	4.20	28.3	0.0
Berardius bairdii	Bbai	III	11	4.24	27.3	0.0
Mesoplodon densirostris	Mden	III	6	4.37	27.6	0.0
Feresa attenuata	Fatt	III	3	4.65	27.8	0.0
Lissodelphis borealis	Lbor	III	15	4.21	26.5	6.7
Peponocephala electra	Pele	III	6	4.42	27.7	16.7
Mirounga angustirostris	Mang	III	49	4.21	20.4	18.4
Phocoena sinus	Psin	III	20	4.09	24.6	45.0
Kogia breviceps	Kbre	IV	56	4.35	19.9	0.0
Lagenodelphis hosei	Lhos	IV	56	4.22	18.9	1.8
Grampus griseus	Ggri	IV	30	4.33	24.3	3.3
Stenella longirostris	Slon	IV	56	4.32	19.6	3.6
Kogia sima	Ksim	IV	28	4.43	24.1	3.6
Ziphius cavirostris	Zcav	IV	26	4.43	25.4	3.8
Stenella attenuata	Satt	IV	62	4.14	19.0	4.8
Stenella coeruleoalba	Scoe	IV	37	4.22	23.6	5.4
Globicephala macrorhynchus	Gmac	IV	28	4.33	25.6	7.1
Physeter macrocephalus	Pmac	IV	39	4.44	22.5	10.3
Orcinus orca	Oorc	V	59	4.53	16.0	8.5
Pseudorca crassidens	Pcra	V	20	4.56	24.3	15.0

are much less than the false negatives under the assumption that marine mammals do not take in Mexican waters preys that take elsewhere. We have thus included the Galapagos fur seal (*Arctocephalus galapagoensis*) in our analysis as it has been sighted in the Mexican Pacific Ocean repeatedly for more than 2 decades, and its diet includes items recorded in this region. We have not included the Galapagos sea lion (*Zalophus wollebaeki*), the Steller sea lion (*Eumetopias jubatus*), or the Southern elephant seal (*Mirounga leonina*) as they were recently registered in Mexican waters, apparently associated with unusual environmental variation (Gallo-Reynoso *et al.* 2020; Elorriaga-Verplancken *et al.* 2022; Barba-Acuña *et al.* 2024), and as we are not aware yet of these species feeding in the region.

Prey were grouped in 8 sets following Pauly *et al.* (1998) as benthic invertebrates (BI), large zooplankton (LZ), small cephalopods, mainly squid (SS), large cephalopods (LS), small pelagic fish (SP), mesopelagic fish (MP), miscellaneous fish (MF), and marine amniotes (HV in the Pauly *et al.* terminology). To properly compare diets that have been described with different taxonomic classifications and prey names along several years, we validated and updated prey identity and occurrence in the Mexican Pacific for the mammals studied by consulting the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/), the World Register of Marine Species (WoRMS, http://www.marinespecies.org/), and several expert researchers at the *Instituto de Ciencias del Mar y Limnología* and *Instituto de Biología, Universidad Nacional Autónoma de México*.

Comparison of diet compositions. We determined a diet similarity index for pairwise comparisons (S_{ij}) between the 40 marine mammal species, looking to build a network with marine mammals as nodes and their similarity comparisons as edges. Our similarity index is based on the mass composition of the 8 prey types by Pauly *et al.* (1998), with slight modifications to the biomass fraction due to the absence or presence of prey types not identified by these authors on marine mammals from the Mexican Pacific Ocean. For Mexican Pacific mammals not in the Pauly *et al.* list, we defined their biomass fractions by averaging the fractions in listed marine mammals with the same foraging habits, distribution in the Eastern Pacific Ocean or adjacent regions, and belonging to the same genus or subfamily. Therefore, we estimated the biomass fractions of *Pseudorca crassidens* as the mean of *Orcinus orca* and *Feresa attenuata*, and the fractions of *Mesoplodon peruvianus* (East Tropical Pacific) as the average of *Mesoplodon densirostris* (tropical and subtropical oceans worldwide), *Mesoplodon layardii* (Southern Ocean), *Mesoplodon hectori* (Southern Ocean), *Mesoplodon stejnegeri* (North Pacific Ocean), and *Mesoplodon carlhubbsi* (Northeastern Pacific Ocean). Our similarity index also compares the number of shared prey species within each prey type. Diet similarity between the 40 marine mammal species analyzed is thus defined as follows:

$$S_{ij}=\Big(\sum_{x=1}^{8}[(R_{xi} \cap R_{xj})/(R_{xi} \cup R_{xj})] + [1-abs(f_{xi} - f_{xj})]\Big)/8$$

where x indicates the 8 prey types described by Pauly *et al.*, $R_{xi} \cap R_{xj}$ are the prey species of type x shared by mammals i and j, $R_{xi} \cup R_{xj}$ are the total prey species taken by both predators, and f_{xi} y f_{xj} are the biomass fractions that prey type x represents in mammals i and j. Notice that the occurrence of a prey species in two marine mammals contributes to their similarity, but the absence of a prey species in two mammal species does not. Adding and not multiplying the similarity factors of biomass fractions and shared species allows detection of a degree of similarity when there are no shared prey species, which might occur in poorly known diets. For the network analysis, our index design also avoids artificial modules created by diets with very few prey species, as Benavidez Gómez (2016) observed in her classification by cluster analysis based on shared prey only. We calculated the similarity matrix among the 40 marine mammal species in the format of an adjacency list, by developing the program DIETSIM in the LAZARUS Integrated Development Environment (https://www.lazarus-ide.org/; available upon request).

Network analysis. We determined the trophic level for each marine mammal species following equation 1 in Pauly *et al*. (1998), based on prey biomass fractions with the modifications described above for Mexican Pacific marine mammals. We used the program GEPHI 0.10 (https://gephi.org/) to build a network with marine mammals as nodes and their similarity comparisons as edges with weight and no direction for the similarity matrix having $(40^2-40)/2 = 780$ pairwise comparisons between all species. Notice that in such a network, nodes are all connected, even if their similarity could be zero. We used GEPHI to determine network attributes such as average degree, with and without weight, density, diameter, transitivity, nodes' degree with and without weight, and nodes' centrality. A node's degree is the sum of its connections (pairwise comparisons) in the network; a weighted connection is a pairwise similarity value –S_{ij}– as described above. See Menczer *et al.* (2020) for an introduction to network analysis.

We examined modularity with resolution 0.95, which better reproduced the cluster analysis of prey diversity by Benavidez Gómez (2016) that yielded the traditional marine mammal guilds: planktophagic, ichthyophagic, teuthophagic, and sarcophagic. Statistical significance of modularity was tested by the Erdös-Renyi procedure, which consists of randomly permuting edges and calculating modularity for each shuffle to build a random distribution of connections between nodes, which was then compared against actual modularity. A total of 1000 edge shuffles were made with the program SHUFFLEDAT developed in the LAZARUS Integrated Development Environment (available upon request), calculating raw modularity with equation 1 in Blondel *et al.* (2008), which is the one used by GEPHI. Statistical significance of modularity was accepted if its actual value was greater than the top 5% of its randomized distribution. We have termed statistically significant modules as trophic guilds.

Marine mammal species were accommodated in the network following modularity and trophic level; edges were plotted with thickness proportional to their weight and the color of the destination module. Marine mammal diets, organized by trophic guilds (network modules), were described with the biomass fractions of the 8 prey types, uniformly subdivided by prey species within each type. To know the position of each marine mammal on the scales of prey richness, weighted degree, and trophic level, and thus to identify species groupings and scale relationships, we examined the cumulative distributions of such scales, distinguishing trophic guilds. We particularly examined the relationship between the nodes' weighted degree and trophic level by marine mammal species and trophic guild.

Trophic overlap with fisheries. We consulted the Mexican National Fisheries Chart (Diario Oficial de la Federación 2012), which lists 298 species exploited by five fishery types: shrimp, squid, cartilaginous fish, minor pelagic fish, and finfish. We examined the trophic overlap between these fisheries and marine mammal diets in terms of shared species, looking to provide a minimum approach for the increasing problems of competition and operational interaction between marine mammals and fisheries worldwide that cause bycatch, depredation of fishing gear, and other ecosystemic impacts that are poorly understood biologically and socially (Jusufovski *et al.* 2019; Jog *et al.* 2022).

Results

Diet composition and trophic guilds. We registered 380 prey species that were validated to compare the diets of 40 marine mammals from the Mexican Pacific Ocean for 1062 trophic relationships in total. Taxonomically, such prey are 21 crustaceans, 74 cephalopods, 3 tunicates, 246 fish, 3 turtles, 5 birds, and 28 mammals. Per ecological type, prey are: BI, 9; LZ, 20; SS, 58; LS, 11; SP, 17; MP, 67; MF, 162; and HV, 36 (Supplementary file). Similarity between marine mammal diets varied from 0.02 between *Zalophus californianus* and *O. orca*, to 0.95 between *Balaenoptera physalus* and *Balaenoptera borealis*. Since the network is completely connected, its density, diameter, and average length are 1. Among nodes, the clustering coefficient is 1 for all; all have unweighted degree 39, all have eccentricity 1, all have centralities 1 (closeness, harmonic closeness, betweenness, and eigen), and all are part of 741 triangles. The average weighted degree was 23.77, ranging from 10.05 for *Z. californianus* (the least connected in the network) to 28.28 for *M. peruvianus*. Modularity after resolution 0.95 was 0.005 with raw calculation of 0.015 using equation 1 in Blondel *et al* (2008). The random distribution of edges in 1000 shuffles yielded a total raw-modularity interval of -0.006 – 0.008 for actual modularity be statistically significant with $p < 0.001$. Trophic level ranged from 3.20 in *Eubalaena japonica* to 4.65 in *Feresa attenuata* (Table 1; Figures 1, 2). Mind that trophic levels are not biased by very few prey species in the diet because the trophic level is calculated from the prey types' biomass fraction, not prey richness (Pauly *et al.* 1998).

GEPHI identified five marine mammal modules that were already identified by Benavidez Gómez (2016) from a cluster analysis based only on the species richness. However, those modules and clusters are not equal in terms of species assignments. We call these modules trophic guilds that, beyond their diet composition, are also distinguished by their trophic level and mean weighted degree. Such guilds, ordered by increasing trophic level, are:

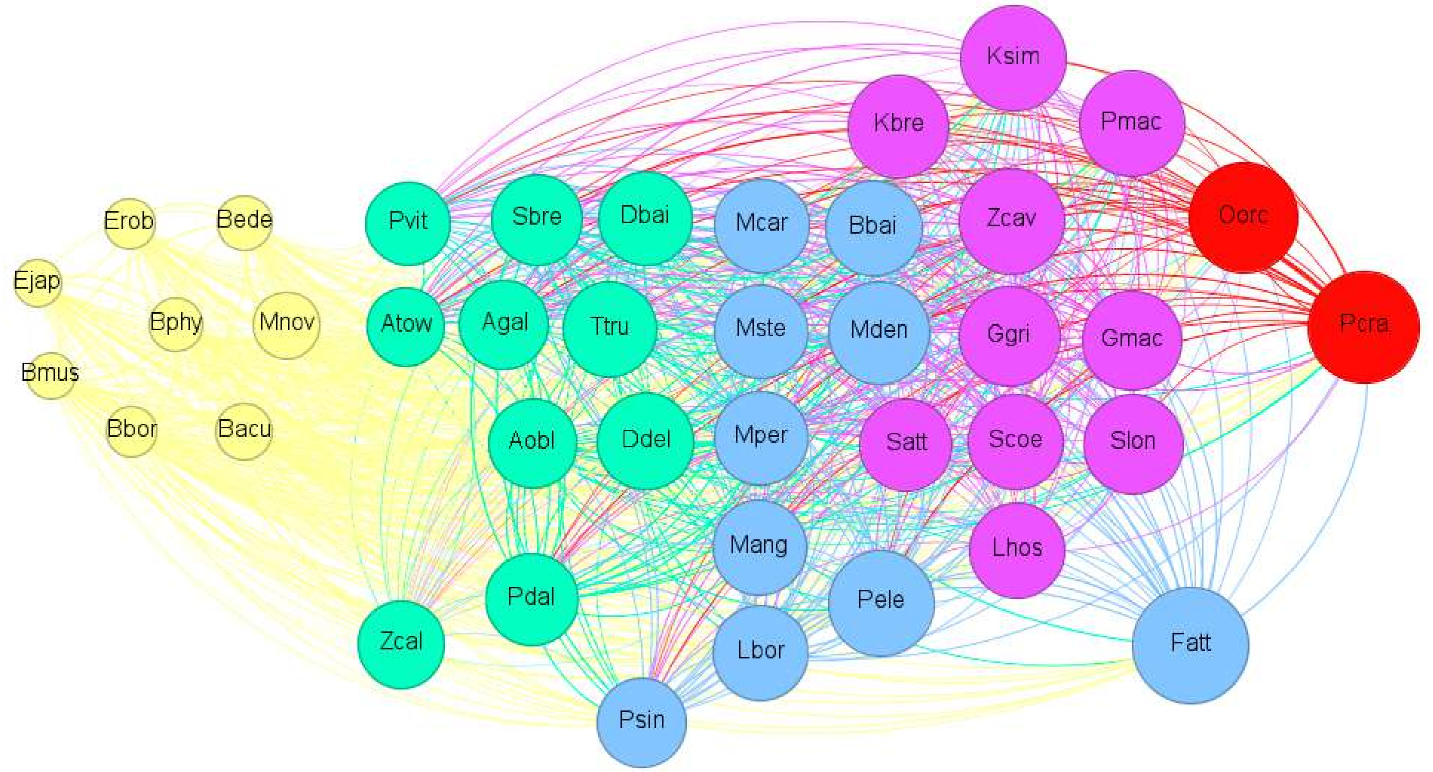

Figure 1. Network of diet similarities (edges) among 40 marine mammal species from the Mexican Pacific Ocean (nodes). Node colors correspond to the 5 modules identified by GEPHI, and their size to trophic level. The network is also oriented rightwards in parallel to the trophic level. Edge colors match the modules that GEPHI identified as their destinations, and their thickness to diet similarity. The network shows only the edges with similarity equal to or larger than 0.5, which is the lowest level at which all nodes are connected. The acronyms for marine mammal identities are formed by the first character of the genus and the first 3 characters of the species names.

I) mainly planktophagic, II) mainly ichthyophagic, III) mainly teuthophagic of low trophic level, IV) mainly teuthophagic of high trophic level, and V) mainly sarcophagic. Guild I includes eight mysticete species (*E. japonica, Balaenoptera musculus, Eschrichtius robustus, B. borealis, B. physalus, Balaenoptera edeni, Balaenoptera acutorostrata*, and *Megaptera novaeangliae*). Guild II is formed by four pinniped and six odontocete species (*Arctocephalus townsendi, Phoca vitulina richardii, Z. californianus, Aethalodelphis obliquidens, A. galapagoensis, Steno bredanensis, Phocoenoides dalli, Delphinus bairdii*, *Tursiops truncatus*, and *Delphinus delphis*). Guild III accounts for one phocid and nine odontocete species (*Phocoena sinus, M. carlhubbsi, M. stejnegeri, M. peruvianus, Mirounga angustirostris, Lissodelphis borealis, Berardius bairdii, M. densirostris, Peponocephala electra*, and *F. attenuata*). Guild IV contains 10 odontocete species (*Stenella attenuata, Lagenodelphis hosei, Stenella coeruleoalba, Stenella longirostris, Grampus griseus, Globicephala macrorhynchus, Kogia breviceps, Kogia sima, Ziphius cavirostris*, and *Physeter macrocephalus*). Guild V includes two delphinids (*O. orca* and *P. crassidens*; Table 1; Figures 1, 3).

Prey richness and diet similarities. Prey richness varied from 1 for *M. peruvianus* to 102 for *Z. californianus*, which combines different knowledge and actual prey diversity variation among the marine mammals studied. The cumulative profile of marine mammal species with reference to prey richness showed discontinuities and asymptotes defining 3 general sets (Figure 4). The first set includes 20 mammal species with 1 – 20 different prey items, the second set is formed by 11 mammal species with 26 – 39 preys, and the third set contains 9 mammal species with 49 – 102 preys. These groups seemingly correspond to marine mammals with poorly known, partially known, and well-known diets, as well as to mammals with really few (*e.g.*, mysticetes) and several prey items (*e.g.*, the California sea lion). Most Mexican Pacific mammals with few prey species after validation (richness < 20), include the false killer whale, and several species from guilds I and III, *i. e.*, several mysticetes that feed mainly in cold-temperate waters of the North Pacific Ocean or in the Eastern Tropical Pacific, and low trophic level teuthophagous, mainly beaked whales, about which there is little biological information. Guilds I and III also include a few poorly known pelagic dolphins, and the Galapagos fur seal, which has been registered repeatedly in Mexican waters for 2 decades (Aurioles-Gamboa *et al.* 2004; Medrano González *et al.* 2008; Tamayo-Millán *et al.* 2021), but whose diet is known for its regular distribution off Mexican waters (Clarke and Trillmich 1980; Dellinger and Trillmich 1999). Mammals with better-known diets (richness ≥ 26) are mainly in guilds II and IV, and include the killer whale from Guild V (richness = 59; Figure 4).

The cumulative profile of marine mammal species concerning weighted degree exhibited discontinuities defining 4 groups (Figure 5). *Z. californianus* alone composes the first set with weight 10.05; *O. orca*, also alone, composes the second set with weight 16.03. Seven species

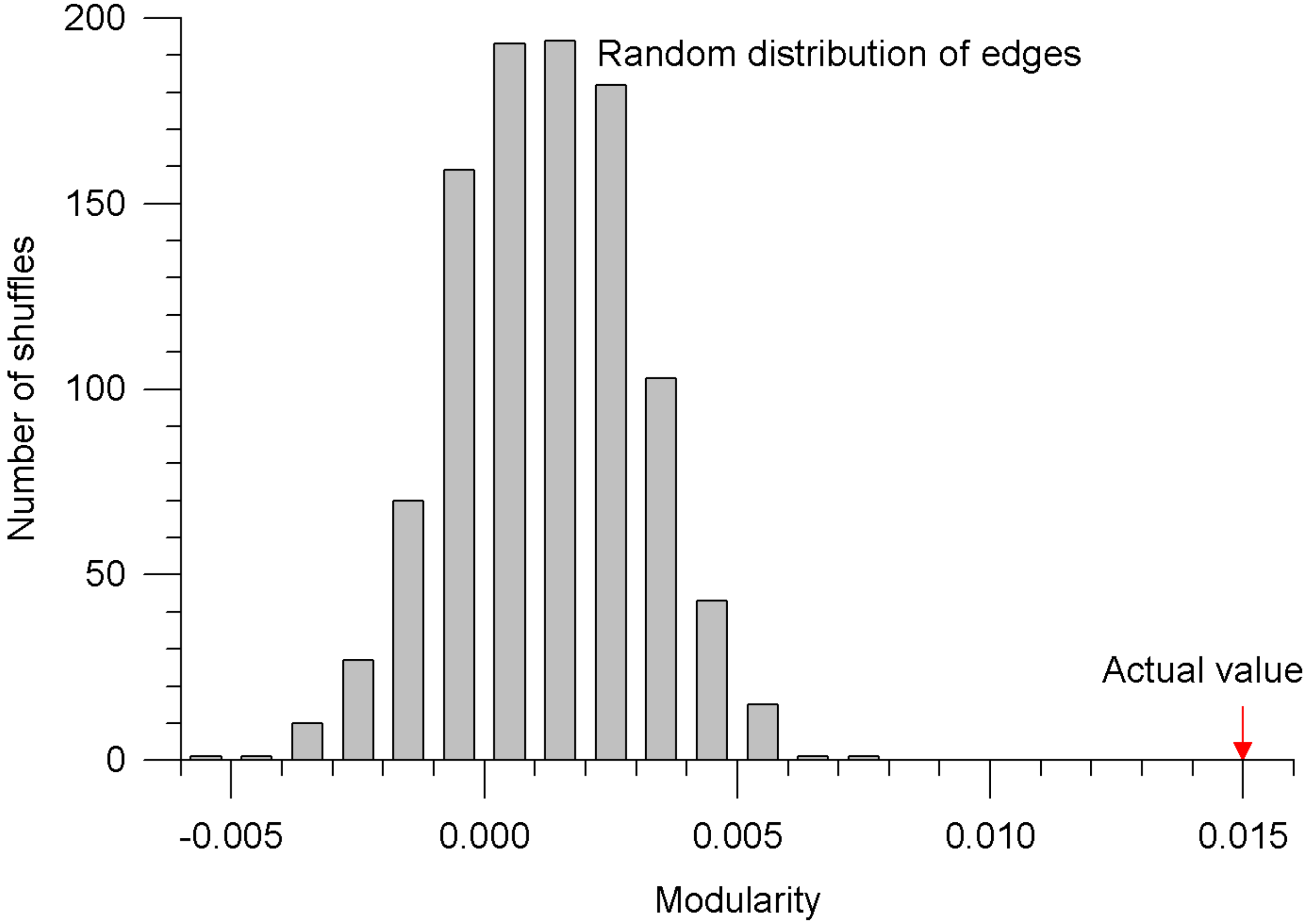

Figure 2. Actual raw-modularity (red arrow) for the network of diet similarities among 40 marine mammals from the Mexican Pacific Ocean and its statistical distribution after 1000 random permutations of the similarity connections between them (gray bars).

are included in the third set with weighted degrees 18.58 – 20.55, 4 from guild IV, 2 from guild II, and 1 from guild III. The fourth set is composed by 31 species with weighted degree varying in the range 22.48 – 28.28, with 8 out of 10 species from guild III being in the highest weight range, *M. peruvianus* in the top (Figure 5).

These results indicate that ordinations for the distributions of prey richness and weighted degree of the 40 marine mammals studied are inversely related. When both attributes were directly compared, an inverse relationship was indeed observed (Figure 6) with a high linear regression coefficient (-0.875) and slope of -0.152. *M. peruvianus* had the lowest prey richness and the highest weighted degree, whereas *Z. californianus* had the highest prey richness and the lowest weighted degree. *O. orca* appeared apart with a slightly lower prey richness and a higher weighted degree compared to *Z. californianus*. Marine mammals with low prey richness and high weighted degree were mainly in guilds I and III, whilst guilds II and IV occurred in the range of high prey richness and low weighted degree (Figure 6).

Trophic level and diet similarities. The cumulative profile of marine mammal species in trophic level exhibited discontinuities defining 4 general groups (Figure 7). The first group is formed only by trophic guild I in the trophic level range 3.2 – 3.6, well below the other guilds. Guilds II, III, and IV overlapped in the second group, with most species of guild II around trophic level 4.1, most species of guild III around trophic level 4.2, and guild IV species in subgroups around trophic levels 4.2, 4.3, and sperm whales by 4.45. The third group was composed only of guild V around trophic level 4.55. Only *F. attenuata* from guild III is in the fourth group with the highest trophic level of 4.65 (Figure 7).

Trophic guilds exhibited increasing trophic level from I to V (Figure 3), with the following means and standard deviations: I, 3.34 ± 0.13; II, 4.10 ± 0.12; III, 4.28 ± 0.16; IV, 4.32 ± 0.10; and V, 4.55 ± 0.02. The relationship between trophic level and weighted degree at the species level seemed inverse. Still, the linear regression index was very low (-0.013) since most species in guild III had high values of both trophic level and weighted degree (not shown). Trophic level and weighted degree showed a better-defined relationship at the guild level, with a linear regression coefficient of -0.279 and a slope of -2.83, with guild I having the lower average trophic level and higher average weighted degree, whilst guild V has the higher average trophic level and lower average weighted degree (Figure 8).

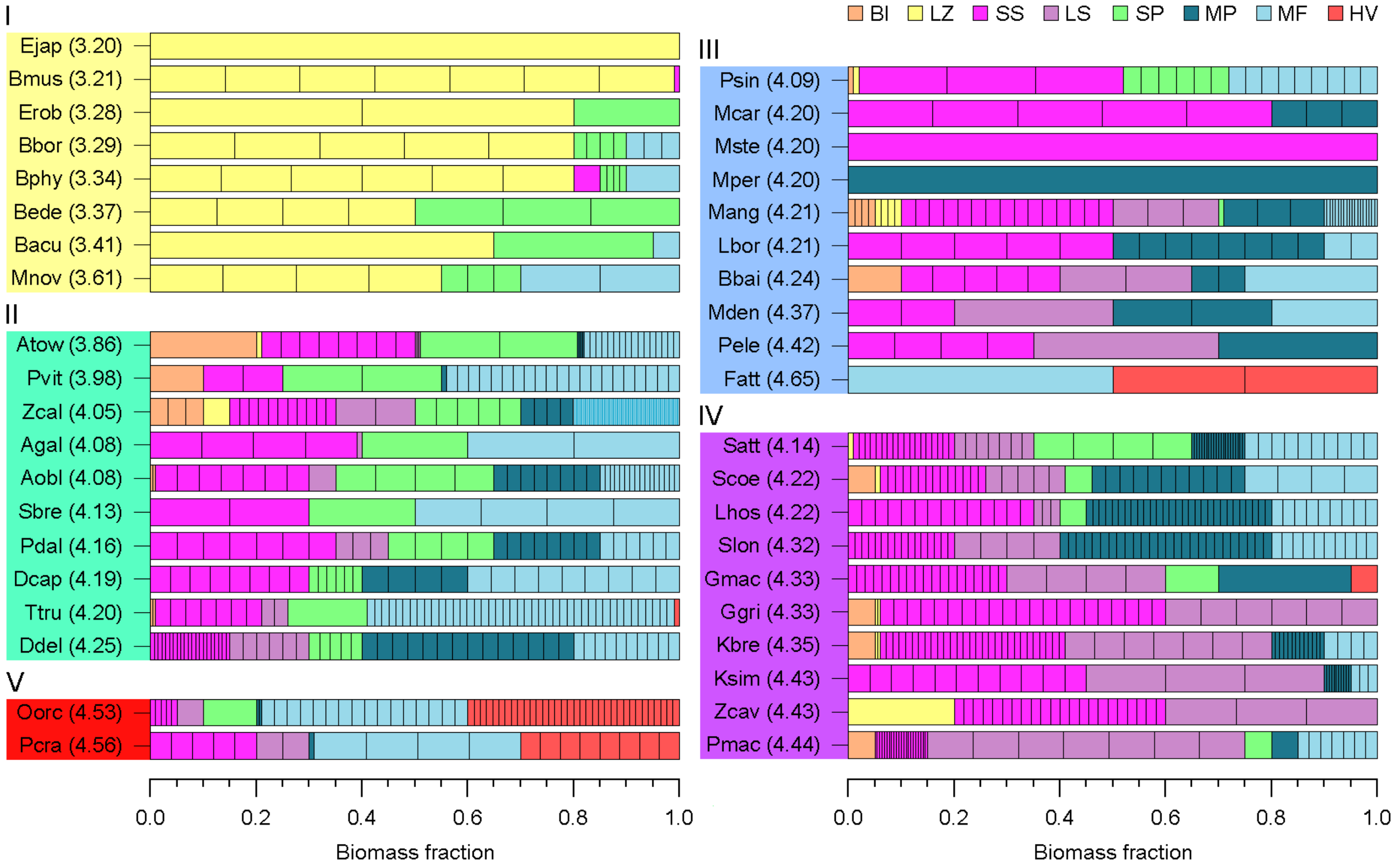

Figure 3. Diet composition of 40 marine mammal species from the Mexican Pacific Ocean. Mammals are grouped in the trophic guilds identified by the modularity analysis and are indicated by Roman numbers. Species are identified by acronyms as in Figure 1. Numbers in parentheses indicate the species' trophic level calculated in the Mexican Pacific Ocean. Bar colors indicate the biomass fraction of the 8 prey types defined by Pauly *et al.* (1998) and are subdivided equally among the prey type's species.

We found no relationship between prey richness and trophic level at the species or guild level. Average prey richness per guild is: I, 7.5 ± 3.5 (SD); II, 38.4 ± 27.8; III, 12.1 ± 14.3; IV, 41.8 ± 14.2; and V, 39.5 ± 27.7. Only within guilds III and IV do the species exhibit an apparent negative relationship between prey richness and trophic level, with r^2 = -0.122 and *r*2 = -0.374, respectively.

Trophic overlap with fisheries. Of the 298 species subjected to fisheries according to the Mexican National Fisheries Chart, 70 are also consumed by Mexican Pacific marine mammals (23.5%) and represent 180 of the 1062 trophic relationships in total (16.9%) validated for the 40 marine mammals and their 380 prey species examined here. 9 species exhibit trophic overlap over 30% of species in their diets shared with fisheries, including 4 mysticetes: *B. acutorostrata* with 2 preys shared with fisheries out of 3 in its diet (2/3 = 66.7%), *B. edeni* (3/7 =42.9%), *B. physalus* (5/12 = 41.7%), and *B. borealis* (4/12 = 33.3 %). The highest trophic overlaps with fisheries among odontocetes are: *P. sinus* (9/20 = 45.0%), *T. truncatus* (22/56 = 39.3%), and *D. bairdii* (10/27 = 37.0%). For pinnipeds, higher trophic overlaps are for *Z. californianus* (43/102 = 42.2%) and *A. galapagoensis* (3/8 = 37.5%). *B. musculus*, *E. robustus*, *E. japonica*, *F. attenuata*, *B. bairdii*, *M. carlhubbsi*, *M. stejnegeri*, *M. densirostris*, *M. peruvianus*, and *K. breviceps* showed no prey shared with fisheries in the Mexican Pacific Ocean (Table 1). Among guilds, higher trophic overlaps with fisheries are, on average: I, 25.8% ± 24.7 (SD); II, 28.1 ± 9.9; III, 8.7 ± 14.7; IV, 4.4 ± 2.8; and V, 11.7 ± 4.6 (Table S1). For fishery type, trophic overlaps with marine mammals are: minor pelagic fish (7/12 = 58.3%), finfish (56/228 = 24.6%), cartilaginous fish (6/52 = 11.5%), squid (1/1 = 100%), and shrimp (0/5 = 0.0%; Table S2).

Discussion

It is well recognized that marine mammals' diets are poorly and differentially known across species, time, and space (Trites 2019). Here, we compiled and validated 380 prey species to properly compare 40 marine mammal species from the Mexican Pacific Ocean. Validation of prey identities and geographic distributions after reviewing literature and our data implies that prey accounts here are in general lower, than the partial accounts in the literature treating marine mammals from the Mexican Pacific Ocean. Variation of prey richness (1 – 102) appears thus to result from differential knowledge of diets and actual prey diversity variation among species. We buffered the incomplete data in diet diversity by adding the similarity factors of biomass fractions and shared species, to minimize errors on similarity and thus network connections by diets with few validated prey items.

Mexican Pacific mammals with few validated prey species include poorly known species, mainly ziphiids and pelagic delphinids, but also well-known species such as

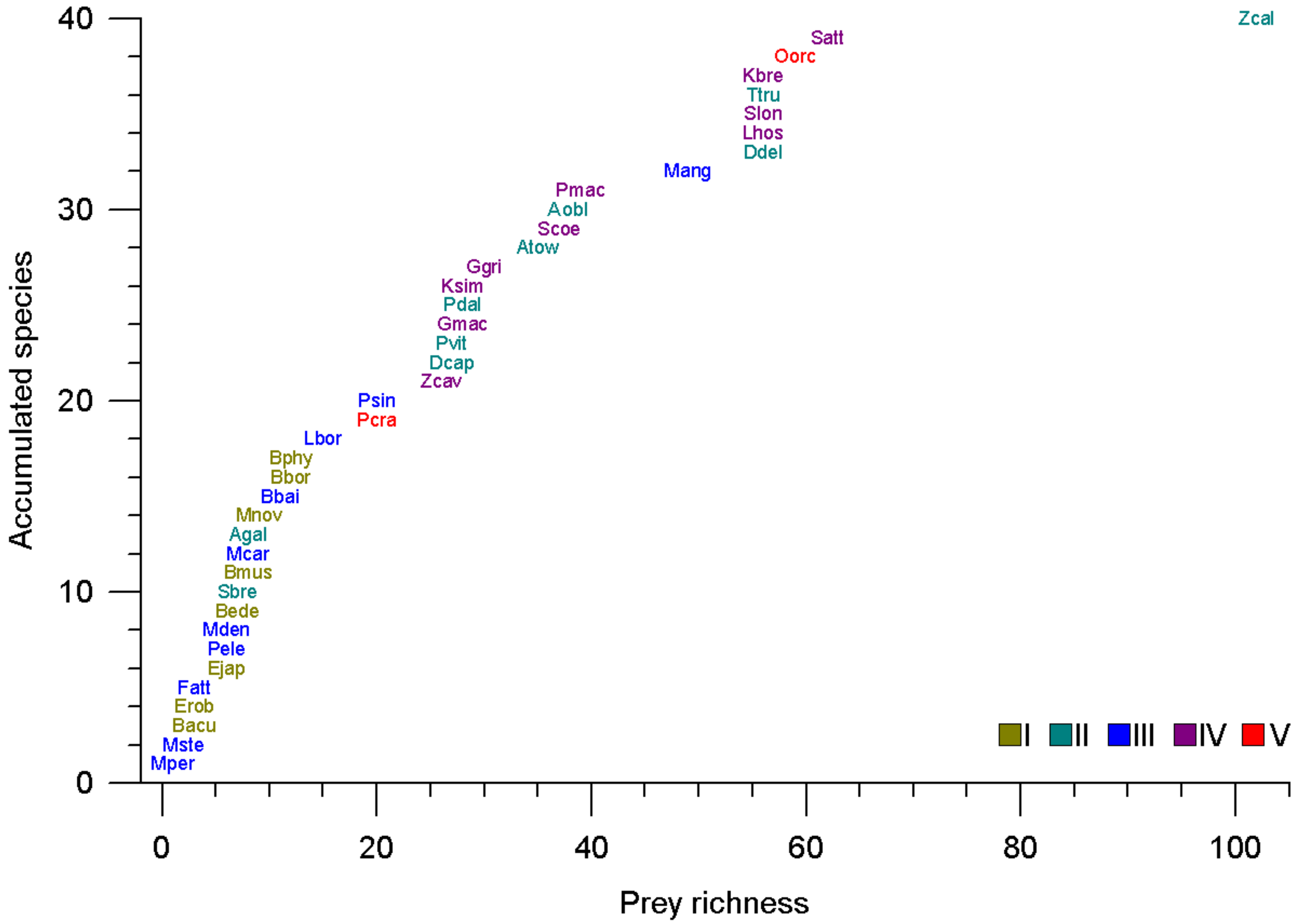

Figure 4. Cumulative distribution of prey richness for 40 marine mammals from the Mexican Pacific Ocean. Identity acronyms are as in Figure 1, and their colors correspond to the trophic guilds also in Figure 1.

the vaquita of which we validated for comparison 20 out of ca. 30 reported species, mysticetes that feed mainly in cold-temperate waters or in the Eastern Tropical Pacific, and the Galapagos fur seal, whose known diet is off Mexican waters. Average prey richness *ca*. 40 has been registered for guilds II, IV, and V, whilst guild I exhibited 7 – 8 prey, and guild III 12 prey. Prey richness appears thus underestimated in guild III, mainly by the few regional data for *M. peruvianus*, *M. stejnegeri*, *M. densirostris*, *F. attenuata*, and *P. electra*. *F. attenuata* is outstanding for it exhibits the highest trophic level among the studied mammals while pertaining to guild III (low trophic-level teuthophagous). First, such a high trophic level results from the higher biomass fraction of amniotes in the *F. attenuata* diet registered in the Mexican Pacific (*D. delphis* and *S. attenuata*), together with the fish *Merluccius* spp. that belong to the fish type with the highest trophic level (Sekiguchi *et al.* 1992; Perrin 2009). Second, to our knowledge, the diet of *F. attenuata* shows no published records of birds, large cephalopods, or small pelagic fish in the Mexican Pacific Ocean, causing us to overestimate the regional trophic level to 4.65 instead of the world value 4.4 by Pauly *et al.* (1998). Third, the diet of *F. attenuata* is in guild III, apparently because of artificial similarities with the diets of other poorly known mammals. The average and standard deviation of similarity between *F. attenuata* and other mammals in guild III was 0.82 ± 0.09, whilst similarity with guild II was 0.66 ± 0.15 and with guild V was 0.69 ± 0.16.

Because of the pairwise comparisons among marine mammal species, our network for diet similarities is all connected, exhibiting variation only in attributes dependent on weighted degree, besides prey richness and trophic level, which are not intrinsic network properties. The modularity analysis showed 5 statistically significant modules that we defined as trophic guilds and that very much correspond to the guilds identified by Benavidez Gómez (2016) from a cluster analysis of prey diversity that did not account for prey type biomass as we did here. Notice that we identified guilds by modules emerging from the trophic network. Such modules apparently assemble species that overlap their ecological niches, as using the same environmental resources in a similar way irrespective of their taxonomic position, as guilds are defined by Simberloff and Dayan (1991). Whether such guilds are generic in marine mammal trophic networks beyond the Mexican Pacific Ocean is uncertain, though we suspect that they might be, given that diet-biomass partition among the eight prey types should be similar at least in tropical and subtropical seas, as indicated by

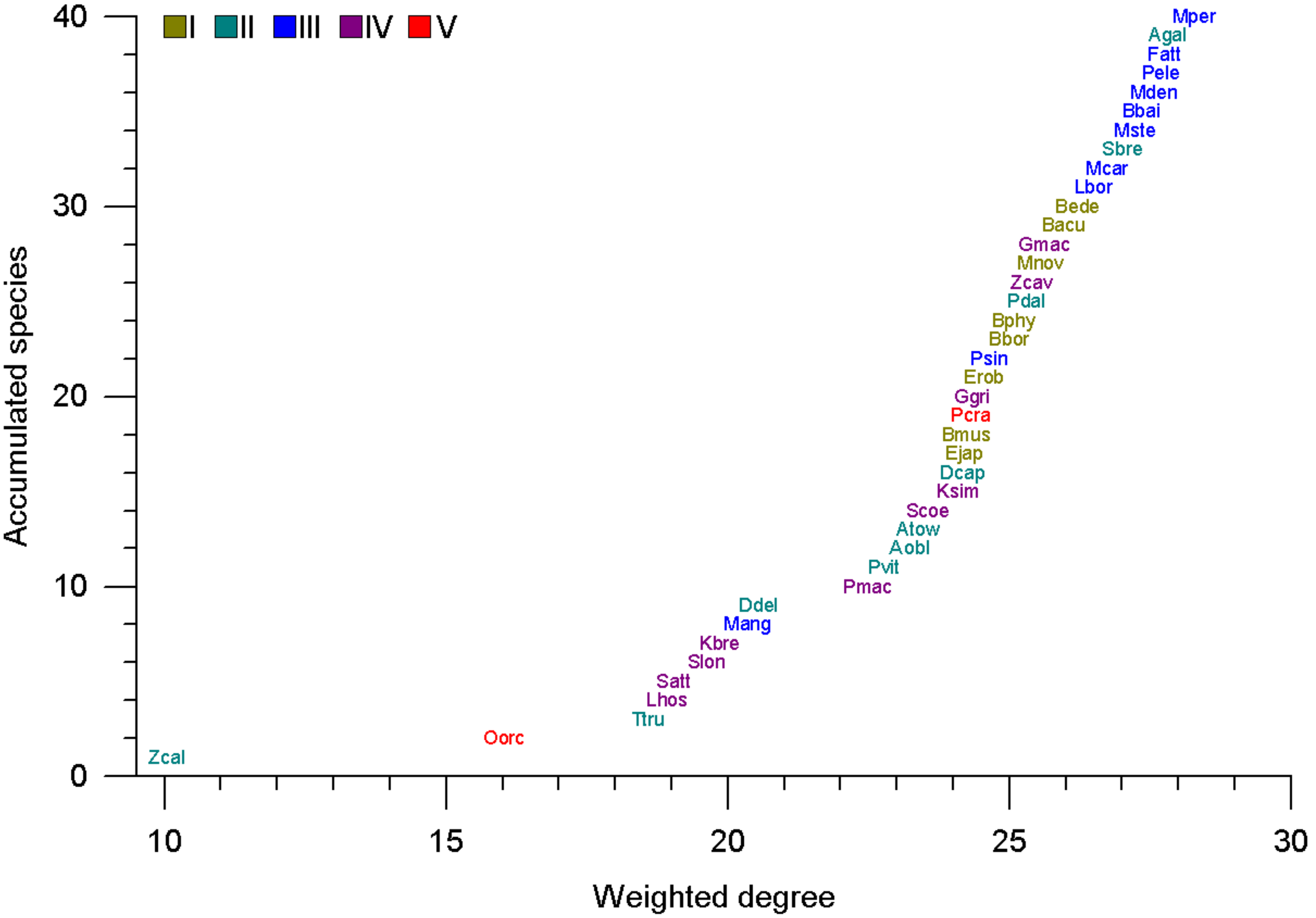

Figure 5. Cumulative distribution of the weighted degree for 40 marine mammals from the Mexican Pacific Ocean. Identity acronyms are as in Figure 1, and their colors correspond to the trophic guilds also in Figure 1.

the minor corrections done for the Mexican Pacific Ocean from the Pauly *et al.* (1998) data.

Despite the incomplete diet data, we determined a clear negative relationship between weighted degree and prey richness at the level of mammal species that results from the term in the similarity definition for which increasing prey richness of a mammal species conveys greater increments in the denominator, decreasing thus similarity which is the network's weighted degree; this does not depend from underestimations of prey richness for some species. This means that taking more prey species decreases the chances of not sharing such prey, *i. e.*, that prey richness of Mexican Pacific marine mammals is determined by competitive exclusion. For deviations in the prey richness vs weighted degree relationship, *Z. californianus*, *O. orca*, and the 8 mysticetes, especially *E. japonica*, exhibited the most negative residuals, *i. e.*, a weighted degree lower than expected from prey richness. Slightly positive residuals, *i. e.*, weighted degree higher than expected from prey richness, were observed for *A. galapagoensis*, *G. macrorhynchus*, *S. coeruleoalba*, and *D. delphis*. This means that the diets of *Z. californianus*, *O. orca*, and the 8 mysticetes are more unique among Mexican Pacific mammals in general, and these species indeed stand out among the other in several of the data comparisons, *e. g.*, the lowest pairwise diet similarity is between *Z. californianus* and *O. orca*. On the other hand, the diets of *A. galapagoensis*, *G. macrorhynchus*, *S. coeruleoalba*, and *D. delphis* share more prey items with other mammals. Larger negative residuals are greater than larger positive residuals, meaning that marine mammals may have unique diets rather than similar ones.

The average trophic level increases with guild ordination from I to V, paralleling a general decrease in guilds' average weighted degree. Guild III (low trophic level teuthophagous) had a trophic level slightly lower than guild IV (high trophic level teuthophagous). Still, guild III exhibited the highest average weighted degree, suggesting that this species group may be part of a different trophic system that permits greater trophic overlap –as indicated by diet similarity and thereon weighted degree– among the mammal species on it. However, if guild III prey richness is underestimated, as discussed above, increasing its prey richness could decrease its average weighted degree for a better fit of its relationship with trophic level, for perhaps integrating guilds III and IV as one, and possibly moving *F. attenuata* to guild V. If marine mammals from the surface ecosystem, segregate in guilds with clearly different trophic levels, marine mammals in the deep might too conform

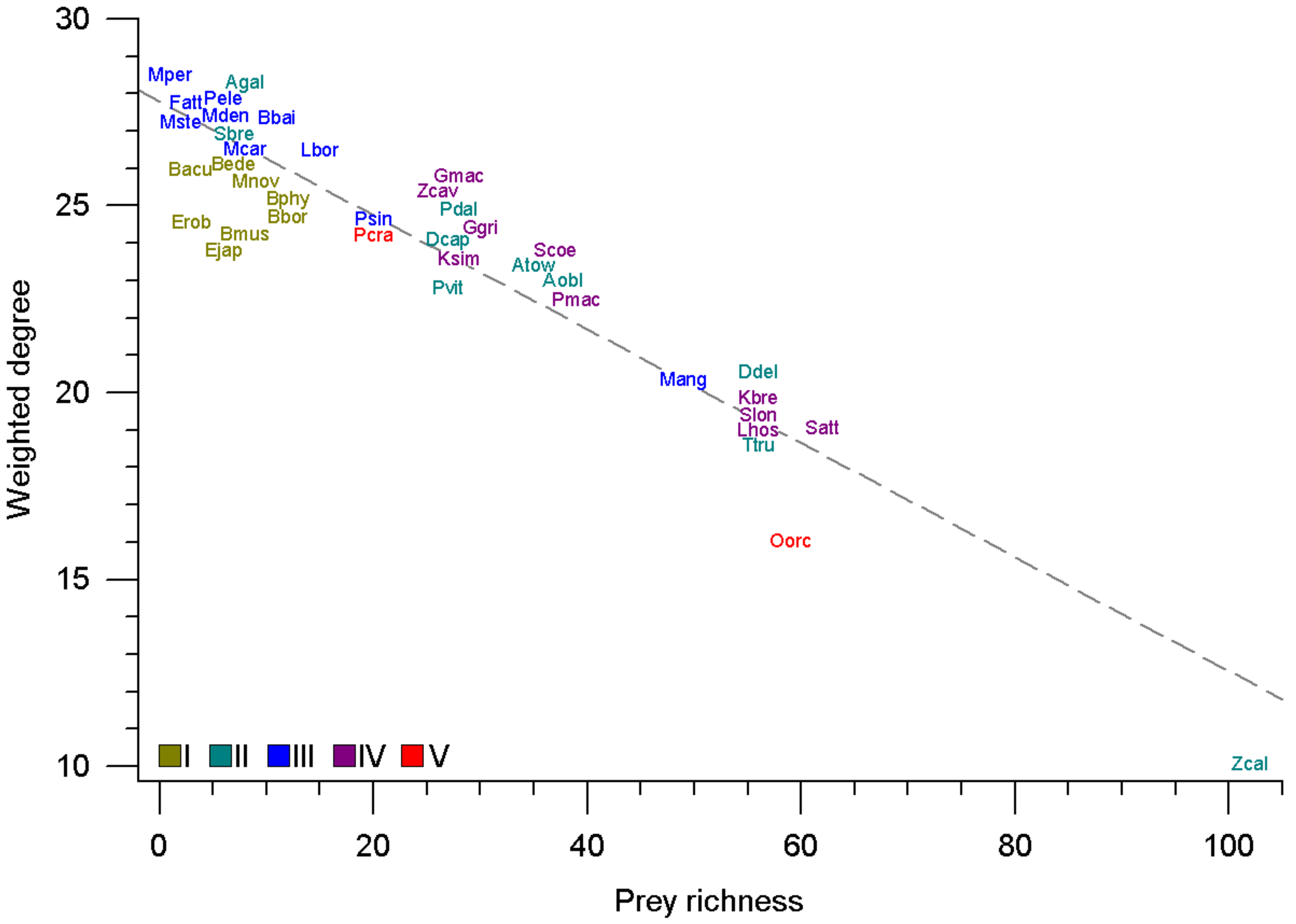

Figure 6. Relationship between prey richness and weighted degree for 40 marine mammals from the Mexican Pacific Ocean. Identity acronyms are as in Figure 1, and their colors correspond to the trophic guilds also in Figure 1. The dashed line indicates regression with parameters slope, -0.152; ordinate, 27.79; and r_2, -0.875.

guilds differentiated by trophic level, although maybe not so apart, exactly as Figure 8 shows. Independent of whether guilds III and IV are the same one or not, teuthophagous marine mammals indeed belong to a trophic system of deep waters different than that of guilds I, II, and V, which feed mainly in surface waters. We cannot define such trophic systems as different webs, as we do not know how much their trophic connections are separated, nor what their trophic bases are. A degree of trophic overlap between deep-water and surface-water marine mammals exists at least because teuthophagous mammals feed in part in surface waters when they are there for breathing. Another indication of different trophic systems for surface- and deep-water marine mammals comes from their different responses in distribution and occurrence to environmental variation, as observed by Arroyo Sánchez (2023) in the Gulf of California during the summer of years 2012 – 2019, which included two La Niña events (2012, 2017) and one strong El Niño event (2015 – 2016).

Over the statement that lower trophic levels have larger available biomass for feeding, the inverse relationship between average trophic level and weighted degree for marine mammals in the Mexican Pacific Ocean suggests stronger competitive exclusion at higher trophic levels and therefore lesser trophic overlap among mammal species, part of which is given by higher prey richness.

The Mexican Pacific is a region of high biodiversity and high marine productivity for which seasonal (*e. g.*, winds regime) and annual variations (*e. g.*, El Niño/La Niña) are important. The region's high productivity sustains an abundant, diverse, and singular marine mammal fauna with different biogeographic and ecological affinities (Rosales-Nanduca *et al.* 2011) by which species distributions and abundances fluctuate due to lowly known combinations of species plasticity and preferences, environmental variation, and anthropic impacts (Fiedler and Reilly 1994; Reilly and Fiedler 1994; Ballance *et al.* 2006; Fiedler *et al.* 2017). Human impacts on the Mexican Pacific ecosystems are not among the largest worldwide (Halpern *et al.* 2008, 2015), but exhibit concerning trends in some regions, including synergic effects of climate change (the anthropogenic warming of the ocean and atmosphere; Gates 1993) over anthropic impacts to marine mammals, especially by fisheries (Escobar Briones *et al.* 2015).

Largest diet similarities, *i.e.*, trophic overlaps, (0.90 – 0.95) occurred between mysticetes that feed mainly in the

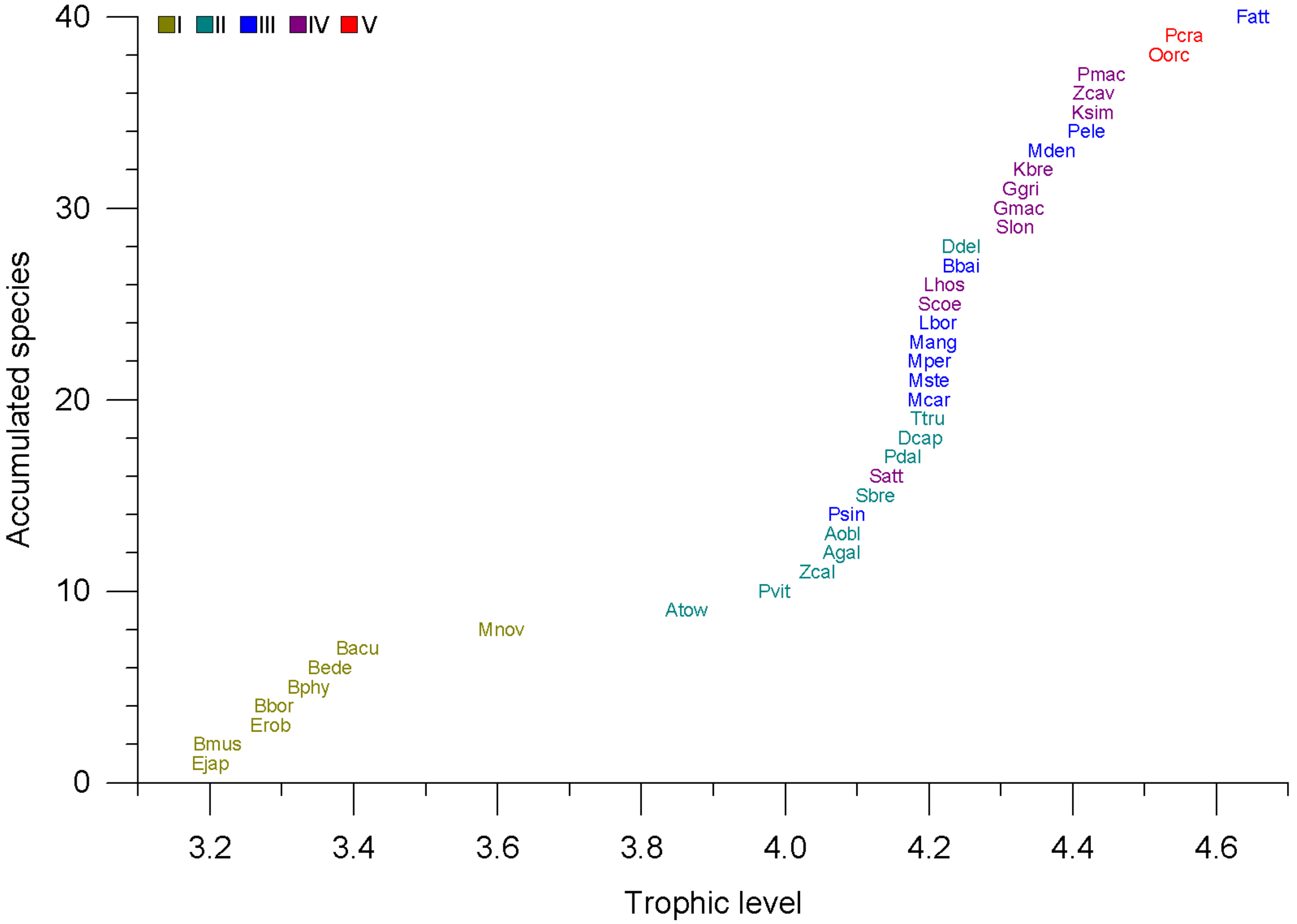

Figure 7. Cumulative distribution of the trophic level for 40 marine mammals from the Mexican Pacific Ocean. Identity acronyms are as in Figure 1, and their colors correspond to the trophic guilds also in Figure 1.

productive temperate and cold waters of the North Pacific or in the Eastern Tropical Pacific with different patterns of spatial and seasonal occurrence, as well as between *Mesoplodon* species, whose regional diet diversities seem quite incomplete and whose geographic distributions are separated by the California Stream, *e. g.*, *M. stejnegeri* vs *M. peruvianus*, or *M. carlhubbsi* vs *M. peruvianus*. Apparent geographic and seasonal overlap between mysticetes for feeding in Mexican waters occurs in the Gulf of California, which is a region of extraordinarily high productivity that sustains a diverse and abundant marine mammal fauna, outstanding at global level (Schipper *et al.* 2008; Arellano-Peralta and Medrano-González 2013, 2015). Therefore, the potential for competition between marine mammals should be assessed considering their geographic and seasonal distributions.

Trophic overlap between marine mammals and fisheries regarding shared species implicates operational and potential competitive interactions as well as operational interactions not mediated by shared prey, such as the tuna purse-seine fishery. Arellano-Peralta and Medrano-González (2015) reviewed published operational interactions between *T. truncatus* and five fisheries, *G. macrorhynchus* and five fisheries, as well as 12 marine mammal species and four fisheries, as the most relevant among others. The 12 referred species are: *Z. californianus*, *D. delphis*, *D. bairdii*, *S. attenuata*, *S. longirostris*, *S. coeruleoalba*, *A. obliquidens*, *G. griseus*, *Z. cavirostris*, *M. densirostris*, *M. peruvianus*, *P. macrocephalus*, and *K. sima*. Fisheries interacting with more mammal species are finfish (25), cartilaginous fish (17), shrimp (16), and minor pelagic fish (14). Given the incompleteness of diet data, trophic overlaps with fisheries are underestimated here. In our data, marine mammals with greater trophic overlap with fisheries in the Mexican Pacific Ocean occur in guilds I and II (surface water), mainly *P. sinus*, *Z. californianus*, *T. truncatus*, and *D. bairdii*. Together with *G. macrorhynchus*, these four species appeared as the most frequent in remains with indication of anthropogenic death along Mexican coasts (Zavala-González *et al.* 1994). Morant *et al.* (2025) have identified high values for potential feeding sites of marine mammals in the Gulf of California and the Pacific coast of the Baja California Peninsula, whilst Benavidez Gómez (2016) identified both, high values of marine mammal foraging and interaction with fisheries in these regions.

High trophic overlaps with fisheries also occur in *B. edeni*, *B. physalus*, and *B. borealis* that feed importantly in Mexican waters over 7 – 12 prey, of which the Pacific sardine (*Sardinops sagax*) and Northern anchovy (*Engraulis mordax*)

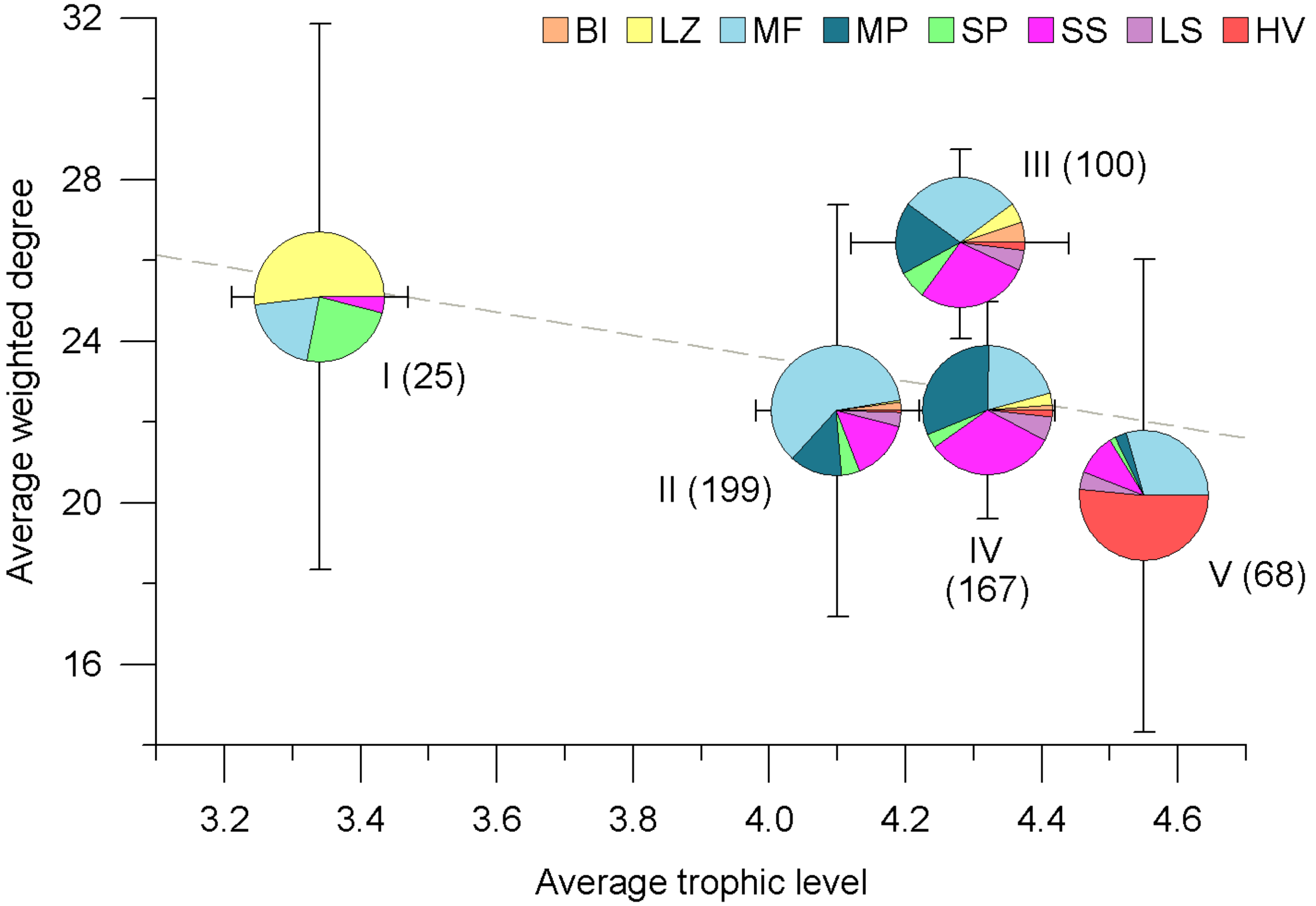

Figure 8. Relationship between average trophic level and average weighted degree among the 5 trophic guilds identified for marine mammals from the Mexican Pacific Ocean. Variation intervals indicate standard deviation among guild species. Guild identity and total prey richness in parentheses are indicated near the pies, which depict the richness of the 8 prey types.

are important food items in the Gulf of California and Pacific coast of Baja California (Tershy 1992). In the Eastern North Pacific, *Balaenoptera* whales are known to entangle only in offshore drift nets for sharks and swordfish, especially *B. acutorostrata*, though this is underestimated (Barlow *et al.* 1997; Reeves *et al.* 2013). Mysticetes are neither attracted to settings for minor pelagic fish (Morales-Bojórquez *et al.* 2021). Interaction between fisheries and *Balaenoptera* whales could occur by competition for minor pelagic fish that are subjected to large fluctuations associated with El Niño/La Niña oscillation; however, such variation in the Gulf of California seems not to affect the functional relationships of the pelagic trophic system (Del Monte-Luna *et al.* 2011; Velarde *et al.* 2013). For these reasons, we now consider that the high trophic overlap between *Balaenoptera* whales and fisheries in the Mexican Pacific is potentially relevant. Minor pelagic fishery appears important anyway for small cetaceans and pinnipeds, with 7 shared species among 12 fished in total, together with the finfish fishery, with 56 shared species among 228 fished.

An ultimate network analysis still shall wait for several diets to get sufficient data, as well as variation values for biomass fractions and prey diversities across space and time. Beyond the data constraints discussed above, our network analysis proved being useful for detecting marine mammal community structure in the Mexican Pacific Ocean over the patterns of diet diversity, diet similarity, and trophic level, which subsequently need examination for their relation with the spatial and temporal segregation between species. In summary, marine mammals from the Mexican Pacific Ocean compose 4 – 5 trophic guilds of at least 2 trophic systems, mainly structured by competitive exclusion over the regional biodiversity that these mammals feed on. How trophic relationships among marine mammals can be determined in their geographic distributions, and their responses to environmental variation, as well as how exclusion relationships are affected by human activities in the sea, principally fisheries, appear as relevant issues to investigate after the early attempt by Benavidez Gómez (2016). Under the principle of investigating marine mammals as indicators of marine ecosystems' condition, *i. e.*, as sentinels, getting actual data straight from the sea is the most necessary work to do nowadays.

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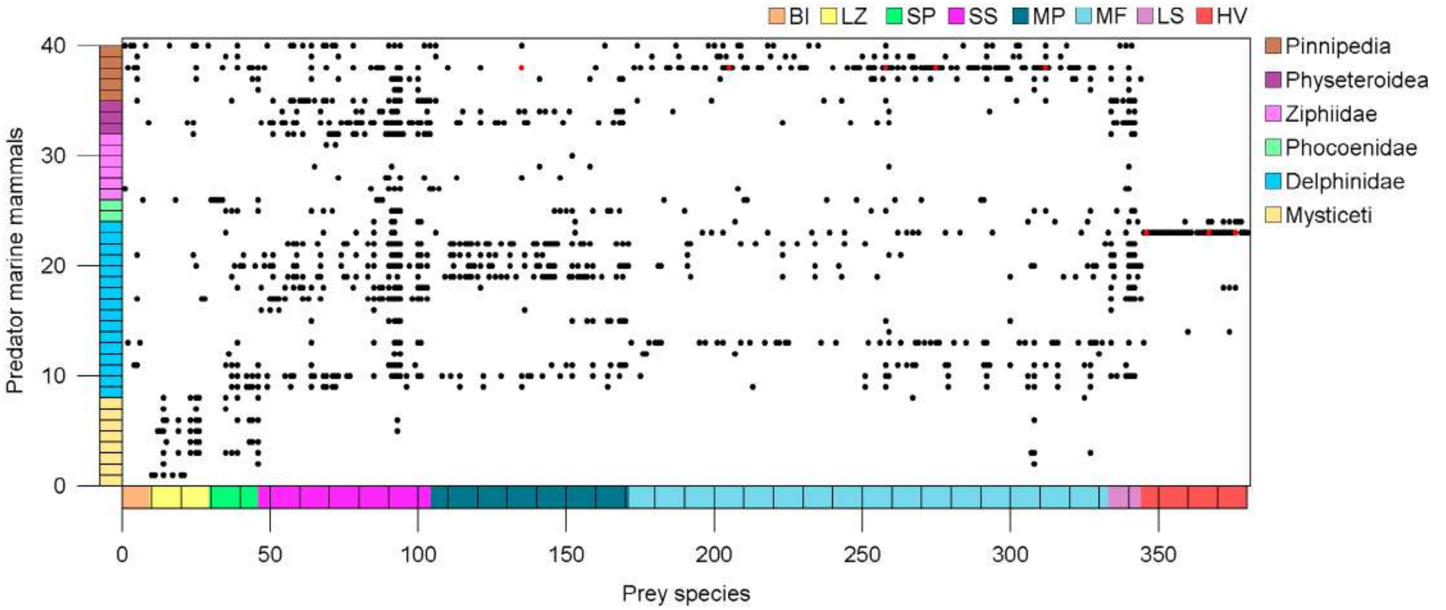

Figure S1. Graphic table of the validated 380 prey items taken by 40 marine mammal species for 1062 trophic relationships in the Mexican Pacific Ocean. Prey type abbreviations are defined in the Methods section. The 8 red points indicate trophic relationships from our group not recorded in the literature for the region.

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Fisiología del epitelio vaginal en hembras de *Anoura latidens* (Chiroptera: Phyllostomidae), anotaciones sobre la estructura social de la colonia en refugios artificiales, Calarcá, Quindío, Colombia

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Documentamos por primera vez la fisiología reproductiva y la estructura social de *Anoura latidens* en los Andes Centrales de Colombia, a partir del seguimiento de una colonia establecida en túneles artificiales en Calarcá, Quindío. El análisis citológico de 52 hembras reveló un ciclo monoestral con dos picos de ovulación entre agosto y septiembre, coincidiendo con la transición entre periodos de lluvia. La proporción de sexos mostró variaciones temporales, con machos en mayor proporción al inicio de la temporada de cópula en el mes de agosto, disminuyendo drásticamente hacia octubre y noviembre, cuando las hembras conformaron colonias de maternidad. La segregación espacial de sexos dentro del refugio estuvo asociada a la estabilidad microclimática, lo que sugiere una partición funcional del espacio durante la reproducción. Estos hallazgos aportan evidencia inédita sobre la ecología reproductiva de *A. latidens* y resaltan la importancia de refugios artificiales en la dinámica poblacional de murciélagos nectarívoros andinos.

Palabras clave: Chiroptera, ciclo estral, polinizadores, reproducción, servicios ecosistémicos.

We report, for the first time, the reproductive physiology and social structure of *Anoura latidens* in the Central Andes of Colombia, based on the monitoring of a colony established in artificial tunnels in Calarcá, Quindío. Cytological analysis of 52 females revealed a monoestrous cycle with two ovulatory peaks between August and September, coinciding with the transition between rainy periods. Sex ratios showed temporal variation: males were abundant at the beginning of the mating season in August, but decreased sharply toward October and November, when females established maternity colonies. Spatial segregation between sexes within the roost was associated with microclimatic stability, suggesting a functional partitioning of the space during reproduction. These findings provide novel evidence on the reproductive ecology of *A. latidens* and highlight the importance of artificial roosts in the population dynamics of Andean nectarivorous bats.

Key words: Chiroptera, ecosystem services, estrous cycle, pollinators, reproduction.

El estudio de la reproducción en murciélagos neotropicales ha permitido identificar una amplia diversidad de estrategias ecológicas, fisiológicas y comportamentales (Taddei 1980; Kunz y Fenton 2005; Ocampo-González *et al.* 2021). Entre estas se incluyen diferentes sistemas de apareamiento —desde promiscuidad hasta estructuras tipo lek— cuya expresión depende de la disponibilidad de recursos y del gremio trófico de cada especie (Heideman *et al.* 1996; Dorrestein *et al.* 2024). En ambientes con alta oferta de recursos, se han documentado especies con múltiples eventos reproductivos a lo largo del año (Findley 1993; Ocampo-González *et al.* 2021), mientras que, en ecosistemas más restrictivos, como los de alta montaña, predominan ciclos monoestrales (Zortéa 2003; Morales-Rivas 2016; Tirira 2017).

En este contexto, los murciélagos nectarívoros del género *Anoura* constituyen un grupo clave en ecosistemas altoandinos, al desempeñar un rol esencial como polinizadores (Voigt y Speakman 2007). Para varias especies del género (*A. geoffroyi, A. caudifer, A. aequatoris, A. cultrata*), se han reportado ciclos reproductivos monoestrales asociados a la estacionalidad de los recursos florales y a la estabilidad microclimática de los refugios (Galindo-Galindo *et al.* 2000; Zortéa 2003; Morales-Rivas 2016). Asimismo, se ha descrito que estas especies suelen ocupar sistemas kársticos naturales o túneles artificiales, aprovechando las condiciones de temperatura y humedad que facilitan la reproducción (Torres-Flores y López-Wilchis 2010; Zafra 2021).

A pesar de ello, no existen estudios previos sobre la biología reproductiva de *Anoura latidens*, especie distribuida en Colombia y Venezuela y considerada típica de los ecosistemas andinos (Gardner 2007). Esta falta de información contrasta con la creciente transformación de los ambientes altoandinos, donde la pérdida y fragmentación del hábitat comprometen la persistencia de especies nectarívoras y, con ello, procesos ecosistémicos críticos como la polinización (Carvajal-Ortiz *et al.* 2025).

En este trabajo caracterizamos por primera vez el ciclo reproductivo y la estructura social de una colonia de *A.*

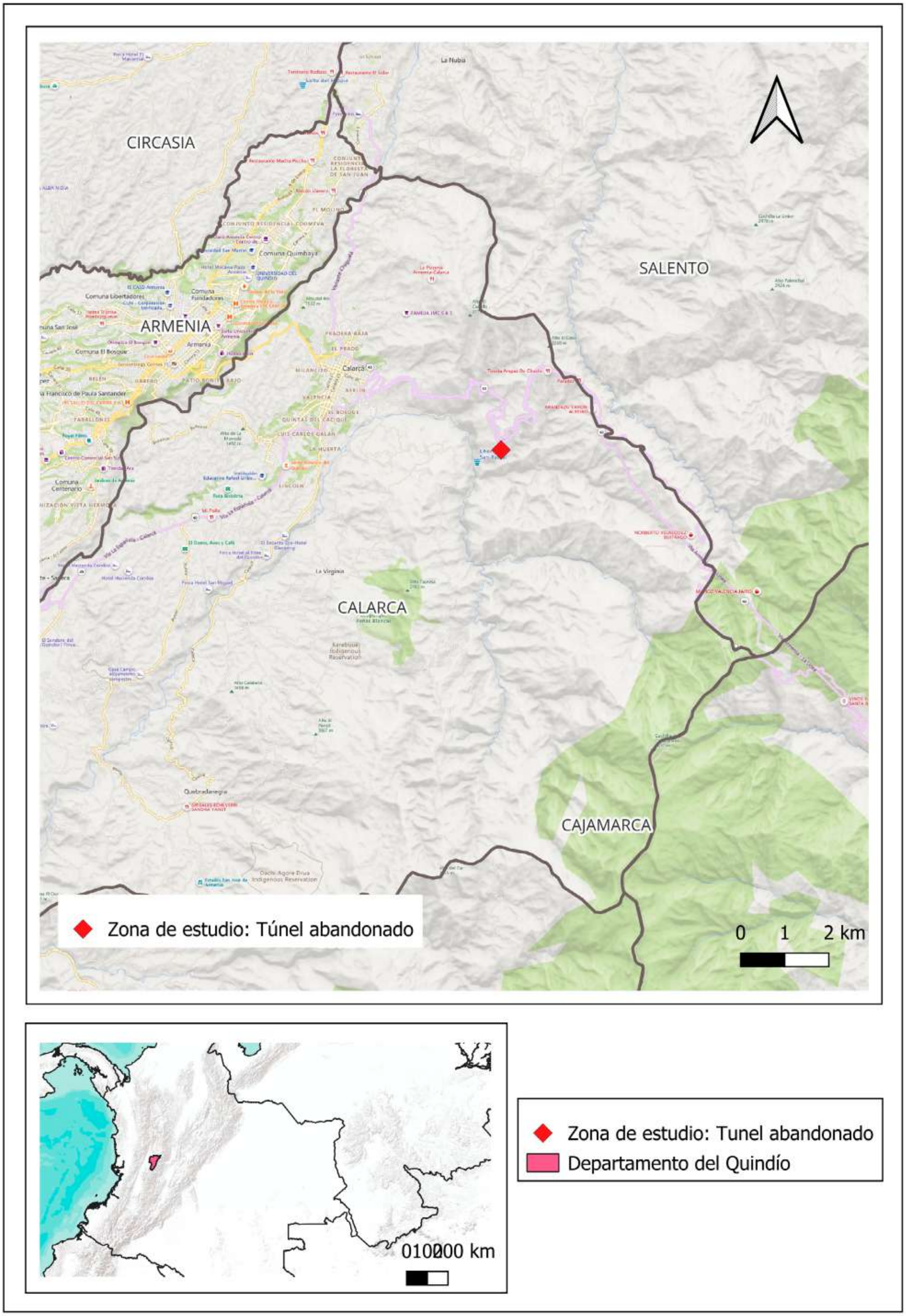

Figura 1. Localización de área de estudio. Túneles abandonados, vereda el Túnel, Calarcá Quindío, Colombia.

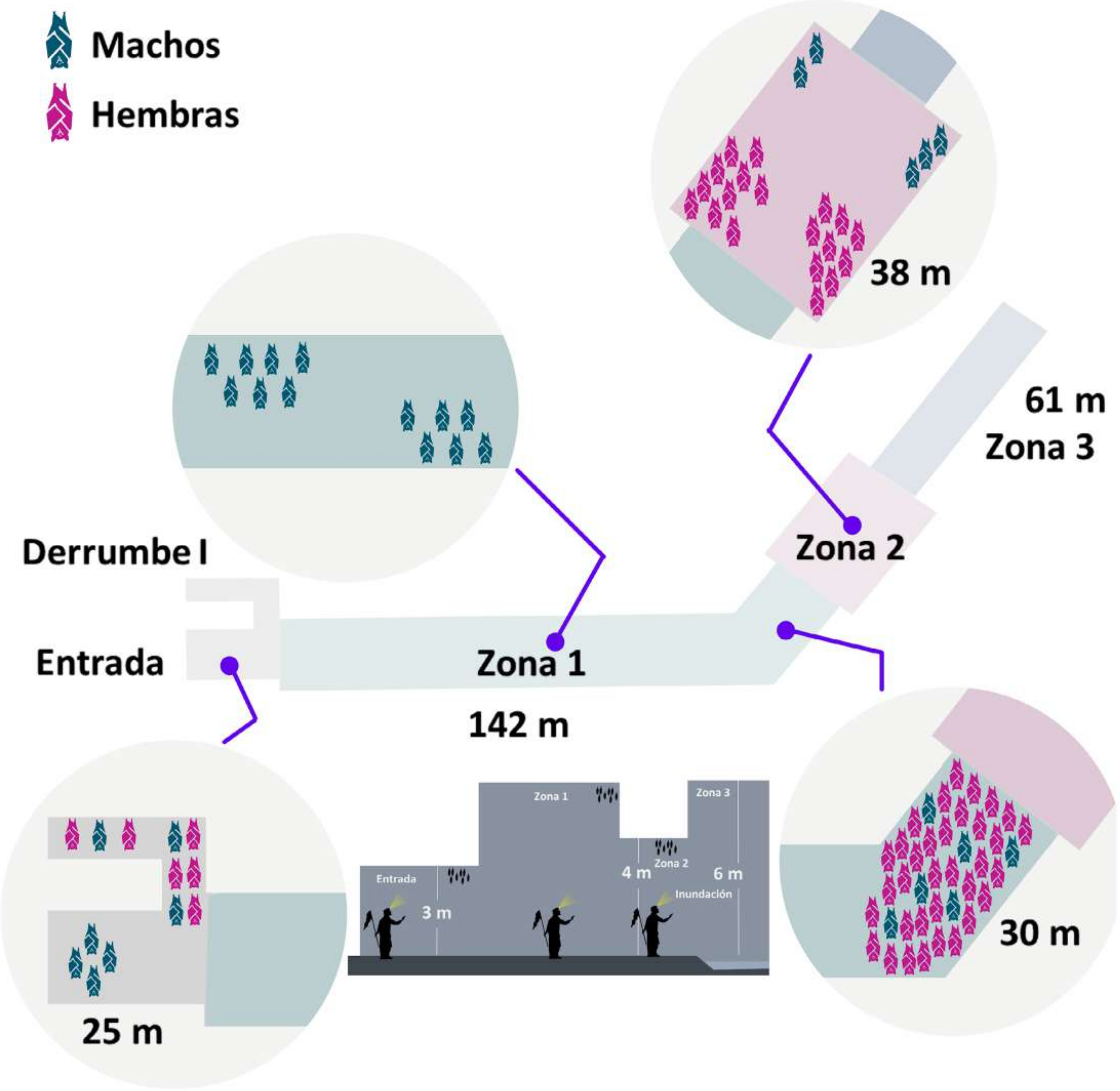

Figura 2. Zonificación del refugio: Entrada (E), 23m; Zona de derrumbe I (D1) 12m; Zona I (Z1), 142m; Zona de derrumbe II (D2), 30m; Zona II (Z2), 38m; Zona III (Z3), 61m.

latidens establecida en túneles artificiales en los Andes Centrales de Colombia. Específicamente, describimos la variación temporal de la proporción de sexos y los patrones de maduración ovulatoria en hembras, a fin de aportar evidencia sobre la ecología reproductiva de esta especie y discutir el papel de los refugios artificiales en su dinámica poblacional.

Materiales y métodos

Área de estudio. Este estudio se desarrolló en tunel artificial, correspondiente a una excavación inconclusa realizada en la etapa inicial de las obras del proyecto vial "Túnel de la Línea", que se encuentra ubicada en el flanco occidental de la Cordillera Central de los Andes Centrales de Colombia a 2600 msnm, en la vía Calarcá (Quindío)– Cajamarca (Tolima), (4.514925 N; -75.607796) (Figura 1). El túnel abandonado tiene una longitud total de 220 metros con una morfología lineal de poca pendiente, ramificaciones y zonas diferenciadas por procesos de erosión, derrumbes e inundaciones.

La zonificación del túnel se realizó por medio de recorridos descriptivos, en los que se evaluó: la estructura, estabilidad y configuración espacial, alto y ancho de cada sección, de acuerdo a estas características el túnel se

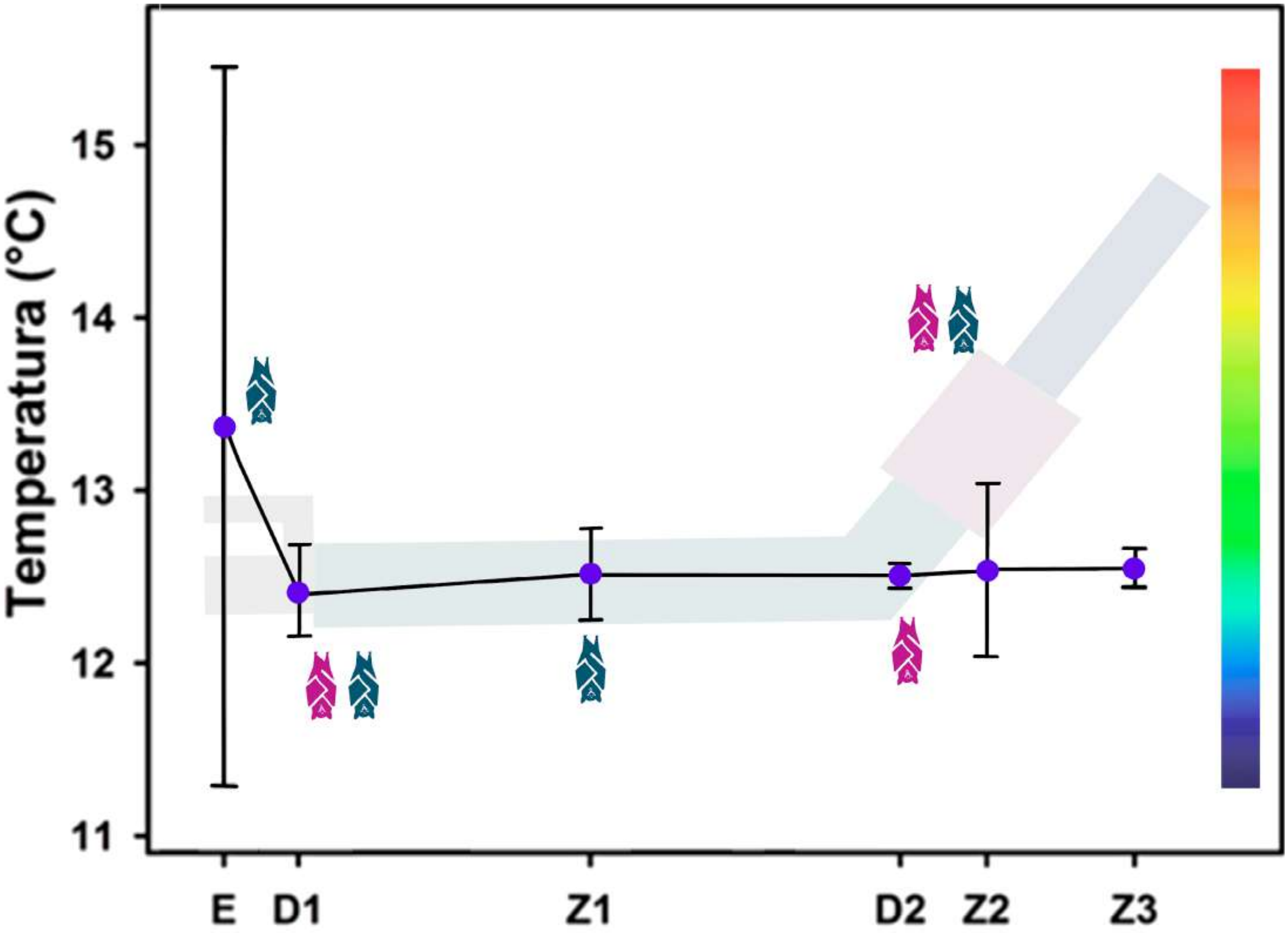

Figura 3. Promedio de temperatura (pt) para cada una de las zonas del refugio. (pt) = 12.55 C°.

dividió en las siguientes secciones: Entrada: un pasaje de 23 metros que conecta el interior y exterior, la entrada en su inicio es estrecha y se va expandiendo a un diámetro de 4 metros y una altura de 3 m; Zona de derrumbe I (D1): un tramo inestable con acumulación de piedras y detritos provenientes del techo colapsado, ubicado en el costado izquierdo de la entrada; La Zona I (Z1) corresponde al sector más extenso del sistema, con 142 m de longitud, presenta morfología de galería alargada de amplitud relativamente constante; Zona de Derrumbe II (D2), longitud de 4 m, conformada por acumulaciones de escombros que interrumpen parcialmente la continuidad del pasaje; Zona II (Z2) con una longitud de 38 metros y seis metros de ancho, seguida de la Zona III (Z3) con una longitud de 61 metros, se caracteriza por nivel de inundación superior a 40 cm. (Figura 2).

La temperatura y humedad relativa fueron monitoreadas en cada sección del túnel con el uso de datta loggers (Hobbo pro 8.02) lo cuales fueron calibrados para tomar datos cada 30 minutos en periodos de 20 días por sección entre los meses de agosto y noviembre del año 2016 (Figura 3).

La descripción del paisaje circundante corresponde a ecosistemas altoandinos, bosques de galería y pastizales dedicados a la ganadería, que se alternan con áreas de explotación maderera de pino (*Pinus patula*). El clima de la zona se determinó de acuerdo con la clasificación de Koppen como (Af) tropical ecuatorial, tropical semihúmedo con reportes de precipitaciones.

Se realizaron un total de siete salidas de campo a intervalos de 15 días con dos días efectivos de trabajo entre agosto y noviembre del año 2016, cubriendo los periodos de altas y bajas precipitaciones para la zona; durante la fase de campo se realizaron procedimientos citológicos para reconocer el estado de maduración ovulatoria de las hembras en la colonia y conteos quincenales para establecer la proporción de hembras:machos y número de individuos en cada sección del túnel.

Citologías y obtención de índices de maduración ovulatoria. De la totalidad de individuos capturados se seleccionaron hembras adultas y pre adultas para la realización del procedimiento citológico siguiendo el protocolo propuesto por Vela-Vargas (2013), inoculando 1.5 ml de solución salina en la entrada vaginal con una micropipeta HUAWEI de 10 mm, procedimiento que se repitió durante dos minutos para obtener células escamadas del epitelio vaginal. Posteriormente, se extrajo la solución

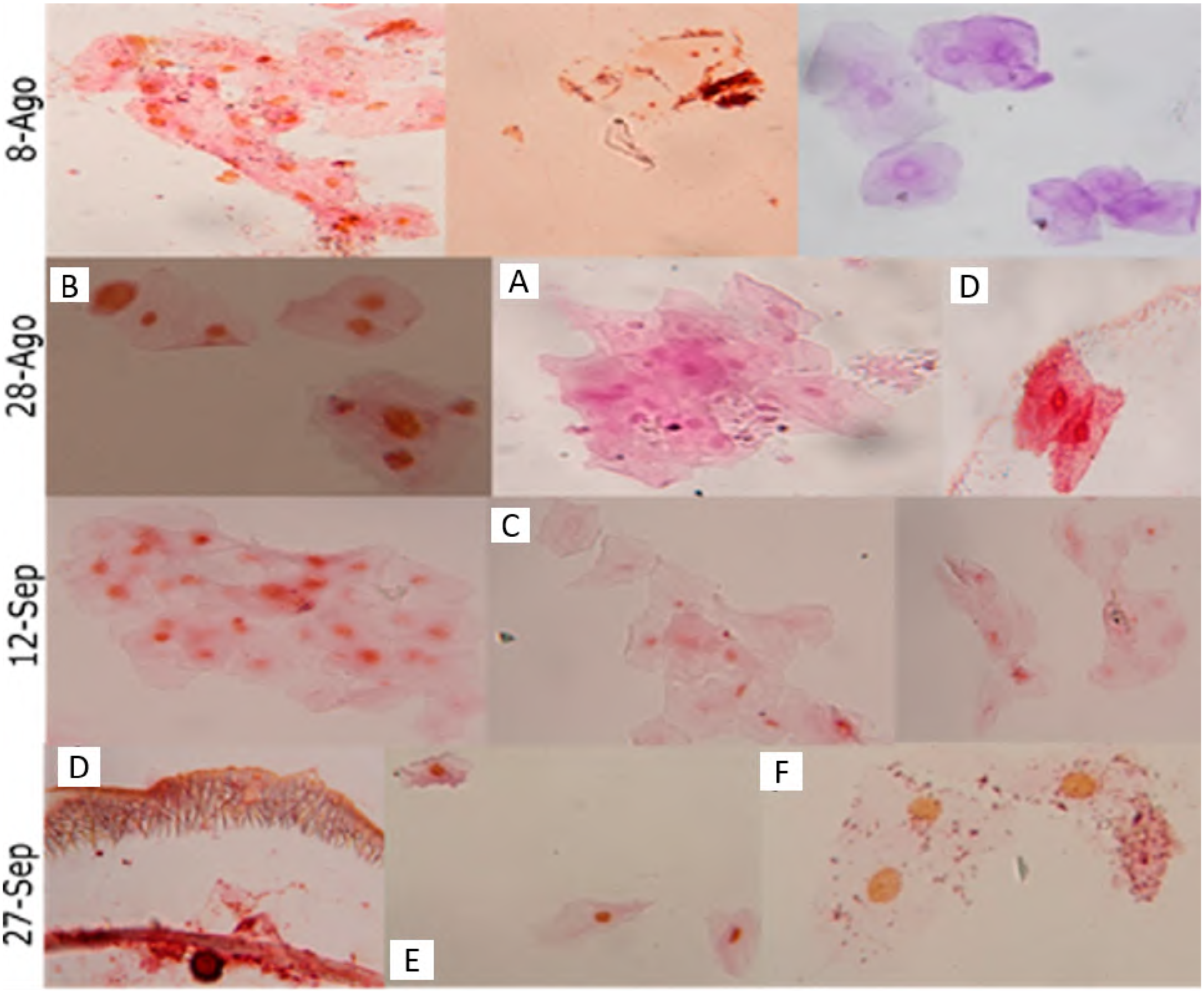

Figura 4. Muestras citológicas de tracto vaginal de *A. latidens*. Células superficiales agrupadas en estratos (A), citoplasmas transparentes con bordes plegados (B) células con núcleos desnudos y núcleos picnóticos (C), formación de cristales de estrógeno (D), células naviculares con núcleos picnóticos (E) núcleos granulados presentan queratohialina (F).

salina, dejando secar alícuotas del líquido recuperado en láminas de vidrio que fueron ubicadas para su secado inicial, en una caja portaobjetos esterilizada. Las muestras fijadas en etanol al 95%, fueron llevadas al laboratorio de la universidad del Quindío donde se realizó tinción de Wright, siguiendo el protocolo propuesto por Zerpa (2003). Las hembras evaluadas fueron tatuadas con un número en la parte inferior del ala izquierda, siendo luego liberadas (Torres-Flores y López-Wilchis 2010).

Para la diagnosis citológica los frotis con descamación del epitelio vaginal fueron observados al microscopio, se estableció el valor de maduración de Meisel (VMM) (Meisel 1967) y el índice de maduración de Frost (IMF) (Frost 1961) con la asignación de un valor numérico para cada tipo de célula observada (células superficiales = 1; células intermedias = 0.5; células parabasales = 0) de acuerdo a lo propuesto por Frost (1961) y se calculó la distribución del tipo de células en cada fase (Pelea y González 2003). La intensidad de la ovulación en la colonia se analizó a través de estadísticos de series de tiempo con medias móviles para datos no suavizados y un análisis de varianza. Adicionalmente, se evaluó la normalidad y la independencia de los datos a través del test de Kolmogorov Smirnov y Box. test en R studio (Team 2015).

Proporción de sexos en perchas. En cada una de las secciones del túnel, se identificaron sitios de percha y se realizaron capturas con una jama de longitud máxima de 4 metros, los individuos capturados fueron identificados siguiendo las claves de Gardner (2007), anotando medidas morfológicas externas, identificando sexo, estado reproductivo, estado de desarrollo y peso. Con la información compilada finalmente se estableció el número y proporción de hembras y machos para cada una de las zonas del túnel y para el total del refugio durante el periodo agosto-noviembre, a través de un análisis de varianza en SPSS 15.0.

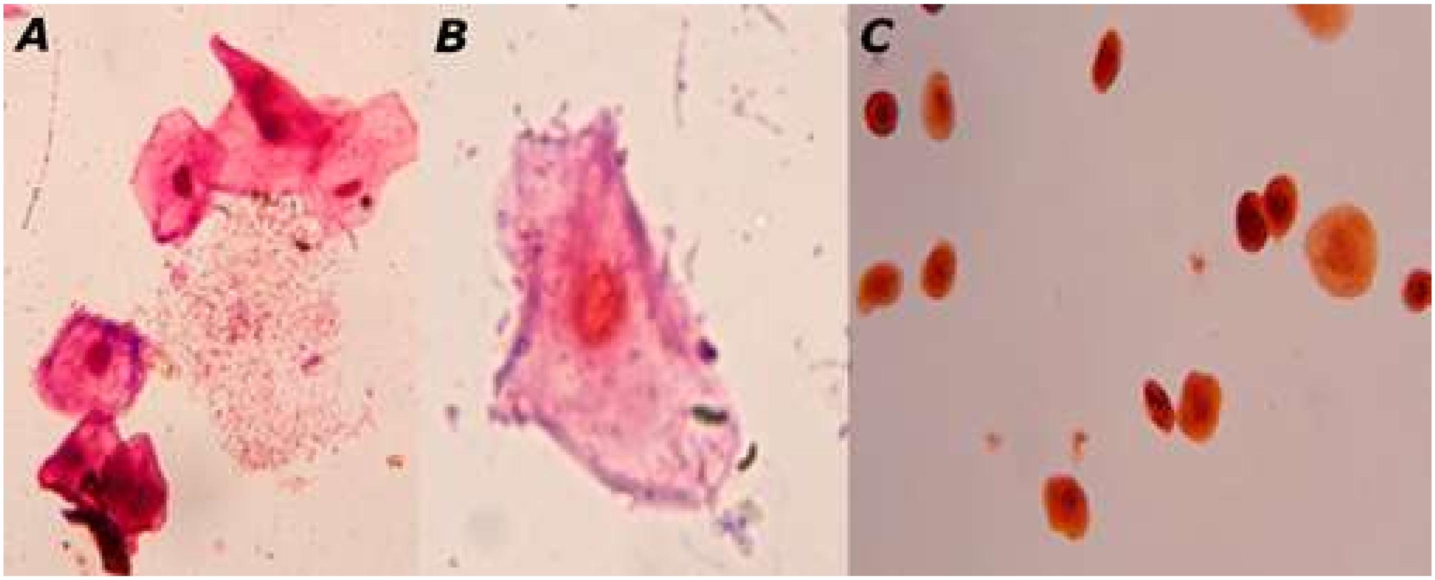

Figura 5. A) citoplasma y células intermedias; B) células superficiales con acumulación de glucógeno; C) células trofoblásticas.

Resultados

Maduración celular y ovulación. Los análisis citológicos muestran una alta acción trófica de los estrógenos en las células del epitelio vaginal durante las primeras semanas de agosto y finales de septiembre, con frotis que se componen principalmente por células intermedias y células superficiales agrupadas en estratos, citoplasmas transparentes con bordes plegados, células con núcleos desnudos y núcleos picnóticos (Figura 4). Se observan dos picos de acción estrogénica; el primero, ocurrido a comienzos de agosto con un VMM = 0,77 y el IMF en proporciones 1/58/39 para células parabasales, intermedias y superficiales respectivamente, y un segundo pico de ovulación, ocurrido durante la última semana de septiembre (VMM = 0,88 y el IMF en proporciones 2:63:65 para células parabasales, intermedias y superficiales respectivamente), el valor de desviación típica para los valores de maduración durante la temporada de agosto septiembre fue 0.001 (Tabla 1). El test de Kolmogorov muestra que los datos son independientes y presentan una distribución normal.

Tabla 1. Valores promedio de maduración celular (VM) quincenal y la desviación estándar (DV)

Fecha	% Maduración	DV
Agosto 8	0.77	0.27
Agosto 38	0.62	0.25
Septiembre 12	0.41	0.24
Septiembre 27	0.88	0.19
Octubre 27	0.55	0.25
Noviembre	0.22	0.25
Noviembre 21	0.15	0.02

Durante la temporada post reproductiva que comprende los meses de octubre y noviembre, el valor de desviación típica fue de 0.3 y los valores de maduración celular decrecen drásticamente con un VMM = 0,30 y el IMF en proporciones 93:22:0 para células parabasales, intermedias y superficiales respectivamente. Los frotis se caracterizan por presentar células intermedias con acumulación de glucógeno en los bordes del citoplasma (Figura 5**)**. Las muestras citológicas obtenidas para el mes de noviembre muestran los valores de maduración más bajos con promedios iguales o cercanos a cero, debido a la disminución de células superficiales (Figura 6). El porcentaje de células intermedias estuvo por debajo del 10%, lo que concuerda con los reportes de embarazos durante el último muestreo.

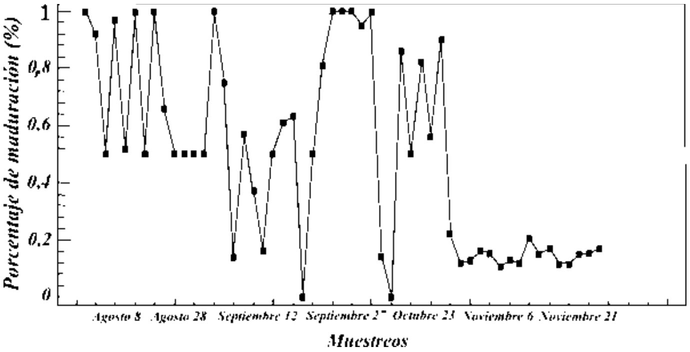

Figura 6. Estadístico de series de tiempo: se observan dos picos de actividad ovulatoria reportados durante la primera semana de agosto y la última semana de

Proporción de sexos en perchas. Durante la fase de campo fueron capturados un total de 444 individuos de *A. latidens*, con un total de 339 hembras de las cuales el 72 % fueron hembras adultas (Adultos = 244; juveniles = 95), un total de 105 machos de los cuales el 90% fueron adultos escrotados (Adultos = 94; Juveniles = 11) (Tabla 2), la distribución y proporción de sexos vario significativamente en función de la zona: la Entrada tuvo en general menor número de individuos con una proporción promedio 1:1, la Zona I presento una mayor proporción promedio de machos en perchas con promedios de cinco a seis individuos, en su mayoría machos, la zona Derrumbe II (D2) y Zona II

(Z2) presentaron la mayor concentración de individuos, cinco a diez individuos y la mayor proporción de hembras promedio = 7; la Zona 3 se caracterizó por la ausencia de individuos de *A. latidens* durante toda la fase de campo, en cambio se registraron tres individuos de *Myotis keasy* (hembras = 2; macho =1) (Figura 2).

Tabla 2. Parámetros poblacionales durante el periodo reproductivo y gestación entre agosto-septiembre.

	Agosto	Septiembre	Octubre	Noviembre
Hembras	87	78	83	91
Machos	38	32	25	10
Proporción h/m	2:1	2:1	3:1	9:1

La proporción entre hembras y machos varió a través del tiempo con una disminución significativa del número de machos presentes en los meses de octubre y noviembre con proporciones respectivas: i) agosto = 2:1 (87/38); ii) septiembre = 2:1 (78/32); iii) octubre = 3:1 (83/25) y iiii) noviembre = 9:1 (91/10).

Discusión

La obtención de índices de maduración ovulatoria mediante identificación y recuento de células del epitelio vaginal constituye un método válido para establecer la fisiología y duración del periodo reproductivo en especies con diferentes intensidades del ciclo estral. Estudios en ovinos, donde se caracterizaron morfológicamente los distintos tipos celulares durante protocolos de sincronización, demostraron que la frecuencia relativa de células parabasales, intermedias y superficiales se correlaciona con los momentos más cercanos a la ovulación (Retamozo *et al.* 2021). Para quirópteros los estudios sobre reproducción a través de citologías han sido utilizados para establecer periodos reproductivos en ensamblajes de especies frugívoras y nectarívoras (Vela-Vargas *et al.* 2016). Más recientemente, en murciélagos frugívoros de la familia Phyllostomidae de la cordillera Central, Antioquia, Colombia, la citología vaginal permitió identificar fases y patrones de ciclo mono y poliéstrico en hembras que externamente fueron clasificadas como no reproductivas (Mejía *et al.* 2024).

Rasweiler *et al.* (2000), reconocen la aparición de moco cervical con cristales de estrógenos en muestras citológicas para trece especies de murciélagos filostómidos; estos autores, mencionan que la acumulación de moco cervical durante la ovulación está relacionada con el transporte de esperma a través del tracto reproductivo, por lo cual se considera como un indicador de ovulación o de acción estrogénica durante el ciclo estral. Por su parte, Castillo-Navarro *et al.* 2016; Vásquez-Mejía *et al.* (2024), reportan la aparición de moco cervical durante los periodos de ovulación de *Artibeus jamaicensis*; Greiner *et al.* (2011) reportan una alta concentración de estrógenos para más del 50% de hembras de *Saccopteryx bilineata* evaluadas durante dos momentos del periodo de cópulas, basados en la concentración de metabolitos secundarios en heces fecales.

Para los murciélagos del género *Anoura* (*A. geoffoyi, A. cultrata, A. peruana*) se ha descrito ciclos monoestrales con un momento reproductivo al año (Heideman *et al.* 1992). Zortéa (2003) reportan diferencias en la proporción de sexos durante el periodo copula post cópula lo cual, coincide con nuestros resultados, siendo este el primer estudio sobre la fisiología reproductiva de *A. latidens* y el primer reporte de una colonia estable en el departamento del Quindío. Consideramos como un aporte de nuestro estudio a la descripción de la actividad hormonal a partir de citologías en quirópteros, la implementación de categorías de maduración celular del epitelio vaginal, asignando valores numéricos a cada tipo celular para la construcción de índices hormonales, como ha sido previamente implementado en citologías humanas (Frost 1961; Meisel 1967; Pelea y González 2003).

Proporción de sexos en perchas. La proporción y distribución de sexos al interior del refugio en nuestra colonia de *A. latidens*, corresponde a un comportamiento anteriormente descrito para otros representantes del género, como *A. caudifer* (Tirira 2017) y *A. geoffroyi* (Heideman *et al.* 1992, Galindo-Galindo *et al.* 2000, Morales-Rivas 2016; Saldaña-Vázquez *et al.* 2020), y se asocia a una partición funcional de roles según el estado de desarrollo reproductivo de los individuos, según Bradbury (1977); Kunz (1982); Gallardo y Lizcano (2014), la variación en el número de individuos y proporción de sexos en perchas es una conducta social típica en filostómidos que puede estar relacionada a : i) aspectos comportamentales, con machos dominantes formando harenes de varias hembras y machos juveniles formando grupos pequeños y aislados (McCracken y Bradbury 1981; McCracken y Wilkinson 2000; Senior *et al.* 2005) y ii) a requerimientos fisiológicos diferenciales con hembras grávidas ocupando zonas con microclimas más estables (Kunz 1982; Racey y Entwistle 2000).

De manera interesante, la itinerancia en nuestro estudio está referida al abandono del refugio por parte de los machos durante el periodo post cópula. Se ha sugerido que la proporción de individuos en los refugios es una función de la disponibilidad de recursos (Kunz 1982; Villalobos-Chaves 2016; Kelm *et al.* 2021); toda vez que, las hembras lactantes presentan mayores demandas fisiológicas (Kurta *et al.* 1989). En este sentido, se podría plantear como hipótesis a ser desarrollada en trabajos futuros, que, para sistemas de oferta alimenticia más constante, como los encontrados en la zona ecuatorial, las hembras de *A. latidens* tiendan a permanecer, durante la lactancia, en los mismos refugios de cópula, mientras que la presencia intermitente de los machos se debe a su comportamiento de exploración de otras colonias de hembras en disponibilidad para la reproducción.

Para la región central de los Andes colombianos, no se presenta una estacionalidad climática marcada, situación que se asocia a la hipótesis planteada (Melo y Ruiz 2024). No obstante, es necesario: i) ampliar los tiempos de monitoreo de la colonia al ciclo anual; y ii) complementar los mismos con marcaje espacial de hembras y machos para determinar la escala de los movimientos de individuos marcados, entre refugios a lo largo del año.

La colonia de *A. latidens* aquí estudiada presenta un ciclo reproductivo monoestral, caracterizado por segregación espacial de sexos y zonas de fusión para la cópula; con machos itinerantes y conformación de colonias de maternidad. Nuestro estudio documenta por primera vez el ciclo reproductivo para *Anoura latidens*, especie típica de los Andes de Colombia y Venezuela y propone la necesidad de realizar estudios que permitan entender: a profundidad: i) el efecto de las dinámicas reproductivas en la estructuración social de los murciélagos en ecosistemas altoandinos; y ii) el rol y efecto de los refugios naturales y artificiales en la ecología de las especies andinas de quirópteros.

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