# Therya

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## La portada

La mara (*Dolichotis patagonum*) tambine conocida como liebre patagónica en realidad es una especie de roedor (Rodentia) de la familia Caviidae y no tiene ninguna relacion con las liebres (Lagomorpha). Es considerado como el roedores más grandes del mundo llegando a pesar hasta 16 kg. Las maras son endemicas de planicies patagonicas de Argentina asociadas a las estepas semiáridas y desiertos de arbustos espinosos. Son una especie considerada como vulnerable principalmente por la pérdida de hábitat debido al desarrollo agrícola y competencia con las liebres europeas (*Lepus europaeus).*

## Nuestro logo "Ozomatli"

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

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Volumen 14, número 2 mayo 2023

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# Therya

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# Letter to the Editor:

## A single sample is not enough to claim systematic conclusions, much less for taxa of conservation concern: comments on Jaramillo and Ruiz-García (2022)

Jaramillo and Ruiz-García (2022) provide new information on aspects of the phylogeography of *Nasua narica*, particularly for Central America and northern South America. Nevertheless, we disagree with these authors on some fundamental points and emphatically reject their conclusion that "*the molecular evidence seems to reject the validity of* Nasua narica nelsoni (N. nelsoni)." Here we point out errors in interpretation and scope that the authors attribute to their data, as well as to previously published information on the Cozumel dwarf coati, *Nasua nelsoni*.

Since conservation efforts in México, and worldwide, tend to prioritize species and, to a lesser extent, subspecies, or populations (Garner *et al.* 2005; Gippolliti and Amori 2007; Ceballos *et al.* 2017), such errors of interpretation are not trivial. In fact, they can have negative consequences because their conclusions are directly linked to the assessment and attention, in terms of conservation, that can and should be given to a taxonomic entity whose geographic distribution is extremely restricted and has a critically endangered population scenario.

When Jaramillo and Ruiz-García (2022) point out that their evidence does not support the validity of the specific identity and even of the singularity at the subspecies level of the Cozumel dwarf coati (*Nasua nelsoni*) they make an interpretation and an assertion that is too categorical for the amount and quality of their data. Moreover, it is based on an incorrect and incomplete consideration of the previously published evidence in this regard, as we demonstrate here.

*A single sample is not enough.* The categorical conclusions of Jaramillo and Ruiz-García (2022) regarding *N. nelsoni* are based on data from a single specimen. Moreover, they do not specify the origin of this individual, including the specific collection locality. That is, the authors do not present the most basic information in mammalogical research, including whether this specimen was captured alive and belonged –or not– to a wild population; or if it was an opportunistic sampling of a dead animal, was a captive individual or a museum specimen. Furthermore, the genetic data (mitochondrial sequence) of this specimen is not publicly available (as of the time we submitted this letter), while the GenBank accession numbers they provide (page 4; MT587713-MT587788, MW410859-MW410914, and MW419814-MW419853) are of 306 bp length (not the 2,153 and 16,200 bp they claimed were analyzed). In addition, it should be noted that captive mainland coatis (*N. narica*) have been introduced and released onto Cozumel Island (Cuarón *et al.* 2004; J. C. González Malpica, pers. com.). Genetic evidence supports this assertion (Flores-Manzanero *et al.* 2022). Thus, it cannot be discarded that Jaramillo and Ruiz-García (2022) may have analyzed a sample of a *N. narica* specimen on Cozumel Island. In fact, *N. narica* is among the species of mammals with highest cultural value in México, and very commonly used as pet, particularly in southern México (Cuarón 1997; Ávila-Nájera *et al.* 2018; Herrera-Flores *et al.* 2019). Some of these animals are taken to places well beyond their site of capture within the country (Cuarón 2005).

Jaramillo and Ruiz-García (2022) cite Nigenda-Morales *et al.* (2019), indicating that we used nine coati specimens from Cozumel and that we did not find obvious differences (at the molecular level) between these individuals and other samples from Yucatán or northern Guatemala. However, in Nigenda-Morales *et al.* (2019) we made a rather relevant precision to avoid over interpretation of the data: "*Nonetheless, the number of samples from Cozumel was low (eight samples) and seven of the samples were from pet or captive raised animals that may have been derived from the mainland and transported to the island. Therefore, we suggest caution in interpreting our results regarding the genetic status of the coati samples from Cozumel. Given the uncertainty about the taxonomic status of the coati population on Cozumel, a more extensive analysis, including more samples and additional loci, will be required to reach any conclusions that could affect the conservation efforts of this population*".

Furthermore, Jaramillo and Ruiz-García (2022) incorrectly cite and interpret Glatston (1994) when they say: "*We agree with Glatston (1994) that this coati was introduced to Cozumel by the Mayans, although we do not agree that* N. nelsoni *is a full species*". What Glatston (1994) states is: "*It has been suggested that these animals were introduced to Cozumel by the Maya (Bixler, unpubl.)*", although she does not present empirical evidence in this respect.

Regarding the possible introduction of *N. nelsoni* to Cozumel, by the early Mayas, there is crucial information that Jaramillo and Ruiz-García (2022) do not consider. On the one hand, in McFadden *et al.* (2008) we clearly indicate that our data (with $n = 2$) do not enable asserting whether *N. nelsoni* colonized Cozumel before or after the Maya. On the other hand, there is a critical reference on this matter, not considered by Jaramillo and Ruiz-García (2022). That study is Hamblin (1994), in which the author presents detailed information on the remains of wild fauna found in Mayan archaeological sites at Cozumel Island. The author reports relevant data on the size of the bones and on the abundance of the remains of the different species found, to derive conclusions about the possible pre-Maya colonization of the island. She found that *N. nelsoni* bones accounted for 92 % of all procyonid bones and estimated that these remains represented at least 88 individuals with dimensions consistent with very small animals (adults and juveniles); she also found evidence that they were hunted locally and were well distributed, suggestive of their settlement on the island prior to the Mayan colonization of Cozumel.

Finally, our recent key study, Flores-Manzanero *et al.* (2022; available online since September 2021) is, to date, the most updated and complete on the genetic diversity and divergence, both on a historical and contemporary scale, of the Cozumel dwarf coati. Thus, the statement by Jaramillo and Ruiz-García (2022) about *"... an original population persists in the island, which had not been sampled..."*, is wrong.

In Flores-Manzanero *et al.* (2022) we analyzed samples from 46 individuals of the Cozumel dwarf coati (that were captured alive on site) and performed a comprehensive battery of analyzes based on both mitochondrial (cytochrome b) and nuclear (microsatellite) information. The results show a clear genetic differentiation between Cozumel dwarf coatis and *Nasua narica* individuals from the mainland. These results support that, at least following the precautionary principle, the Cozumel dwarf coati should be considered and managed as a Significant Evolutionary Unit (*sensu* Moritz 1994). Thus, we presented evidence that supports its uniqueness and our urgent proposal to change its conservation status in the IUCN Red List of Threatened Species to Critically Endangered (see the Appendix in Flores-Manzanero *et al.* 2022).

Based on all of the above, it is relevant to emphatically reiterate that the conclusion of Jaramillo and Ruiz-García (2022) about the invalidity of *Nasua narica nelsoni* (*N. nelsoni*) does not hold.

*Other considerations.* Jaramillo and Ruiz-García (2022) indicate, in the introduction of their article, that *N. n. nelsoni* is a recognized subspecies, but they incorrectly associate that point with the work of Merriam (1901). It was Decker (1991) who suggested categorized the Cozumel dwarf coati as a subspecies (*N. n. nelsoni*). This author analyzed cranial morphometric characteristics for *Nasua narica*, *N. nelsoni* and *N. nasua*. She considered data from more than 100 individuals of *N. narica* and *N. nasua*, but only 11 of *N. nelsoni*. In addition, in most of the statistical comparisons made, the cranial morphometric data of *N. narica* and *N. nasua* were compared against data from only 1 to 3 *N. nelsoni* individuals, significantly reducing the statistical power of the results that sustains Decker's (1991) conclusions. Merriam (1901) described the Cozumel dwarf coati (*Nasua nelsoni*) as a distinct species, based on the minute size of Cozumel coatis in comparison to those from the mainland. This dwarfism pattern is observed with several other taxa from Cozumel Island (Martínez-Morales 1996; Cuarón *et al.* 2004, 2009; McFadden and Meiri 2013), a pattern consistent with Foster's Rule (McFadden and Meiri 2013).

Jaramillo and Ruiz-García (2022) specify, *"McFadden et al. (2008) designated the insular subspecies as a different species (*N. nelsoni*) based on a morphometric analysis"*. This statement is incorrect. In McFadden *et al.* (2008) we start by considering *N. nelsoni* as a distinct species based on Merriam (1901). Later, based on the molecular analyzes carried out, and being always cautious with our assertions (particularly due to the small sample size used for *N. nelsoni*, $n = 2$), we specify that some of the analyzes used support a population differentiation between individuals from the Yucatán peninsula and those from Cozumel (*e. g.* the global AMOVA), but other results did not (*Fst* was not significant). We clearly stated too in McFadden *et al.* (2008) that *"... although our data do not unequivocally suggest unique species status, we believe that the body of evidence does suggest a precautionary approach which allows these taxa (allopatric populations or species) to be managed as distinct management units"*, based on a holistic perspective, where we consider estimated divergence time and haplotype diversity data, among others.

*Taxonomic uniqueness and conservation.* Any conclusions about the identity and taxonomic uniqueness of *Nasua nelsoni* (and any other species, particularly those facing conservation threats) should be based on the most robust information and on careful interpretation of it. If *N. nelsoni* is again recognized as a distinct species, as we stress it should be, along with its categorization as Critically Endangered, better attention and resources for its conservation will be feasible. The currently available evidence supports that the Cozumel dwarf coati is genetically distinct (especially in mitochondrial markers) with respect to continental congenerics, that its population has been historically small since its foundation and that the current total number of individuals is extremely small and decreasing due to anthropogenic pressures. Finally, that it faces population, ecological and evolutionary challenges that threaten its persistence.

Indeed, the Cozumel dwarf coati faces serious threats derived from the increase of tourism infrastructure, land-cover change and habitat fragmentation, from the introduction of exotic species and continental congeneric individuals to the island, and from a greater anthropization of Cozumel Island, which increases the negative effects on this and many other endemic species (e.g., deaths by car run over, pathogens and disease spillover from feral and domestic animals, risks of genetic introgression and hybridization; Cuarón *et al.* 2004, 2009; Flores-Manzanero *et al.* 2022).

Paradoxically, Ruiz-García *et al.* (2021) argue that having an exhaustive sampling including as many individuals as possible is the most important condition for both delineating conservation units and developing management and conservation plans for many species. Therefore, the conclusion by Jaramillo and Ruiz-García (2022) regarding Cozumel's Dwarf coati based on one sample only, is totally contradictory and lacking scientific support.

Therefore, we consider it of the utmost importance to be cautious, concise and objective in using adequate evidence to support the taxonomic and genetic uniqueness of the Cozumel dwarf coati and to generate the most appropriate scenario for the conservation of this procyonid. Systematics requires robust data and interpretations, while effective conservation requires robust systematic data and conclusions.

David Valenzuela-Galván[1], Ella Vázquez-Domínguez[2], Alfredo D. Cuarón[3]*, Luis-Bernardo Vázquez[4], Alejandro Flores-Manzanero[2] and Sergio Nigenda-Morales[5]

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# Letter to the Editor:

## Response to Valenzuela-Galvan *et al*. 2023: It is not necessary to "create" a new species for the sake of conservation: the case of the Cozumel's coati

Although we acknowledge the conservation efforts of McFadden *et al.* (2008), we suggest caution in suggesting the existence of a new species without providing sufficient evidence. Recall the work of Zachos (2016), and Zachos *et al.* (2013a, b), which stated that species are such fundamental units that they should not be introduced carelessly and that descriptions and splitting of species based on simple morphometric differences (even significant ones) or phylogenetic relationships derived from limited molecular datasets (for instance, only one or few mtDNA genes) should be strongly discouraged. They may serve to support conclusions derived from larger and more complete datasets, but are not enough on their own. The case of the Cozumel's coati (*Nasua nelsoni*, *Nasua narica nelsoni*, or a special population of *Nasua narica*) is an emblematic example in which multiple authors have considered a taxon to be a full species without providing strong justification.

From our perspective, there are two relevant points to consider in clarifying the issue of whether or not Cozumel's coati is a full species: the null hypothesis we presented and which definition of species should be used. The null hypothesis is that the Cozumel coati and the coati living in the nearby continental México (Campeche, Quintana Roo, Yucatán) are the same species (Cozumel island is separated from the Mexican mainland by 18 km). In Jaramillo and Ruiz-García (2022), we discuss the analysis of a complete mitogenome of a road specimen sampled on Cozumel Island. The specimen was a male with developed testicles, but its overall size was smaller than the coatis we had observed on the Yucatán Peninsula. Based on analysis, the specimen's mitogenome was not significantly different from the mitogenomes of specimens of *N. narica* sampled in the Yucatán Peninsula. In other words, we couldn't reject the null hypothesis and concluded that Cozumel's coati is not a different species from the Yucatán and Quintana Roo mainland coati. Authors claiming that this island coati is a different species, must reject the null hypothesis and demonstrate that the alternative hypothesis —that there are two different species— is more acceptable. However, based on the paper by McFadden *et al.* (2008) and others, there is insufficient evidence to reject the null hypothesis. Next, we discuss the shortcomings of the paper authored by these authors.

1. Valenzuela-Galvan *et al.* (2023) wrote "*Merriam (1901) described the Cozumel dwarf coati* (Nasua nelsoni) *as a distinct species, based on the minute size of Cozumel coatis in comparison to those from the mainland.*" and "*In* McFadden *et al.* (2008) *we start by considering* N. nelsoni *as a distinct species based on Merriam (1901).*" These quotes confirm that the study by Valenzuela-Galvan *et al.* (2023) was initially based on the typological species concept similar to studies conducted by many zoologists during the 19th century as well as the beginning of the 20th century. Because the quote specifically mentions Merriam (1901), we need to determine the criteria Merriam used in distinguishing different species. We find the answer in Merriam (1918). In this study, Merriam distinguished 86 different bear species just in North America. He based these "species" by noting minimal differences in the pelage and skulls of the sample that he analyzed. Today, the majority of these 86 bear taxa are forgotten. As Osgood (1943) claimed, Merriam ordered his findings and labeled them without any effort to interpret them. These zoologists ignored the within phenotypic and genetic variability which naturally occurred within a species, especially if a species has a wide geographical distribution. This mentality was eventually overcome with the arrival of synthetic neo-Darwinism (Dobzhansky 1937, 1970; Mayr 1942, 1963, 1970; Simpson 1944, 1953). Nevertheless, many biologists, including molecular ones, maintain a typological view of biological processes.

There are many examples of island dwarfism in mammals that are not representative of different species (anagenesis or phyletic evolution). For example, the extinct Japanese wolf (*Canis lupus*) and the extinct Balis's tiger (*Panthera tigris balica*) were significantly smaller than their continental counterparts. Mitochondrial DNA analysis showed that the Japanese wolf was indifferentiable from North American wolf lineages (Ishiguro *et al.* 2010; Matsumara *et al.* 2014). Similarly, mtDNA evidence indicates that the Balis's tiger is indifferentiable from the Sumatra tiger (*Panthera tigris sondaica*; Kitchener *et al.* 2017). There are other examples too, such as the goats (*Capra hircus*) introduced into the Juan Fernández archipelago during the 16th and 18th centuries by conquerors and pirates. These goats are half of the size of the goats from which they were derived (Muñoz-Pedreros *et al.* 2003). Recently, Ruiz-García *et al.* (2022) showed that the agouti from the Roatán Island (*Dasyprocta ruatanica;* Honduras), also considered a different species by its size, is molecularly indifferentiable from the Centro-American agouti, *Dasyprocta punctata*. Indeed, both forms of agoutis can breed without problem (Ruiz-García, unpublished observations). It has been shown that artificial, natural, and sexual selection can drastically change the morphology of different organisms without speciation in a just a small number of generations (the foxes of Belyaev, Dugatkin 2018; *Podarcis sicula*, Herrel *et al.* 2008; or *Poecilia reticulata*, Endler 1980, 1983, 1986; Reznick *et al.* 1997). Additionally, the average height of people within indigenous populations of different Southeast Asian islands (Andaman, Luzon, Panay, and Mindanao islands) is considerably less than that of humans from the Asian continent (Stock 2013; Endicott *et al.* 2003; Deng *et al.* 2022; Zhang *et al.* 2022). However, no one, considers each one of these insular dwarf human populations as different species. Thus, insular dwarfism in mammals is not a sufficient requirement to differentiate a species as is the case of the Cozumel's coati.

2. McFadden *et al.* (2008) claimed the Cozumel's coati as a full species, but they did not demonstrate this affirmation (*i. e.*, they never defined a possible species concept). None of the analyses they carried out showed the Cozumel's coati as a full species (This holds true whether we use the Biological Species Concept, BSC, Mayr 1942, 1963, 2004; or different variants of the Phylogenetic Species Concept, PSC: PSC1, Cracraft 1989, Wheeler and Nixon 1990;PSC2, De Queiroz and Donoghue 1988; PSC3, Baum and Donoghue 1995; Shaw 1998).

McFadden *et al.* (2008) analyzed mt control region data and calculated genetic heterogeneity statistics comparing the Cozumel's coati and the coati found in the Yucatán Peninsula. None of the statistical values they presented were less than 0.05; in other words, the statistical results were not significant ($F_{ST} = 0.166$, $p = 0.061$; $F_{ST} = 0.089$, $p = 0.050$). Therefore, the results presented by McFadden *et al.* (2008) are insufficient to suggest that there is a new species. Additionally, McFadden *et al.* (2008) stated that a global AMOVA supported a significant difference between the Cozumel coati population and other Mexican mainland coati populations. Yet again, statistical evidence did not support a clear difference ($F_{ST} = 0.108$, $p = 0.050$). The genetic distance that they obtained between the Cozumel's coati and the Yucatán counterpart was 0.51 %. Kartavtsev (2011) analyzed sequences of mt *COI* from 20,731 vertebrate and invertebrate animal species and obtained 0.89 % ± 0.16 % for populations within species, 3.78 % ± 1.18 % for subspecies or semispecies, and 11.06 % ± 0.53 % for species within a genus. Bradley and Baker (2001) claimed, for mt *Cytb*, that values less than 2 % would equal intra-specific variation, values between 2 % and 11 % would merit additional study, and values greater than 11 % would be indicative of specific recognition. Avise (1994) determined 5 to 7 % of differences at the mt control region for different species and around 2 % for subspecies in mammals. Thus, the genetic distance between the Cozumel's coati and the Yucatan's coati is at most typical of intra-specific variation. Additionally, McFadden *et al.* (2008) estimated the average temporal split between the Cozumel coati population and the populations of coati from Yucatán peninsula and Belize, respectively. They yielded a temporal value of 6,300 years ago (ya) with the population of Yucatán and around 12,000 ya with the population of Belize. Even, with the most rapid mutation rates, these temporal splits should be around 1,300 ya and 2,400 ya, respectively, which agree well with Decker (1991), Glaston (1994), and Zeveloff (2003) that the coati was introduced when Mayas colonized Cozumel Island around 2,500 years ago. In fact, the bones of Cozumel's coati found in excavations in the island were dated to the Mayan Classic Period (*ca.* 1,300 to 1,700 years bp; Hamblin 1984). In that period, the Cozumel's coati was widespread. Archaeological excavations have found them to be located at several sites on the island with a high number of coati remains found at each site (Hamblin 1984). However, we don't have information about older skeletal remains of this coati. Thus, it is possible that humans introduced this coati taxon in historical times.

Based on text and statistical data in McFadden *et al.* (2008) and McFadden (2004) there is no compelling evidence of a new species. For example, McFadden *et al.* (2008) wrote "…*it is difficult to make conclusions about this population's haplotype diversity or species level uniqueness*…", and, "…*our analyses suggest that the Cozumel taxa are most closely related to their Yucatan and Belize conspecifics*…", and "…*we believe small sample size and low level of population differentiation are responsible for the insignificant Fst value.*" Additionally, McFadden (2004) analyzed sequences of a nuclear gene (*CHRNA1*) which were indifferentiable for Yucatán and Cozumel coatis. Despite of this, the authors considered that the Cozumel's coati was a full species for conservation considerations. Again, based on the presented data and statistical analysis there is insufficient information to reject the aforementioned null hypothesis.

3. Nigenda-Morales *et al.* (2019) carried out a very interesting research project on *N. narica*. They included nine specimens of coatis sampled on Cozumel island. All of the analyses that they carried out (Phylogenetic tree based on maximum likelihood and Bayesian inference of 2,201 base pairs of concatenated mt sequences from three genes, *Cytb*, *NADH5*, and *16S rRNA*; timetree showing divergence times among the 21 mtDNA haplotypes that they found within *N. narica;* median-joining network with these 21 haplotypes of *N. narica*; genetic clustering [Structure] and neighbor-joining tree based on DA distance based on genotypes of 11 microsatellite loci in 85 specimens) showed the same results. The Cozumel's coati specimens formed a group together with the specimens from Yucatán, Belize, and part of Guatemala. This group is clearly differentiated from the groups of coatis from Morelos, Jalisco, and Arizona-New Mexico, which are all well differentiated from each them. It is interesting to note that if we follow the reasoning of Valenzuela-Galvan *et al.* 2023 these other *N. narica* clusters should be named as full new species of coatis. The rebuttal letter by Valenzuela-Galvan *et al.* 2023 cited Nigenda-Morales *et al.* (2019) and quoted several sentences: "*Nonetheless, the number of samples from Cozumel was low (eight samples) and seven of the samples were from pet or captive raised animals that may have been derived from the mainland*

*and transported to the island. Therefore, we suggest caution in interpreting our results regarding the genetic status of the coati samples from Cozumel. Given the uncertainty about the taxonomic status of the coati population on Cozumel, a more extensive analysis, including more samples and additional loci, will be required to reach any conclusions that could affect the conservation efforts of this population".* However, Valenzuela-Galvan *et al.* (2023) curiously forgot to comment on the following paragraph in the work of Nigenda-Morales *et al.* (2019): "*We did not find significant evidence indicating coatis from Cozumel Island represent a distinct lineage and therefore a different subspecies* (N. n. nelsoni) *from those on the Yucatan peninsula* (N. n. yucatanica*; table A.4, A.9; figure 1B, 3, 6). These results are largely consistent with the findings of* McFadden *et al.* (2008) *based on mtDNA control region sequence data that coatis on Cozumel Island may have colonized the island during the Late Pleistocene or Holocene (possibly through human-mediated dispersal)".* Therefore, Nigenda-Morales *et al.* (2019) also did not reject the null hypothesis that was mentioned earlier. It is always possible that the two specimens of McFadden (2004) and McFadden *et al.* (2008), the nine specimens of Nigenda-Morales *et al.* (2019), and the specimen of Jaramillo and Ruiz-García (2022), although sampled in the Cozumel island, do not represent the "true" Cozumel's coati because all of them may have been derived from the mainland and transported to the island, but the likelihood of that happening would have been extremely low.

4. When we wrote the article of Jaramillo and Ruiz-García (2022), we ignored the existence of the work of Flores-Manzanero *et al.* (2022). Flores-Manzanero *et al.* (2022) analyzed samples from 46 individuals of the Cozumel's coati (that were captured alive on site) and performed an analysis of mt *Cytb* and nuclear microsatellites. The authors obtained two main results. By using the Structure Program applied to microsatellite data, the authors demonstrated that the Cozumel's coati population was differentiated from the populations in Jalisco and Morelos (figure 2b). However, this result was not new. Nigenda-Morales *et al.* (2019) had already communicated this result to the scientific community. Curiously, the analysis by Flores-Manzanero *et al.* (2022) did not include coati specimens from Quintana Roo, Yucatán, and Campeche, which were genetically similar to the Cozumel's coati (Nigenda-Morales *et al.* 2019; Jaramillo and Ruiz-García 2022). Obviously, if they had introduced specimens from these nearby Mexican mainland areas, the differentiation of the Cozumel's coati would have been less conspicuous (conservation prejudice). On the other hand, multiple populations of the same species can significantly diverge for a set of microsatellites but this does not mean that they are different species. For instance, populations of pink river dolphins (*Inia geoffrensis*) from two Colombian rivers (Putumayo and Caquetá) and from different Peruvian rivers are significantly different based on microsatellite data (Ruiz-García 2010; Ruiz-García *et al.* 2018). Similarly, different populations of the Andean cat (*Leopardus jacobita*) from Perú, Bolivia, and Argentina are considered significantly different based on seven microsatellites (Cossíos *et al.* 2012; Ruiz-García *et al.* 2013a). Additionally, different populations of ocelots (*Leopardus pardalis*) throughout South American are significantly different at 10 microsatellites (Ruiz-García *et al.* 2013b). The last example we mention here from a very long list of examples is humans. Indigene human populations in South America are significantly different for 15 microsatellites (Demarchi 2009). Does this mean that they are different species because they have significant differences for nuclear microsatellites? The answer is no. In reference to the mt*Cytb*, Flores-Manzanero *et al.* (2022) showed a phylogenetic tree in figure 3. The authors found that the specimens from Jalisco and Morelos conformed well differentiated clades from the clade of the Cozumel's coati. Again, the scientific community had already been informed about this finding three years earlier (Nigenda-Morales *et al.* 2019). Within the Cozumel's coati clade, Flores-Manzanero *et al.* (2022) found three sub-clades. One of them included a few specimens from Quintana Roo that the authors included in their analysis. Therefore, no reciprocal monophyly exists between the Cozumel's coati and the coatis from the nearby Mexican mainland. Additionally, if Flores-Manzanero *et al.* (2022) had enclosed more specimens from Quintana Roo, Yucatán, or Campeche, surely more specimens of these Mexican mainland areas would have been clustered with the Cozumel's coati group. In other words, similar to McFadden (2004), McFadden *et al.* (2008), Nigenda-Morales *et al.* (2019), and Jaramillo and Ruiz-García (2022), Flores-Manzanero *et al.* (2022), did not reject the null hypothesis that *N. narica* and the Cozumel's coati are different species. In fact, Flores-Manzanero *et al.* (2022) indicated that they knew it was not a full species when they wrote "*These results support that, at least following the precautionary principle, the Cozumel dwarf coati should be considered and managed as a Significant Evolutionary Unit (sensu Moritz, 1994)".* Notwithstanding, the Cozumel's coati does not fit the definition of an ESU (sensu Moritz 1994). An ESU should be reciprocally monophyletic for mtDNA and this is not the case with the Cozumel's coati. The Cozumel's coati is, more likely, an example of a management unit (MU). Moritz (1994) originally defined an MU as a population that showed significant differences in allele frequencies at nuclear or mtDNA loci. This can show that their population dynamics depend more on local birth and death rates than on immigration (demographical independence; Bentzen 1998).

There are many definitions of a species besides those previously cited (BSC, PSC1, PSC2, PSC3) such as the Genotypic Cluster Species Concept (GCSC; Mallet 1995), Recognition Species Concept (RSC; Paterson 1985; Lambert *et al.* 1987), Cohesion Species Concept (CSC; Templeton 1989), etc. The Cozumel's coati does not meet most of these definitions. But, the most universally accepted concept is the BSC, in part because it is the one we apply to our own species. We agree with the explanation about concepts offered by Mayr (2004): "*it is very important to understand what the* *word concept means when combined with the word species. It implies the meaning of the species in nature. A population, or group of populations, is a species, according to BSC, if it configures a reproductive community and does not reproduce with members of similar communities. BSC, as defined, plays a concrete role in nature and differs in this respect from all those other so-called concepts of species that are nothing but instructions, based on human judgment, about how to delimit specific taxons."* Clearly, the Cozumel's coati is not "above" the BSC. It would not be surprising that the authors who support the Cozumel's coati as a full species, have some doubts about their suggestion. Why? The *N. narica* from the Mexican mainland is known to have been brought over to Cozumel (historically as pets), and it is suspected that they may have interbred with the island coati (Gomper, and others). There are no reproductive isolation barriers between the Cozumel's coati and the nearby Mexican mainland coati. In other words, the Cozumel's coati is the same species of coati that is found in the Mexican mainland.

Nevertheless, conservation biological prejudice exists (see McFadden 2004): "*Depending on how strictly one interprets the biological species definition (O'Brien and Mayr 1991), the island taxa of* N. nelsoni *could thus be classified as the same species as the mainland taxa of* N. narica. *If the criterion of reproductive isolation is generally applied, the taxa of dwarf carnivores may go unrecognized as evolutionarily differentiated populations and thus separate conservation units."* But it is not necessary to define new species to conserve "special" populations such as the Cozumel's coati (even biological conservation can be endangered by partition of a species in different supposed species; see Zachos *et al.* 2013b). For example, the US Endangered Species Act (ESA) of the United States has been a major stimulus to develop criteria for identifying intraspecific population units for biological conservation. The ESA provides full legal protection for subspecies and for Distinct Population Segments (DPSs) of vertebrate species, as if they were full species.

In conclusion, we agree with Valenzuela-Galvan *et al.* 2023 that the Cozumel's coati should be actively and urgently protected by Mexican institutions, but we urge caution in accepting the existence of a new species unless statistical analysis (p value less than 0.05) rejects the null hypothesis (*i. e.*, no difference).

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# Monogamy or monogamish? Re-examining monogamy in *Peromyscus californicus*

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California mice have been widely recognized as one of the few examples of 'true' genetic monogamy in mammals and are one of only four mammalian species considered to be both genetically and socially monogamous. The mating system of this species, first described by David O. Ribble (1991), was initially investigated in a single population by integrating both behavioral data and DNA fingerprinting to classify *P. californicus* as monogamous. Here, we investigated the parentage of field sampled litters of California mice across 4 populations using both classic field methods and modern microsatellite analyses. We putatively identified male-female mouse pairs in the field using capture localities and transfer of fluorescent pigment between individuals. We then used microsatellite loci to genotype pregnant adult females, their embryos, and the adult males identified in the field as the partners of those females. We identified occurrences of extra-pair paternity in 3 out of the 4 populations of California mice, calling in to question the designation of this species as genetically monogamous (Figure 1 and Table 1). We suggest a careful re-examination of the mating system of this species using modern molecular methods to analyze a greater number of samples representing multiple sampling localities. Future studies of this species should prove particularly informative regarding the correlates of extra-pair mating and, hence, the adaptive bases for the maintenance of male-female pair bonds in the absence of true genetic monogamy.

El ratón de California ha sido ampliamente reconocido como uno de los pocos ejemplos de monogamia genética "verdadera" en mamíferos y son una de solo cuatro especies de mamíferos consideradas genética y socialmente monógamas. El sistema de apareamiento de esta especie, descrito por primera vez por David O. Ribble (1991), se investigó inicialmente en una sola población mediante la integración de datos de comportamiento y huellas dactilares de ADN para clasificar a *P. californicus* como monógamo. Aquí, investigamos la paternidad de camadas de ratones de California muestreadas en 4 poblaciones salvajes utilizando métodos de campo clásicos y análisis de microsatélites modernos. Identificamos pares de ratones macho-hembra en el campo usando localidades de captura y transferencia de pigmento fluorescente entre individuos. Luego usamos loci de microsatélites para determinar el genotipo de las hembras adultas preñadas, sus embriones y los machos adultos identificados en el campo como las parejas de esas hembras. Identificamos casos de paternidad extra-pareja en 3 de las 4 poblaciones de ratones de California, lo que cuestiona la designación de esta especie como genéticamente monógama (Figura 1 y Tabla 1). Sugerimos una re-examinación cuidadosa del sistema de apareamiento de esta especie utilizando métodos moleculares modernos para analizar un mayor número de muestras que representen múltiples localidades de muestreo. Los estudios futuros sobre esta especie deberían resultar particularmente informativos con respecto a los correlatos del apareamiento extra-pareja y, por lo tanto, las bases adaptativas para el mantenimiento de los lazos de pareja macho-hembra en ausencia de una verdadera monogamia genética.

***Keywords:*** California deermouse; genetic monogamy; monogamy; paternity; *Peromyscus californicus*.

## Introduction

Monogamous mating systems have long puzzled behavioral ecologists given that males – and in many species females – appear to gain fitness benefits by mating with multiple members of the opposite sex (Bateman 1948; Trivers 1972). Studies that integrate behavioral and molecular data have revealed that monogamy consists of two distinct but related components. Social monogamy is characterized by the formation of an exclusive behavioral bond between a male and a female (Kleiman 1977). In contrast, genetic monogamy refers to the number of partners whose gametes contribute to production of an individual's offspring (Dolotovskaya *et al.* 2020; Kappeler 2019). The extent to which these two forms of monogamy coincide varies, as evidenced by interspecific differences in the frequency of extra-pair copulations and fertilizations in socially monogamous taxa (Waser *et al.* 1994; Sillero-Zubiri *et al.* 1996; Girman *et al.* 1997; Goossens *et al.* 1998; Fietz *et al.* 2000; Solomon *et al.* 2004; Cohas and Allainé 2009, 2009; Huck *et al.* 2014; Dolotovskaya *et al.* 2020). The duration of monogamous relationships also varies, ranging from a single round of reproduction to lifetime reproductive partners (Kleiman 1977; Lukas and Clutton-Brock 2013). Quantifying these sources of variation is critical to identifying the combination of selective factors favoring monogamous mating systems across diverse species.

Social monogamy is often inferred from behavioral data (*e. g.*, spatial relationships, evidence for pair bonds; Ribble and Salvioni 1990; Sabol *et al.* 2018). In contrast, demonstrating genetic monogamy typically requires molecular data regarding the parentage of young (Lambert *et al.* 2018). Studies that incorporate both types of information suggest that truly monogamous – that is, both socially and genetically monogamous – systems are rare among vertebrates (Lambert *et al.* 2018). Among mammals, only a handful of species are thought to be truly monogamous (3 to 5 % (Kleiman 1977), including the Malagasy giant jumping rat (Sommer and Tichy 1999), Kirk's dik-dik (Brotherton *et al.* 1997), and some populations of coyotes (Hennessy *et al.* 2012). Consistent with this designation, genetic analyses confirm that in each of these taxa all offspring are sired by a female's social partner.

One of the best-studied examples of monogamy in mammals is the California deermouse (*Peromyscus californicus*). This species was first described as socially monogamous by Ribble and Salvioni (1990), who used a combination radiotelemetry and fluorescent powder tracking to demonstrate that members of a male-female pair share a home range and a nest site but do not typically overlap spatially with neighboring pairs of animals. Subsequent analyses based on multi-locus DNA fingerprinting (Ribble 1991) revealed no evidence of extra-pair parentage, suggesting that social partners are genetically monogamous. Building on this foundation, studies of California mice have been used to examine the ecological, life history, endocrine, and neural correlates of mammalian monogamy (*e. g.* Gubernick and Nordby 1993; Bester-Meredith *et al.* 1999; Trainor and Marler 2001; Campi *et al.* 2013; Johnson *et al.* 2015; Pultorak *et al.* 2015; Petric *et al.* 2021).

Characterization of free-living *P. californicus* as socially and genetically monogamous is based on data collected from a single population studied in oak savannah habitat in Monterey County, California. The geographic distribution of this species, however, extends from the San Francisco Bay area south to Baja California and encompasses habitats ranging from mesic coastal woodlands to considerably more arid chaparral (Grinnell and Swarth 1913; Grinnell and Orr 1934; King 1968). Given this geographic and ecological variation and given intraspecific variability in rates of extra-pair paternity in other socially monogamous species (Cohas and Allainé 2009), we chose to explore the occurrence of monogamy in populations of *P. californicus* from multiple locations in California. Specifically, we used a combination of live-trapping, fluorescent powder tracking, and microsatellite analyses of parentage to determine if male-female pairs identified on the basis of spatial relationships were the genetic parents of offspring reared by the female in each pair. These analyses generate important new insights into the occurrence of extra-pair young in this species, thereby contributing to efforts to understand the adaptive bases for social versus genetic monogamy in free-living populations of mammals.

## Materials and methods

*Field sites, trapping, and marking of animals.* Mice were captured at 4 localities – 2 in the northern and two in the southern portion of the range of *P. californicus* (Figure 1). The northern sites sampled were at the Hastings Natural History Reservation, Carmel Valley, California and the Landels-Hill Big Creek Reserve, Big Sur, California. The two southern sites were located at the Emerson Oaks Reserve, Temecula, California and the Torrey Pines State Natural Reserve, La Jolla, California. These are the same locations sampled by Melendez-Rosa *et al.* (2020). As described by these authors, the two northern sites are characterized by greater annual rainfall; for both northern and southern localities, the more coastal site receives greater rainfall than the more inland site. Collectively, these sampling localities span much of the range of habitats and environmental conditions in which *P. californicus* is known to occur (Meléndez-Rosa *et al.* 2020).

All trapping of mice was conducted between February and April 2016. At each sampling locality, animals were captured using Sherman live-traps baited with rolled oats and containing a small ball of synthetic batting that the animals used as nesting material. A total of 180 traps per locality were set, with traps placed in pairs at 10 m intervals to create a grid measuring 150 m x 60 m and containing 90 trap stations (pairs of traps). At each sampling locality, traps were opened at 16:00 hrs and closed 3:00 hrs for 20 consecutive nights. Individuals captured were identified to species using standard pelage and body size characters (Jameson and Peeters 2004). At the time of first capture, each animal was permanently marked by attaching a uniquely numbered metal tag (Monel 1005-1, National Band and Tag Company, Inc.) to the right ear pinna. In addition, each animal was weighed and its sex and reproductive status were assessed based on the appearance of the external genitalia. Upon completion of these procedures, each animal was released at the location at which it had been caught.

*Field identification of male-female pairs via pigment transfer.* Putative male-female pairs were identified based on capture localities and transfer of fluorescent pigment between individuals. A male and female were considered probable reproductive partners if they were captured in adjacent (paired) traps on more than three occasions during the same 20-night trapping effort. Physical contact between putative partners was confirmed using fluorescent powder tracking (Ribble and Salvioni 1990; Kalcounis-Rüppell *et al.* 2001). Previous studies of *P. californicus* have demonstrated that when a female whose pelage has been coated with fluorescent powder returns to her nest, some of the powder is transferred to the adult male with which she lives (Ribble and Salvioni 1990; Ribble 1991). By recapturing the female and her partner on the following night, transfer of powder can be detected visually (either directly or with a hand-held black light; Figure 1: photo A), thereby confirming physical contact between the adults in question. Accordingly, the female in each putative pair was covered from neck to tail in one of six colors of non-toxic Eco Pigments$^{Tm}$ (DayGlo, Cleveland, OH) fluorescent powder just prior to release at the point of capture. In the few cases in which the male was not caught the following night, additional powder was applied to the female and the process was repeated. A male displaying significant powder transfer was determined to be a female's putative reproductive partner; significant transfer was defined as powder that was visible without the assistance of a UV light. Although transfer of powder could be detected on any part of a male's body, it was most common on the pinnae, muzzle, and around the eyes as well as on the feet, tail, and genitalia. As a final check on our assignments of individuals to reproductive pairs, the fluorescent powder tracking process was repeated for each putative pair using a different color of powder.

All fieldwork involving mice was approved by the Animal Care and Use Committee at the University of California, Berkeley, and was consistent with the Guidelines for the Use of Wild Mammals in Research published by the American Society of Mammalogists (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016).

*Microsatellite analyses of paternity.* Prior to this study, genetic monogamy had been assessed only for *P. californicus* at HNHR (Figure 1) based on multi-locus DNA fingerprinting (Ribble 1991). To confirm the reported genetic monogamy of this population and to determine patterns of parentage at the other localities sampled, we used microsatellite loci to genotype pregnant adult females, their embryos, and the adult males identified in the field as the partners of those females; we focused these analyses on pregnant females because use of known mother-offspring pairs increased our confidence in the associated assignments of paternity. At each locality, the subset of females that were determined to be pregnant and whose putative male partners had been identified using the trapping and powder transfer criteria described above were euthanized via overdose with Isoflurane, after which a sample of the female's liver and each embryo were frozen separately in liquid nitrogen until they could be transferred to a -80° C freezer on the Berkeley campus. Similarly, we euthanized and collected liver samples from all putative adult male partners.

Allelic variation was assessed at nine microsatellite loci. Primers for four loci (PO-9, PO-88 PO-26, PO-16) had been developed for *P. polionotus* by Prince *et al.* (2002). Primers for the remaining five loci (5477, 5411, 5142, 5466, 5334), were developed for *Peromyscus* by Weber *et al.* (2010). PCR

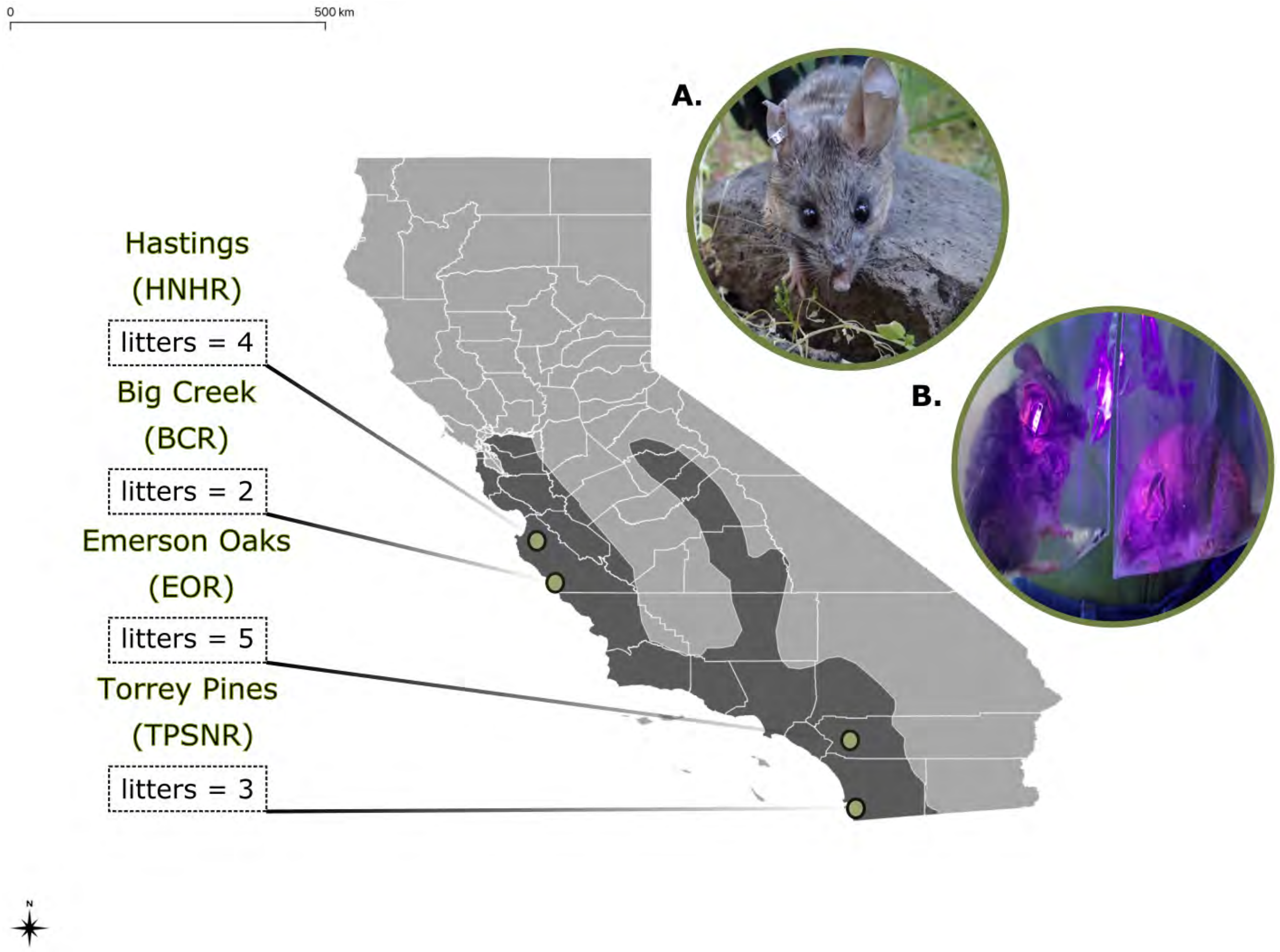

**Figure 1.** Locations of the populations of Peromyscus californicus sampled during this study. The geographic distribution of this species is shown in dark gray. The sites sampled were: Hastings Natural History Reservation (HNHR), Big Creek Reserve (BCR), Emerson Oaks Reserve (EOR), and Torrey Pines State Natural Reserve (TPSNR). For each site, the total number of litters (= number of male-female pairs) for which paternity was determined is indicated. Photo A: adult P. californicus from HNHR. Photo B: male (left) and female (right) P. californicus demonstrating transfer of fluorescent pigment; pigment revealed using a handheld UV lamp.

amplification of loci isolated from *P. polionotus* was accomplished using the same master mix employed by Meléndez-Rosa *et al.* (2019) to amplify the *cyt-b* locus from our study populations; thermocycling conditions were the same as those described by Prince *et al.* (2002). The master mix for the remaining loci consisted of 6.76µL of $ddH_2O$, 1.25µL of 10x buffer, 1µL of $MgCl_2$ (25µM), 1.25µL of BSA (Bovine Serum Albumin), 0.1875µL of dNTPs (10µM), 0.475µL (20pmol) of each primer (fluorescently tagged forward primer; Table 1), 0.10µL of Taq polymerase (New England Bio Labs), and 1µL of the DNA template. Amplification conditions for these loci consisted of an initial denaturation at 95 °C for 4:00 min followed by 40 cycles of denaturation at 95 °C for 0:30 min, annealing at 55 to 56 °C for 0:30 min, and extension at 72 °C for 0:30 min. Specific annealing temperatures for each locus are provided in Table 1.

To assess allelic variability at each microsatellite locus, amplicons were electrophoresed on an ABI 3730 sequencer, with 500 LIZ size standard (GeneScan) included in each lane. Allele sizes were determined using Geneious 7.1.7 (Kearse *et al.* 2012), after which estimates of allelic diversity, heterozygosity, and polymorphic information content (PIC) were generated and departures from Hardy-Weinberg expectations (HWE) were assessed using CERVUS 3.0.7. Pairwise estimates of linkage disequilibrium (LD) were calculated for all loci using GENEPOP v4.7.5 (Raymond and Rousset 1995; Rousset 2008). To determine the paternity of individual fetuses, genotypes for females, their offspring, and all males sampled were compared using CERVUS 3.0.7 (Kalinowski *et al.* 2007). This software package calculates likelihood ratio scores (LOD) for each candidate sire, after which the difference in LOD scores between the two most likely sires is used to assign parentage at a 95% confidence level.

## Results

*Identification of social partners.* A total of 23 male-female pairs ($n$ = 46 individuals) were identified based on trapping locations and the transfer of fluorescent powder from females to males. The number of pairs identified per trapping locality was 7 at BCR, 5 at EOR, 7 at HNHR, and 4 at TPSNR (Figure 1). In no case did we capture non-paired individuals in adjacent traps or detect pigment transfer from a female to more than one male.

*Microsatellite genotyping.* Of the 23 females for which a male partner was identified, 14 (60.8 %) were determined to be pregnant. This included 2 females at BCR, 5 at EOR, 4 at HNHR, and 3 at TPSNR. Based on the number of embryos detected, mean litter size was 2.2 ± 0.6 offspring per female (range = 1-3 embryos, $n$ = 31 embryos recovered from 14 females). The ages of embryos varied, with the result that litters for three females (21.0 %; all from HNHR) contained offspring that were too small to yield DNA that was not contaminated with maternal tissue. As a result, genotypes were generated for 11 females and their 26 offspring (Table 2).

All nine microsatellite loci employed were variable, with the number of alleles per locus ranging from 3 to 16 (*mean* = 9.4 $\pm$ 4.7; Table 1). CERVUS was unable to evaluate departures from HWE expectations for five loci due to the limited number of individuals genotyped (Table 1); two of the remaining loci revealed significant departures from Hardy-Weinberg expectations (Table 1). Given the demonstrated effects of small samples sizes on accurate estimates of departures from HWE (Elston and Forthofer 1977; Wittke-Thompson *et al.* 2005), all loci were retained in downstream analyses of paternity because they were variable and informative. Only one pair of loci revealed potential LD for samples from EOR (loci PO-16 and 5466; $p$ = 0.045); no significant LD was detected for any other pairwise comparisons of loci ($p > 0.05$; mean $p = 0.73$) and thus, again, all loci were retained in downstream analyses. Locus-specific estimates of polymorphic information content (PIC) ranged from 0.343 to 0.908 (mean = 0.742 $\pm$ 0.180), indicating highly polymorphic fragments appropriate for paternity testing (Table 1).

*Paternity analyses.* For each of the 26 embryos genotyped, only a single sire was identified with $\geq$ 95% confi-

**Table 1.** Summary of microsatellite markers used to determine paternity for embryonic litters of *P. californicus*. For each locus, the annealing temperature used in PCR amplifications is indicated, as is the fluorescent dye used during screening of variability at each marker. A total of 60 individuals were genotyped using these markers; for each locus, the number of alleles detected in this sample is given, as are the values for observed heterozygosity, expected heterozygosity, and the polymorphic information content (PIC). Significant departures from Hardy–Weinberg expectations are indicated (NS = not significant at $p < 0.05$; *** $p < 0.001$); departures from expectation could not be evaluated (ND = not estimated) for 5 loci due to the limited number of individuals genotyped.

| Locus | Annealing temperature | Dye | No. of alleles | $H_o$ | $H_e$ | PIC | HWE | Source |
|---|---|---|---|---|---|---|---|---|
| PO-9 | 55 | HEX | 13 | 0.8194 | 0.8573 | 0.839 | NS | Prince *et al.* (2002) |
| PO-88 | 55 | 6-FAM | 16 | 0.9583 | 0.9206 | 0.908 | ND | |
| PO-26 | 58 | VIC | 7 | 0.2083 | 0.3564 | 0.343 | ND | |
| PO-16 | 58 | 6-FAM | 3 | 0.338 | 0.6668 | 0.588 | *** | |
| 5477 | 56 | 6-FAM | 5 | 0.4722 | 0.7583 | 0.713 | *** | Weber *et al.* (2010) |
| 5411 | 55 | NED | 7 | 0.6806 | 0.8153 | 0.781 | ND | |
| 5142 | 55 | PET | 7 | 0.8451 | 0.7881 | 0.749 | NS | |
| 5466 | 55 | VIC | 11 | 0.7361 | 0.8721 | 0.852 | ND | |
| 5334 | 55 | PET | 16 | 0.5352 | 0.9173 | 0.904 | ND | |

dence. Based on these paternity assignments, 8 (72.3 %) of the litters genotyped were sired exclusively by the mother's social partner (Table 2). Litters belonging to two (18.2 %) other females were sired by more than one male; in both cases the social partner was assigned as the sire of at least one embryo. The final litter examined was sired entirely by a male who was not the social partner of the litter's mother (Table 2). Thus, overall, three (27.3 %) of 11 litters contained young that were not sired by the mother's social partner, with a total of three (11.5 %) of the embryos genotyped being sired by extra-pair males. This included litters from three of our four sampling localities, indicating that extra-pair paternity was not restricted to a single population. For each of the litters containing extra-pair young, both the mother's social partner and the extra-pair sire of her offspring were captured during this study; in all cases the extra-pair sire was trapped within 300 m of the capture localities for the female and her social partner.

## Discussion

Our analyses indicate that although *P. californicus* has been described as genetically monogamous (Ribble and Salvioni 1990; Ribble 1991), extra-pair paternity of young does occur. Despite our limited sample size, we detected extra-pair young in multiple litters, including a litter from HNHR, the site of the studies that led to the original description of *P. californicus* as genetically monogamous (Ribble and Salvioni 1990; Ribble 1991; Ribble and Stanley 1998). Overall, extra-pair young were detected at three of our sampling localities, indicating that this phenomenon was not population specific. The occurrence of extra-pair paternity in *P. californicus*, even at low frequency, raises intriguing questions regarding the factors contributing to both extra-pair mating and the maintenance of strong social male-female pair bonds in the absence of true genetic monogamy.

**Table 2.** Results of paternity assignment analyses for *P. californicus*. Data are based on microsatellite analyses ($n = 9$ loci) of 11 embryonic litters obtained from females whose social partner had been identified based on live capture and fluorescent marking data. For each litter, the identity of the mother and putative sire (female's social partner) are indicated, as are the LOD score and delta score for all candidate sires identified by CERVUS. The results for each litter are summarized with respect to the number (multiple paternity, yes or no) and identities of sires (social partner or other male). Data are organized by study site to facilitate comparisons of results across the populations sampled.

| Site | Mother ID | Social partner ID | Offspring ID | Candidate sire ID | LOD score | Delta Score | Multiple paternity | Sire type |
|---|---|---|---|---|---|---|---|---|
| EOR | A | 1 | $A_1$ | 1 | 4.18 | 2.59 | N | partner |
| | | | $A_2$ | | 2.79 | 2.79 | | |
| | B | 2 | $B_1$[a] | 2 | 3.46 | 3.46 | N | partner |
| | | | $B_2$ | | 6.82 | 6.82 | | |
| | C | 3 | $C_1$ | 3 | 2.55 | 2.55 | N | partner |
| | | | $C_2$ | | 3.00 | 3.00 | | |
| | | | $C_3$ | | 3.66 | 3.66 | | |
| | **D**[†] | 4 | $D_1$ | 1 | 6.66 | 6.66 | Y | partner & other |
| | | | $D_2$ | 4 | 5.81 | 1.47 | | |
| | | | $D_3$ | 2 | 7.95 | 6.28 | | |
| | E | 5 | $E_1$ | 5 | 5.65 | 5.65 | N | partner |
| | | | $E_2$ | | 6.44 | 6.44 | | |
| | | | $E_3$ | | 7.82 | 7.82 | | |
| TPSNR | F[a] | 6 | $F_1$ | 6 | 5.10 | 5.10 | N | partner |
| | | | $F_2$ | | 1.12 | 1.12 | | |
| | G | 7 | $G_1$ | 7 | 4.46 | 4.46 | N | partner |
| | | | $G_2$ | | 8.20 | 8.20 | | |
| | | | $G_3$ | | $5.62^{-01}$ | $5.62^{-01}$ | | |
| | H | 8 | $H_1$ | 8 | 2.26 | 2.26 | N | partner |
| HNHR | **I**[†] | 9 | $I_1$ | 12 | 7.06 | 6.89 | Y | partner& other |
| | | | $I_2$ | 9 | 9.88 | 3.41 | | |
| BCR | J | 10[a] | $J_1$ | 10 | 1.49 | 1.49 | N | partner |
| | | | $J_2$ | | 1.49 | 1.49 | | |
| | | | $J_3$ | | 4.29 | 4.29 | | |
| | **K**[†] | 11 | $K_1$ | 10 | 1.77 | 1.77 | N | other |
| | | | $K_2$ | | 1.77 | 1.77 | | |

[a] individuals typed at 8 out of 9 total loci.
[†] females with extra-pair paternity litters.

*Variable rates of extra-pair paternity.* Although we detected extra-pair paternity in several of the litters of *P. californicus* examined here, Ribble (1991) found no evidence of extra-pair young in the 28 litters of California mice that he analyzed. One factor that may have contributed to this apparent disparity in outcomes is the use of different molecular markers to determine parentage. Our analyses of paternity were based on microsatellite loci; in contrast, Ribble (1991) employed multi-locus fingerprinting of mini-satellite DNA regions. These markers differ with respect to multiple features, including the structure of the underlying genetic material, the associated rates of evolutionary change, and the molecular procedures used to detect variability (Flanagan and Jones 2019). Accordingly, it is possible that these markers differ in their ability to detect fine-scale genetic differences among individuals such as those typically used to determine paternity. In particular, given that microsatellite markers can detect single base pair differences in allele sizes, it is possible that these markers reveal more genotypic variation than traditional analyses of mini-satellite regions of DNA (Jones *et al.* 2010). Accordingly, use of microsatellite markers may have contributed to the discovery of extra-pair paternity in our data set but not in that of Ribble (1991).

At the same time, it is possible that the occurrence of extra-pair paternity is dynamic and varies temporally in response to changes in behavioral, ecological, and demographic conditions (Emlen and Oring 1977; Lambert *et al.* 2018). Each extra-pair sire identified during this study was resident near the female with which he produced offspring, suggesting that density- or resource-driven changes in home range size or overlap may influence access to non-partner females and thus the prevalence of extra-pair young (Westneat and Sherman1997; Mayer and Pasinelli 2013). Further, variation in adult sex ratios, in particular the occurrence of male-biased populations, may increase the probability of extra-pair encounters (Fromhage *et al.* 2005). Intra-specific variation in rates of extra-pair paternity has been reported for multiple species of socially monogamous birds (Griffith *et al.* 2008; Botero and Rubenstein 2012; Wan *et al.* 2013; Brouwer and Griffith 2019) and it seems reasonable to expect that similar variation occurs in mammalian species. Clearly, more extensive sampling – in particular sampling conducted over longer time periods – is required to assess potential temporal variation in the prevalence of extra-pair young.

*Monogamy in Peromyscus.* The genus *Peromyscus* contains at least two independent evolutionary origins of social monogamy. One consists of *P. californicus* and, potentially, its sister species, *P. eremicus,* both of which occur in the western US and México (Grinnell and Swarth 1913; Grinnell and Orr 1934; King 1968). The other is *P. polionotus*, which occurs in the southeastern US (King 1968; Foltz 1981). The occurrence of male-female pair bonds is well established in *P. californicus* and *P. polionotus* (Ribble 2003; Jašarević *et al.* 2013). In contrast, the characterization of *P. eremicus* as socially monogamous is more equivocal and is based on largely anecdotal information regarding spatial relationships among opposite-sex individuals (Wolff 1989; Kalcounis-Rueppell and Ribble 2007). No analyses of parentage have been conducted for *P. eremicus* and thus the genetic mating system of this species remains unknown. Based on allozyme analyses, *P. polionotus* has been described as 'overwhelmingly monogamous,' with an estimated frequency of extra-pair paternity of ~ 12 % of offspring (Foltz 1981). The frequency of extra-pair paternity in our dataset was similar, again with ~ 12 % of offspring sired by extra-pair males. Although a larger sample size for *P. californicus* is desirable, available data suggest that this species and *P. polionotus* are similar with respect to degree of genetic monogamy.

The occurrence of two convergent examples of monogamy within *Peromyscus* suggests that comparative studies of these species may offer important insights into the factors favoring this mating system. At the same time, comparisons between monogamous and closely related but polygamous or polygynandrous species of *Peromyscus* provide opportunities to explore the factors associated with the evolution of divergent mating systems. Mating systems theory predicts that monogamy will occur when individual males are unable to monopolize access to more than one potential mate, typically due to either the spatial distribution of females or the need for biparental care to ensure offspring survival (Emlen and Oring 1977; Clutton-Brock 1989; Lukas and Clutton-Brock 2012, 2013). Because this conceptual framework views monogamy as a default strategy that males are forced to adopt under certain ecological, demographic, or life history conditions, it seems reasonable to expect that monogamous animals will pursue extra-pair copulations when such opportunities arise. Future studies that compare *P. californicus* to both socially monogamous and polygynandrous congeners should prove particularly informative regarding the correlates of extra-pair mating and, hence, the adaptive bases for the maintenance of male-female pair bonds in the absence of true genetic monogamy.

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# Increasing the known specific richness of living mammals in Chile

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The Chilean mammal fauna is one of the best known of South America. In spite of this, in the last decade several new species have been described based on specimens collected in the country, while other species previously known elsewhere have been recorded for the first time in Chile. Here we keep on this trend by recording for the first time for Chile a species of long-tailed mouse of the genus *Oligoryzomys*. This mention is based on genetic (cytochrome b gene sequences) and morphological data gathered from several specimens collected at four localities of Quebrada de Camarones, Región de Arica y Parinacota in northern Chile. At one of these localities a specimen was live-trapped; while in the other three localities several osteological remains were recovered from owl pellets. The morphologic and genetic information robustly indicate that the revised specimens belong to the genus *Oligoryzomys*. The phylogenetic analyses show that the trapped specimens belong to *O. flavescens s. l.* However, it remains unsolved to which of two main lineages of *O. flavescens s. l.*, *O. flavescens s. s.* or *O. occidentalis*, belongs the specimens from Camarones. Here we increase the known species richness of Chilean living mammals by showing that northernmost Chile is inhabited by *O. flavescens s. l.* The possibility that the specimens from Camarones represent an undescribed species cannot be ruled out. These new records indicate, once again, that much remains to be learn about basic aspects of the Chilean mammals, including which species form the local assemblages.

La fauna de mamíferos de Chile es una de las mejores conocidas de América del Sur. No obstante, en la última década se han descrito varias especies nuevas a partir de ejemplares colectados en el país, mientras que otras previamente conocidas en otros países se han registrado por primera vez para el país. En este trabajo profundizamos esta tendencia al registrar por primera vez para Chile una especie de ratón colilargo del género *Oligoryzomys*. Esta mención se basa en datos genéticos (secuencias del gen citocromo b) y morfológicos de varios especímenes colectados en cuatro localidades de Quebrada de Camarones, Región de Arica y Parinacota en el norte de Chile. En una de estas localidades se capturó un espécimen; mientras que en las otras tres localidades se recuperaron restos osteológicos de egagrópilas de lechuza. La información morfológica y genética indican de manera robusta que los ejemplares estudiados pertenecen al género *Oligoryzomys*. Los análisis filogenéticos muestran que el especímen colectado en Camarones pertenece a *O. flavescens s. l.* Sin embargo, no es posible esclarecer a cuál de los dos linajes principales de *O. flavescens s. l.*, *O. flavescens s. s.* u *O. occidentalis*, pertenece dicho ejemplar. Aumentamos la riqueza de especies conocidas de mamíferos vivientes chilenos al mostrar que *O. flavescens s. l.* habita en el extremo norte de Chile. No se puede descartar la posibilidad de que los ejemplares de Camarones representen una especie no descrita. Estos nuevos registros indican, una vez más, que queda mucho por aprender sobre aspectos básicos de los mamíferos chilenos, incluyendo qué especies forman los ensambles locales.

***Keywords:*** Hantavirus: Rodentia; Supramyomorpha; Cricetidae; Sigmodontinae.

## Introduction

A recent list of living native mammal species indicates 163 species in Chile (D'Elía *et al.* 2020). Although this number is similar to the figures provided in the latest published lists of species (*e. g.*, 160 in Iriarte 2008 and 157 in Yañéz *et al.* 2009), the composition of the recent list differs substantially from the previous ones. Some of the differences among these lists are due to the exclusion, in the new list, of domestic varieties of wild species and species with an unconfirmed presence in the country. Additionally, most differences among lists refer to taxonomic changes, including the description of new species and the recording in Chile of species previously known from neighboring countries. From 2014 to date, five species — two rodents and three bats — have been recorded in Chile for the first time. Zúñiga and Tancara (2014; see also Valladares *et al.* 2015) and D'Elía *et al.* (2016) reported, the presence of *Abrothrix jelskii* and *Notiomys edwardsii*, respectively (also being the first report for *Notiomys*, as currently delimited, for Chile). On the other hand, Ossa *et al.* (2015, 2018a) reported records of *Eptesicus (Histiotus) laephotis* and *Promops davisoni*. Finally, after the most recent list of Chilean mammals was published (D'Elía *et al.* 2020), Rodriguez-San Pedro *et al.* (2022) reported the presence of the bat *Nyctinomops aurispinosus* in Chile. Similarly, the taxonomic review of the *Phyllotis xanthopygus* complex by Ojeda *et al.* (2021) indicates that, in addition to *P. xanthopygus s. s.* and *P. limatus*, *P. vaccarum* and a species whose name is for the moment unclear (referred to as *P. posticalis-P. rupestris*) are also distributed in Chile. Finally, Novaes *et al.* (2022) recently reviewed the species of *Myotis* present in Chile, concluding that *M. arescens* is distinct from *M. chiloensis*. These recent studies bring to 167 the species of living mammals recorded in Chile.

In line with the studies mentioned above, this work increases the known richness of living native mammals to Chile, reporting for the first time a rodent species of the genus *Oligoryzomys*. Long-tailed mice, as sigmodontines belonging to the genus *Oligoryzomys* are commonly known, form a monophyletic group composed of about 32 living species (Hurtado and D'Elía 2019), distributed from northeast Mexico to the southern tip of Chile and Argentina (Weksler and Bonvicino 2015). The species recorded herein for the first time in Chile belongs to the *Oligoryzomys flavescens s. l.* species complex. This group has a wide known geographic distribution, including areas of Argentina, Bolivia, Brazil, Paraguay, Peru, and Uruguay, and is made up of two candidate species: *O. flavescens s. s.* and *O. occidentalis* (Rivera *et al.* 2018; Hurtado and D'Elía 2019; see below). The report of *Oligoryzomys flavescens s. l.* in Chile is based on a specimen captured and several remains recovered from owl pellets, which together came from four localities in Quebrada de Camarones, Región de Arica y Parinacota, in northernmost Chile.

## Materials and methods

*Study area and samples*. During surveys carried out from 2017 to 2021 in Quebrada de Camarones, Arica y Parinacota Region, Chile, we collected a specimen of the genus *Oligoryzomys* and recovered skeletal remains belonging to this genus from 21 pellets of the American barn owl (*Tyto furcata*). These specimens are the basis of the mention presented in this study. Details of the collection localities are given in the Results section and in Figure 1. The collected specimen was deposited in the Colección de Mamíferos of the Universidad Austral de Chile (UACH), Valdivia, Región de Los Rios, Chile, under the collection number UACH 8477; it was conserved as a fluid specimen after tissue aliquots were preserved in ethanol. The skeletal remains recovered from owl pellets were deposited in the Colección Zoológica de Zonas Áridas y Alto Andinas, Universidad de Tarapacá (CZZA-UTA), Arica, Región de Arica y Parinacota, Chile; the sample includes the remains recovered from 21 owl pellets deposited under the collection number CZZA-UTA 410-430.

*Molecular analyses*. A fragment of 801 base pairs of the mitochondrial gene encoding cytochrome b (*CytB*) of the collected specimen was sequenced using the primers MVZ 05 and MVZ 16 (Smith and Patton 1993), following the laboratory protocol described by Cadenillas and D'Elía (2021a). The amplified fragment was sequenced by a contract laboratory (Macrogen Inc., Korea). The sequence obtained was deposited at GenBank (OP135496) and subsequently integrated into a matrix composed of two sequences of each of the *Oligoryzomys* species delimited by Hurtado and D'Elía (2019). In the case of the forms in the *O. flavescens s. l.*

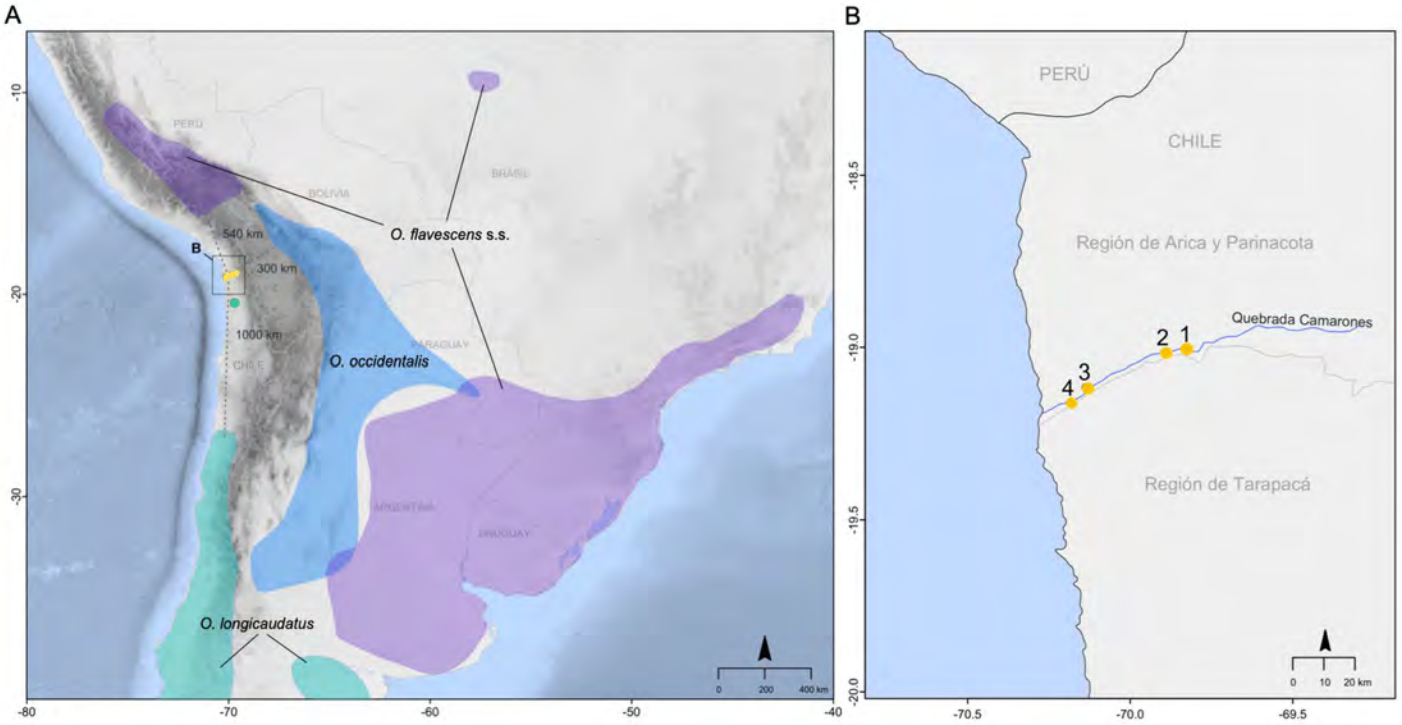

**Figure 1.** A) Partial map of southern South America showing the approximate distribution of the *Oligoryzomys flavescens s. l.* complex and *O. longicaudatus*, the other species of *Oligoryzomys* recognized in Chile. Approximate distances between the Chilean records of *Oligoryzomys flavescens s. l.* and the nearest of *O. flavescens s. s.* and *O. occidentalis* are indicated. The green circle indicates the locality where *O. longicaudatus* was reported by Torres *et al.* (2018) and that we suggest to be disregarded (details in the text). B) Partial map of northern Chile indicating the four collection localities in the Quebrada de Camarones, Región de Arica y Parinacota, Chile, where the specimens of *O. flavescens s. l.* were recorded.

complex, we used a sampling of sequences with a broad geographic coverage. The outgroup was formed following Hurtado and D'Elía (2019) with sequences representative of the main clades of the tribe Oryzomyini (*sensu* Weksler 2006): clade A, *Zygodontomys brevicauda* and *Scolomys ucayalensis*; clade B, *Handleyomys alfaroi* and *Hylaeamys megacephalus*; clade C (to which *Oligoryzomys* belongs), *Microryzomys minutus*, *Neacomys minutus*, *Neacomys paracou*, and *Oreoryzomys balneator*; clade D, *Oryzomys palustris* and *Holochilus sciureus*. Details of the sequences used, including the catalog numbers and collection localities of the sequenced specimens, are provided in the Supplementary material 1. Sequence alignment was performed with Clustal as implemented in MEGA6 (Tamura *et al.* 2013), with the default values for the alignment parameters. Subsequently, a visual inspection was carried out to search for potential reading frame changes. The matrix obtained was analyzed through Maximum Likelihood (ML) and Bayesian inference (BI). The ML analysis was carried out with IQ-TREE (Nguyen *et al.* 2015) using the W-IQ-TREE online tool (http:/iqtree.cibiv.univie.ac.at; Trifinoupoulus *et al.* 2016), with the disturbance intensity set to 0.5, the term rule set to 100, and the molecular evolution model TIM2+F+R4, which was also selected using ModelFinder (Kalyaanamoorthy *et al.* 2017) in IQ-TREE, according to the Bayesian Information Criteria (BIC). Clade support was calculated via 1000 ultrafast bootstrap (UFB) pseudoreplicates. The BI analysis was performed with MrBayes (Ronquist and Huelsenbeck 2003) using two separate runs with four Markov chains for $1 \times 10^6$ generations, which were sampled every 1000 generations. As TIM2+F+R4 cannot be implemented in MrBayes, we used the GTR+G+I model following the recommendations of Ronquist and Huelsenbeck (2003). Convergence in stable log-likelihood values was verified by plotting likelihood values versus the number of generations. The first 25 % of the trees were discarded as *burn-in*; the remaining trees, sampled in the convergence zone of both runs, were used to compute a tree according to the majority rule and obtain a-posteriori probability (PP) values for each clade. Percentage of genetic difference between pairs of sequence samples were estimated based on pdistance using MEGA 6.

*Morphological review*. The collected specimen was identified at the genus level based on its external traits. The bone remains recovered from owl pellets were determined using the key written by Reise (1973) and comparative material from the Colección de Mamíferos UACH.

## Results

A male specimen of *Oligoryzomys* (UACH 8477) was captured in 1) Ruta A-345 km 28, Camarones, Comuna de Camarones, Región de Arica y Parinacota (18.997300° S, 69.827450° W, 790 m; Figure 1). In addition, mandibular remains of at least 10, one, and 13 individuals assigned to the genus *Oligoryzomys* (Figure 2) were recovered from owl pellets collected, respectively, in the following three localities along the Quebrada de Camarones: 2) Approximately 600 meters south of Ruta A-345, km 20, Camarones, Comuna de Camarones, Región de Arica y Parinacota (19.014307° S, 69.892347° W); 3) Hacienda Camarones, 7 kilometers west of Puente Cuya, Cuya, Comuna de Camarones, Región de Arica y Parinacota (19.115264° S, 70.135602° W); And 4) Puente Cuya Ruta 5, Cuya, Comuna de Camarones, Región de Arica y Parinacota (19.158704° S, 70.182612° W; Figure 1).

The mandibular remains recovered from owl pellets assigned to *Oligoryzomys* show a short and high general contour, lunar notch poorly excavated, capsular process well-developed, and upper and lower masseteric ridges converging anteriorly in V-shape under m1 (Figure 2). Similarly, the external traits of the UACH 8477 specimen (Figure 3), including its size and body metrics [(total length: 208 mm; tail length: 115 mm; foot length without/with nail: 25/27 mm; ear length: 14 mm; weight: 17 g); dorsal pelage thick, uniformly orange and brownish, ventral pelage lighter; feet without interdigital membranes; foot sole distally covered with scales, heel bare, hypothenar pad long, interdigital pads small, with pads 1 and 4 in a more proximal position than pads 2 and 4; tail sparsely haired, bicolored, covered with conspicuous epidermal scales and without a long tuft of terminal hairs] unambiguously indicate that this specimen belongs to the genus *Oligoryzomys* (see the diagnosis of the genus in Weksler and Bonvicino 2015). This identification at the genus level was confirmed by the phyloge-

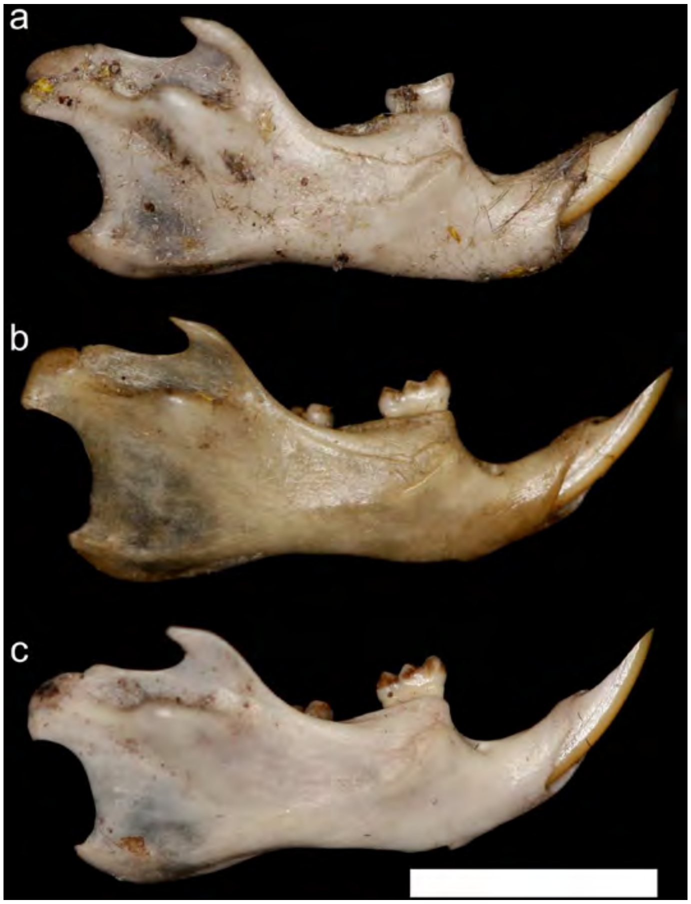

**Figure 2.** Right mandibles of three specimens of *Oligoryzomys flavescens s. l.* collected at three localities (a: 4, b: 3, and c: 2 in Figure. 1) in Quebrada de Camarones, Región de Arica and Parinacota, Chile. The bar indicates 5 mm.

netic analyses. In the trees obtained by BI (Figure 4) and ML, whose topologies are similar, the haplotype recovered from the Camarones specimen is part of the clade (PP = 0.99; UFB = 100) corresponding to the *O. flavescens s. l.* complex. Within this clade, the position of the haplotype from Camarones varies depending on the analysis. In the BI tree (Figure 4b) the haplotype from Camarones (*i. e.*, UACH8477) appears in a relationship lacking significant support (PP = 0.65), as sister to the clade corresponding to *O. flavescens s. s.* (recovered in both analyses: PP = 0.85; UFB = 79). In the ML tree (Figure 4c), the haplotype from Camarones is recovered as sister (UFB = 89) to the clade formed by the haplotypes of *O. occidentalis* (recovered in both analyses: PP = 0.99; UFB = 99). The mean genetic distance observed between the haplotype of the Chilean specimen and the haplotype sample of *O. flavescens s.s.* is 2.92 %, while the mean value of the comparison between the haplotype of the Chilean specimen and the sample of haplotypes of *O. occidentalis* is 3.03 %. Finally, the observed divergence between the samples of *O. flavescens s. s.* and *O. occidentalis* haplotypes is 3.27 %.

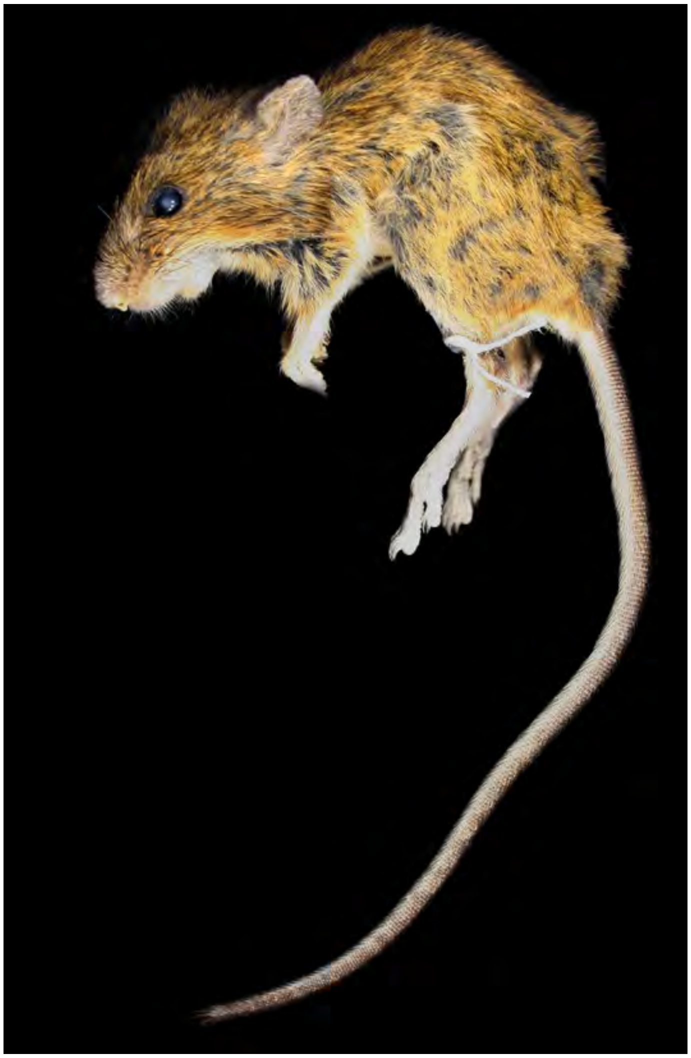

**Figure 3.** Specimen of *Oligoryzomys flavescens s. l.* (UACH 8477) collected in Quebrada de Camarones (locality 1 in Figure 1), Arica y Parinacota, Chile.

The Chilean localities where *Oligoryzomys flavescens s.l.* has been recorded in Chile are in Quebrada de Camarones, in an area within the vegetation unit Desierto Absoluto, in the northern portion of the Atacama Desert. Basically, Quebrada de Camarones is a deep canyon that channels the Camarones River, which runs from the western Andean foothills across the desert and reaches the Pacific Ocean. The local vegetation, a xeric shrubland, limits to a strip a few meters wide that stretches along both sides the river and includes shrubs and herbs (*e. g., Atriplex* spp, *Ephedra breana*, *Cistanthe celosioides, Diplostephium meyenii*, *Senecio reicheanus*) and, in some areas, also trees such as *Acacia cavens*, *Geoffroea decorticans*, or *Prosopis tamarugo*. The collection site of the specimen UACH 8477 is largely impacted by anthropic activities (*e.g.,* human constructions, livestock, crops). In addition to *Oligoryzomys flavescens s.l.*, specimens of *Abrothrix* sp., *Phyllotis* sp., *Thylamys pallidior*, and the exotic species *Mus musculus* and *Rattus norvegicus* were also collected in the same location.

## Discussion

This work recorded a new species of living mammal for Chile. It corresponds to a form of long-tailed mice of the complex of *Oligoryzomys flavescens s. l.*, which is widespread in South America. The taxonomy of this lineage is still unclear. Recent studies indicate that the traditional concept of *O. flavescens* (*e. g.*, Weksler and Bonvicino 2015) comprises two main mitochondrial lineages representing candidate species (Hurtado and D'Elía 2019) that exhibit different climate preferences (Rivera *et al.* 2018). The validation of these candidate species, which should be carried out with analyses based on morphology and/or variation of nuclear genes, is still pending. The two mitochondrial lineages of the *O. flavescens* complex have available names. *O. flavescens s. s.* (including *O. fornesi* as a synonym Massoia, 1973) corresponds to the lineage with the broadest geographic range, with records in central and northeast Argentina, Uruguay, central, southern, and southeastern Brazil, southeastern Paraguay, and southern Peru. *O. occidentalis* corresponds to the lineage distributed in central-western and northwestern Argentina, southern, central, and western Bolivia, and southern Paraguay (Rivera *et al.* 2018; Hurtado and D'Elía 2019). The haplotype of the specimen collected in Quebrada de Camarones appears as sister to the clade corresponding to *O. flavescens s. s.* or *O. occidentalis*, depending on the analysis considered (BI or ML, respectively). However, such relationships have either non-significant (PP = 0.65) or moderate (UFB = 83) support, so the Chilean haplotype cannot be assigned with certainty to one of these lineages. Likewise, since the morphological distinction of the two main mitochondrial lineages of *O. flavescens s. l.* has not yet been evaluated, the mandibles recovered from the owl pellets from Quebrada de Camarones, which are clearly different from those of *O. longicaudatus*, cannot be assigned with certainty to either lineage of the *O. flavescens s. l.* complex. Therefore, in this work, we opted to assign the

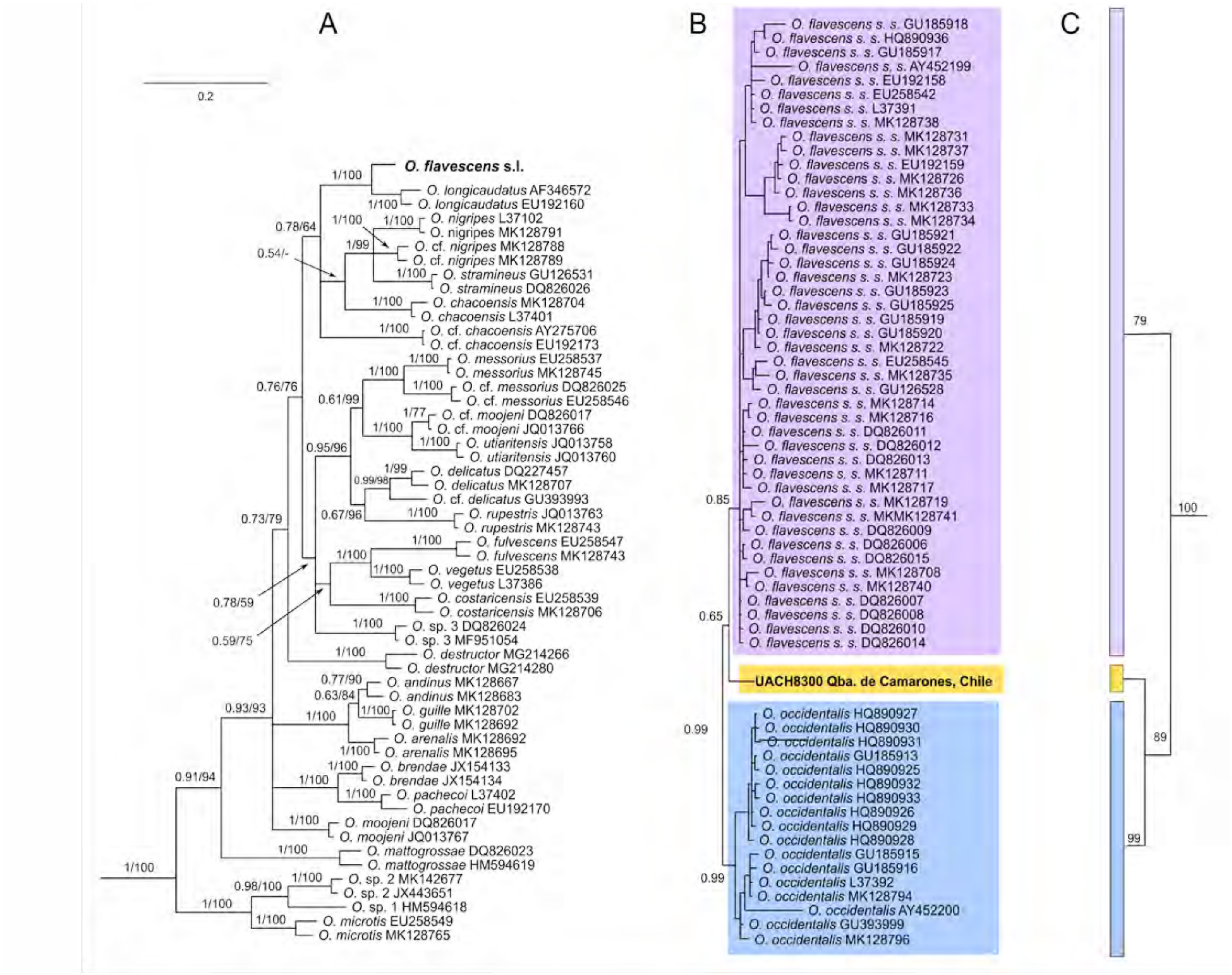

**Figure 4.** Phylogenetic relationships among the species of the genus *Oligoryzomys* (outgroup not shown), based on DNA sequences of the cytochrome b be gene and reconstructed by Bayesian inference. A) Clade composed by species of the genus *Oligoryzomys*. Numbers indicate posteriori probability (left) and ultrafast bootstrap (right) values obtained in the ML analysis (ln = -10641,151). The hyphen (-) indicates that the signaled node was not recovered in the ML tree. Terminal labels indicate species and GenBank access numbers. Localities of the specimens included in the analysis are detailed in the Supplementary material. B) Details of the clade corresponding to *O. flavescens s. l.* Intraspecific support values are omitted for clarity (only posterior probability values are shown). The two major mitochondrial lineages of *O. flavescens s. l.* are indicated by colored rectangles: purple, *O. flavescens s. l.*; blue, *O. occidentalis*). The haplotype of the Chilean specimen (UACH 8477) is indicated by a yellow rectangle. C) Schematic relationships among the main lineages of *O. flavescens s. l.* found in the ML analysis (only ultrafast bootstrap support values are indicated). The colors used are as in B.

records of *Oligoryzomys* from Quebrada de Camarones to *O. flavescens s. l.* We consider that, even with the uncertainty associated with the specific identity of these specimens, it is important to report the existence of an additional species of *Oligoryzomys*, that has not been previously reported for Chile. This finding is even more relevant given that *Oligoryzomys* is a genus in which several species, including those of *O. flavescens s. l.*, are reservoir of hantavirus strains (*e. g.*, Delfraro *et al.* 2003; González-Ittig *et al.* 2014; Rivera *et al.* 2007). Finally, considering the phylogenetic uncertainty of the sample from Quebrada de Camarones and the fact that, genetically, it is almost equidistant to both lineages of the *O. flavescens s. l.* complex (2.92 % and 3.03 % relative to *O. flavescens s. s.* and *O. occidentalis*, respectively), the possibility that it represents a third species of the *O. flavescens s. l.* complex should be explored through the analysis of additional samples. In this context, it is worth noting that in a recent analysis of species delimitation focused on this genus, Hurtado and D'Elía (2019) identified eight candidate species that need evaluating (for the formalization of one of these species see Hurtado 2021). Thus, the possibility that the population of *Oligoryzomys* from Camarones represents a yet undescribed species is not unexpected.

The Chilean records of *Oligoryzomys flavescens s. l.* enlarge *ca.* 540 km to the south and 300 km to the west the know distribution of this species complex from the Peruvian and Bolivian records, respectively (Figure 1). In addition, the Chilean records of *O. flavescens s. l.* are the first of this complex reported on the western slope of the Andes. In turn, these records are located *ca.* 1000 km north of the lowest-latitude record of *O. longicaudatus*, which is in the Región de Atacama of Chile (Weksler and Bonvicino 2015). In this regard, it should be noted that Torres *et al.* (2018) reported the supposed predation of a specimen of *O. longicaudatus* by the passeriform *Agriornis micropterus* in the Reserva Nacional Pampa del Tamarugal, Región de Tarapacá, 150 km south of our records of *O. flavescens s. l.* in Quebrada de Camarones. However, the evidence presented in the work mentioned above — a gray scale photograph of a bird carrying a small, long-tailed mouse in its bill — does not allow us to determine whether it is a specimen of *Oligoryzomys*. Similarly, Torres *et al.* (2018) did not mention whether the mouse specimen was collected and deposited in a biological collection where it can be studied. Therefore we suggest that the mention of *O. longicaudatus* for such northern latitudes of Chile, about 850 km north of the northernmost records of the species confirmed with specimens, be disregarded until an unequivocal record from Pampa del Tamarugal or adjacent localities is reported.

Regarding the areas of Chile where mammal assemblages are better characterized, it is interesting to note that, except for *Notiomys edwardsii*, the mammal species previously known from neighboring countries and that have been recently reported for Chile are from the north of the country. This fact reinforces the known pattern of a higher richness of small mammal species in northern Chile (Samaniego and Marquet 2009; see also Revollo-Cadima *et al.* 2021); therefore, further surveys and collection of specimens should be conducted in that area of the country.

Along with *Oligoryzomys longicaudatus*, *O. flavescens s. l.* is the second species of the genus *Oligoryzomys* known for Chile (refer to D'Elía *et al.* 2020, in which the nominal forms *O. magellanicus* and *O. yatesi* are included as synonyms of *O. longicaudatus*), and the mammal species number 168 with records in the country (D'Elía *et al.* 2020; Ojeda *et al.* 2021; Novaes *et al.* 2022; Rodríguez-San Pedro *et al.* 2022). This new record, together with those presented in recent years and the new species that have recently been proposed based on specimens collected in Chile (see a synthesis in D'Elía *et al.* 2020; see the proposal of candidate species of *Octodon* in Cadenillas and D'Elía 2021b), indicate that the mammalian fauna of Chile is still not completely characterized. There are knowledge gaps related to basic aspects of it, such as to clarify which species constitute the mammal assemblages in several geographic areas (*e. g.*, Storz *et al.* 2020). Therefore, we close this publication by reminding government officers in charge of issuing collection permits, institutional animal care and use committees, and research funding agencies that to obtain adequate characterization of mammal assemblages requires facilitating and enhancing scientific collection and taxonomic work based on collections.

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P. Teta shared with us his knowledge about *Oligoryzomys*. Members of the local communities of Quebrada de Camarones allowed us to sample in their land. FONDECYT 1180366 and 1221115 (GD) and UTA Major Internal Research Project 4725/21 (PV) provided financial support.

*Authors' contributions*. MQC, AG and GD did the fieldwork where the specimen was collected. PV collected the owl pellets. AG did the lab work. MQC, PV, NH and GD did the collection-based work. GD did analyses and wrote the manuscript. All authors edited and approved the text.

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## Supplementary material 1

List of specimens, including their GenBank accession numbers and collection localities, analyzed in the phylogenetic analyses.

https://www.revistas-conacyt.unam.mx/therya/index.php/THERYA/article/view/2217/Supplementary%20material_2217

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# From the bottom up – attributes of small vesper bats' activity sites in an upland ecosystem in Chiapas, México

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We studied bats in a natural protected area and the influence zone in Chiapas, México. Focusing on small vesper bats (Vespertilionidae: Myotinae) in water sinkholes, flooded surfaces, and forested locations (sampling sites), our objectives were to: 1) differentiate the sampling sites based on abiotic and biotic variables, 2) compare the relative activity between the contrasting sampling sites, and 3) determine the relative importance of surrounding land covers amounts on the relative activity. We expected the following: 1) that sampling sites would be differentiable based on an interaction of environmental conditions and nocturnal flying insects' biomass, 2) to find a significantly higher relative activity associated with water bodies as a reflection of potential drinking and feeding supplies, and 3) that forest cover would have a significant association in a positive direction with the relative activity as a reflection of roosting and commuting opportunities. We obtained weather data with a portable station, collected flying nocturnal insects with a passive trap, and recorded bats with ultrasonic detectors. We also used satellite imagery to calculate land covers amounts around the sampling sites. We performed a multivariate analysis to compare sampling sites, and elaborated correlation models of relative activity against surrounding land covers amounts. The only significant variable for differentiating sampling sites was wind speed; in water sinkholes, with lower mean value compared to flooded surfaces and forested locations, along with a comparatively higher percentage of dipterans in the samples. The mean relative activity of bats was significantly higher in water sinkholes, with maximum values reaching 95 %, which we can relate to environmental conditions and resources available. We found associations in a positive direction with forest cover, secondary forest and water surface, and negative with agricultural land and human development. Finally, we argue that some of the water sinkholes meet the criteria of *small natural features* supported by the unusual activity of the studied bats, and that targeted conservation actions will complement other strategies implemented in the area.

Estudiamos murciélagos en un área natural protegida y la zona de influencia en Chiapas, México. Enfocándonos en vespertiliónidos pequeños (Vespertilionidae: Myotinae) en cenotes, superficies inundables y ubicaciones boscosas (sitios de muestreo), nuestros objetivos fueron: 1) diferenciar los sitios de muestreo en función de variables bióticas y abióticas, 2) comparar la actividad relativa entre los sitios de muestreo contrastantes, y 3) determinar la importancia relativa de las coberturas terrestres circundantes en la actividad relativa. Esperábamos lo siguiente: 1) que los sitios de muestreo fueran diferenciables en función de una interacción de las condiciones ambientales y la biomasa de los insectos voladores nocturnos, 2) encontrar una actividad relativa significativamente mayor asociada con los cuerpos de agua como reflejo de fuentes potenciales de bebida y alimentación, y 3) que la cobertura boscosa tendría una asociación significativa en un sentido positivo con la actividad relativa como un reflejo de las oportunidades de refugio y desplazamiento. Obtuvimos datos ambientales con una estación portátil, recolectamos insectos voladores nocturnos con una trampa pasiva, y grabamos murciélagos con detectores ultrasónicos. También utilizamos imágenes satelitales para calcular las cantidades de coberturas terrestres alrededor de los sitios de muestreo. Realizamos un análisis multivariado para comparar los sitios de muestreo, y elaboramos modelos de correlación entre la actividad relativa y las cantidades de coberturas terrestres circundantes. La única variable significativa para diferenciar los sitios de muestreo fue la velocidad del viento; en los cenotes, con un valor promedio menor en comparación con las superficies inundables y las ubicaciones boscosas, junto con un porcentaje comparativamente mayor de dípteros en las muestras. La actividad relativa promedio de los murciélagos fue significativamente mayor en los cenotes, con valores máximos alcanzando 95 %, lo cual podemos relacionar con las condiciones ambientales y los recursos disponibles. Encontramos asociaciones en una dirección positiva con la cobertura forestal, el bosque secundario y la superficie de agua, y negativas con las tierras agrícolas y el desarrollo humano. Finalmente, argumentamos que algunos de los cenotes cumplen con los criterios de *rasgos naturales pequeños* por la actividad inusual de los murciélagos estudiados, y que las acciones de conservación dirigidas complementarán otras estrategias implementadas en el área.

***Keywords:*** Forest loss; hierarchical partitioning; landscape level; local level; *Myotis* spp.; small natural features; targeted conservation.

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## Introduction

The accelerated loss of biodiversity because of human activities is a concern worldwide. Therefore, identifying which attributes in agricultural zones with a high rate of forest loss can back away from the decline of animal populations is crucial (Heim *et al.* 2015). This way, multi-level management may contribute to more suitable habitats for wild species.

For aerial insectivorous bats, localized resources (*e. g.*, water, prey) can represent limiting factors for survival and reproductive success (Findley 1993). At the same time, landscape structure (*e. g.*, forest amount and its spatial arrangement, linear elements, among other attributes) may facilitate commuting toward drinking and feeding sites (Fuentes-Montemayor *et al.* 2017; Mendes *et al.* 2017; Martino *et al.* 2019).

Fahrig (2003) argues that the number of individuals of any species should be, to some point, a positive function of the quantity of habitat available, such as native forests. The strong forest dependency some species of bats exhibit depends on roosting preferences and flight restrictions in open areas (Lacki *et al.* 2007; Fuentes-Montemayor *et al.* 2013; Parreira Peixoto *et al.* 2018; Novella-Fernandez *et al.* 2022). Forest loss can decrease structural connectivity for many species (Fischer and Lindenmayer 2007). However, this topic needs to be better studied in the Neotropics, specifically considering slow-flying bats in upland ecosystems.

Flying away from vegetation cover may impose higher energetic costs for slower species (due to the stronger wind), such as vesper bats (Vespertilionidae), characterized by wings with low aspect ratio and loading (Norberg and Rayner 1987; Heim *et al.* 2015). Moreover, their short and high-frequency modulated echolocation pulses are not well suited to open areas because they are more subject to atmospheric attenuation (Pettersson 2002; Jones and Rydell 2003; Frey-Ehrenbold *et al.* 2013; Heim *et al.* 2015).

We studied small vesper bats (Vespertilionidae: Myotinae) in water sinkholes, flooded surfaces, and forested locations (sampling sites), inside a natural protected area and the influence zone in Chiapas, southeast México. We based the selection of this group of bats on traits such as wing morphology and echolocation system related to the sensitivity to forest loss (Heim *et al.* 2015). They also are essential predators of nocturnal flying insects (*e. g.*, mosquitoes), which are potentially deleterious to humans and become pests, which should be a key consideration in sustainable land management (Gonsalves *et al.* 2013; Heim *et al.* 2015; Puig-Montserrat *et al.* 2020).

Our objectives were to 1) differentiate the sampling sites based on abiotic and biotic variables, 2) compare the relative activity between the contrasting sampling sites, and 3) determine the relative importance of surrounding land covers amounts on the relative activity. We expected 1) that sampling sites would be differentiable based on an interaction of environmental conditions and nocturnal flying insects', 2) to find a significantly higher relative activity associated with water bodies as a reflection of potential drinking and feeding supplies, and 3) that forest cover would have a significant association in a positive direction with the relative activity as a reflection of roosting and commuting opportunities.

## Materials and methods

*Study area and sampling points.* The study area is intermediate between lowlands and highlands, in the comiteca plateau (Meseta Comiteca), Chiapas, México (Figure 1). It includes the Lagunas de Montebello National Park (PNLM, by its initials in Spanish), listed in the RAMSAR convention (no. 1325). The main vegetation association inside the PNLM is a mixed forest dominated by pines (*Pinus* spp.) and oaks (*Quercus* spp.), with a canopy stratum between 20 to 35 m and an interior species-rich tree stratum below 20 m height (González and Ramírez 2013). The predominant land cover outside the PNLM is agricultural land. There is a dry season (approximately March to June), a rainy season (approximately July to October), and a windy season (approximately November to February).

The sampling points were selected based on physiognomy and pre-sampling, with three repetitions each: water sinkholes, which are open areas with surrounding vegetation and have a permanent water surface several meters below the ground (Figure 2a), flooded surfaces, which are also open areas with surrounding vegetation, and have a fluctuating water surface that is superficial, and represent natural and human-induced elements (Figure 2b), and forested locations, such as clearings and trails inside the forest (Figure 2c). There were no considerable elevation differences in the sampling points, which started from 1,460 to 1,540 m, and accounting for extent restrictions for the spatial analysis, the minimum distance between them started from ~ 3.5 km. The samplings took place from July 2014 to July 2015 to obtain data on environmental conditions, insect collections, and bat recordings simultaneously at each event.

*Satellite imagery processing and land covers amounts.* We used multispectral SPOT 5 orthorectified satellite images (spatial resolution of 10-m pixels) of 2015 (dry season). The latter images were subject to radiometric calibration and were provided by the Laboratorio de Información Geográfica y Estadística (LAIGE, by its initials in Spanish) of El Colegio de la Frontera Sur. We performed composition in false color using bands 3 (near infrared), 2 (red), and 1 (green), with a simple linear contrast. The land covers (classes) defined were (Figure 1): forest cover (mature forest), secondary forest (such as coffee crops and orchards), agricultural land (extensive crops such as maize and grasslands), human development (including urbanization and bare soil), and water surface (lakes, water sinkholes, and flooded surfaces). Following Fuentes-Montemayor *et al.* (2013), we grouped distinct forest types to avoid confounding information (*e. g.*, adjacent types with undistinguishable limits).

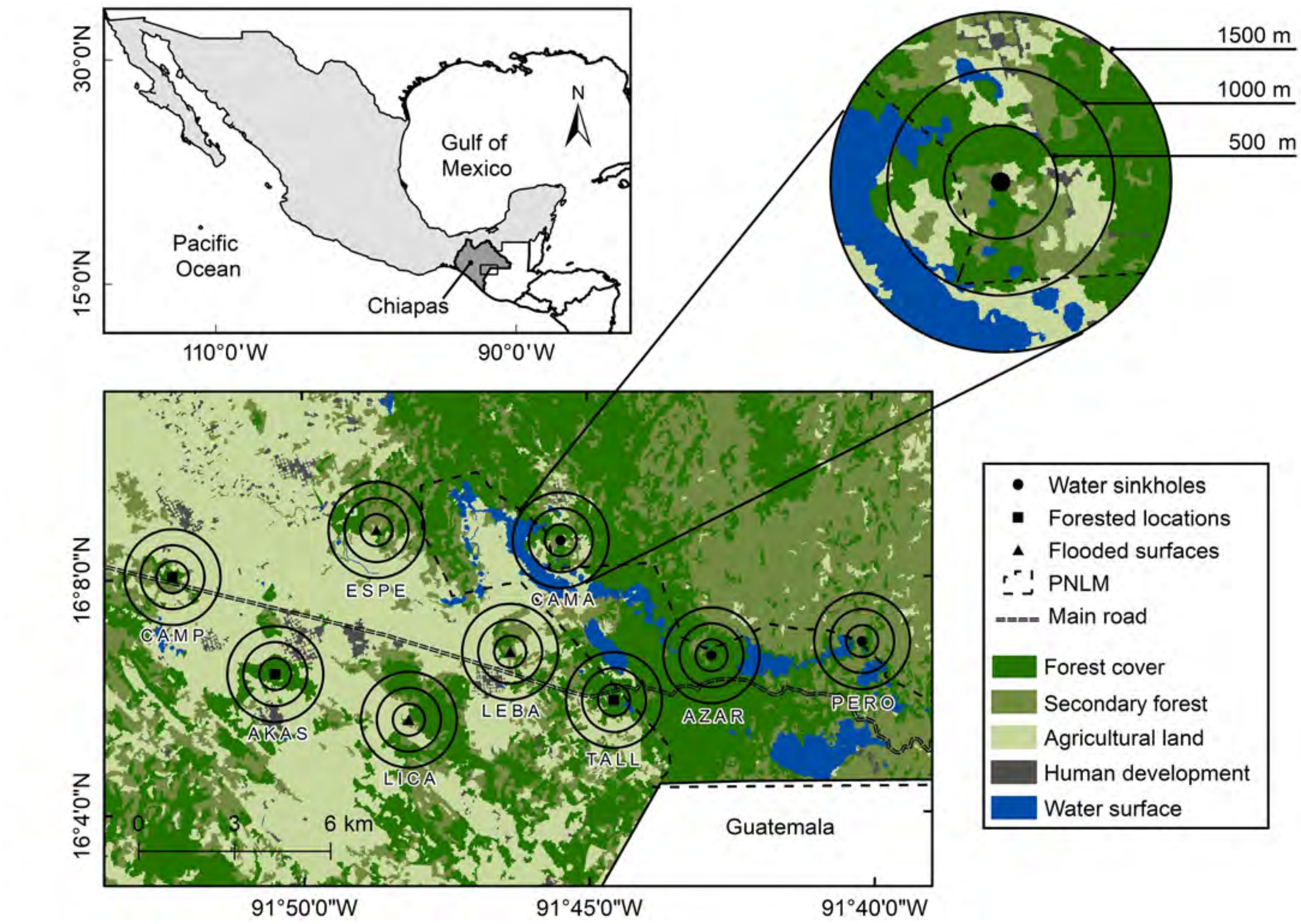

**Figure 1.** Map of the study area in Chiapas, México. We identify sampling points by different symbols, surrounded by concentric buffers in which we calculated land covers amounts.

We performed a supervised classification in Idrisi version 17.0 (Clark Labs, Clark University). Following Lu and Weng (2007) and Eastman 2012, we implemented a segmentation routine that identifies adjacent pixels grouped by spectral similarity, so-called objects. We used a similarity threshold of 30 to obtain homogeneous segments of a size facilitating the selection of training samples. Then, we used the maximum likelihood classifier. Finally, we re-classified the image to improve the precision of the classification and to produce smooth edges between classes by using a distinctive classifier of the segmentation routine. An error matrix (see Verbyla 1995) assessed the overall classification accuracy from 100 field control points spread throughout the study area, obtained during 2014 to 2015, yielding an accuracy of 85 %.

We calculated the area (ha) and percentage of each land cover in concentric buffers of 500-m (78.6 ha), 1,000-m (314.2 ha), and 1,500-m (706.8 ha) radii around the sampling points (Supplementary material 1), delimited in ArcGIS version 10.2.1 (ESRI, Inc.). We were looking to encompass the home range of small vesper bats (*e. g.*, *Myotis* spp.; Owen *et al.* 2003; Coleman *et al.* 2014). Also, the nested design allowed us to explore responses by changing spatial scales and the non-overlapping buffers to avoid re-measuring land covers or pseudoreplication (Popescu and Gibbs 2010; Arroyo-Rodríguez *et al.* 2016).

*Environmental conditions.* We used a multi-function weather meter WM-350 WindMate® (WeatherHawk) to obtain monthly data on temperature (°C), relative humidity (%), and wind speed (KMPH). We took measurements three times per night at the beginning, half, and end of the sampling events; this way, we calculated means for comparison between sampling points. We used the mean calculation per night as input for multivariate analyses.

*Insects' biomass.* For the collection of nocturnal flying insects, we used a Malaise trap. The latter is a passive trap, which we use to not interfere with recordings by attracting prey. We suspended the trap on trees near the sampling points and sampled around water surfaces near the shore. The trap was installed 1.5 m above ground. All specimens were preserved in 70 % alcohol inside plastic containers (Wickramasinghe *et al.* 2004) and labeled for posterior processing and identification.

In the laboratory, we identified the specimens into the orders Diptera (dipterans) and Coleoptera (coleopterans). We pooled Trichoptera and Lepidoptera orders into the superorder Amphiesmenoptera (amphiesmenopterans) because we faced difficulties identifying alcohol-preserved samples. These orders are among the most common food elements for aerial insectivores, particularly this group of bats (see Whitaker 2004; Segura-Trujillo *et al.* 2018), and may reflect potential prey availability.

We counted the number of individuals of each taxon in each sample (see Queiroz de Oliveira *et al.* 2015), which were dried later in a stove at 70 °C for 48 h (Bradley *et al.* 1993), and obtained biomass (g) using an Explorer™ Pro Analytical Balance (EP214C), with a readability of 0.1 mg (Ohaus Corporation). Following Queiroz de Oliveira *et al.* (2015), we divided it by the number of insects for standardized measurement. Finally, we calculated the mean per night at each sampling point.

*Relative activity of bats.* We used an Echo Meter EM3+ Ultrasonic Detector (Wildlife Acoustics, Inc.) to obtain full-spectrum bats recordings. We performed short-term passive recording sessions lasting 4 hrs or else standardized. We configured the EM3+ with 1) sample rate of 256 kHz, 2) WAV audio file format, 3) maximum duration of 15 sec, 4) frequency trigger of 15 kHz, 5) amplitude trigger of 18 dB, 6) trigger window of 1 sec, and 7) gain of 30 dB. We positioned the detector on a 1-m pole with a 45° upward angle, directed toward acoustic space in water sinkholes, flooded surfaces, and forested locations (gaps and trails). Each sampling point was visited twice during three consecutive nights. We stored the recordings in a 32 GB SD card (Kingston© Technology Corporation), and the total recording effort was 432 hrs.

We were interested in small vesper bats flying through the acoustic space of the sampling points. These bats exhibit slow and high-maneuverability flight determined by wing morphology, and high-frequency modulated echolocation pulses of short-range easily attenuated in open areas (Frey-Ehrenbold *et al.* 2013; Bader *et al.* 2015), though facing similar restrictions. Some of the species contained in this group of bats can be acoustically cryptic. Identification of those with similar body sizes can be challenging due to the high similarity of the echolocation pulse's structure, and acoustic parameters overlap (see Jung and Kalko 2011; Williams-Guillén and Perfecto 2011; Estrada-Villegas *et al.* 2012). Therefore, we pooled recordings to avoid misclassifications.

The small vesper bats potentially occurring in sympatry in our study area are *Rhogeessa tumida*, *M. nigricans*, and *M. keaysi pilosatibialis* (Barquez and Diaz 2016; Miller *et al.* 2016; Solari 2019). They belong to the same ecomorphotype and foraging type (Fenton and Bogdanowicz 2002; Segura-Trujillo *et al.* 2018), classified as aerial/trawling insectivores that hunt in background clutter space such as forest edges and gaps (Schnitzler and Kalko 2001; Frey-Ehrenbold *et al.* 2013). Accordingly, we selected echolocation pulses start-

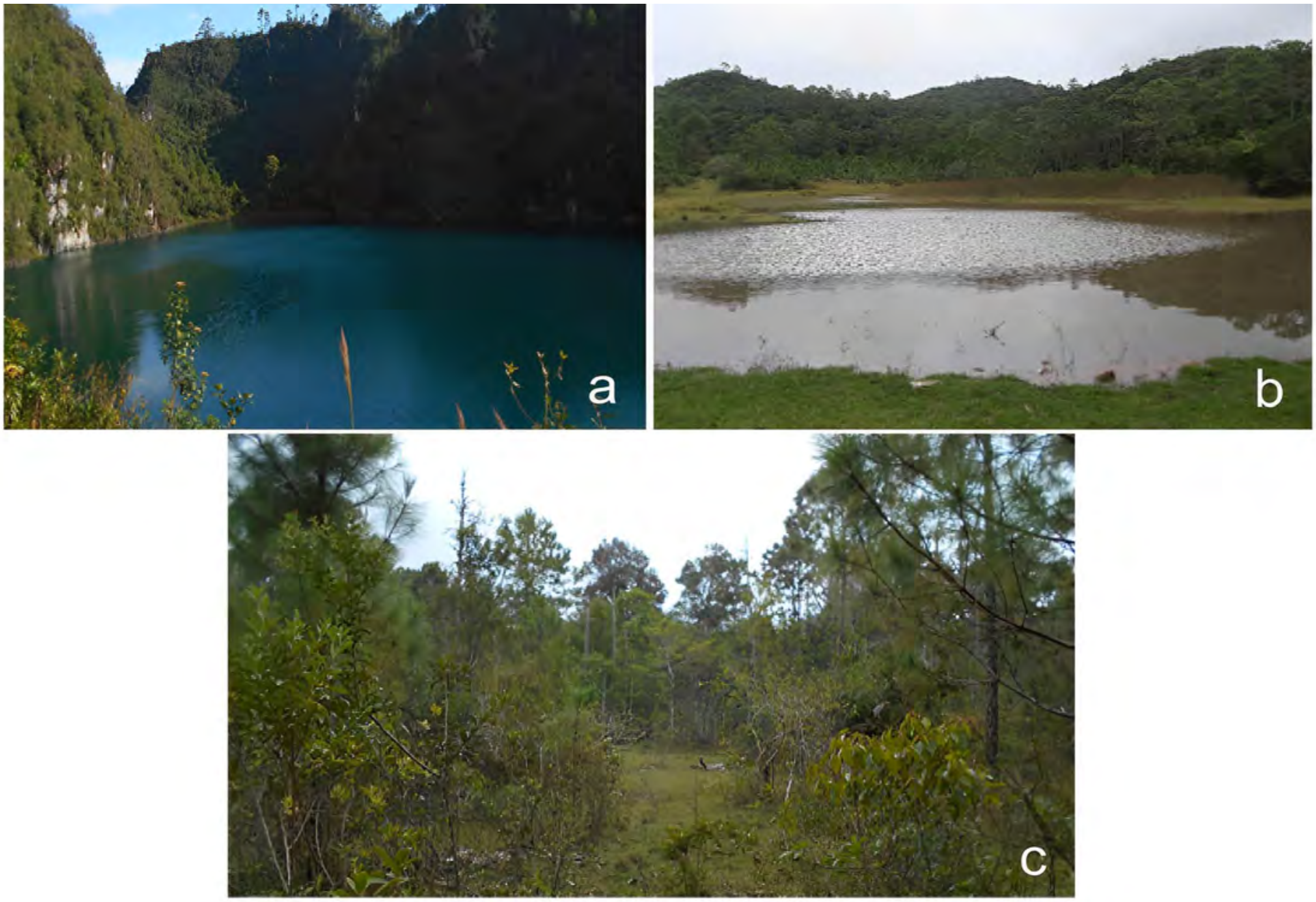

**Figure 2.** Sampling points recognizable by physiognomy in the study area in Chiapas, México: a) water sinkholes, b) flooded surfaces, and c) forested locations.

ing from a final frequency of 45 kHz. Jung and Kalko (2011) and Estrada-Villegas *et al.* (2012) indicate the possibility of erroneously classifying *Rhogeessa tumida* as *M. albescens* or *M. nigricans*. We can also find the distribution limits of *M. albescens* and *M. fortidens* (see www.iucnredlist.org), but they are more associated with lowlands, so we discard them. Other *Myotis* species, such as *M. velifer* and *M. californicus*, produce lower final frequencies (see Orozco-Lugo *et al.* 2013; Zamora-Gutiérrez *et al.* 2016). Finally, *Myotis elegans* emits frequencies above 60 kHz (O'Farrell and Miller 1999), but it was not detected.

For recording processing, in the Bat Analysis Mode, we configured automatic classification using Auto ID for Bats-Bats of the Neotropics (México) with a neutral level of sensitivity, as we were interested in more identifications and not highly accurate ones (see User Guide). We automatically filtered noise files. We processed the recordings in Kaleidoscope Pro v. 5 (Wildlife Acoustics, Inc.). With Kaleidoscope Viewer, we displayed and verified the resulting recordings because automatic identification may produce false positives (see Auto ID for Bats; Rydell *et al.* 2017).

For the calculation of relative activity (a surrogate of bat abundance; Froidevaux *et al.* 2021), we considered the activity index proposed by Miller (2001). The latter index is based on the total 1-min blocks with evidence of echolocation pulses during constant periods; in our case, for a total of 240 min (4 hrs), otherwise standardized to the total minutes sampled. We considered evidence of at least one recording with a minimum of two consecutive echolocation pulses (MacSwiney *et al.* 2009; Heim *et al.* 2015). We expressed the calculations as percentages. For comparisons, we calculated the mean percentage of relative activity at each sampling point for six events.

*Environmental conditions and insects' biomass analysis.* We were interested in differentiating sampling sites based on interacting abiotic and biotic variables. Therefore, we performed a stepwise discriminant analysis. We included the following variables: dipterans biomass, coleopterans biomass, amphiesmenopterans biomass, temperature, relative humidity, and wind speed. We based this analysis on Wilk's Lambda ($\lambda$), the *F* statistic's significance, and the independent contributions of variables in the model through Partial $\lambda$ and the *F-remove* statistic's significance. We performed these calculations in STATISTICA® version 8.0 (StatSoft, Inc.).

*Surrounding land covers amounts analysis.* Hierarchical partitioning (*hp*) is a multivariate exploratory analysis that explains variance in the response variable attributable to univariate correlations with each independent variable (Radford and Bennett 2007). We employed *hp* to measure the relative importance of surrounding land covers amounts (Chevan and Sutherland 1991; Mac Nally 2000). This analysis has the advantage of addressing potential multicollinearity (Olea *et al.* 2010).

We performed *hp* with routine hier.part included in package hier.part version 1.0-6 (Walsh and Mac Nally 2022) ran in R version 3.6.2 (R Core Team 2019). The response variable was the mean percentage of active 1-min blocks, and the independent variables were the percentage of each land cover. The variables were arcsine-transformed [*ASIN(SQRT(x/100)*], and we ran the models specifying a gaussian distribution, with the goodness of fit based on $R^2$. We tested the statistical significance by comparing randomization (1000 permutations) with routine rand.hp included in package hier.part version 1.0-6 (Mac Nally 2002; Walsh and Mac Nally 2022) ran in R version 3.6.2 (R Core Team 2019). We used the generated *Z*-scores to establish statistical significance based on the upper 95 % ($Z \geq 1.65$) confidence limit (Walsh and Mac Nally 2022). The *hp* does not indicate the direction of paired associations, which we determined by non-parametric correlations.

## Results

*Sampling sites.* We provide descriptive statistics of environmental conditions, insect collections, and single-variable statistical comparisons between sampling sites (Supplementary materials 2, 3, and 4). The stepwise procedure resulted in a statistically significant model, including only the wind speed, for the differentiation between sampling sites ($\lambda = 0.5259$, $F_{(2.24)} = 10.8143$, $P = 0.0004$), which exhibited the lowest mean values in water sinkholes in all measurements. We include the calculations for the variables not in the model in Supplementary material 5. The stepwise procedure excluded insect collections. However, we observed a comparatively higher percentage of dipterans in water sinkholes in the samples (Supplementary material 3).

*Relative activity of bats.* We include the calculations of relative activity in the sampling sites in Supplementary material 6. The difference was statistically significant between sampling sites (*K-W* = 28.78, $P = 5.638E\text{-}7$; Figure 3), specifically between water sinkholes and flooded surfaces (Dunn post-test, mean rank difference 14.750, $P = 0.002$), with higher mean value in water sinkholes; between water sinkholes and forested locations (Dunn post-test, mean rank difference 27.250, $P = 5.228E\text{-}6$), with higher mean value in water sinkholes; between flooded surfaces and forested locations (Dunn post-test, mean rank difference 12.500, $P = 0.004$), with higher mean value in flooded surfaces.

*Surrounding land covers amounts.* Invariably, we observed associations in a positive direction with forest cover, secondary forest, and water surface. We observed associations in a negative direction with agricultural land and human development, except for the latter class in the 500-m buffer (9 % of the variance), where a small surface characterized it. In the 500-m buffer, we established that the relative activity is mainly determined by forest cover and secondary forest, explaining 25 % and 26 % of the variance (respectively), and agricultural land explaining 34.7 % of the variance (Figure 4). In the 1,000-m buffer, we established that the relative activity is mainly determined by forest cover and secondary forest, explaining 36.4 % and 20.6 % of the variance (respectively), and agricultural land explaining 23.8 % of the variance (Figure 4). In the 1,500-m buffer, we established that

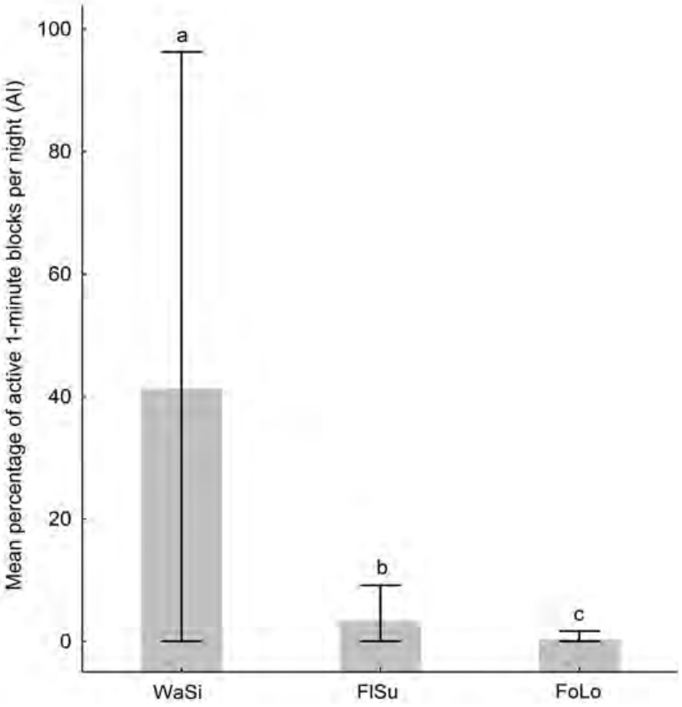

**Figure 3.** Mean percentage of 1-min blocks per night with acoustic evidence of small vesper bats. The whiskers indicate minimum-maximum values. The different letters indicate statistically significant differences with a 95 % confidence level. WaSi = water sinkholes, FlSu = flooded surfaces, FoLo = forested locations.

the relative activity is mainly determined by forest cover and water surface, explaining 20.3 % and 42.9 % of the variance (respectively), and agricultural land explaining 28.7 % of the variance (Figure 4). We did not find statistically significant associations based on the upper 95 % confidence limit (Supplementary material 7).

## Discussion

In the study area, we could differentiate water sinkholes, flooded surfaces, and forested locations based on the wind speed, with the lowest mean values in water sinkholes in all measurements. Although the insect collections did not stand out in the model, we observed a comparatively higher percentage of dipterans in water sinkholes in the samples. Some dipterans are strongly associated with calming water because their pupae and larvae are susceptible to increasing wind speed (Gillies and Wilkes 1981; Rutledge 2008). The characteristics of water sinkholes, specifically the surface several meters below the ground, can be associated with environmental conditions' buffering beneficial for this kind of insect.

Many *Myotis* species consume small and soft prey, specifically the aerial ecomorphotypes such as *M. nigricans* and *M. pilosatibialis*, which exhibit short wavelength echolocation suitable for these targets (Gonsalves *et al.* 2013), and biomechanical limitations (bite strength) correlated to body size (Segura-Trujillo *et al.* 2018). Their diet can be composed of several orders, including dipterans (Aguiar and Antonini 2008; Gamboa Alurralde and Díaz 2019; Ingala *et al.* 2021). However, bats eat a wide range of insects, most representatives seem to have flexible diets (Jones and Rydell 2003), and there is evidence that suggests that the diet responds to local fluctuations, as well as the abundance and type of prey (Salinas-Ramos *et al.* 2015).

In water sinkholes, the small vesper bats exhibited a comparatively high relative activity in two of the three sampling sites and in most of the recording events, reaching up to 95 % of the time, specifically in AZAR (Supplementary material 6), and on average, it was significantly higher compared to flooded surfaces and forested locations. We also obtained a high rate of buzzes, which we determined by the output of the detector and subsequently by visual inspection of the recordings; these might represent drinking and/or feeding buzzes, but a detailed analysis distinguishing them based on their structure is necessary to make adequate interpretations (see Russo *et al.* 2015). The physiological characteristics of bats demand high amounts of water and prey for reproductive success (MacSwiney *et al.* 2009; Seibold *et al.* 2013; López-González *et al.* 2016), and there is presence of at least one maternity colony inside the natural protected area, relate to our observations.

Invariably, we observed associations in a positive direction with forest cover and secondary forest. The latter associations could reflect feeding and commuting opportunities. Other studies report similar results, specifically positive correlations between the activity and abundance of this kind of bats and forest cover (native and planted forests) and higher activity in locations at a smaller distance to

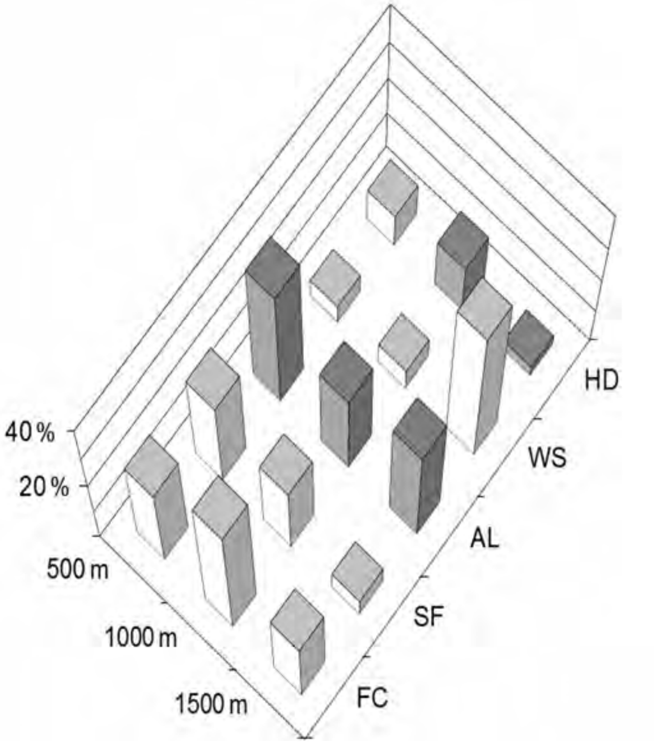

**Figure 4.** Hierarchical partitioning analysis showing the small vesper bats' activity variance attributable to univariate correlations with each independent variable, expressed as percentages, in concentric buffers of 500-m, 1,000-m, and 1,500-m radii starting from the sampling points (centroid). The white bars indicate non-parametric positive correlations, and the gray bars negative correlations. FC = forest cover, SF = secondary forest, AL = agricultural land, WS = water surface, HD = human development.

forested areas (Heim *et al.* 2015; Rodríguez-San Pedro and Simonetti 2015; Fuentes-Montemayor *et al.* 2017; Put *et al.* 2019; Laurindo *et al.* 2020; Falcão *et al.* 2021).

Fuentes-Montemayor *et al.* (2013) make note that the use of forests by aerial insectivorous bats can also be influenced by attributes such as tree density, among other forest structure variables, conditions that Rauchenstein *et al.* (2022) define as the "suitable foraging habitat". Vegetation clutter (*e. g.*, forest canopy openness or increased shrub cover) can influence prey abundance (Froidevaux *et al.* 2021; Rauchenstein *et al.* 2022), restrict mobility and flight maneuverability (Estrada-Villegas *et al.* 2012; Fuentes-Montemayor *et al.* 2013), and forest maturity (*e. g.*, tree sizes) can be a limiting factor for tree roosting bats (Novella-Fernandez *et al.* 2022).

We also observed an association in a positive direction with water surfaces, represented in the study area by lakes, water sinkholes and flooded surfaces. The latter elements could represent stepping-stones for many taxa in agriculture-dominated landscapes (Hunter Jr. *et al.* 2017). Even for highly mobile organisms such as bats, a dense network of connecting elements might be beneficial and promote activity in open areas (Heim *et al.* 2015). In the UK, Fuentes-Montemayor *et al.* (2013; 2017) found positive correlations between the abundance of *Myotis* species to a larger proportion of surrounding water and decreasing distance between water bodies. In the Atlantic Forest of Brazil, Laurindo *et al.* (2020) found that the number of captures of insectivorous bats, including *Myotis* species, was significantly associated with the area covered by water bodies within highly fragmented agricultural landscapes.

We observed associations in a negative direction with agricultural land and human development, except for the latter class in the 500-m buffer, where a small surface characterized it. The latter associations could reflect the effect of an increasingly open area and unfavorable conditions for bats. For instance, Fuentes-Montemayor *et al.* (2017) found higher activity of *Myotis* species in forests immersed in areas with a smaller proportion of urban areas. On the other hand, Laurindo *et al.* (2020) found a negative correlation between the number of captures of insectivorous bats with increased agricultural area.

We explain the associations of relative activity to landscape covers amounts by natural history traits, particularly wing morphology, which is a surrogate for mobility, and echolocation system, which is a surrogate for perceptual range (Frey-Ehrenbold *et al.* 2013; Bader *et al.* 2015; Heim *et al.* 2015; Fuentes-Montemayor *et al.* 2017). The morphological and echolocation system traits of sympatric small vesper bats in the study area point to slow flight with more energetic cost in open areas and short-range high-frequency pulses that are more attenuated in open areas (Frey-Ehrenbold *et al.* 2013; Bader *et al.* 2015; Heim *et al.* 2015). However, Fuentes-Montemayor *et al.* (2017) suggest that low-mobility species are more influenced by local conditions and the landscape becomes more important for high-mobility species, which perceive the environment at a coarser scale. The latter argument could support our observations, specifically the strong local-level response of small vesper bats to the presence of water sinkholes and the lack of significance of the associations at the landscape level.

*Final remarks.* The presence of water sinkholes and environmental conditions such as low wind speed possibly regulating prey availability are important drivers of small vesper bats' activity in the study area. We found no significant associations for flooded surfaces and forested locations. However, flooded surfaces are particularly relevant to other groups of bats (*e. g.*, Mormoopidae) and vertebrate fauna in the influence zone, such as anurans and migratory birds, representing a seasonally limiting resource.

We observed trends of positive correlation between relative activity to forest cover, secondary forest, and water surface and negative correlation to agricultural land and human development. Although we found no significant associations, we provide explanations based on the argument that small vesper bats react negatively to forest loss and increasingly open area, which we explain by wing morphology and echolocation system traits. Other authors suggest that, for low-mobility species, the landscape may become less important than local-level attributes.

Some of the water sinkholes analyzed in the study area meet the criteria of *small natural features* having ecological importance that is disproportionate to their size (Hunter Jr. 2017; Hunter Jr. *et al.* 2017), as we determined for small vesper bats. The latter consideration is valuable for complementing large-scale conservation through targeted actions and should be further evaluated for its implementation in the area. The water sinkholes are located inside and right on the limits of the natural protected area, in the jurisdiction of federal authorities. The flooded surfaces and forested locations studied are communal goods in the influence zone exposed to human activities and their effects, such as pollution derived of the use of pesticides in the agricultural zones nearby, water exploitation for crops irrigation and cattle drinking supply, forest exploitation with consequent degradation and deforestation.

Finally, we point out that the adequate management of these types of landscape elements should be prioritized in the conservation agenda of the area in an effort of a consensus with *ejidatarios* (local authorities and *ejido* members). There is a need for a dialogue that should follow the socio-ecology premises, specifically a transdisciplinary approach to solving environmental problems, looking to benefit biodiversity, the continuity of ecosystem services, and human well-being.

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## Supplementary material

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# Revised checklist and conservation status of the mammals of Costa Rica

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Mammal diversity in Costa Rica is considerably high given the size of the country (51,100 km$^2$), and has increased in recent years. Taxonomic changes together with distribution extension records have contributed to such an increase. Here we present the revised and updated list of mammals confirmed for Costa Rica based on previous lists and recent additions obtained from literature, with notes on endemism and conservation status. This updated list was based on Rodríguez-Herrera *et al.* (2014a) list, compared and matched with the most updated taxonomic review. A total of 256 mammals are now confirmed for Costa Rica, with the order Chiroptera and Rodentia as the most representative. We report 30 endemics for Costa Rica, including those species whose distribution is limited to the country and one of its two neighboring countries, from which 21 are rodents. Compilation on conservation status information reveals 29.6 % of species within the list classified as threatened, either by Costa Rican or international environmental authorities. Increase in new studies on mammals all around the world is leading to the discovery of new species. While systematic and phylogenetic revisions is revealing new taxonomic relationships, and cryptic species. Mainly on highly diverse and taxonomically challenging groups, as bats, rodents, and shrews, as we evidence here. Several threatened and endemic species occur in Costa Rica, where the greatest endemism area is the high elevations, and most endemic species are mice. The creation and establishment of protected areas in a large part of the Costa Rican territory has favored the prevalence of a diverse mammalian assemblage.

La diversidad de mamíferos en Costa Rica es considerablemente alta a pesar del tamaño del país (51,100 km$^2$), y ha aumentado en los últimos años. Los cambios taxonómicos junto a registros de ampliaciones de distribución han contribuido a este aumento. Aquí presentamos la lista revisada y actualizada de mamíferos de Costa Rica, basada en listas anteriores y una revisión bibliográfica, con notas sobre el endemismo y el estado de conservación. Esta lista actualizada se basó en la lista de Rodíguez-Herrera *et al*. (2014a), comparada y cotejada con la revisión taxómica más actualizada. Confirmamos un total de 256 especies de mamíferos para Costa Rica, siendo los órdenes Chiroptera y Rodentia los más representativos. Reportamos 30 endémicas para Costa Rica, incluyendo aquellas especies cuya distribución se limita al país y alguno de sus dos países vecinos, de las cuales 21 son roedores. La compilación del estado de conservación revela que el 29.6 % de las especies en la lista están clasificadas bajo alguna categoría de amenaza, ya sea por las autoridades ambientales locales o internacionales. El aumento en nuevos estudios sobre mamíferos en todo el mundo está llevando al descubrimiento de nuevas especies. Mientras que las revisiones sistemáticas y filogenéticas están revelando nuevas relaciones taxonómicas y especies crípticas. Principalmente en grupos muy diversos y taxonómicamente desafiantes, como murciélagos, roedores y musarañas, como evidenciamos aquí. Varias especies con poblaciones amenazadas y endémicas ocurren en Costa Rica, donde la zona de mayor endemismo son las tierras altas, y la mayoría de las especies endémicas son ratones. La creación y el establecimiento de áreas protegidas en gran parte del territorio costarricense ha favorecido la prevalencia de un conjunto diverso de mamíferos.

***Keywords:*** Central America; conservation status; Costa Rica endemic species; new records; taxonomic changes.

## Introduction

Ecosystems around the world are losing biodiversity at an accelerated rate as a result of habitat loss, overexploitation, introduction of invasive species, and climate change (Naeem *et al.* 2012; Johnson *et al.* 2017; Ceballos *et al.* 2020). This loss of biodiversity has negative repercussions on ecological processes and the services they provide to human populations (Brodie *et al.* 2021). For example, the loss of primate species can have detrimental effects on seed dispersal and consequently on forest regeneration and tree community structure (Gardner *et al.* 2019). Knowing the diversity of mammal species in a given area can not only help to identify the impact of anthropogenic activities on it and its natural habitats (Rocha *et al.* 2014; González-Maya *et al.* 2016; Brodie *et al.* 2021), but also contributes to the creation of baseline studies on different biological aspects (*e. g.*, biogeography, ecology), environmental education, disease prevention or control, and more. From this arises the importance and need to generate updated lists of mammal species at any geographical level, but especially in high diversity areas.

Costa Rica, located in Central America, is a small country with a land area of approximately 51,100 km$^2$, yet it is among the 20 most diverse countries in the world (Obando 2002; Kappelle 2016). Previous work has described a high richness and functional diversity of mammals (González-Maya *et al.* 2015; 2016) that results from its geographical location and geological history (Janzen 1991), and a complex biogeographic history as land bridge and barrier for different groups of mammals that originated in the north and south of the American continent, giving rise to a species composition with origins in both hemispheres (Carrillo *et al.* 2010; Rodríguez-Herrera *et al.* 2014a; Wilson *et al.* 2014; González-Maya *et al.* 2016).

Since Rodríguez-Herrera *et al.* (2014a) mammal's list, where 249 species were reported for the country, there have been changes in the number of species and their taxonomy. This is remarkable, given that information about mammals in the country has been collected for more than 150 years (Rodríguez-Herrera *et al.* 2005, 2014b). The ongoing effort to document the mammalian community of Costa Rica has resulted in several updates to the list (*e. g.*, Wilson 1983; Rodríguez and Chinchilla 1996; Rodríguez-Herrera *et al.* 2002; Rodríguez-Herrera *et al.* 2014a), such that it reflects current taxonomic and systematic classifications (*e. g.*, Pérez Consuegra and Vázquez-Domínguez 2015; Lim *et al.* 2020), accounts for new records and species descriptions (*e. g* Woodman and Timm 2017; Salas-Solano *et al.* 2020; Villalobos-Chaves *et al.* 2018), and changes in distribution range extensions (*e .g.*, González-Maya *et al.* 2017; Ramírez-Fernández *et al.* 2020). The continued addition of mammal species to the list over the years reflects increased efforts on biological research in the country. This study presents an updated list of the mammals of Costa Rica, including notes on their global and local conservation status.

## Materials and methods

This updated list of the mammals of Costa Rica was based on the list of Rodríguez-Herrera *et al.* (2014a), compared and matched with the most updated taxonomic review made for the class Mammalia in the Mammal Diversity Database (MDD 2022), from where the original sources were obtained and consulted. Comments on species taxonomic changes and new additions to the list were provided according to a bibliographic review for the taxa differing between both lists. For changes in the total number of species and species by order, we reviewed previous species lists for the country (Frantzius 1869; Alfaro 1897; Harris 1943; Goodwin 1946; Wilson 1983; Rodríguez and Chinchilla 1996; Rodríguez-Herrera *et al.* 2002; Rodríguez-Herrera *et al.* 2014a). Information on the global conservation status of each species was compiled from the latest International Union for Conservation of Nature Red List assessment (IUCN 2022), and the national conservation status according to Costa Rican environmental authorities (SINAC 2017). Common names, and the phylogenetic order of the different orders, families, subfamilies and tribes follows the most recent publication of the Handbook of the Mammals of the World series (Mittermeier *et al.* 2013; Wilson *et al.* 2016, 2017; Wilson and Mittermeier 2009, 2011, 2014, 2015, 2018, 2019), with genera and species listed in alphabetical order.

## Results

*Species and family richness.* In this study we report a total of 256 extant mammal species for Costa Rica, increasing the number of species by seven according to the last list published (Rodríguez-Herrera *et al.* 2014a; Figure 1). The order Chiroptera accounts for almost half (46 %) of the total number of species documented in Costa Rica with 118 species (9 families), followed by Rodentia (51 species, 8 families), Artiodactyla (31 species, 7 families) and Carnivora (25 species, 6 families; Figure 2). About 88 % of the species are primarily terrestrial environments and 12 % are fully adapted to an aquatic life. In terms of evolutionary distinctiveness (*i. e.*, a measurement of the uniqueness of a species evolutionary history), the updated list includes two artiodactyls *Physeter macrocephalus* and *Balaenoptera musculus*, the perissodactyl *Tapirus bairdii*, and the sirenian *Trichechus manatus* (May-Collado and Agnarsson 2011; Isaac *et al.* 2007).

*Endemism.* Within the list, we listed 30 mammal species that are endemics to some specific region within the geographic area ranging from southern Nicaragua to western Panama, as follows. A total of 20 species have a distribution that encompasses Costa Rica and Panama. These species include members of the orders Didelphimorphia (1 species), Lagomorpha (1), Rodentia (13), Primates (1), Eulipotyphla (2), and Chiroptera (2). The Nicaraguan woolly mouse opossum *Marmosa nicaraguae*, and two species of rodents *Reithrodontomys brevirostris* and *R. paradoxus* are distributed in northern Costa Rica and are shared only with Nicaragua. Seven species are exclusively found in Costa Rica, six rodents *Heterogeomys heterodus*, *Heteromys nubicolens*, *H. oresterus*, *Reithrodontomys cherrii*, *R. musseri*, *R. rodriguezi*, and one shrew *Cryptotis monteverdensis*. Among these species, the taxonomic group with the greatest endemism corresponds to the order Rodentia, with 21 species, followed by Eulipotyphla with three, and Chiroptera with two (Supplementary material).

*Conservation status.* According to the IUCN (IUCN 2022), eight species are reported as Endangered. These include the manatee *Trichechus manatus*, the tapir *Tapirus bairdii*, the primates *Aloautta palliata* and *Saimiri oerstedii*, the pinnipeds *Arctocephalus galapagoensis* and *Zalophus wollebaeki*, and the artiodactyls *Balaenoptera borealis*, and *B. musculus*. Six species are considered Vulnerable, including the giant anteater *Myrmecophaga tridactyla*, the primates *Ateles geoffroyi* and *Cebus imitator*, the oncilla *Leopardus tigrinus*, the white-lipped peccary *Tayassu pecari*, and the

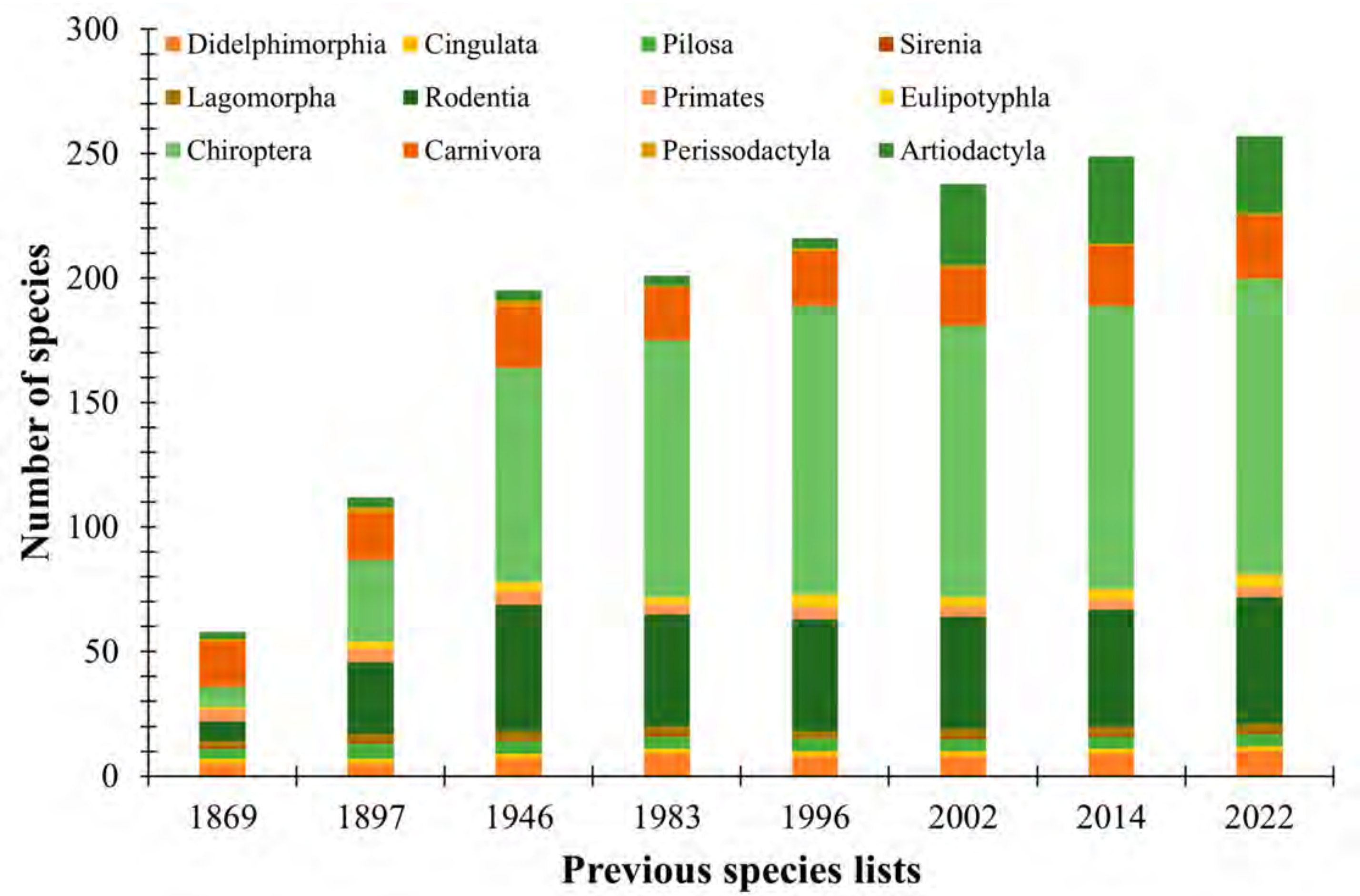

**Figure 1**. Changes in the number of mammal species from previous species lists for Costa Rica ranging from 1869 to date; orders taxonomic classification follows this work. References: Frantzius (1869), Alfaro (1897), Goodwin (1946), Wilson (1983), Rodríguez and Chinchilla (1996), Rodríguez-Herrera *et al.* (2002), Rodríguez-Herrera *et al.* (2014a).

endemic lagomorph species *Sylvilagus dicei*; nine species are listed as Near Threatened, and 11 as Data Deficient, with six endemics among them. Nationally, according to environmental authorities (SINAC 2017), 21 Costa Rican mammal species are endangered with extinction (Supplementary material). These include all six species of felids, three out of four monkey species, and the endemic rodents *Reithrodontomys musseri* and *Rheomys raptor*. In addition, a total of 42 species, including nine endemics, have seen their populations reduced or threatened (Supplementary material).

*Changes in distribution.* In terms of the distribution of localities of the recently recorded species *Cryptotis monteverdensis*, *Ichthyomys tweedii*, *Cynomops greenhalli*, *Diplomys labilis*, *Micronycteris tresamici*, *Molossus alvarezi*, *Mormoops megalophylla*, *Myotis armiensis*, *Nyctinomops laticaudatus*, and *Speothos venaticus* there is no clear pattern. Most of the new species are the result of taxonomic changes, although some indicate range expansion towards both borders of the country, with most of the new ones occurring in lowlands (Figure 3).

*Taxonomic changes.* Several taxonomic changes have occurred in the last decade at various taxonomic levels. These include:

### Orders

Eulipotyphla. Based on phylogenetic analysis using molecular data (Douady *et al.* 2002), and following the classification proposed by Wilson and Mittermeier (2018), we acknowledge the inclusion of the former orders Soricomorpha (shrews) and Erinaceomorpha (hedgehogs) in the widely accepted order Eulipotyphla.

Artiodactyla. Following the International Code of Zoological Nomenclature (Asher and Helgen 2010), and according to the most recent morphological and molecular phylogenetic analyses (*e. g.*, Agnarsson and May-Collado 2008; Hassanin *et al.* 2012; Gatesy *et al.* 2013, 2017), we recognized Cetacea as an infraorder of the order Artiodactyla, along with their respective parvorders and families.

### Families

Chlamyphoridae. Molecular phylogenetic analysis including fossils and extant species supports the division of the traditional family Dasypodidae into the families Chlamyphoridae and Dasypodidae (Delsuc *et al.* 2016; Gibb *et al.* 2016). Dasypodidae is currently restricted to the genus *Dasypus*, while all other modern armadillos, including *Cabassous*, and the extinct glyptodonts are grouped in Chlamyphoridae.

Choloepodidae. Two-toed sloths of the genus *Choloepus* are now placed in a new family, Choloepodidae, instead of the family Megalonychidae. This new placement is based on phylogenetic analysis of DNA data from fossils and extant species (Delsuc *et al.* 2019; Presslee *et al.* 2019).

### Subfamilies

Former Phyllostominae. We follow the proposal of Baker *et al.* (2016), and Cirranelo *et al.* (2016), who recognized 11 subfamilies and 12 tribes within the family Phyllostomidae, adding the following subfamilies to the list: Glyphonycterinae, Lonchophyllinae, Lonchorhininae, and Micronycterinae.

### Genera

*Heterogeomys*. Following the latest systematic revision of the genus *Orthogeomys* (Spradling *et al.* 2016), we recognize the genus *Heterogeomys* for the different species of the family Geomyidae occurring in Costa Rica. The genus *Orthogeomys* is restricted to the species *O. grandis*, distributed from México to Honduras.

*Heteromys*. Phylogenetic analysis based on molecular data of the family Heteromyidae shown that the genus *Liomys* is paraphyletic with respect to *Heteromys* (Hafner *et al.* 2007). Therefore, the formal taxonomy of the group suggests a synonymy between the two genera, and the name *Heteromys salvini* is accepted.

*Coendou.* According to the latest research in systematics and phylogeny of the family Erethizontidae (Voss 2011; Voss *et al.* 2013; Menezes *et al.* 2021), the correct and most commonly used genus is *Coendou* and not *Sphiggurus*, for *Coendou mexicanus*.

*Gardnerycteris.* According to phylogenetic analysis, the new genus *Gardnerycteris* is recognized for the former members of *Mimon* under the taxon "*Anthorhina*" (represented by *M. crenulatum* and *M. koepckeae*; Hurtado and Pacheco 2014). Thus, *Gardnerycteris crenulatum* is accepted as a valid species.

*Artibeus*. In accordance with the most recent morphological and molecular phylogenetic analysis (Baker *et al.* 2016; Cirranelo *et al.* 2016; Cirranelo and Simmons 2020), the genus *Artibeus* is used for the forms formerly assigned to the genus *Dermanura*.

*Dasypterus.* We follow the proposal of Baird *et al.* (2015, 2021) of three separate genera within the tribe Lasiurini: *Lasiurus* (red bats), *Dasypterus* (yellow bats), and *Aeorestes* (hoary bats). Thus, we recognize de genus *Dasypterus* for the yellow bats *L. ega* and *L. intermedius*.

*Herpailurus*. The species *Puma yagouaroundi* was reassigned to the genus *Herpailurus* according to morphometric analyses by Segura *et al.* (2013). Although some authors

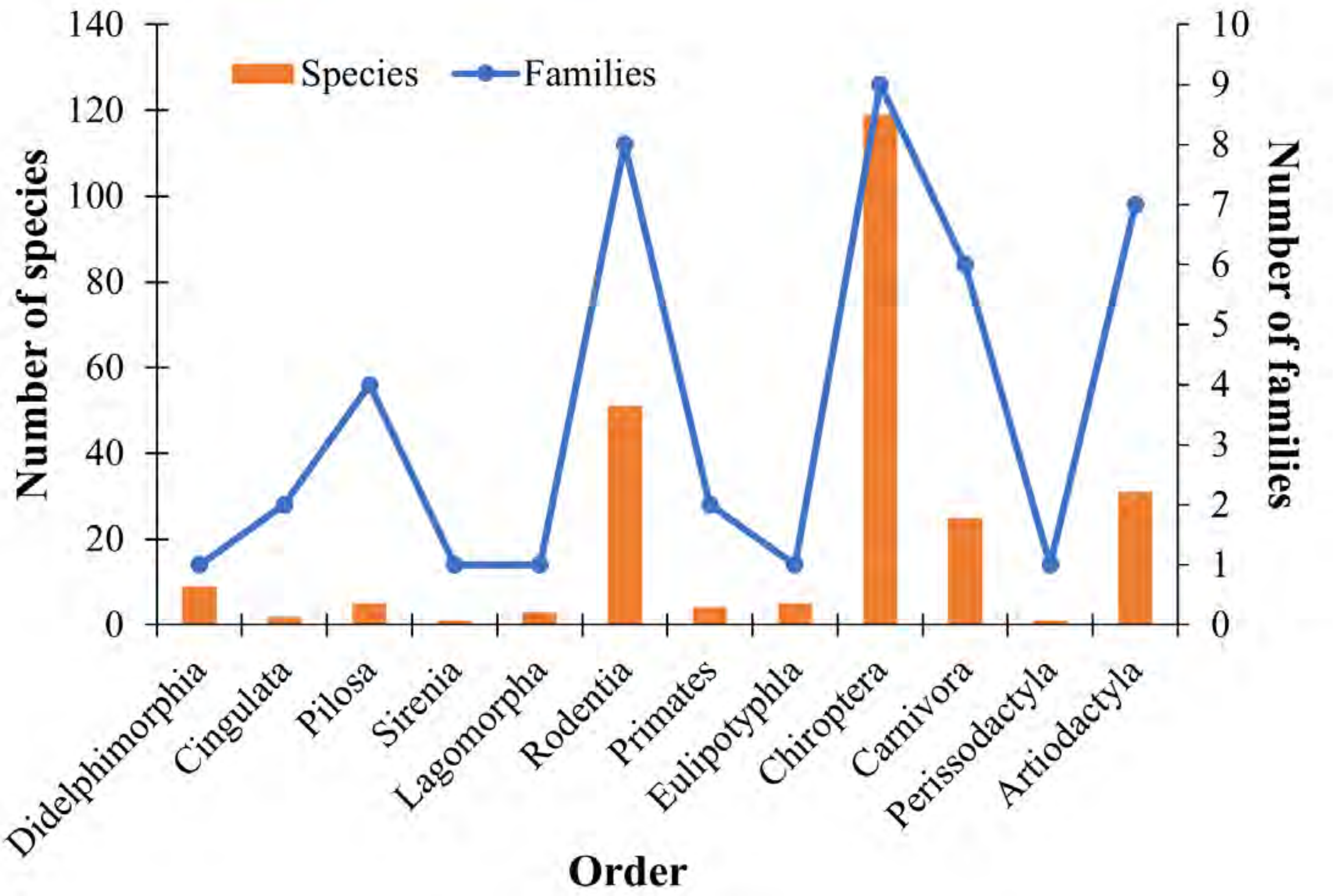

**Figure 2**. Distribution of the number of mammal species and families according to orders in Costa Rica.

still use the genus *Puma* for the species (*e. g.*, Li *et al.* 2016; Tamazian *et al.* 2021), we follow the designation from the Cat Classification Task Force of the IUCN Cat Specialist Group (Kitchener *et al.* 2017).

*Neogale*. According to the latest review of the phylogeny and nomenclature of the genus *Mustela*, *M. frenata* should now be recognized in the genus *Neogale* (Patterson *et al.* 2021).

*Dicotyles*. In accordance with the International Code of Zoological Nomenclature the genus *Dicotyles* is accepted as valid, and the former genus *Pecari* becomes a junior synonym of it (Acosta *et al.* 2020).

### Species

*Marmosa nicaraguae*. According to the latest phylogenetic revision using molecular data of the didelphid marsupial genus *Marmosa*, *M. nicaraguae* was separated and recognized as a valid species from *M. alstoni* (Voss *et al.* 2021). Therefore, both species deserve an endemic status, *M. nicaraguae* with a distribution limited to Nicaragua and Costa Rica, and *M. alstoni* distributed in Costa Rica and Panama (Voss *et al.* 2021; Carter 2022).

*Philander melanurus*. This species was splitted from the *Philander opossum* species complex and validated as a species after phylogenetic and morphological analysis (Voss et al. 2018).

*Cyclopes dorsalis*. Based on phylogenetic analyses using molecular data, coalescent analyses of species delimitation, cranial diagnostic characters, and patterns of coloration and fur structure, we accept the proposal of Miranda *et al.* (2018) to separate the species *Cyclopes didactylus* into seven equally valid species. Therefore, the species recognized for Costa Rica, with a Mesoamerican distribution, corresponds to *C. dorsalis*, while *C. didactylus* is restricted to the northeastern region of South America.

*Cryptotis orophilus*. Following the International Commission on Zoological Nomenclature (2006) the specific epithet changes from *orophila* to *orophilus* because *Cryptotis* gender is masculine.

*Peromyscus nicaraguae*. This species was separated from the *P. mexicanus* species complex and revalidated as a species by Pérez Consuegra and Vázquez-Domínguez (2015). The distribution reported by the authors includes only Honduras and Nicaragua, however two subspecies of *P. nudipes*, *hesperus* and *orientalis*, are included as synonyms of *P. nicaraguae* (Bradley *et al.* 2016), which have been reported in the past for the northern and central mountain

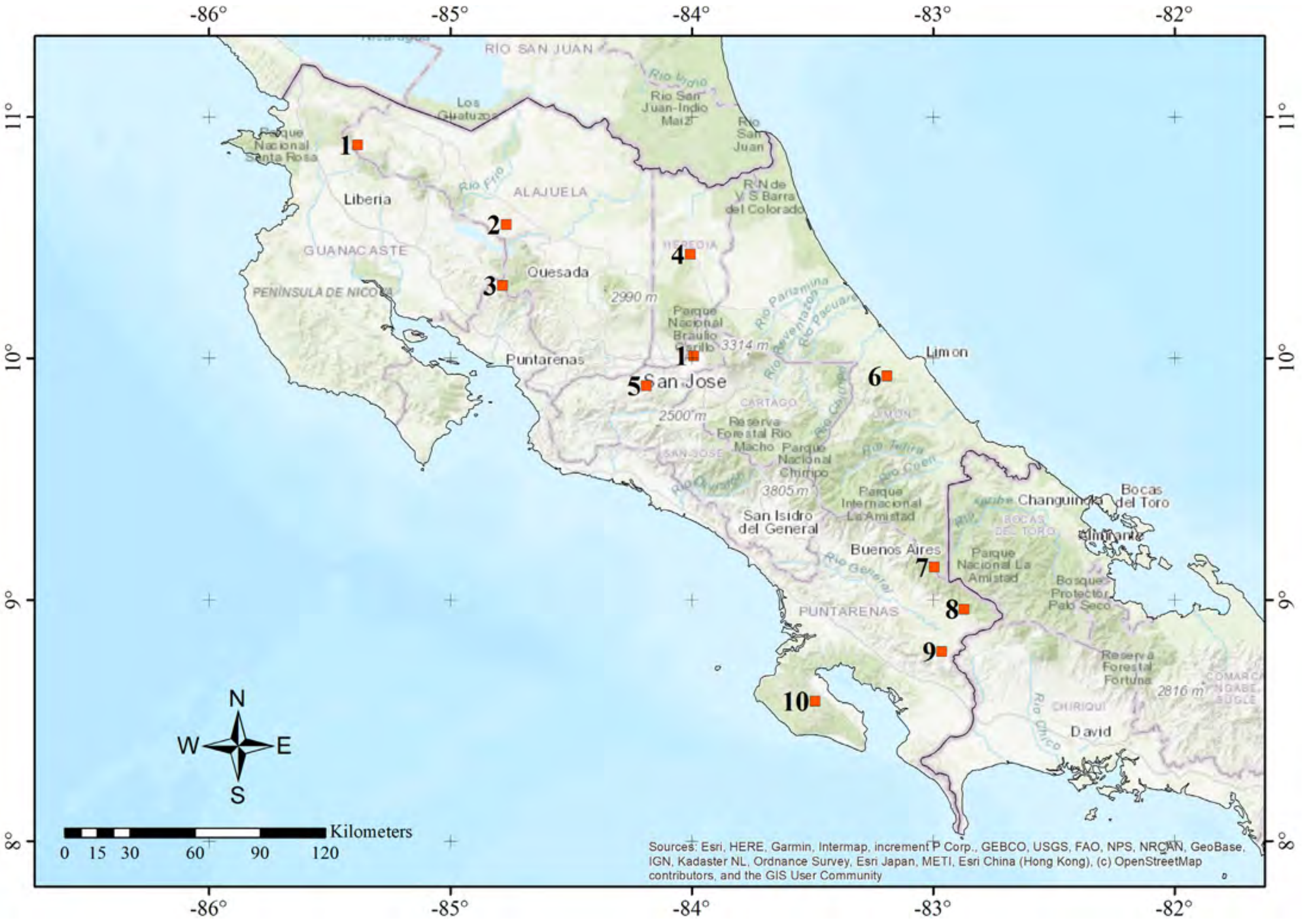

**Figure 3.** Map showing localities of recorded new mammal species for Costa Rica since Rodríguez-Herrera *et al.* (2014) list: 1, *Micronycteris tresamici*; 2, *Mormoops megalophylla*; 3, *Cryptotis monteverdensis*; 4, *Molossus alvarezi*; 5, *Nyctinomops laticaudatus*; 6, *Cynomops greenhalli*; 7, *Myotis armiensis*; 8, *Speothos venaticus*; 9, *Ichthyomys tweedii*; 10, *Diplomys labilis*.

ranges of Costa Rica respectively, confirming its presence in the country.

*Peromyscus nudipes*. Mitochondrial cytochrome-*b* gene data revealed that the *Peromyscus mexicanus* species complex has a polyphyletic origin (Pérez Consuegra and Vázquez-Domínguez 2015). Therefore, it was decided to elevate *P. nudipes* to specific level, due to its monophyletic origin (Bradley *et al.* 2016). This species has a restricted distribution to the highlands of the Talamanca Mountain Range in Costa Rica and Panama.

*Reithrodontomys cherrii*. According to molecular (Arellano *et al.* 2005), and morphological data (Gardner and Carleton 2009), *R. cherrii* was separated from *R. mexicanus* and is recognized as a valid species. Recently, this condition was also reinforced with ecological data (Martínez-Borrego *et al.* 2022). *R. cherri* is an endemic species and its distribution is restricted to the highlands of central Costa Rica.

*Reithrodontomys garichensis*. This species was recognized as such and separated from the *R. mexicanus* species complex based on morphological data (Gardner and Carleton 2009). *R. garichensis* is distributed in the Talamanca Mountain Range in Costa Rica and Panama.

*Melanomys chrysomelas*. Using mitochondrial DNA nucleotide sequences of the cytochrome-*b* gene, phylogenetic relationships were inferred in *Melanomys caliginosus* (Hanson and Bradley 2008). This study included samples from populations in Nicaragua, Costa Rica, Panama, Venezuela and Ecuador, resulting in the elevation of these four groups to a specific level. Thus, the accepted species for Costa Rica is *M. chrysomelas*, and is distributed in Honduras, Nicaragua and Costa Rica (Hanson and Bradley 2008; Wilson *et al.* 2017).

*Oligoryzomys costaricensis*. This species was separated from the *Oligoryzomys fulvescens* species complex according to Bayesian analyses of the mitochondrial cytochrome-*b* gene (Hanson *et al.* 2011). According to this study the form of this species complex accepted for Nicaragua, Costa Rica and Panama is *O. costaricensis*.

*Gardnerycteris keenani*. Based on genetic analyses and morphological comparisons, it was proposed to elevate the subspecies *Gardnerycteris crenulatum keenani* to specific level (Hurtado and D'Elía 2018).

*Diaemus youngii*. The correct original spelling of the species epithet is *youngii* and not *youngi* (Kwon and Gardner 2008).

*Lophostoma silvicola*. The specific epithet changes from *silvicolum* to *silvicola* because it is an invariable noun (MDD 2022).

*Tonatia bakeri*. Based on descriptions and morphometric analyses, and a phylogenetic reconstruction, the three recognized subspecies of *T. saurophila* were recognized and elevated to species level (Basantes *et al.* 2020). *Tonatia bakeri* is distributed from southeastern México to northern South America (Wilson and Mittermeier 2019).

*Glossophaga mutica*. Based on morphometric analyses with cranial characteristics and their relationship with environmental variables, the four subspecies of *Glossophaga soricina* were elevated to specific level (Calahorra-Oliart *et al.* 2021). The distribution of *G. mutica* includes Central America.

*Artibeus aztecus* and *A. toltecus*. It is appropriate to use the specific epithets *aztecus* and *toltecus* instead of *azteca* and *tolteca* because *Artibeus* gender is masculine.

*Chiroderma gorgasi*. According to the latest phylogenetic revision of the genus *Chiroderma* (Lim *et al.* 2020), based on morphological and molecular analyses, the trans-Andean populations of *C. trinitatum* should be elevated to specific level, adopting the name *C. gorgasi*.

*Uroderma convexum*. The trans-Andean subspecies of *U. bilobatum* was elevated to specific level according to a review of the genus *Uroderma*, through analyses of morphological, karyotypic, and molecular variation, and taxonomic affinities between geographic variants (Mantilla-Meluk 2014).

*Eumops ferox*. Based on morphological and genetic analyses, the *Eumops glaucinus* species complex was separated into four entities (McDonough *et al.* 2008). The species *E. ferox* was defined for the Caribbean region, México and Central America, while *E. glaucinus* has a South American distribution.

*Molossus nigricans*. Based on the most recent phylogenetic analysis of the genus *Molossus* (Loureiro *et al.* 2020), *M. nigricans* is revalidated and recognized as a distinct species from *M. rufus*. The former has a Mesoamerican distribution, and the latter is restricted to South America.

*Lasiurus frantzii*. According to a molecular systematic review of the tribe Lasiurini, *L. frantzii* was recognized as a distinct species from *L. blossevillii*, leaving the former with a Mesoamerican distribution (Baird *et al.* 2015), including Costa Rica.

*Myotis pilosatibialis*. The subspecies *Myotis keasy pilosatibialis* was elevated to specific level and separated from *M. keaysi* according to morphological and phylogenetic studies (Mantilla-Meluk and Muñoz-Garay 2014; Carrion-Bonilla and Cook 2020); this being the species present in Costa Rica.

*Kogia sima*. The specific epithet changes from *simus* to *sima* (McGowen *et al.* 2020).

*Recent records since Rodríguez-Herrera et al. (2014) list*

*Cryptotis monteverdensis*. We add a new species of shrew, belonging to the *Cryptotis thomasi* group, described for the Monteverde Cloud Forest Biological Reserve, Puntarenas Province (Woodman and Timm 2017).

*Ichthyomys tweedii*. This species was recorded for southern Costa Rica, at the Las Cruces Biological Station (Ramírez-Fernández *et al.* 2020). This record represents the northern distribution limit for the genus *Ichthyomys*.

*Diplomys labilis*. Reported for the Osa Peninsula, Puntarenas Province, in the south of the country (Ramírez-Fernández *et al.* 2015).

*Mormoops megalophylla*. Captured at the Venado Caves, Alajuela Province (Vicente and Ledezma comm. pers.; see York *et al.* 2019).

*Micronycteris tresamici*. A new species described based on morphometric analysis, and cariological and morphological comparisons (Siles and Baker 2020). Confirmed for Alajuela and Guanacaste provinces in Costa Rica, and Honduras.

*Molossus alvarezi*. Described by González-Ruiz *et al.* (2011), apparently this species used to be misidentified as *Molossus sinaloae*, from which it differs in size, fur coloration, and other morphological characteristics. *M. sinaloae* distribution is restricted to México, therefore we replaced it with the new species *M. alvarezi*.

*Cynomops greenhalli*. Reported for Veragua Rainforest, Liverpool, Limón Province, and confirmed in scientific collections by Salas-Solano *et al.* (2020). This represents a distribution range extension into the Caribbean for the species in Central America.

*Nyctinomops laticaudatus*. Reported for Santa Ana, San José Province (Villalobos-Chaves *et al.* 2018).

*Myotis armiensis*. This species was described by Carrion-Bonilla and Cook (2020). Its distribution includes the premontane and montane forests of Chiriquí (Panama), Valle del Silencio (Costa Rica), and the Cordillera Oriental (Ecuador).

*Speothos venaticus*. Reported for Zona Protectora Las Tablas, Talamanca Mountain Range (González-Maya *et al.* 2017).

## Discussion

Increasing interest in studying mammals, not only abroad, but also locally in Costa Rica (Rodríguez-Herrera *et al.* 2005, 2014b) is leading to the discovery of new species. Ongoing systematic and phylogenetic revisions using a combination of fossils, morphology, and molecular data is revealing cryptic species, and more powerful hypotheses about the evolutionary relationships of mammals at various taxonomic levels. This is particularly evident in the orders Chiroptera, Rodentia and Eulipotyphla which are not only small-sized mammals from very diverse groups, but also more taxonomically challenging (*e. g.*, cryptic species, species complexes) than other orders of mammals. This is reflected in the new additions to the list, where six bat species, two rodent species, and one shrew are among the ten recent records. Of these, four correspond to new species descriptions to science: the bats *M. tresamici*, *M. alvarezi*, and *M. armiensis*; and the shrew *C. monteverdensis*. The latter stands out for being an endemic species for Costa Rica, known only for the Monteverde region, Puntarenas Province (Woodman and Timm 2017).

Another species that stands out among the new records, belonging to the well-studied group of the carnivores, is the bush dog *Speothos venaticus*. This poorly known species, reported to be rare throughout its distribution, was recorded in camera traps for southern Costa Rica (González-Maya *et al.* 2017). According to the authors, the low frequency of records of this species in the study area is consistent with and reinforces the idea of its cryptic habits and natural rarity. This also highlights the great importance and scope of modern technologies for field work, such as camera traps, and long-term research, in developing complete mammal inventories, making it possible to record species that would otherwise go unnoticed.

Regarding aquatic species, as expected the cetaceans are the most diverse group of marine mammals within the Costa Rican Exclusive Economic Zone with 27 species. However, it is important to highlight that this is likely an underestimation. While monitoring efforts in terrestrial habitats continue to grow in Costa Rica, the aquatic environments remain largely unexplored. Deep diving and offshore species are likely missed in opportunistic boat surveys. To this day, no dedicated effort to evaluate the species richness and abundance of cetaceans in Costa Rican waters have taken place. Most survey efforts are near the coast, and temporally and spatially limited (May-Collado *et al.* 2018).

Regarding the last list of mammals published for the country (Rodríguez-Herrera *et al.* 2014a), two bat species, *Sturnira hondurensis* and *M. sinaloae*, and four cetaceans, *Kogia breviceps*, *Mesoplodon europaeus*, *M. ginkgodens* and *Stenella clymene*, were removed from our list. The bats were previously included, most likely given they are cryptic species with respect to *S. burtonlimi* and *M. alvarezi*, respectively, and having little knowledge about their taxonomy, their correct identification has been mistaken since the first records. In the case of cetaceans, these were included in the list based on distribution maps from various sources, that generalize their presence throughout the Pacific or Indian Ocean (*e. g.*, MacLeod *et al.* 2006; Jefferson and Braulik 2018; Kiszka and Braulik 2020). However, these species have not yet been documented in the field or in strandings in Costa Rican territory.

The main areas of mammal endemism in Costa Rica correspond to the high parts of the different mountain ranges, with 21 of the 30 endemic species restricted to these regions, mainly in the mountainous extension of the Talamanca Mountain Range in Costa Rica and Panama. This mountain range presents the highest elevations in the country, reaching a maximum of 3820 masl (Kappelle and Horn 2005), is considered one of the five great forests of Mesoamerica, and is home to the largest forest extension in the country, with more than 400,000 ha of protected areas. These high elevation regions are considered endemism hotspots because of its historical biogeographical formation that has served as a refugee for some species, due to the isolation of relatively small areas with specific climate ranges enhancing speciation rates (Obando 2002; Savage 2002; Kluge and Kessler 2006). It is important to highlight rodents among the endemic species in the country, which comprises 70 % of the total, being at the same time one of the least known groups and with most uncertain conservation status.

Among the Mesoamerican countries, Costa Rica has historically stood out as a leader in conservation. Costa Rica has 166 protected areas that cover approximately 25 % of the national territory (Alvarado *et al.* 2012; González-Maya *et al.* 2015). These protected areas include 50 % of the coastline and 20 of them are exclusive marine protected areas (Alvarado *et al.* 2012). In addition, the land administration has favored the prevalence of wildlife populations in much of the country, including species categorized as threatened and with a special conservation status, both nationally and globally (SINAC 2017; IUCN 2022). In groups such as primates and felids, all species are under some category of threat, and the same happens with the tapir *T. bairdii*, the largest land mammal in the region. In the case of the giant anteater, listed by both SINAC (2017) and IUCN (2022), this has been considered as extirpated from Costa Rica by some authors (Ruiz León 2019).

For other members of the Costa Rican mammal assemblage, long-term monitoring efforts are urgently needed to help identify the impact of direct and indirect anthropogenic activities on their populations. In particular for endemic species, most of which are not included under any threat category despite poor knowledge on its biology and its reduced global distribution range, so its conservation status should be carefully reviewed. Management of protected areas in Costa Rica over time has provided space for the mammal diversity recorded in this study; however, it is necessary to invest in conservation efforts, especially on species that receive less attention, to guarantee their survival and presence in Costa Rican territory in the future.

Although some researchers consider Costa Rican mammalian diversity well known, still new species are continuously being described and some are even periodically reported for the first time for the country. This is reinforced not only by cientific research, but it encompasses constant informal field work, observation and documentation of wildlife by local naturalists; furthermore, growing citizen science and participatory research initiatives (*e. g.*, camera-trapping monitoring, bird counts) are now surveying many areas across the country and will provide new information about multiples groups in the coming years (Gómez Hoyos *et al.* 2021). Moreover, contributions on systematics have proved their need and value as taxonomic boundaries between species from complex groups, as bats and rodents, are being clarified adding more changes to the list.

Although multiple threats still exert pressure over many mammal populations in the country (González-Maya *et al.* 2015, 2016), Costa Rica stood globaly as a leader in conservation, and the country's economy benefits largely from ecotourism (Echeverri *et al.* 2022). This is reflected in the recent creation of new protected areas (Presidencia de la República de Costa Rica 2022), where more than 15,000,000 hectares were added. So we expect that the country will continue towards a conservation-minded path for the future, maintaining committed efforts for safeguarding its biodiversity for the long term, although still many challenges remain to be covered by local government and policies.

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## Supplementary material

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# Distribution and habitat of Ocelot (*Leopardus pardalis*) in Sonora, México

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The ocelot (*Leopardus pardalis*) ranges from northeastern Argentina to southern Texas and southeastern Arizona. It is listed as Endangered in Mexico and the United States. Previous works on ocelots in Sonora, found them occupying different habitats, including tropical deciduous forest, thornscrub, desert grassland, oak woodland, and pine-oak forest, avoiding the driest areas of the Sonoran Desert. The majority of our records are from camera traps, with indirect records from tracks. We analyzed images and videos of more than 100 camera traps used to monitor wildlife in ranches and natural protected areas of Sonora. Cameras were set at an altitude of 70 to 100 cm above ground, some were baited with a sardine-tomato mixture, others were not, the majority were set in areas where other species were the main objective. We obtained 147 recent records from 2015 to 2021 and 28 previous records of ocelots in Sonora. Ocelots occur in the eastern half of Sonora, avoiding areas with less than 400 mm of annual rainfall in the eastern and northwestern Sonoran Desert. Ocelots have been recorded from 53 to 2,151 m elevation (av. 840 m). 21 images in nine localities show females with kittens, indicating breeding populations. In southern and east-central Sonora, ocelots live in tropical deciduous forest, and foothills thornscrub. At Maycoba east of Yécora, in Sierra Huachinera and in the Sky Islands Mountain ranges in northeastern Sonora, ocelots live in temperate vegetation, including desert grassland, oak woodland, and pine-oak forest. Ocelots have been recorded in riparian habitats, transecting other vegetation types in the principal rivers of the state: Ríos Bavispe-Yaqui, Mayo, and Sonora, which drain the Sierra Madre Occidental, the Sky Island Region and southeastern Arizona to the Gulf of California, providing dispersal corridors. Other medium sized rivers provide corridors for the dispersal of ocelots, such as Río Mátape in central Sonora, and Ríos Cocóspera, San Pedro, and Santa Cruz in the north. The nearest Sonoran populations to Arizona are in the Sierra El Alacrán and Río Cocóspera on Rancho El Aribabi.

El ocelote (*Leopardus pardalis*) habita del noreste de Argentina hasta el sur de Texas y sureste de Arizona. Está clasificado como Especie en Peligro de Extinción en México y en los Estados Unidos. Trabajos anteriores han registrado al ocelote en Sonora en varios hábitats, incluyendo selva baja caducifolia, matorral espinoso, pastizal de desierto, bosque de encino y bosque de pino-encino, evitando zonas áridas del Desierto Sonorense. La mayoría de nuestros registros son de cámaras trampa y registros indirectos de rastros. Analizamos imágenes y videos de más de 100 cámaras trampa usadas para monitorear fauna silvestre en ranchos y áreas naturales protegidas de Sonora. Las cámaras se pusieron de 70 a 100 cm sobre el suelo, algunas fueron cebadas con sardina entomatada, otras no. La mayoría fueron puestas donde otras especies eran el objetivo principal. Obtuvimos 147 registros recientes del 2015 al 2021 y otros 28 registros anteriores de ocelotes en Sonora. Los ocelotes ocurren en la mitad oriental de Sonora, evitando el oeste y noroeste del Desierto Sonorense, zonas con menos de 400 mm de precipitación anual. Los ocelotes se registraron de los 53 a los 2,151 m de elevación (prom. 840 m). 21 imágenes de nueve localidades muestran hembras con crías, indicativo de poblaciones reproductoras. En el sur y centro sureste de Sonora, los ocelotes habitan en bosque tropical deciduo y en laderas de matorral espinoso. También habitan en vegetación templada, como pastizal desértico, bosques de encino y bosques de pino-encino en Maycoba al este de Yécora, en Sierra de Huachinera y en las Islas del Cielo, Archipiélago Madrense al noreste de Sonora. Los ocelotes se han registrado en hábitats riparios, en varios tipos de vegetación en los ríos: Ríos Bavispe-Yaqui, Mayo, y Sonora, que drenan la Sierra Madre Occidental, las Islas del Cielo de Sonora y sureste de Arizona hacia el Golfo de California, proveyendo corredores para la dispersión. Otros ríos de tamaño mediano proveen corredores para la dispersión, como el Río Mátape al centro de Sonora, y los Ríos Cocóspera, San Pedro, y Santa Cruz al norte. La población más cercana a Arizona está en la Sierra el Alacrán y el Río Cocóspera en el Rancho El Aribabi.

***Keywords:*** Arizona; Carnivore; distribution; Sonora; vegetation types.

## Introduction

The ocelot (*Leopardus pardalis*) is a medium-sized tropical feline that ranges from northeastern Argentina, northern Paraguay, and southern Brazil north to southern Texas and northwest to southeastern Arizona (Grigione *et al.* 2007; Paviolo *et al.* 2015; Rorabaugh *et al.* 2020; Amador-Alcala *et al.* 2022). Ocelots in Sonora have been found in different habitats, including tropical deciduous forest, thornscrub, desert grassland, oak woodland, and pine-oak forest, avoiding the driest areas in the Sonoran Desert (Brown and López-González 2001; López-González *et al.* 2003; Rorabaugh *et al.* 2020; Amador-Alcala *et al.* 2022). The state has a diverse physiography, from the heights of the Sierra Madre Occidental (SMO) in eastern Sonora and the isolated mountains in northeastern Sonora westward to the western lowlands and the Gulf of California (Castillo-Gámez *et al.* 2010). The SMO reaches its northern limit in the Sierra de Huachinera in northeastern Sonora (30.25° N). There are 55 isolated Island Mountain ranges or complexes of several ranges (González-León 2010) connected by oak woodland corridors in the Madrean Archipelago or Sky Island Region in northeastern Sonora between the SMO and the Mogollon Rim in central Arizona (Van Devender *et al.* 2013a). These Sky Islands crowned with oak woodland or pine-oak forest emerge from lowland 'seas' or inter-montane valleys with desert grassland, tropical deciduous forest (Figures 1 A and B), or foothills thornscrub (Figures 1 C and D). The principal rivers of the state, including the Ríos Bavispe and Río Aros which at their junction forms the Río Yaqui, which together with Río Mayo, drain the Sierra Madre Occidental; Río Sonora drains the Sky Island Region and southeastern Arizona tributaries. All these rivers are dispersal corridors through much of Sonora to the Gulf of California. The primary river corridors for the northward dispersal of ocelots are the Río Bavispe in the east, the Río Sonora in west, the Ríos Cocóspera, San Pedro, and Santa Cruz near the Arizona border, the Río Cuchujaqui (affluent of the Rio El Fuerte) is a corridor between the southern areas of Sonora and the SMO. In Sonora this tropical cat lives in habitats that range from tropical in the southern to temperate in the eastern and northeastern parts of the state.

The ocelot is listed as a least concern species by the IUCN due to its wide distribution in America (Paviolo *et al.* 2015); as an endangered species in the United States (US Fish and Wildlife Service 2022. https://ecos.fws.gov/ecp/species/4474) due to its restricted distribution. In Mexico, it is also listed as endangered "En Peligro" status under NOM-059-ECOL-2010, the Mexican endangered species law (López-González *et al.* 2003; Castillo-Gámez *et al.* 2010; Avila-Villegas and Lamberton-Moreno 2013; Gómez-Ramírez *et al.* 2017). Brown and López-González (2001) and López-González *et al.* (2003) reported ocelots from 28 localities in Sonora. Ocelots are rare in Arizona, with only six individuals seen between 2009 and 2021 (Culver 2016; Sabra Tonn, pers. comm. 2018). Apparently occasional ocelots reach Arizona from the Sonoran populations.

Interest in the current distribution and abundance of ocelots in Sonora was stimulated by the study of López-González *et al.* (2003) that presented a model of the ocelot distribution in Sonora, enhancing the understanding of their distribution in a variety of different habitats and relationship with the vegetation. The Project WILDCAT (PW) wildlife camera study in the lower Rio Bavispe Valley south of Granados (Figure 1D) just north of the Northern Jaguar Reserve (Gómez-Ramírez *et al.* 2017) and south of Nácori Chico yielded additional information on the general distribution of ocelots in Sonora. From 2015 to 2021, ocelots were photographed 180 times at 21 localities on six PW ranches. From 2016 to 2021, ocelots were photographed in Greater Good Charities' (GGC) Madrean Discovery Expedition Wildlife project at 20 localities in the Sierras Alacrán, Aurora, Batamote, Huachinera, and Pajarito, and Rancho Chairababi near Cucurpe, Ranchos Pino Seco and San Manuel near Nacozari de García, and Ranchos Peñascal and Las Playitas near Bacoachi. Other projects that are currently going on in several areas of Sonora and have also provided records of ocelots as in the upper Bavispe, in Sierra de Huachinera and near Bacanora.

López-González *et al.* (2003) reported on a kitten from near Rosario de Tesopaco in southern Sonora. A female with a kitten was photographed on Rancho El Aribabi in February 2011 (Avila-Villegas and Lamberton-Moreno 2013; Rorabaugh *et al.* 2020). Here we report females with kittens photographed during Project WILDCAT on Rancho Carrizal (29.651° N) in January 2018 and three times in two localities on Pueblo Viejo (29.628° N) in November 2016 and May and November 2018 (Figure 4D). Breeding likely also occurs in the Nácori Chico area (29.419 to 29.479° N) as 21 images in nine widely spaced localities is too many for wandering individuals. Our goals are to summarize the distribution of ocelots in Sonora and analyze the relationships of vegetation type, the amount of rainfall, and altitude on distribution.

*Previous mammal studies mention ocelots in Sonora.* Ocelots are not specifically mentioned in Ignaz Pfefferkorn's (2008) descriptive accounts of Sonora around 1756 to 1767 but were somehow mixed in with the description of bobcats (*Lynx rufus*). Burt (1938) reported ocelots at Güirocoba, south of Álamos. William Caire's 1978 doctoral dissertation at the University of New Mexico on "The Distribution and Zoogeography of Mammals of Sonora, Mexico" mentions only two ocelot specimens from southern Sonora but gives a map of its probable distribution that reaches Arizona. His state of Sonora mammal checklist (Caire 1997) and doctoral dissertation publication (Caire 2019) also mentioned those records. A list of mammals in Schwalbe and Lowe (2000) on the amphibian and reptiles of the Sierra de Álamos region in southern Sonora included ocelots. Several ocelot records are mentioned in Brown and López-González (2001). López-González *et al.* (2003) reported early accounts of hunting of ocelots in Sonora and other records of the species, these authors summarized the distribution of ocelots in Sonora and included a

Genetic Algorithm for Rule Set Production (GARP) model of their distribution. Ceballos and Oliva (2005) presented limited descriptions of Sonoran mammal species, including the distribution of ocelots. Castillo-Gámez *et al.* (2010) presented an updated ocelot distribution in Sonora mostly based on López-González *et al.* (2003) with several new records. Avila-Villegas and Lamberton-Moreno (2013) documented ocelots in oak woodland at Rancho El Aribabi, including the northernmost breeding population in Sonora. Coronel-Arellano *et al.* (2016) reported a camera image of an ocelot from the Sierra Los Ajos. Gómez-Ramírez *et al.* (2017) documented 33 individual ocelots on the Northern Jaguar Reserve and calculated their survival and density. Rorabaugh *et al.* (2020) on Rancho El Aribabi and Amador-Alcala *et al.* (2022) on Northern Jaguar Reserve discussed the ecology of ocelots, based on photographs obtained with camera traps.

## Materials and methods

We analyzed 149 recent records of Sonoran ocelots compiled from wildlife camera and track studies in Sonora, including the Reserva Monte Mojino near Álamos (Figure 1B); the Sierra de Álamos; in the lower Río Bavispe Valley (Project WILDCAT) and south of Nácori Chico (Figure 1E); Madrean Discovery Expedition Wildlife cameras in the Sierras Alacrán (Figure 2B), Aurora, Batamote, Huachinera, and Pajarito (Figure 1F); Ranchos Peñascal and Las Playitas near Bacoachi, Ranchos Pino Seco and San Manuel near Nacozari de García, and Rancho Chairababi near Cucurpe; Universidad de la Sierra study areas near Moctezuma; CIAD-Guaymas study at Rancho Bamochi, Rancho Agua Caliente at Huachinera, Rancho Teiserobabi, and Rancho La Joya near Bacanora; other ranchos near Ures; near Santa Ana west of Yécora; Sierras Los Ajos Buenos Aires, and La Madera in APFF Bavispe; Rancho El Aribabi (Figure 2A) and the Río Cocóspera in Sierra Azul; and Cajón Bonito (Figure 2C) near the Arizona border. No camera traps were set up for ocelot distribution in areas where ocelots have never been reported like the dry western part of Sonora with a Sonoran desertscrub vegetation *i. e*. Hermosillo, Caborca, San Luis Río Colorado or at Desierto de Altar.

Previous 28 ocelot records in Brown and López-González (2001) and López-González *et al.* (2003) were added to the Madrean Archipelago Biodiversity Assessment (MABA) database (accessible in the Madrean Discovery Expeditions [MDE] database). They were also used for this study. All the recent ocelot records and images in the present study are publicly available online in the MDE database (madreandiscovery.org). The Bavispe APFF monitoring records are in the Bavispe Flora and Fauna Protected Area database (accessible in the MDE database). And a single record from UNISIERRA database (http://csvcoll.org/portal/collections/harvestparams.php).

Camera traps of different make were set either to take still photographs or in video mode; the majority of the cameras were set at an altitude of 70 to 100 cm, above ground, some of them were baited with a sardine-tomato mixture,

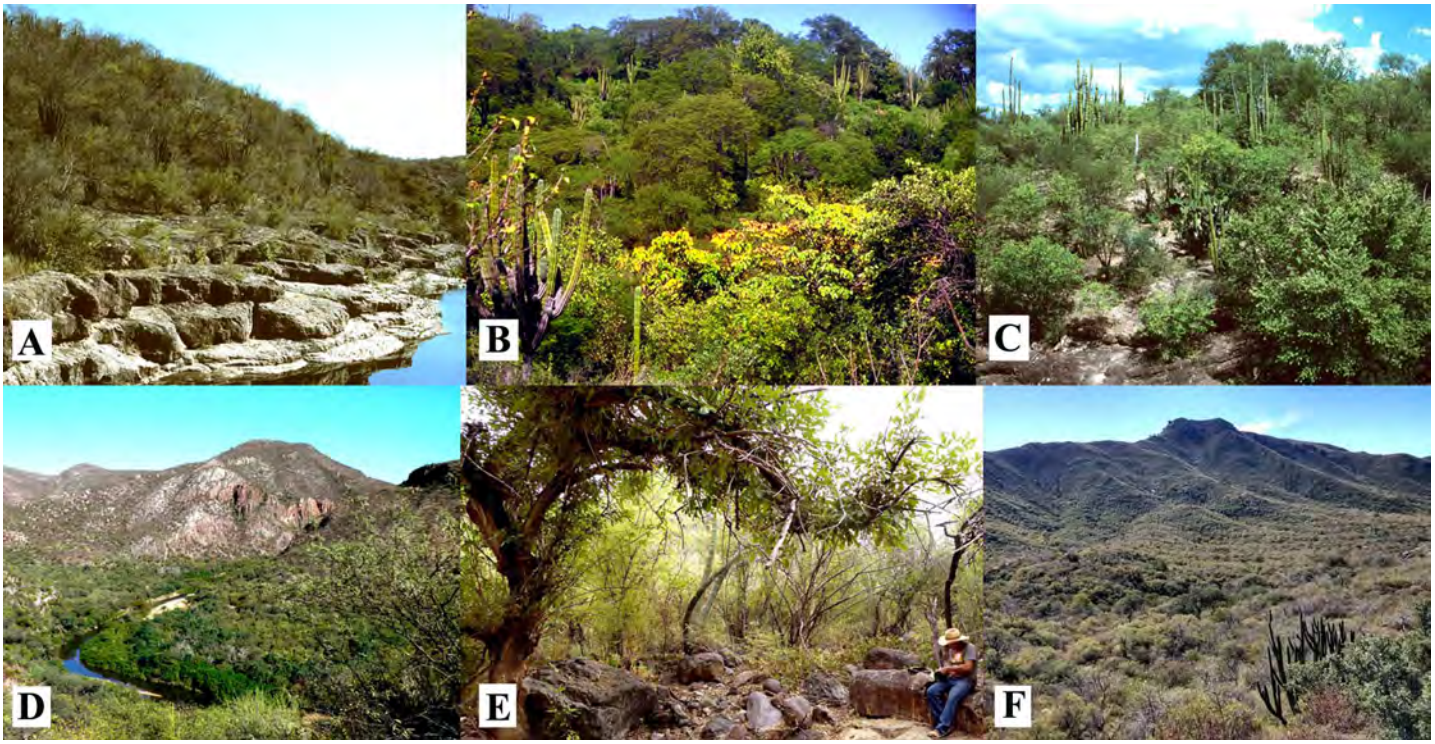

**Figure 1.** Tropical ocelot habitats in Sonora. A. Tropical deciduous forest along the Río Cuchujaqui east of Álamos. January 1984 (Photo T. R. Van Devender). B. Tropical deciduous forest near Álamos. Summer 1991. *Hecho* cacti (*Pachycereus pecten-aboriginum*) are prominent (Photo M. A. Dimmitt). C. Foothills thornscrub near Curea. September 1998. Organ pipe cacti/*pitahaya* (*Stenocereus thurberi*) are common (Photo T. R. Van Devender). D. Foothills thornscrub above the Río Bavispe on Rancho Pueblo Viejo east of Divisaderos. November 2016 (Photo T. R. Van Devender). E. J. M. Galaz-Galaz checking wildlife camera in foothills thornscrub on Rancho Pueblo Viejo. November 2016 (Photo A. L. Reina-Guerrero). F. Foothills thornscrub at Puerta del Sol, Sierra Pajarito east of Ures. November 2018. Visible on lower right are an organ pipe cactus and a fish poison tree/*palo blanco* (*Piscidia mollis* gray leaves) (Photo N. L. Villanueva-Gutiérrez).

some others were not baited and set in areas where other species were the objective, *i. e.* neotropical otters (*Lontra longicaudis*) in riparian habitats (Gallo-Reynoso *et al.* 2019). More than 100 places were used for the monitoring of ocelots, each site had a camera trap (to complete that number several cameras were moved to different locations along the study time), camera traps were set for the record of multiple species in most of the ranches and natural protected areas in which they were used for monitoring wildlife, ocelot records were extracted from those monitoring efforts.

We analyzed the frequency of observations effect of precipitation, vegetation type, and altitude on the distribution of *L. pardalis* in Sonora, the data set used for this analyzes comprised 175 out of 177 records that were complete having the four variables, of which we removed two duplicated records (curtailing the effect of multiple records produced by any camera trap). Original data were obtained from GPS location records with notes on the surrounding vegetation type in each one of the records. Precipitation was obtained from Gallo-Reynoso *et al.* (2018) armadillo (*Dasypus novemcinctus*) distribution in Sonora by superposing location data of Ocelots on the precipitation/ vegetation type SIG data of Sonora.

## Results

The distribution of ocelot in Sonora is complex as shown by the distribution of the 175 records considered herein (Figure 3). These records represent five years (2015 to 2021) of camera trap studies, and 28 previous records of ocelots in Sonora (Brown and López-González 2001; López-González *et al.* 2003). Ocelots occur in the eastern half of Sonora, generally avoiding lower areas with less than 400 mm of annual rainfall and the arid Sonoran Desert, therefore records were found in the southeastern, central, and northeastern part of the state. There are 72 municipalities in Sonora, and ocelots have been documented in 28 of them: Agua Prieta, Álamos, Arizpe, Bacadéhuachi, Bacanora, Bacoachi, Cajeme, Cananea, Cucurpe, Cumpas, Divisaderos, Fronteras, Granados, Huachinera, Huásabas, Ímuris, Moctezuma, Nácori Chico, Nacozari de García, Navojoa, Quiriego, Rosario de Tesopaco, Sahuaripa, San Pedro de la Cueva, Soyopa, Suaqui Grande, Tepache, Ures, and Yécora. Ocelots are likely present in another 22 municipalities in southern and eastern Sonora.

Ocelot distribution in the study area were obtained on mean altitude above sea level (m) of 840 m (± 458 m, range: 53 to 2,151 m), its modal distribution was 1,326 m, although denoting some preference for higher areas over lower ones with 93 (53.1 %) occurrences in areas higher than 1,000 m, and with 82 (46.9 %) occurrences below 1,000 m. Their distribution on Sonora is affected by the amount of rainfall in the different areas of central and eastern Sonora, preferring areas with a mean of 515 mm of rain (± 107 mm, range: 400 to 800 mm), modal distribution was found at 500 mm of rain. No record was obtained in less than 400 mm of rain in the area occupied by Sonoran desertscrub (Figure 3). The areas preferentially occupied by ocelots in Sonora had different frequencies of occurrence related to the amount of rain (mm), 43 occurrences (24.6 %) between 400 to 499 mm of rain; 81 occurrences (46.3 %) between 500 to 599 mm of rain; 21 occurrences (12 %) between 600 to 699 mm of rain; 25 occurrences (14.3 %) between 700 to 799 mm of rain; there were only 5 occurrences (2.8 %) above 800 mm of rain, there were no records below 400 mm of rain.

Ocelots occur in tropical deciduous forest and foothills thornscrub in the southern Sonora (Figure 1 B and C) and desert grassland, oak woodland, and pine-oak forest in the east and northeast (Figure 2 B). The vegetation in which Ocelots occurred preferentially was Sierra Madrean woodlands and forests with 86 (49.1 %) occurrences, followed by foothills thornscrub with 47 (26.9 %) occurrences, tropical deciduous forest with 32 (18.3 %), grasslands / Chihuahuan desertscrub with 7 (4 %) occurrences and by Sonoran desertscrub with 3 (1.7 %) occurrences (Figure 1F).

Ocelots are commonly photographed in foothills thornscrub habitats at localities in the lower Río Bavispe Valley (Figure 1 A, B and D). Ocelots are also photographed with regularity as far north as Rancho El Aribabi in the Sierra Azul in oak woodland (Figure 2 A and B; Figure 4 E and F) and along the Río Cocóspera (30.856° N) in riparian habitats. Additionally, ocelots shift from tropical lowland habitats into upland desert grassland, oak woodland, and pine-oak forest north of the Neotropics. Ocelots have been recorded from 53 to 2,151 m (average 840 m) in Sonora but are not expected to be found near sea level in the southern coastal areas. Ocelots probably use riparian habitats to disperse between areas and vegetation types.

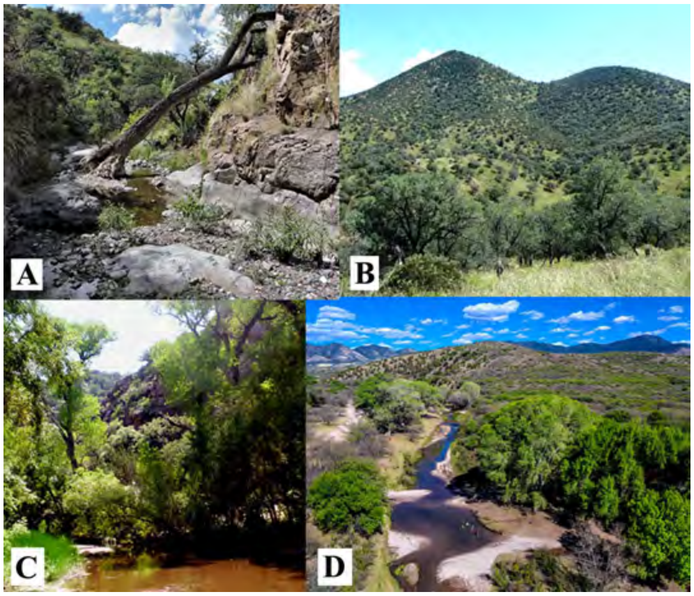

**Figure 2.** Temperate ocelot habitats in Sonora. A. Rocky stream canyon in oak woodland. Arroyo Las Palomas, Rancho El Aribabi, Sierra Azul, east-northeast of Ímuris. October 2015 (Photo J. C. Rorabaugh). B. Oak woodland dominated by Arizona white and Mexican blue oaks (*Quercus arizonica, Q. oblongifolia*). Sierra Alacrán southeast of Cananea. September 2018 (Photo A. L. Reina-Guerrero). C. Cottonwood-willow (*Populus fremontii-Salix* spp.) riparian deciduous forest, desert grassland on slopes. Cajón Bonito, Rancho los Ojos Calientes east of Agua Prieta. August 2007 (Photo T. R. Van Devender). D. Cottonwood-willow riparian gallery forest along Río Santa Cruz in desert grassland. Aerial view to the north of Paseo del Cajón northeast of San Lázaro. April 2019. Only 18 km south of the border, this is a likely corridor for ocelots to the Huachuca Mountains in Arizona (Photo L. Gutiérrez, NortePhoto, Inc).

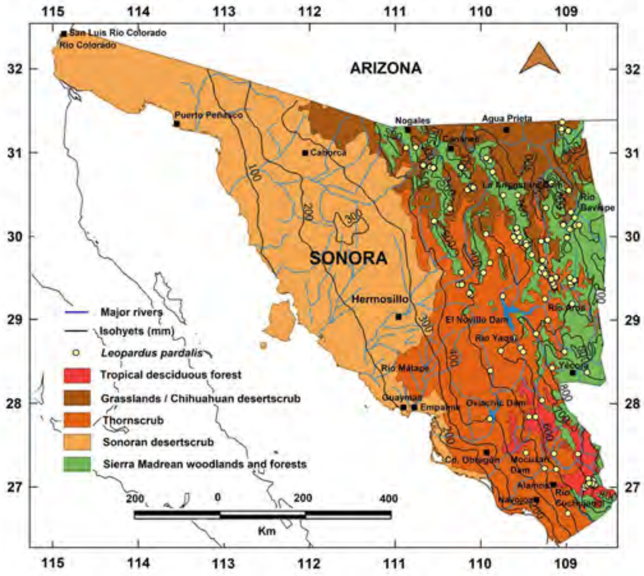

**Figure 3.** Distribution of ocelot records in Sonora and their occurrence in different vegetation types. Areas with a precipitation less than 400 mm are not occupied by ocelots, unless they occupy riparian habitats adjacent to canyons and rivers. Inter-montane valleys and riparian habitats are widely used for ocelot dispersal corridors.

Comparison by frequency analysis shows that precipitation above 400 mm (*n* = 132) explained the 75.4 % of the presence of Ocelots in Sonora. Vegetation type (Sierramadrean woodlands and forests (*n* = 86) explained the 49 %. Elevation above 800 m (*n* = 109) explained the 62.3 % of occurrences. Therefore, a combination of precipitation-elevation-vegetation type were the variables that defined the presence and distribution of Ocelots in Sonora (Figure 5). Unaccounted variables such as seasonality might also be important for the presence and distribution of Ocelots in Sonora but was not analyzed in this study.

## Discussion

The distribution of ocelot records is much wider than expected and occupies more geographic area than predicted by the GARP analysis in López-González *et al.* (2003), mostly based on tropical deciduous forest and thornscrub. Our records still fall inside the predicted area in their southern distribution but extend in other regions not reported previously that include several vegetation types such as desert grassland, oak woodland, and pine-oak forest to the north and northeast. The distribution of ocelots in Sonora follows the distribution of precipitation, similar to what has been demonstrated with armadillos in Sonora (Gallo-Reynoso *et al.* 2018).

There are still some gaps in their distribution in more remote areas in Sonora, where it needs to develop field surveys and assessment with the use of wildlife cameras. Also, ocelots are expected to occur in tropical deciduous forest in the foothills of the Sierra Madre Occidental from the Sinaloa border north through the Álamos area north to Yécora. Ocelots are also likely to occur in foothills thornscrub along the Río Yaqui from Ónavas to Tónichi north to San Felipe de la Cueva, Sahuaripa, and Mátape. They are also expected in foothills thornscrub in the Río Sonora Valley from the Sierra Aurora north to the Sierras Aconchi and Los Locos and to the Rancho El Charababi area just south of the Sierra Azul. To the west, they are likely to be in foothills thornscrub in the Sierra Bacatete east of Guaymas on Yaqui First Nation land, Sierra El Aguaje corridor to Sierra Libre north of Guaymas where an intermittent creek bears the name of "El Ocelote", between Guaymas and Hermosillo. Considering the records of ocelots in Arizona, they likely also occur in Sonora close to the Arizona border in the Sierras Las Avispas, Chivato, Pinito, and San Antonio. Note that our report does not include recent records from

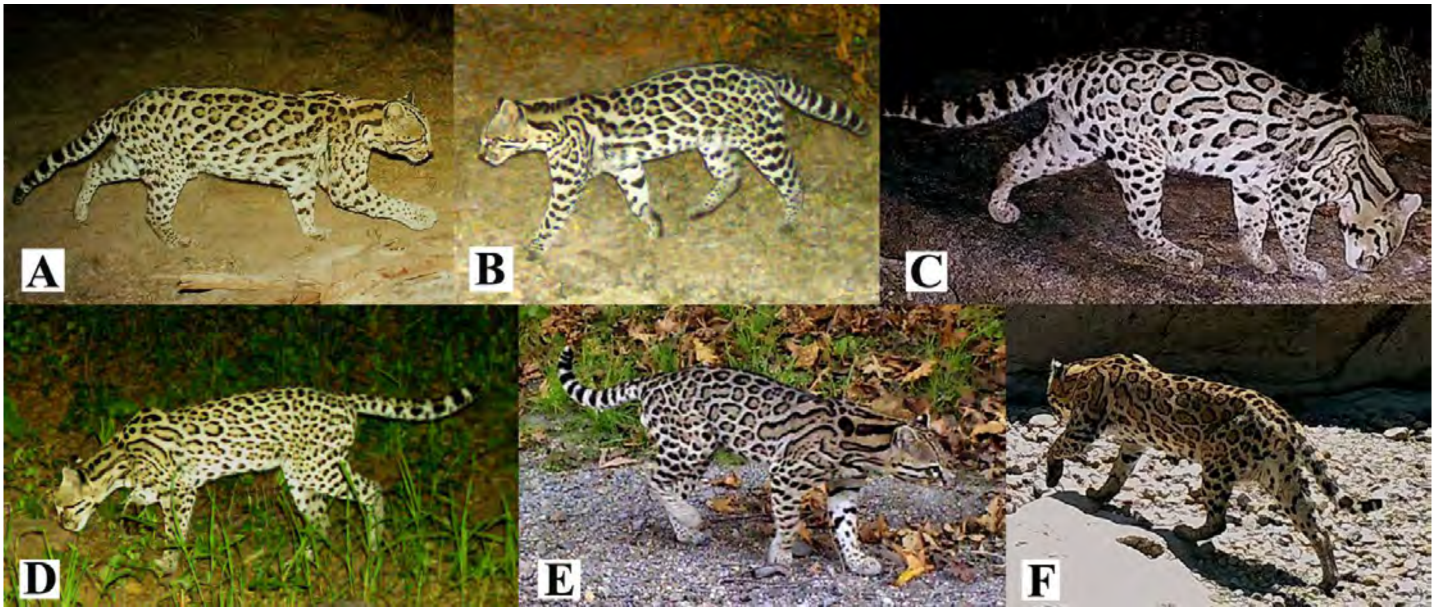

**Figure 4.** Ocelot images at different places. A. Arroyo Las Palomas 28 km ENE Ímuris November 2015 (Photo by J. C. Rorabaugh). B. Rancho Hoyo, Arroyo Bacadéhuachi east of Divisaderos. February 2016 (Photo by J. M. Galaz-Galaz). C. Puerta del Sol, Sierra Pajarito east of Ures, April 2019 (Photo by J. M. Cirett-Galván). D. Rancho Los Pescados south-southeast of Nácori Chico, August 2018 (Photo by J. M. Galaz-Galaz). E. Female with kitten. Pozo del Indio, Rancho Pueblo Viejo, November 2015 (Photo by J. M. Galaz-Galaz).

the Northern Jaguar Reserve, although Gómez-Ramírez *et al.* (2017) reported 381 wildlife camera images of which 33 were individually identified ocelots during 2010-2012, and Amador-Alcalá *et al.* (2022) reported ocelot abundance and home range based in 54 camera traps stations, obtaining 135 photographic records of which 17 were individually identified ocelots during 2015.

Ocelots are present in the northernmost area of the SMO in the Sierra Huachinera. There is an observation of an individual in the SMO from the Río Maycoba east of Yécora near the Chihuahua border. Probably ocelots use riparian corridors to reach higher areas of the Sierra Madre Occidental, similar to a nine-banded armadillo (*D. novemcinctus*) record from Los Pilares in a nearby locality (Gallo-Reynoso *et al.* 2018) likely reached the area from tropical vegetation in the Río Mayo drainage near Moris, Chihuahua (36 km to the southeast), a route likely followed by ocelots. Wildlife camera-trap surveys in pine-oak forest in the Mesa Tres Ríos area in Sonora near the Chihuahua border were carried out in 2018 but did not find ocelots. López-González *et al.* (2015) added ocelots to the fauna of the SMO in Chihuahua based on a wildlife camera study on oak woodland east of Nuevo Casas Grandes. The extent of the ocelot distribution in the Sierra Madre Occidental (SMO) needs further detailed study.

*Biogeography.* Four different biotic provinces merge in the Madrean Archipelago in northeastern Sonora and southeastern Arizona – the Rocky Mountains, California Mediterranean, Great Plains/Chihuahuan Desert, and Sierra Madre Occidental, and there is also the transition zone between the Nearctic, and Neotropical Biogeographic regions (Van Devender *et al.* 2013a). Although the Tropic of Cancer (23.43° N) just north of Mazatlán, Sinaloa is often considered the northern limit of the New World Tropics, tropical deciduous forest extends 680 km to the northwest to the Sierra San Javier, Sonora (28.62° N; Van Devender *et al.* 2013a). The transition between the Neotropical and Nearctic provinces starts in Sonora. In southern Sonora, coastal thornscrub is transitional between tropical deciduous forest (Figure 1, A and B) and Sonoran desertscrub, and in central Sonora foothills thornscrub (Figure 1, C and D) is between Sonoran desertscrub and oak woodland. The northernmost thornscrub in Sonora is found near Arizpe in the Río Sonora Valley (30.35° N) and at Presa La Angostura on the Río Bavispe (30.44° N; Van Devender *et al.* 2013b). At its northern edge where thornscrub transitions into desert grassland, it is limited by freezing temperatures during fall - winter.

The distribution of ocelot in Sonora reflects the effect of precipitation and vegetation types mentioned above but does not occur in desertscrub in the Sonoran Desert. In Sonora, ocelots occur in areas with 400 mm or greater annual precipitation, unless they are in riparian habitats adjacent to canyons and rivers. Inter-montane valleys in this precipitation range regardless of their vegetation type are also occupied by ocelots, meaning that these riparian habitats are widely used for ocelot dispersal (Figure 2 C and D).

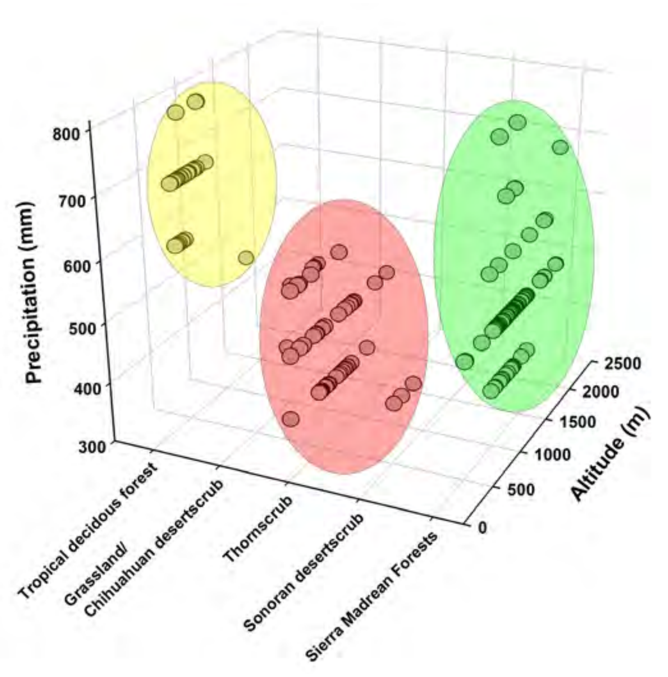

**Figure 5**. Influence of the vegetation type, rainfall, and elevation on the distribution frequency of *Leopardus pardalis* in Sonora. Vegetation types: Sierramadrean forests with 86 occurrences (49.1 %), Sonoran Thornscrub and desertscrub (Chihuahuan and Sonoran) with 57 occurrences (32.6 %), and tropical deciduous forest with 32 occurrences (18.3 %) were the type of vegetation more frequented by ocelots. Precipitation above 400 mm accounted for 132 occurrences (75.4 %), and 43 records in precipitation between 300 to 400 mm (24.6 %). Elevations from 800 to 2,200 m accounted for 109 records (62.3 %), 52 occurrences in elevations between 400 to 800 m (29.7 %) and 14 records in elevations less than 400 m (8 %).

Many tropical species reach their northern distributional limits in desert grassland and oak woodland in northern Sonora or southern Arizona (Van Devender *et al.* 1994). Examples include Neotropical reptiles such as vine snake/bejuquillo (*Oxybelis aeneus*), and thornscrub hooknose snake (*Gyalopion quadrangulare*); and Neotropical plants such as coralbean/chilicote (*Erythrina flabelliformis*), and kidneywood/palo dulce (*Eysenhardtia orthocarpa*). The latitudinal wedge effect caused by increasing aridity at lower elevations and colder temperatures at higher elevations is a general biogeographic pattern in the western half of the continent. Neotropical otter (*Lontra longicaudis*; Gallo-Reynoso *et al.* 2019), Jaguar (*Panthera onca*), ocelot (*L. pardalis*), coati/chulugo (*Nasua narica*), collared peccary/ cochi jabalí (*Pecari tajacu*), Sonoran possum/ tlacuache (*Didelphis virginiana californica*; Babb *et al.* 2004) and nine-banded armadillo *D. novemcinctus* (Gallo-Reynoso *et al.* 2018) are Sonoran mammals that fit this pattern.

Foothills thornscrub in the Granados-Divisaderos area in the lower Río Bavispe Valley and the Bacadéhuachi-Nácori Chico area to the east, and the Río Sonora to the west are likely the sources for ocelots dispersing to the mountains of northern Sonora *e. g.*, Sierras Huachinera, Buenos Aires, de Los Ajos, and Azul. From these areas, ocelots disperse into some areas of southeastern Arizona, including the Huachuca, Pajaritos, Santa Rita, and Whetstone Mountains (Culver 2016).

One important result of this study is the documentation of the use of riparian habitats by ocelots. River corridors for northward dispersal of ocelots are likely the Río Bavispe in the east, the Río Sonora in the west, the Río Mátape in the center, the Río Mayo and Río Cuchujaqui in the south, and Ríos Cocóspera, San Pedro, and Santa Cruz (Figure 2 D) near the Arizona border. However, considering that ocelots primarily live in tropical habitats in southern Sonora, habitat connectivity is more important than river corridors. They will likely thrive unless there is drastic habitat loss or unavailable prey. In northeastern Sonora where foothills thornscrub merges into desert grassland and ocelots move up into woodlands, river corridors are likely more important for their dispersal.

Near the Arizona border, ocelots are found in oak woodland in the Sierras Alacrán, Avispas, and Azul but were not found in the Sierra Chivato close to the border with Arizona, USA. In 1964, Sewell Goodwin killed an ocelot at 2,583 m elevation in a pine tree on the summit of Pat Scott Peak in the Huachuca Mountains, Arizona (Brown and López-González 2001). Recently, an ocelot was seen in the Huachuca Mountains in Arizona (A. Niels, pers. comm, 2021), it is probable that the ocelot dispersed from Sierra Chivato 35 km southwest of where the southern end of the Huachuca Mountains reaches the border. It is also possible that ocelots use the Río Santa Cruz as a dispersal corridor north into the San Rafael Valley or at Sierra El Pinito (not yet studied with camera-traps), near there are the Patagonia Mountains, a northern extension of the Sierra El Pinito and just south of the Santa Rita Mountains. The Canelo Hills at the north end of the San Rafael Valley is a woodland corridor between the Patagonia Mountains and the Huachuca Mountains to the east used by ocelots. The ocelot population in the Rancho Las Playitas area near Bacoachi, indicates that ocelots also live in desert grassland in the valley between the Sierra Azul and the Arizona mountains.

This remarkable connectivity of ocelot habitat depending on factors such as precipitation, vegetation type and elevation should be protected as corridors for the benefit of ocelots and other tropical Mexican endangered species such as jaguar (*P. onca*) that as well as ocelots reach their northern distribution limits in America in Sonora and/or southern Arizona.

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# Density and abundance estimate of Antillean manatees (*Trichechus manatus*) within a landlocked lake in Southeastern México

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Manatees are threatened along their range. In México, this species is listed as endangered. Manatee conservation strategies require density or occupancy estimates, especially in areas where species face survival risks. On turbid waters, like those found in rivers and lakes of the Southern Gulf of México coast, visual methods used to detect and count manatees underestimate actual numbers. Our goal was to estimate the density and abundance (N) of Antillean manatees in a small State Natural Protected Area, where a manatee population of unknown size inhabits. We performed line transects using a side scan sonar to detect animals and mark-recapture in the isolated population of manatees within Laguna de las Ilusiones, México, a landlocked lake that excludes transit. Using distance sampling from 14 boat trips, estimates of density and abundance were 15.5 manatees km$^{-2}$ and 27 ± 5 manatees in the lake (CV ≈ 16.6 %). With MARK, from six capture events and 19 individual encounter records, the abundance estimated was 24 manatees (CV ≈ 16 to 24 %). Previous number of manatees were based only on visual surveys, which reported at least seven manatees. Density is lower than other similar studies along narrow waterways in important areas in México and other Central and South America countries. Studying this endangered subspecies is limited by cryptic habits, turbid waters, poor funding, and low densities, making density or abundance estimates difficult. However, within particular areas and established monitoring areas, these methods could be useful to generate baselines for conservation strategies.

El manatí está amenazado en todo su área de distribución, en México se encuentra enlistado en peligro de extinción. Para implementar estrategias de conservación del manatí se requiere de estimaciones de densidad u ocupación, especialmente en áreas en las que enfrentan riesgos. En aguas turbias, como en ríos y lagunas de la costa del sur del Golfo de México, los métodos visuales de detección y conteo muy probablemente subestiman los números. Se estimó la densidad y abundancia de manatí antillano en una pequeña Área Natural Protegida Estatal donde habita una población de número desconocido de manatíes. Se usaron transectos lineales empleando un sonar de barrido lateral de imágenes para detectar animales y modelos de captura-recaptura de una población aislada de manatíes en Laguna de las Ilusiones, México, un cuerpo de agua cerrado que impide la entrada y salida de manatíes. Usando muestreo por distancias de 14 navegaciones estimamos una densidad y abundancia de 15.5 manatíes por km$^{-2}$ y 27 ± 5 manatíes habitando la laguna. Utilizando datos de recapturas con MARK, con seis eventos de captura y 19 historias de encuentro individual, la abundancia estimada fue de 24 manatíes (CV 16 a 24 %). Estudios previos en la laguna reportaban al menos siete animales. La densidad estimada es menor que la encontrada en otros estudios con métodos similares, pero realizados sobre cursos de agua estrechos, en zonas importantes en México y en otros países de Centro y Sudamérica. Los estudios con esta subespecie amenazada se dificultan por los hábitos crípticos de los animales, aguas turbias y escasez de financiamiento; aunado a las bajas densidades, haciendo difícil la estimación de la densidad y abundancia, sin embargo, dentro de áreas específicas y en zonas establecidas de monitoreo, estos métodos pueden ser útiles para generar una línea base para estrategias de conservación.

**Keywords:** Distance sampling; freshwater environment; mark-recapture; side scan sonar; turbid waters.

## Introduction

The West Indian manatee (*Trichechus manatus*) is distributed from the Southeastern United States to Northeastern Brazil, including the Major Antilles and Trinidad and Tobago (Lefebvre *et al.* 2001). This species is considered vulnerable by the International Union for Conservation of Nature, IUCN and the Convention on International Trade in Endangered Species of Wild Fauna and Flora, CITES. *Trichechus manatus manatus*, the Antillean subspecies, is distributed outside the United States and listed as Endangered due to the lack of data on its status. This subspecies has been listed as a conservation priority species in México (SEMARNAT 2018) and in the Wider Caribbean region (UNEP 2010). Among the key recommendations in the Regional Management Plan for the West Indian Manatee (UNEP 2010) is the assessment of manatee status and distribution, including abundance and population trend estimations whenever possible.

Abundance estimates are prone to difficulties when working with Sirenians, given the high variability in environmental conditions and site occupancy (Marsh *et al.* 2011). For the Antillean manatee, especially within freshwater inland systems, given the complexity of its habitat, high water turbidity in most areas, and its elusive behavior, aerial surveys yield poor results in manatee counts and density estimates. Interviews and opportunistic sightings are alternatives to aerial surveys, but they are biased and yield irreproducible information. New sighting methods include side scan sonar on boat platforms, which can become acceptable if standardized procedures and data interpretation improve (UNEP 2010).

The use of Side Scan Imaging Sonar beams (SSI) has been explored to detect and count manatees on turbid or dark waters with promising results (González-Socoloske *et al.* 2009; González-Socoloske and Olivera-Gómez 2012; Arévalo-González *et al.* 2014; Puc-Carrasco *et al.* 2016, 2017; Guzmán and Condit 2017; Castelblanco-Martínez *et al.* 2017). This sonar has also been used to detect other aquatic fauna (Davy and Fenton 2013; Flowers and Hightower 2013).

The capture and tagging of Antillean manatees had been conducted in specific regions in México, mainly for health assessment purposes, but also to monitor their movements and preferred areas (*e. g.*, Castelblanco-Martínez *et al.* 2013; Morales-López *et al.* 2012; Aragón-Martínez *et al.* 2014). Capture information can complement interviews and aerial and boat surveys, to draw distribution and abundance information. Along with line transects on aerial and boat platforms, mark-recapture models (Otis *et al.* 1978; White *et al.* 1999) are reliable and well-established methods for estimating abundances when constrained by specific conditions, like those of closed populations or long-term population follow-up. However, the high cost and the logistic demands required to obtain reliable results from large and cryptic species, such as manatees, limit their use in developing countries.

Most of the studies for the Antillean manatees have logistic and funding limitations, producing minimum counts on distribution surveys (*e. g.* Morales-Vela *et al.* 2000). There are a few studies that have dealt with area-specific abundance or density estimates of Antillean manatees (La Commare *et al.* 2012; Guzmán and Condit 2017; Puc-Carrasco *et al.* 2017) or abundance over extended regions (*e. g.* De Olivera-Alves *et al.* 2015; Collazo *et al.* 2019).

The Grijalva-Usumacinta River basin is assumed to have México's densest manatee population. Conservation actions in this region must have a high impact on the country's species (UNEP 2010; SEMARNAT 2018). However, density and abundance estimates are constrained by the high turbidity of its interior waters. Counts to estimate relative abundance on specific waterways provide few insights into the whole population of this region (Puc-Carrasco *et al.* 2016, 2017). Combining Mark-recapture, distance sampling and interviews could produce better estimates. The lake of Laguna de las Ilusiones is located within the Grijalva-Usumacinta Rivers basin and presents conditions usually found in most of the basin's water bodies. This lake is currently landlocked and hosts an isolated manatee population (Pablo-Rodríguez and Olivera-Gómez 2012), which brings the opportunity to explore and standardize methods to estimate population density. In this study, we explore the estimation of the abundance of manatees in Laguna de las Ilusiones through boat line transects, using sonar sightings and through data on manatee mark-recapture procedures performed for telemetry and health assessment purposes. This research is useful to highlight limitations of methods and variance associated with detection. Once accepted and standardized, these methods could be readily replicated in other wetlands.

## Materials and methods

*Study area.* Laguna de las Ilusiones (17° 59.7' N, -92° 56.3' W; hereafter LI) is a lake covering 189.1 ha with a 41 km perimeter and a depth mostly less than 3.5 m (Ricárdez-de la Cruz *et al.* 2016). LI hosts an isolated manatee population in the state of Tabasco in Southeastern México (Figure 1; Pablo-Rodríguez and Olivera-Gómez 2012). LI, like most of the lakes in the Mexican Gulf plain, is a quaternary fluvial and lacustrine alluvium deposit from the Grijalva River, that has a meandric origin from contact with the Mezcalapa river. In the past, LI was connected to the Mezcalapa river by the "El Espejo" stream; however, a dam was built at the mouth of the creek, blocking water flow. Currently, the water input to LI is mostly through rainfall. In LI, the submerged vegetation is nearly absent, and the floating plants, mostly *Pistia stratiotes* and *Eicchcornia crassipes,* are present but scarce because they are actively controlled. Bank vegetation is the primary food source for manatees (Ponce-García *et al.* 2017), composed mostly of grasses, other associated plant species, and isolated trees.

LI was declared a State Natural Protected Area in 1995, but the growth of Villahermosa city has resulted in a highly urbanized lake perimeter (Pablo-Rodríguez and Olivera-Gómez 2012; Ramírez-Jiménez *et al.* 2017). Fishing in LI is prohibited; however, small artisanal fishing remains. The boat traffic in the lake is highly reduced, and usually the manatee population remains undisturbed. Preliminary limited surveys in LI reported the presence of at least seven manatees on the lake (Pablo-Rodríguez and Olivera-Gómez 2012).

*Abundance and density estimate by boat trips.* Between April and June 2011, we conducted 14 boat trips (*ca.* 3 h each) in LI during daylight hours along an 18 km zigzag-like fixed transect, with a minimum distance of 20 m to the banks (Figure 1). During all the surveys, the boat path never changed. We used a 1.3 m length aluminum boat with a 15 HP four-stroke outboard engine (Honda, BJ 15, Japan), with a sustained speed between 2.5 and 7.0 km/h. During the surveys, we monitored the manatees observed with a side scan imaging sonar (Humminbird 987c SI, Eufaula, Alabama, USA). Two observers participated in the boat trips, one was focused on the sonar, and the other piloted the boat path and took notes. The observers were trained to detect manatees with the sonar. The sonar was operated at 450 kHz, and the beam had an 85° detection angle on the water column and was set to detect manatees within 20 m on each side of the boat. Screen images of all suspected manatees in the sonar were saved to verify later, using the procedure described by Gonzalez-Socoloske *et al.* (2009).

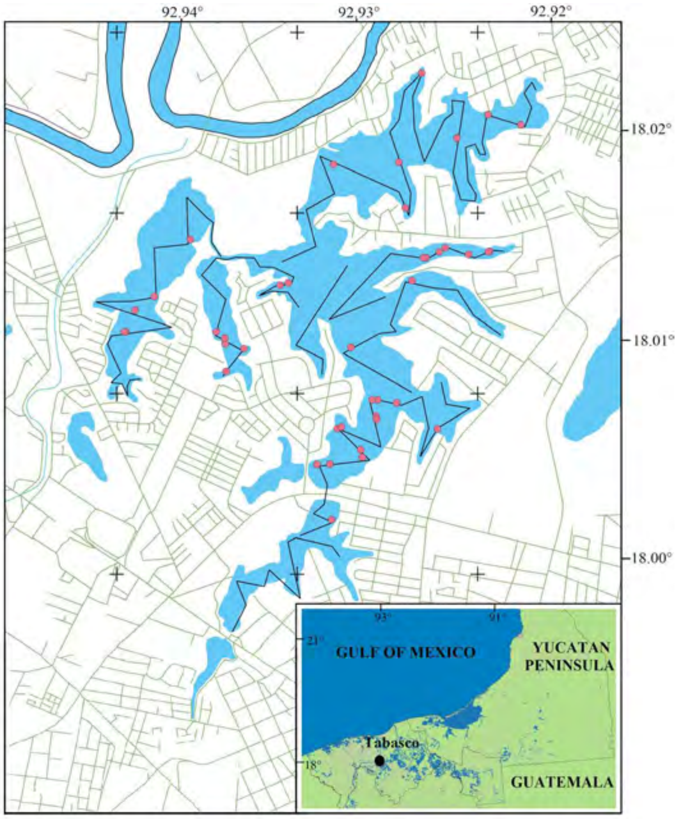

**Figure 1**. Study area in the lake of "Laguna de las Ilusiones", Villahermosa, Tabasco, México. The solid black line marks the boat route followed in the manatee surveys from April to June 2011. Red points indicate the location of detections with the Side Scan Imaging Sonar.

Coordinates and manatee group size were taken from all the sonar sightings. The perpendicular distances were estimated directly on the saved screen images. In the case of multiple individuals, the distance to the approximate geometric center of the group was estimated. Sighting depths were obtained directly from the sonar, and distance to the nearest bank was calculated on the polygon map of the lake using the software QGIS version 3.12.3-Bucuresti (QGIS 2021). We used the perpendicular distance to sighted individuals, with depth and distance to the nearest bank as covariates, to fit data distributions to several typical models using DISTANCE package (Miller 2022) in R (vers. 4.1.2). We pooled the distance of the 14 surveys to improve the fitting of the detection functions. Best models were selected using the Akaike Information Criteria for small samples (AICc) (Thomas *et al.* 2010), when the sample size is small or the number of parameters is large relative to sample size, AICc is recomended over AIC (Strindberg 2012). The estimated probability of detection was used to calculate the effective width of transects. Encounter rate was calculated manually for each survey, dividing the number of manatees sighted in a survey by the accumulated line kilometers traveled and then, density was obtained multiplying the encounter rate by the effective transect width obtained earlier in DISTANCE. Finally, the mean and standard error among all surveys were taken as the estimated parameter, and abundance (N) was calculated for the lake using the proportion of area sampled to the most recent calculation of the water surface area of the of LI made by Ricardez-de la Cruz *et al.* (2016).

*Mark-recapture models.* We used data from six capture events in LI conducted during the ten-year period between July 2006 and June 2016, with 19 individual encounter histories. Of the 19 individuals, three died at some moment during the ten-year period. Manatees were captured for radio tagging and health assessment (*e. g*., Morales-López *et al.* 2012; Aragón-Martínez *et al.* 2014; Ramírez-Jiménez *et al.* 2017). Captures were done with purse seine nets, and manatees were pulled up to the boat and towed carefully to a nearby lake shore where they were measured, biological samples obtained, and equipped with a standard VHF telemetry system (Morales-López *et al.* 2012; Ramírez-Jiménez *et al.* 2017). After the procedure, the animals were taken back to the capture site. All captures were conducted under federal permits (SGPA/DGVS/04060/06, 01103/07, 01754/09, 04675/10, 00646/16). A subcutaneous PIT tag (AVID, Norco, California, USA) was implanted in all the captured individuals in the dorsal area between the scapula and the occipital condyles, following Bonde *et al.* (1983). Manatee's recaptures were confirmed with a manual PIT tag reader (AVID, Norco, California, USA).

Mark-recapture data were stored in a binary matrix, where the rows are individuals and the different sampling events are columns, additional columns were added for sex and length at first capture event, the first for grouping and the last as a continuous covariate. This matrix was entered into the MARK software 8.2 (White and Burnham 1999). There is no individual migration to or from LI, but new individuals were born, and others died along the ten-year interval of sampling used for capture histories, reason because we used the Jolly-Seber approach, CJS model, for open populations in MARK with the POPAN formulation to explore variation when estimating manatee abundance. An average of nearly one manatee dies in the lake per year; activities of cleaning the lake and people frequenting the shores ensure that practically all carcasses are found.

Parameters set in the models were: *phi* (probability of passing over to the next capture event), *p* (probability of capture), *pent* (probability of entrance), and *N* (population number). MARK estimates of parameters, CV (coefficient of variation), and CI (confidence intervals) were computed from the best-fit models. In the structure of models in MARK, sex of individuals was incorporated as grouping variable, as well as one continuous covariate: length at first capture. The simple model was that with all parameters set as constant (phi{.}p{.}pent{.}N{.}) and variants of it are those where parameters are a function of time(t), group (g) or covariate (cov). Models in MARK were compared using Akaike´s Information Criterion for small samples, AICc, and model likelihood (Burnham and Anderson 2002) and the best models were selected.

## Results

*Abundance and density estimate by boat trip.* We detected an overall of 59 manatees in 45 groups. The mean number of manatees detected per survey was 4.2 ± 2.7, with a maximum number of manatees per survey of nine and a minimum of one. We detected single manatees on 80 % of the sonar sightings, pairs were 13 %, and the rest were groups of three and four individuals. The mean depth where the manatees were detected in sonar sightings was 2.1 ± 0.6 m.

The best model for the distance function was the Half Normal key function (Figure 2). We observed that the likelihood ratio between the best model and the next was 0.9, and the transect width was truncated to 15.2 m, as it was the maximum distance for an observed manatee. The density and abundance estimates using the Distance detection function were 15.546 ± 2.575 manatees km$^{-2}$ (CV = 16.6%) and 27.1 ± 4.5 manatees in the lake.

*Mark-recapture models.* Table 1 shows the best two models that converged in MARK. In the best model, *phi* and *pent* were set as constant over time; *p* dependent on covariate (length at first capture encounter), and *N* was set to depend on group (gender). The abundance estimate from the best model was 24 manatees (CI 20 to 42; Table 1).

**Table 1**. Mark models, parameters, and estimates from six capture events and 19 individual encounter histories of manatees in Laguna de las Ilusiones, Tabasco, México. A is the best model and B is the second-best model.

| Model | N | S.E. | CI95 % | deltaAICc | likelihood |
|---|---|---|---|---|---|
| A)<br>phi{.} p{.,cov} pent{.} *N*{g}<br>phi= 0.997 ± 0.003,<br>p = 0.381 ± 0.088,<br>pent = 0.999 | Females 17<br>Males 7<br>Total 24 | 2.78<br>1.74 | 14 - 27<br>6 - 15<br>20 - 42 | 0.000 | 1.000 |
| B)<br>phi{.} p{.,cov} pent{.} *N*{.}<br>phi = 0.997 ± 0.003,<br>p = 0.381 ± 0.087,<br>pent = 0.999 | Total 15 | 1.70 | 14 - 21 | 0.642 | 0.725 |

phi = survival probabilities between successive occasions, p = capture probability, pent = probability of entrance, *N* = population number, g = grouping variable (gender), cov = covariate (length at first capture encounter).

## Discussion

There was no previous estimation of the density nor the abundance of manatees in the LI protected area; reports only gave the maximum number of manatees sighted along navigations in the lake (Pablo-Rodríguez and Olivera-Gómez 2012). Our results will serve as a baseline for management purposes in this lake, 19 manatees were captured in LI from 2006 to 2016, and 16 remained with pit tags, and an estimate of 22 to 32 individuals in the lake are likely. The density of 15.5 manatees km$^{-2}$ is lower than that reported in a hotspot area in the lower basin of the Usumacinta River (21.7 manatees km$^{-2}$, Puc-Carrasco *et al.* 2017). Compared with LI, where manatees are confined to a small but rounded area, surveys performed in other sites within Grijalva- Usumacinta River basin were done over stretch water courses, not much wider than the sonar reaching width increasing manatee encounter probability along the transect, as we noted that in this cases manatees behave remaining in the bottom or swimming parallel to path of the water course, and probably had an effect on the density calculation.

Even though this study estimates are helpful, there are several concerns about the proper method to gather information about population density or abundance in this kind of shallow environment with low water transparency and complex contours and with the assumptions the method requires, because we observed that manatees tend to be distributed towards some embayment and not following a desirable random dispersal.

Manatees fairly unreacted to boat approach, and this is part of the problem with collisions (Lima *et al.* 2015). Manatees react more when boat pass less than 15 m from the animal, if a deeper area is near, usually manatees heading toward it (Miksis-Olds *et al.* 2007) but heading, or reactions depend much on the activity level of the animal (Rycyk *et*

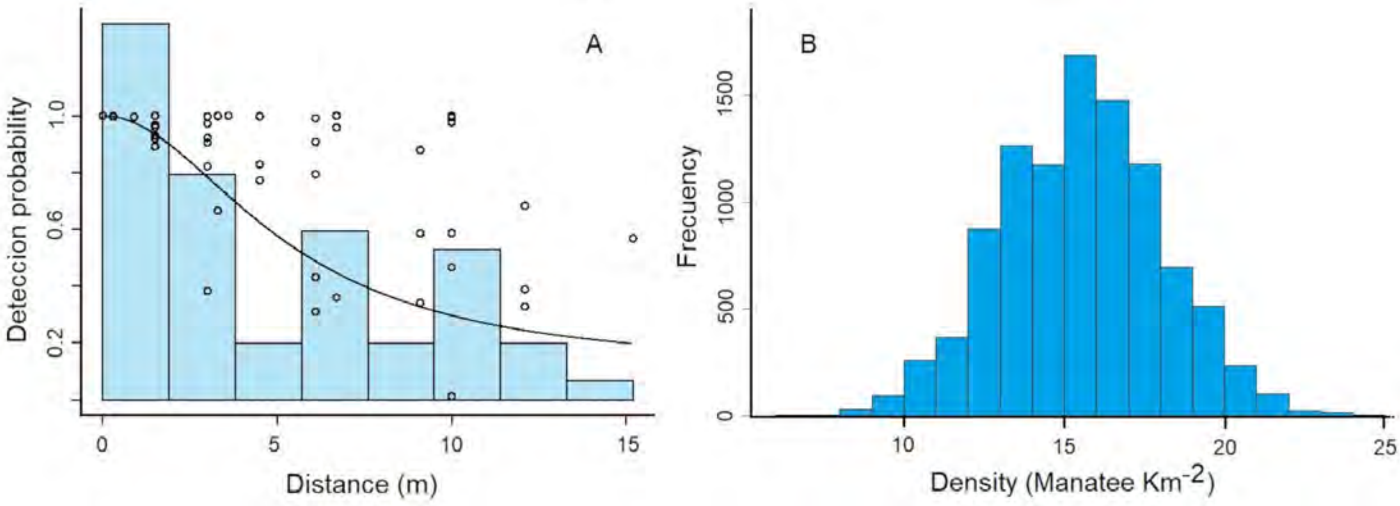

**Figure 2**. (A) Best fitted detection function (solid line) for manatee sightings from boat surveys, estimated in Distance in R from surveys conducted in the lake "Laguna de las Ilusiones" in Tabasco, México, between April and June 2011. Lake detpth and distance to the nearest bank were used as covariates. (B) Distribution of densities in the lake using 10,000 bootstrap sample means from the observed 14 survey mean densities of manatees km$^{-2}$.

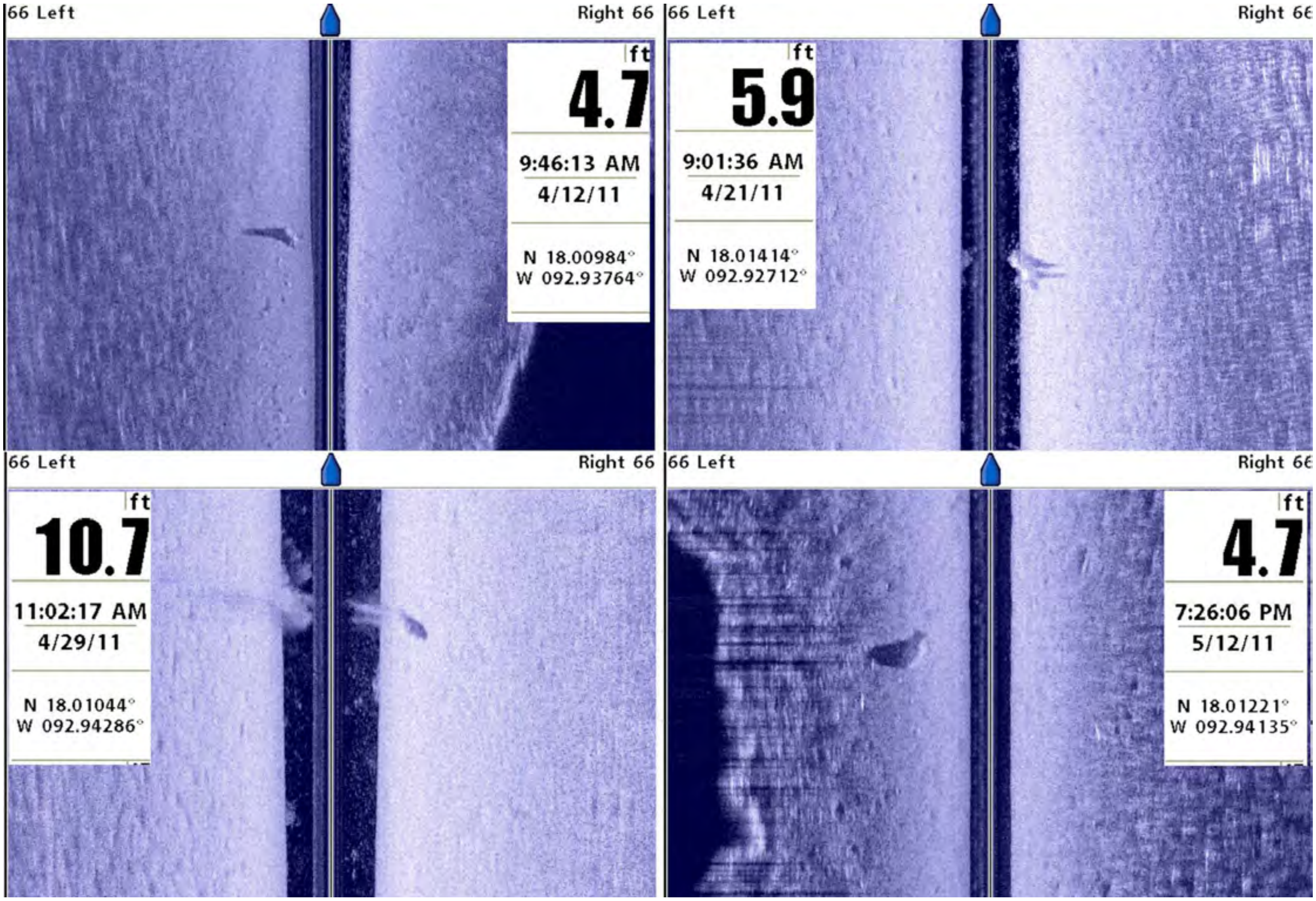

**Figure 3**. Examples of manatees sighted using side scan sonar (screen savings) on different dates along this study.

al. 2018). When manatees detected the boat, we observed two behavioral responses: the first, usually swimming away from the boat axis, but occasionally parallel or towards the ship; the second, remaining passive at the bottom of the lake. An observer using the visual method would be unable to detect manatees that adopted the passive behavior because the animals would not leave the distinctive sediment plumes or bubbles associated with swimming; sonar observers were able to detect these passive manatees, this being the main advantage of the method; boat speed must assure that manatees along the transect remain on it before the sonar pass. The relative position of manatees with respect to the boat and sonar beam axis could result in variations in the strength and quality of the object represented in the sonar screen.

Availability bias could also explain variation (Packard *et al.* 1985; Walker *et al.* 2000; González-Socoloske *et al.* 2009). Puc-Carrasco *et al.* (2016) estimated a correction factor of 20 % opportunistically during their sonar survey in a nearby area, based on a two-observers' procedure. Manatee´s differential daily and seasonal use of the study site, and being different from a random distribution, would also account as a source of variation (Ramírez-Jiménez *et al.* 2017), considering strata will serve to lower this effect, but it is difficult to implement them in a small area.

Estimates with mark-recapture and line transects produced close numerical results and a similar coefficient of variation. However, distance sampling is preferable because it is cheaper and less time-consuming. Recent studies obtained reliable results using sonar (*e. g.*, Puc-Carrasco *et al.* 2017; Guzmán and Condit 2017). The side scan sonar also shows advantages over other methods when estimating group size and discriminating small calves from larger individuals when they are together (González-Socoloske and Olivera-Gómez 2012). However, combining methods could bring insights into low-density populations (Gerrodette *et al.* 2011).

Capture-recapture model assumptions are difficult to meet with small groups of animals; compared to distance sampling, mark-recapture is known to be more sensitive to assumption violations (Thomas *et al.* 2002). The survival probabilities between occasions in the different models were high, 0.997 monthly and 0.965 yearly, in full accord with the mean number of deaths in the lake, almost one individual per year in data from 1984 to 2016.

Including the gender of the captured animals as a group improves the mark-recapture models. The model that did not account for gender produced an estimated 15 animals: a number smaller than the total of the tagged manatees alive at the last capture event. The length at first capture of each individual as a covariate contributed to the variance in capture probabilities, with young animals being more likely to be trapped than older ones along this study. The mark-recapture model was helpful in LI but is hard to replicate in other localities in the region. This method is expensive and laborious given that the low visibility environment restricted the use of individualized permanent marks such as those derived from boat collisions (Beck and Reid 1995), necessitating the capture of individuals to insert PIT tags. This approach could be considered as a long-term project for well-suited areas and with the proper funding.

It is a logistic and funding challenge to design surveys for the manatee´s inland populations in México and the rest of the range of Antillean manatees. Still, appropriate index localities, using available methods, could lead to viable regional conservation strategies, and the estimate of density could be a better parameter for comparison of overabundance.

The group of isolated manatees in LI is currently under high pressure due to the rapid growth of the Villahermosa urban area. Threats associated with urban development include the presence of pathogens such as *Leptospira* spp. (Aragón-Martínez *et al.* 2014), and genetic problems resulting from an isolated inbreeding group (Gómez-Carrasco *et al.* 2018). The undisputable presence of a couple of dozen animals at the time of this study should be considered in a management plan for this lake.

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# Updated distribution of the Texas kangaroo rat (*Dipodomys elator*) and patterns of rodent species associations from county road surveys in Texas

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The Texas kangaroo rat (*Dipodomys elator*) is a rare species of conservation interest at both the state and federal level. Therefore, an updated understanding of distribution and abundance of *D. elator* is critical for initiating informed decisions about its conservation status and subsequent management strategies. We surveyed more than 850 locations along unpaved county roads across the historical range of this species in north-central Texas to identify sites of *D. elator* presence and examine patterns of rodent species associations. We determined that *D. elator* presently occurs in five counties in Texas within its historical range and was the eighth most abundant species of the 14 species that we captured. Moreover, we found that the majority of pairwise species associations, including those involving *D. elator*, were random and there was no strong evidence that pairs of rodents were aggregating or segregating with respect to each other. We did observe negative associations between *D. elator* and both *Dipodomys ordii* (Ord's kangaroo rat) and *Sigmodon hispidus* (hispid cotton rat). Nonetheless, these patterns indicate that interspecific interactions do not play a strong role in influencing the distribution of *D. elator*. However, the restricted and temporally dynamic distribution of this species suggests that a metapopulation perspective should be considered when making future conservation considerations.

La rata canguro de Texas (*Dipodomys elator*) es una especie rara, de interés para la conservación tanto a nivel estatal como federal. Por lo tanto, un conocimiento actualizado de la distribución y abundancia de *D. elator* es fundamental para tomar decisiones informadas sobre su estado de conservación y subsecuentes estrategias de manejo. Estudiamos más de 850 localidades a lo largo de carreteras rurales sin pavimentar en el área de distribución histórica de esta especie en el centro-norte de Texas para identificar los sitios con presencia de *D. elator* y examinar los patrones de asociación de especies de roedores. Determinamos que *D. elator* se encuentra actualmente en cinco condados de Texas dentro de su área de distribución histórica y que es la octava especie más abundante de las 14 que capturamos. Además, descubrimos que la mayoría de las asociaciones de especies por parejas, incluyendo las que implicaban a *D. elator*, eran aleatorias y no hay evidencia sólida de que las parejas de roedores se estuvieran agregando o segregando entre sí. Observamos asociaciones negativas entre *D. elator* y *Dipodomys ordii* (rata canguro de Ord) y *Sigmodon hispidus* (rata algodonera crespa). No obstante, estos patrones indican que las interacciones interespecíficas no juegan un papel importante en la distribución de *D. elator*. Sin embargo, la distribución restringida y temporalmente dinámica de esta especie sugiere que debe tenerse en cuenta una perspectiva meta poblacional a la hora de realizar futuras consideraciones de conservación.

**Keywords:** *Dipodomys elator*; distribution; road surveys; small mammal; Texas kangaroo rat.

## Introduction

Accurate knowledge of species distributions is fundamental to conservation biology. Occurrence data are a valuable source of information for managers to guide and assess conservation planning. These data especially are important for rare species that pose additional logistical challenges due to low density, which may be further exacerbated by inaccessible habitat (McCain and Childs 2008; Kéry *et al.* 2010). Furthermore, many rare species may be highly temporally dynamic in terms of presence and abundance across their distribution (Hanski 1991), and continually updated information regarding species occurrences is important for promoting the most effective management strategies.

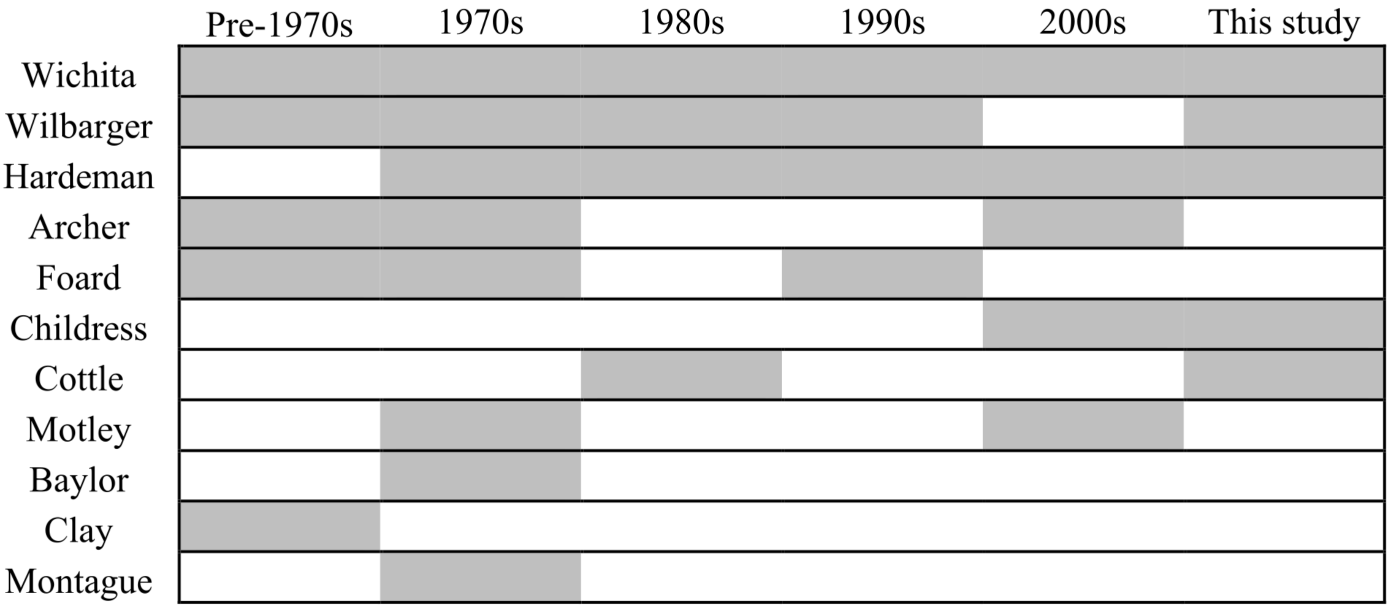
The Texas kangaroo rat (*Dipodomys elator*) is a rare, semi-fossorial rodent that has historically been documented in 11 counties in north-central Texas and two counties in southern Oklahoma, United States (Carter *et al.* 1985; Martin 2002; Schmidly and Bradley 2016). However, this species has not been observed in Oklahoma in over a century, except for one questionable record immediately north of the border with Texas, and is believed to be extirpated from that part of its historical range (Bailey 1905; Baumgardner 1987; Braun *et al.* 2021). The present distribution of *D. elator* within Texas is uncertain, in part because new records that expand the distribution of this species continue to be identified (*e. g.*, Martin 2002), while resurveys of previously inhabited sites have often failed to document the continuous presence of any *D. elator* individuals (*e. g.,* Nelson *et al.* 2013).

Because *D. elator* can be easily observed on unpaved roads at night (Martin and Matocha 1972), surveying county roads may be an effective means of discovering sites of occurrence. Prior to this study, *D. elator* had been known from six Texas counties: Clay (Merriam 1894; Bailey 1905), Wilbarger (Blair 1949; Dalquest and Collier 1964), Archer (Dalquest and Collier 1964), Foard (Packard and Judd 1968), Wichita (Packard and Judd 1968), and Baylor (Baccus 1971) (Figure 1). Martin and Matocha (1972), based on county road surveys, documented *D. elator* again in Archer, Foard, Wichita, and Wilbarger, as well as in two new counties: Hardeman and Motley, both to the west of the prior geographic range. Jones *et al.* (1988), also based on county road surveys, surveyed a total of 14 counties in Texas and documented *D. elator* in only four: Cottle (*i. e.,* a new county record), Hardeman, Wichita, and Wilbarger. More recently, Martin (2002) visited all of the counties in the historical range of *D. elator* and documented the species in five: Archer, Childress, Hardeman, Motley, and Wichita (Figure 1).

Although there appears to be consensus as to the general geographic range of *D. elator*, the results of previous surveys suggest a dynamic distribution, in that (1) hotspots of abundance were found in different portions of its geographic range at different time periods and (2) across many of these locations *D. elator* was encountered only sporadically through time. This, coupled with a decade-and-a-half long hiatus since the last range-wide survey, suggests that an update is paramount to understanding the present-day status of *D. elator*. Such an update via county road surveys would provide an important comparison to earlier studies. Furthermore, although interspecific interactions can have an important influence on distribution and abundance of rodent species (*e. g.,* Brown and Munger 1985), relatively little research has been conducted on patterns of rodent species associations with *D. elator* or their potential influence on distribution of this species.

## Materials and methods

Between June 2015 – August 2017, we surveyed 808 locations along dirt and gravel roads within the 11 counties in Texas where *D. elator* has been documented (Figure 2). An additional 60 sites were surveyed in Hall County between 24 – 26 March 2017 (Figure 2). While *D. elator* has never been documented in Hall County, Martin (2002) recommended additional surveys at the periphery of the historical range, particularly to the west, and Hall County is located directly to the northwest of the historical geographic range of *D. elator* (Figure 2). Although *D. elator* is believed to be active year-round (Dalquest and Collier 1964), we completed our surveys primarily during the spring, summer, and fall to avoid logistical challenges and potential mortality events associated with trapping rodents in below-freezing temperatures. We selected survey locations based primarily on vegetation preferences described for *D. elator*, which includes short, sparse grasses (Goetze *et al.* 2007; Sikes et al. 2016; Nelson *et al.* 2013), as well as sites in which burrows were present. At each site we placed a Sherman live trap (7.6 × 7.6 × 25 cm; H.B. Sherman Traps, Inc., Tallahassee, Florida) every 10 m along the side of the road over a total distance of 100 m. Traps were baited with rolled oats for one night and checked the following morning, for a total of 11 trap nights per site. Given the primary objective of this study, we opted for one night at each site in order to maximize the spatial coverage of our survey efforts. All transects were separated by a minimum of 200 m. Rodent handling adhered to Texas Tech



| | Pre-1970s | 1970s | 1980s | 1990s | 2000s | This study |
|---|---|---|---|---|---|---|
| Wichita | ▨ | ▨ | ▨ | ▨ | ▨ | ▨ |
| Wilbarger | ▨ | ▨ | ▨ | ▨ | | ▨ |
| Hardeman | | ▨ | ▨ | ▨ | ▨ | ▨ |
| Archer | ▨ | ▨ | | | ▨ | |
| Foard | ▨ | ▨ | | ▨ | | |
| Childress | | | | | ▨ | ▨ |
| Cottle | | | ▨ | | | ▨ |
| Motley | | ▨ | | | ▨ | |
| Baylor | | ▨ | | | | |
| Clay | ▨ | | | | | |
| Montague | | ▨ | | | | |

**Figure 1.** Summary of the county-level distribution of *D. elator* in Texas organized by decade, determined by the results of status surveys and other publications. Darker cells indicate detections of *D. elator* within a given county. Counties are arranged in order of the number of times *D. elator* has been detected.

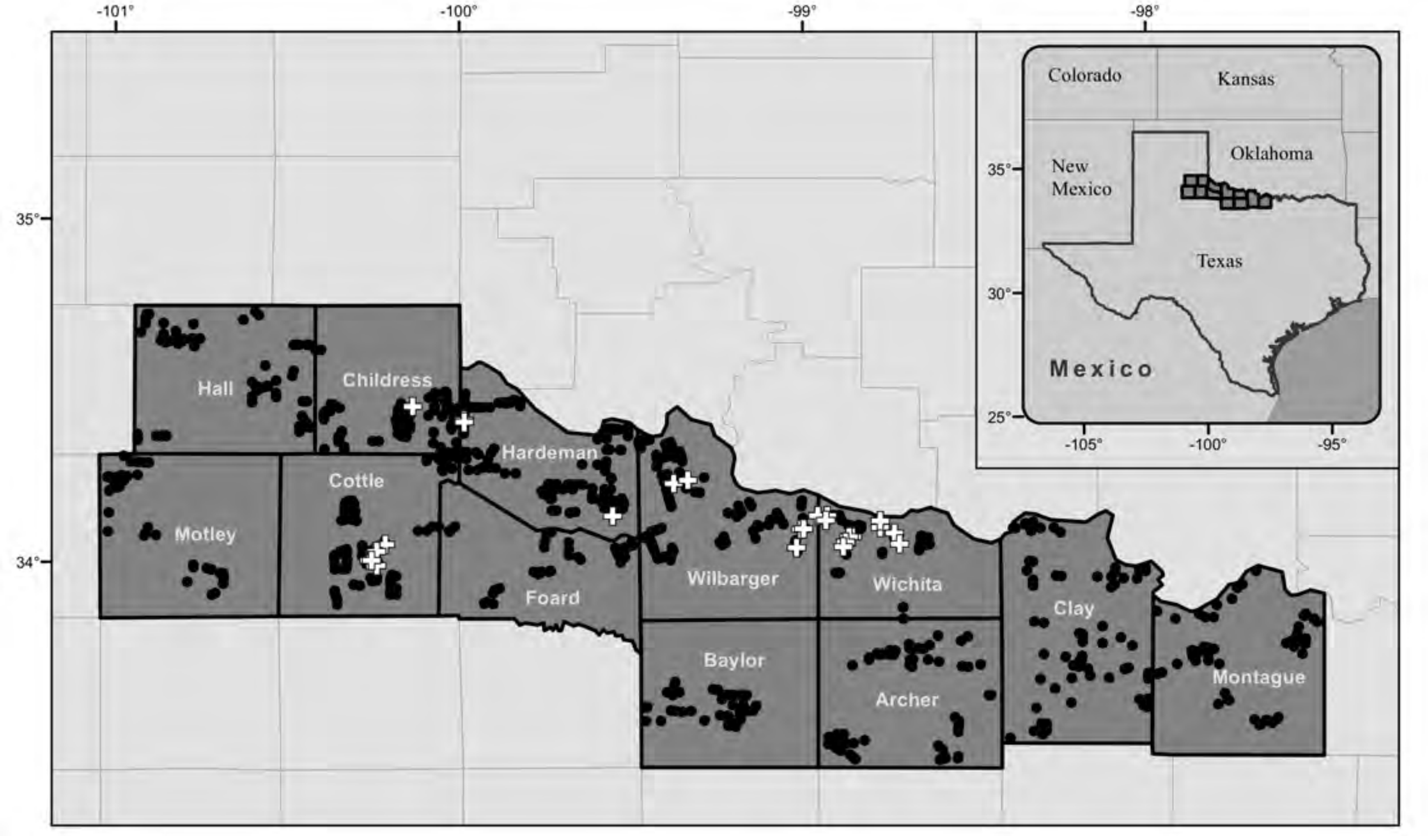


**Figure 2.** Results of county road surveys (n = 868 sites) across the historical distribution of *D. elator*, including Hall County, between 2015 – 2018. Dark circles indicate areas where traps were deployed but the species was not captured (*i. e.*, "absence" sites), whereas light crosses indicate areas where *D. elator* was captured (*n* = 26 localities).

University Institutional Animal Care and Use Committee Protocol 18013-02 based on guidelines approved by the American Society of Mammalogists (Sikes *et al.* 2016). We identified captured individuals to species (Schmidly and Bradley 2016) and collected and deposited voucher specimens in the collection at the Natural Sciences Research Laboratory (NSRL), Museum of Texas Tech University.

We examined spatial structure of rodent species composition based on a Canonical Correspondence Analysis (CCA; Ter Braak 1986). Geographic coordinates of latitude and longitude formed the independent matrix, and rodent species presence or absence across sites formed the dependent matrix. We examined the final solution of the CCA to determine if it accounted for more variation than expected by chance based on 10,000 permutations of the original data. If the amount of variation accounted for by the CCA based on the real data was greater than in 95 % of the applications to permuted data this was considered significant.

We analyzed patterns of species associations based on a site-by-species presence/absence matrix, excluding sites at which no species was detected, as well as the results from Hall County because we were primarily interested in species associations within the known geographic range of *D. elator*. After filtering, 481 sites were used for the species association analysis. We used the package *cooccur* (Griffith *et al.* 2016) in R (R Core Team 2020), which is based on the probabilistic model of species co-occurrence (Veech 2013). The probabilistic model determines the probability that the observed frequency of co-occurrence is significantly large and greater than expected (*i. e.,* a positive association), significantly small and less than expected (*i. e.,* a negative association), or not significantly different and approximately equal to expected (*i. e.*, a random association; Griffith *et al.* 2016). The expected co-occurrence of any two species is the product of the two species' probability of occurrence multiplied by the number of sampling sites (Griffith *et al.* 2016).

## Results

We captured 35 *D. elator* at 26 (3 %) of 868 surveyed sites (Figure 2) in five counties: one site in Childress, six sites in Cottle, three sites in Hardeman, ten sites in Wichita, and six sites in Wilbarger. Seven of these sites had more than one *D. elator* individual (maximum: three individuals), and 16 of these sites had other species (*i. e.*, 1-2) present. We captured fourteen rodent species in total (Figure 3). The five most abundant and widely distributed species were *Sigmodon hispidus*, *Dipodomys ordii*, *Chaetodipus hispidus*, *Peromyscus maniculatus*, and *Peromyscus leucopus*, respectively (Figure 3, Table 1). *Dipodomys elator* occurred at the eighth most sites (Table 2) and was the eighth most abundant species (Figure 3, Table 1). Furthermore, *D. elator* shared six sites each with *P. leucopus* and *P. maniculatus*, four sites each with *C. hispidus* and *S. hispidus*, and one site each with *D. ordii*, *P. laceianus*, and *R. fulvescens* (Table 1). The *D. ordii* individual that occurred at the same site as *D. elator* was a juvenile that we believe was dispersing.

**Table 1**. Species list from the road surveys indicating the total number of individuals captured, number of sites each species was captured at, and the number of sites at which a particular species co-occurred with *D. elator*.

| Species | Total Individuals | Number of sites | Co-occurrences w/ *D. elator* |
|---|---|---|---|
| *Baiomys taylori* | 7 | 6 | 0 |
| *Chaetodipus hispidus* | 182 | 134 | 4 |
| *Dipodomys elator* | 35 | 26 | -- |
| *Dipodomys ordii* | 210 | 119 | 1 |
| *Neotoma leucodon* | 9 | 8 | 0 |
| *Neotoma micropus* | 8 | 7 | 0 |
| *Onychomys leucogaster* | 37 | 27 | 0 |
| *Perognathus merriami* | 38 | 32 | 0 |
| *Peromyscus attwaterii* | 3 | 3 | 0 |
| *Peromyscus laceianus* | 2 | 2 | 1 |
| *Peromyscus leucopus* | 116 | 85 | 6 |
| *Peromyscus maniculatus* | 159 | 99 | 6 |
| *Reithrodontomys fulvescens* | 13 | 13 | 1 |
| *Sigmodon hispidus* | 318 | 154 | 4 |

Although weak, significant spatial structure was exhibited by species within the study area. The first two canonical axes accounted for only 2.66 percent of the variation among sites in presence/absence of species but this was significantly greater than expected by chance alone. *Dipodomys elator* exhibited essentially no spatial structure across its geographic range (Figure 4). All other species exhibited varying degrees of spatial structure. *Baiomys taylori*, *Peromyscus attwateri*, and *P. laceianus* exhibited the greatest spatial structure with the former two species more common in the eastern portion of the study area and the latter more common to the west (Figure 4).

Out of 91 possible species pairwise combinations characterizing the rodent community, 28 pairs (30.8 %) were removed from the analysis because the expected co-occurrences of these pairs was less than one site, indicating that many species were too rare to meaningfully use in analyses. Of the remaining 63 pairs, 35 of the associations were random, one was positive, and 27 were negative. For *D. elator* specifically, there were no positive associations with other species but significant negative associations with *D. ordii* and *S. hispidus* (Figure 5). This means that the species occurred at the same sites as these species less often than expected based on their overall presence across all sites (*D. ordii*: expected number of sites: 7.4, observed number of sites: 1; *S. hispidus*: expected number of sites: 10.1, observed number of sites: 4). In contrast, *D. ordii* displayed significant negative associations with eight other species and one significant positive association with *Onychomys leucogaster* (Figure 5).

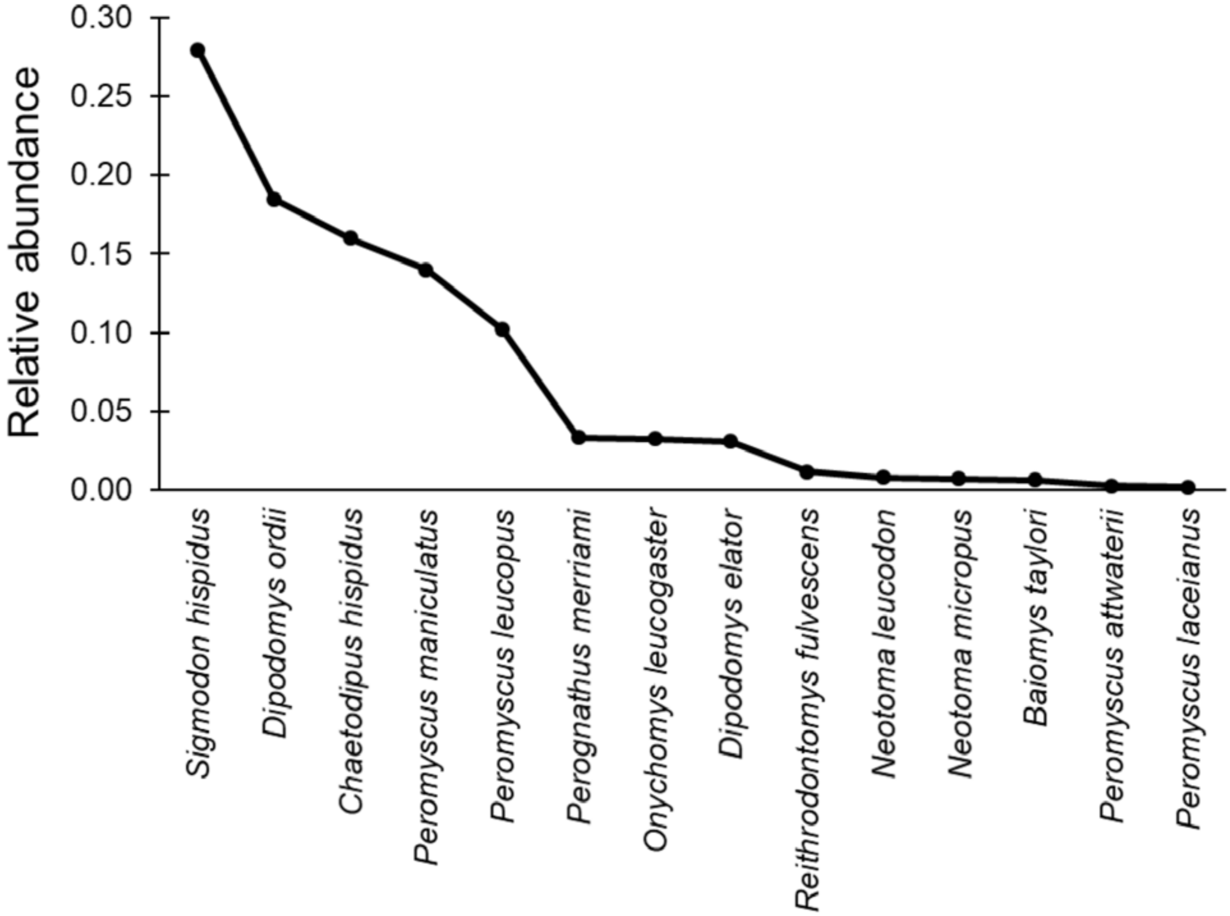


**Figure 3.** Rank-abundance curve for the rodent community (*n* = 14 species) based on the county road surveys. The y-axis denotes the proportional abundance of every species (along the x-axis) within the overall species pool.

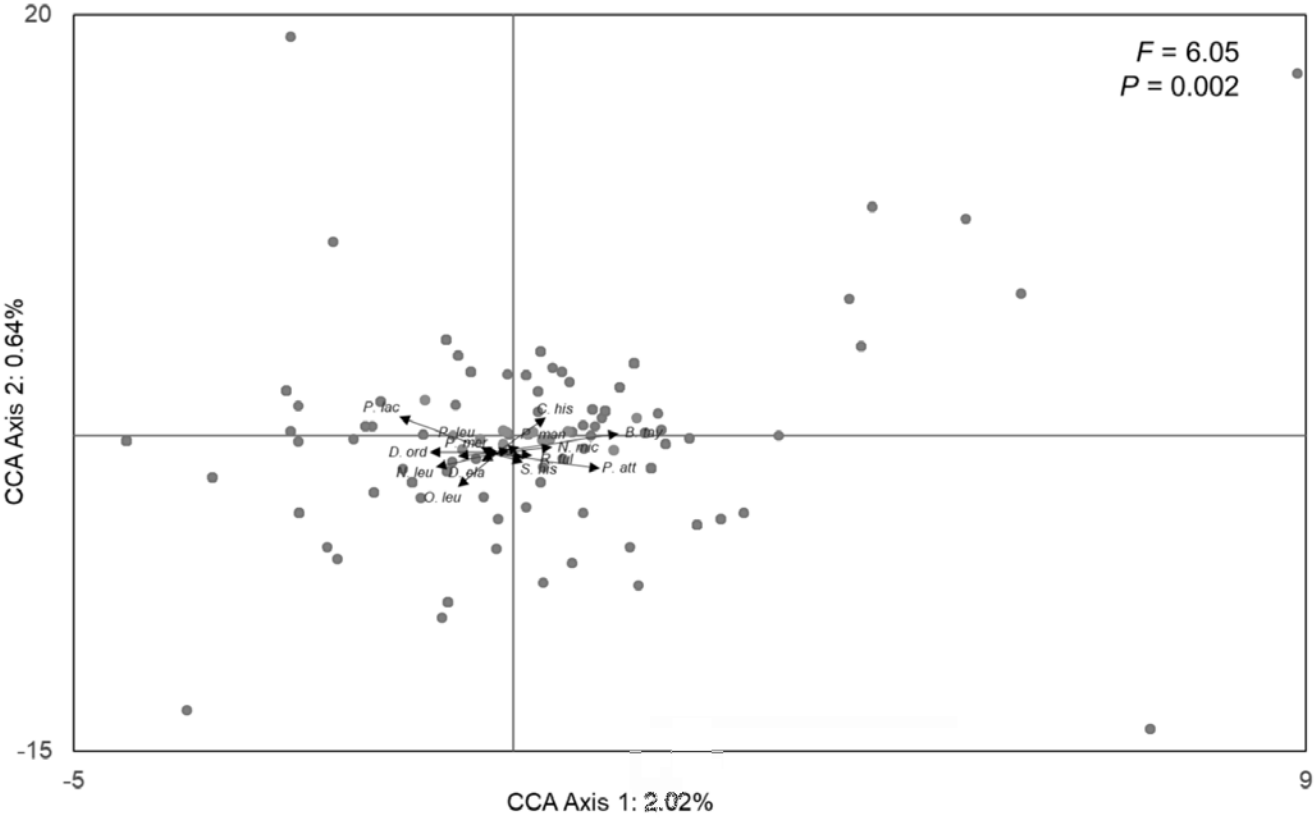


**Figure 4.** Results from canonical correspondence analysis (CCA) depicting the amount of spatial structure in the distribution of rodent species occurring in the geographic range of *D. elator*. CCA Axis 1 corresponds to a west to east (small to large values) gradient whereas CCA Axis 2 corresponds to a south to north axis (small to large values). Length and orientation of arrows indicated how correlated species are to a particular axis. A long arrow that is parallel to a particular CCA axis indicates a strong correlation.

## Discussion

We conducted roadside surveys for *D. elator* across its historical geographic range (Figure 1). Our results suggest that this species (1) presently occupies less than half of the Texas counties from which it was previously documented and (2) occurs sporadically in both space and time throughout its distribution. Furthermore, a majority of possible pairwise species associations were random, including for *D. elator*, suggesting that interspecific interactions do not strongly structure rodent communities within the distribution of the Texas kangaroo rat.

The spatial distribution of *D. elator* described by the current study was similar to that reported by recent surveys (Martin 2002; Ott *et al.* 2019). Importantly, *D. elator* was encountered in the same five counties as Ott *et al.* (2019), a study that was conducted over a time period corresponding to our study, and four of the same five counties as Martin's earlier study (2002). This suggests that the regional distribution of *D. elator* has remained relatively stable over the last two decades, although site-level persistence may be much more variable (*e. g.*, Nelson *et al.* 2013). Martin (2002) suggested that *D. elator* may be shifting its distribution to the periphery of its historical geographic range, and in particular to the west, but we did not find support for this hypothesis. Although *D. elator* was not captured in Clay or Montague Counties, where the species has not been detected for several decades (*e. g.*, Martin 2002), Wichita County had the highest number of *D. elator* capture sites (Figure 2). Similarly, Wilbarger County also had several sites of presence (Figure 2). In contrast, *D. elator* was only captured on the eastern edge of Childress County, near the border with Hardeman County, and no Texas kangaroo rats were captured in Motley County or during ancillary surveys in Hall County (Figure 1). Based on these results, there is no indication that the species is shifting to the western portion of its range. Because much of the research on *D. elator* has been conducted at small scales (*e. g.*, Martin and Matocha 1991; Stangl *et al.* 1992; Nelson *et al.* 2009, 2011; Goetze *et al.* 2007, 2016), future investigations should incorporate comparisons of habitat and population characteristics across the entire region (*e. g.*, Nelson *et al.* 2013). Such an approach would identify differences between the eastern and western portions of the species' range and persistence of *D. elator* in these areas.

There was no evidence that interspecific interactions are strongly influencing *D. elator* distribution patterns. As with most other species, a majority of the interspecies associations with *D. elator* were random, and only two were negative (*i. e.*, *D. ordii* and *S. hispidus*; Figure 4). Although anecdotal accounts suggest that *D. elator* may be a relatively docile, unaggressive species (Goetze *et al.* 2008), and that *D. ordii* is comparably more aggressive (*e. g.*, Perri and Randall 1999), it is more likely that the negative associa-

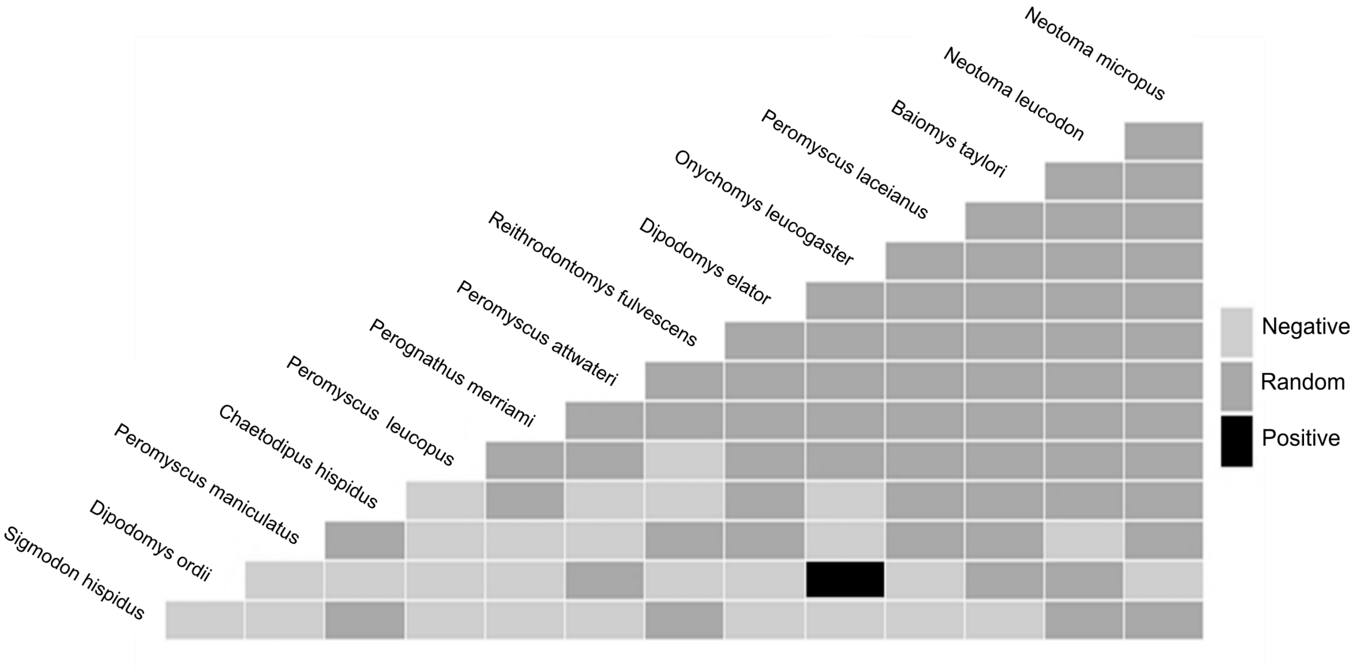


**Figure 5.** Species co-occurrence matrix from the road survey sites, excluding Hall County, for which there were significant positive, negative, or random associations based on the probabilistic model of species co-occurrence. Note that associations for which there was not sufficient data to detect are also categorized as random.

tions documented herein are a product of differences in habitat associations between *D. elator* and both *D. ordii* and *S. hispidus*. In particular, *D. elator* is often associated with clay-loam soils (Roberts and Packard 1973; Goetze *et al.* 2007), whereas *D. ordii* is associated with habitats with sandy soils (Garrison and Best 1990; Schmidly and Bradley 2016). Moreover, while *D. elator* is typically found in sparse, short grassland habitat (Roberts and Packard 1973; Stangl *et al.* 1992; Nelson *et al.* 2009), *S. hispidus* utilizes grass-dominated habitats (Cameron and Spencer 1981). This is notable because *S. hispidus* and *D. ordii* were the two most abundant species in this region (Figure 3) and occurred at the highest and third highest number of sites, respectively (Table 1). The pervasiveness of these two species in the rodent community, given their different habitat associations in relation to *D. elator*, suggests a lack of suitable habitat for *D. elator* along roadsides and in adjacent pastures across this region (Goetze *et al.* 2016). Such unsuitable habitat conditions along roadsides and within pastures could have consequences to dispersal patterns because *D. elator* likely uses roadsides and pasture margins as movement and/or dispersal corridors (Roberts and Packard 1973; Stangl *et al.* 1992).

Most of the earlier surveys for *D. elator* were performed along roads to verify presence within its current range (*e. g.*, Jones *et al.* 1988; Martin 2002). However, Goetze *et al.* (2016) found more frequent use of pastureland relative to adjacent roadsides, likely because dense concentrations of introduced grasses along roadsides negatively affected *D. elator* (*e. g.*, impeding burrow construction and/or movements). Nelson *et al.* (2013) suggested that, despite not documenting *D. elator* at any of the same sites as Martin (2002), there were large amounts of potential habitat on private land. Greater effort should therefore be given to accessing private land to obtain complementary estimates of *D. elator* distribution and abundance. Nevertheless, access to private land remains difficult and there is little public land in this portion of Texas, such that county roads remain the best available option for both studying and managing this species in a range-wide context. More focus should therefore be given to understanding how *D. elator* utilizes different road types for movement, foraging, etc. (*e. g.*, Roberts and Packard 1973; Brock and Kelt 2004) as well as the suitability of roadsides as habitat for *D. elator* (Goetze *et al.* 2016). Such information will be critical for developing management strategies.

A number of range-wide surveys for *D. elator* have been performed over the last four decades (*e. g.*, Martin and Matocha 1972; Jones *et al.* 1988; Martin 2002; Nelson *et al.* 2013; Ott *et al*. 2019). Although these surveys have provided updates as to the distribution of *D. elator*, and despite indications that the distribution is changing (*e. g.*, Martin and Matocha 1991; Martin 2002), no study has evaluated these changes. This is significant because these changes suggest that *D. elator* forms a metapopulation that exhibits local extinction and recolonization dynamics (Hanski 1991). Directly incorporating a metapopulation perspective when investigating distribution and abundance of threatened and endangered species can improve our understanding of such dynamics and our ability to manage these species (Hanski 1991). Thus, future studies should examine *D. elator* within a metapopulation framework to better understand the importance of different characteristics to *D. elator* persistence across the landscape (Halsey *et al.* 2022).

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# Taxonomic, functional, and phylogenetic diversity of primate communities in Ecuador

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One of the fundamental objectives of ecology is to study the relative importance of ecological, evolutionary, and stochastic processes in determining local community structure. Many studies have focused on taxonomic diversity, paying relatively little attention to other dimensions of biological diversity, such as phylogenetic and functional diversity. Little is known about how these dimensions relate to each other, and the ecological processes that influence their variation. In this study, we characterize these three dimensions of biodiversity in 14 primate communities from different ecosystems in Ecuador to understand possible mechanisms responsible for their assembly. Results show that coastal communities are taxonomically less diverse than those from the Amazon and present different functional groups. On the other hand, phylogenetic diversity is higher in Amazonian communities, showing a tendency for overdispersion (high values of $MPD_{PD}$ = mean phylogenetic distance per pair and low PSC = degree to which coexisting species are related). This indicates a possible influence of biological factors, such as competition, on community assembly. For all three dimensions, climatic variables were the most significant predictors of community structure, while vertical forest structure contributed significantly to variation in the phylogenetic dimension. The high functional diversity reported in this study highlights the importance and vulnerability of this group and the ecosystems they inhabit. Macroecological studies, such as the one presented here, allow a better understanding of community structure and provide important information for the development of conservation strategies.

El estudio de la importancia relativa de los procesos ecológicos, evolutivos y estocásticos en la determinación de la estructura de las comunidades es un objetivo fundamental de la ecología. Muchos de los estudios se han centrado en la diversidad taxonómica y prestan relativamente poca atención a otras dimensiones de la diversidad biológica, tales como la filogenética y la funcional. Se conoce poco sobre cómo se relacionan estas dimensiones entre sí, y sobre cuáles son los procesos ecológicos que influyen sobre su variación. En el presente trabajo se caracterizan estas tres dimensiones de la diversidad en 14 comunidades de primates de diferentes ecosistemas del Ecuador, buscando entender los posibles mecanismos responsables de su ensamblaje. Los resultados muestran que las comunidades de la costa son taxonómicamente menos diversas que las de la Amazonia y presentan grupos funcionales diferentes. Por otro lado, la diversidad filogenética es mayor en las comunidades amazónicas, mostrando una tendencia a la sobre dispersión (altos valores de $MPD_{PD}$ y bajo PSC), lo que indica la posible influencia de factores biológicos, y particularmente la competencia como posibles determinantes del ensamblaje de esas comunidades. Para las tres dimensiones, el factor climático influencia la estructura de las comunidades de primates, mientras que para la dimensión filogenética la estructura vertical del bosque afecta en gran parte su variación. La gran diversidad funcional reportada en este estudio pone en evidencia la importancia y vulnerabilidad de los primates y los ecosistemas que habitan. Los estudios macroecológicos permiten comprender mejor los factores que influyen en la composición de las comunidades animales y proveen información importante para el desarrollo de estrategias de conservación.

***Keywords:*** Community structure; functional traits; Neotropical primates; phylogenetic relationships; predictive variables.

## Introduction

One of the main objectives of ecology is to understand the coexistence patterns of species and identify the mechanisms regulating the assembly of biological communities (Llorente-Bousquets and Morrone 2003). In this context, several hypotheses have been proposed regarding the relative importance of deterministic and stochastic processes in community assembly (Schöener and Haken 1986; Hubbell 2001) which varies depending on the spatial and temporal scales of measurement (Gavilanez and Stevens 2013; Plasencia-Vázquez *et al.* 2014; Stevens and Gavilanez 2015; Aguirre *et al.* 2016). Deterministic hypotheses propose that community composition is determined by niche differentiation according to the principles of competitive exclusion. This hypothesis prioritizes deterministic biotic interactions or abiotic filtering mediated by niche conservatism (Weiher *et al.* 2011). Environmental filtering (stress tolerance) proposes that the similarity of species within a given community increases due to abiotic restrictions (Cornwell *et al.* 2006). On the other hand, ecological differentiation (niche partitioning, limitation of similarities) proposes that ecological interactions prevent similarities between coexisting species (MacArthur and Levins 1967; Chesson 2000). On the other hand, stochastic models consider processes such as dispersal limitation and demographic drift, which produce assemblage patterns that can explain spatial autocorrelation in the presence of species, regardless of environmental variables. Particularly, dispersal limitation proposes that the presence of species in a community is limited by their ability to reach the site (Hurtt and Pacala 1995; Beaudrot and Marshall 2011).

Recently, studies focused on the multiple dimensions of diversity have been developed (Webb *et al.* 2002; Petchey and Gaston 2006; Cadotte *et al.* 2011; Srivastava *et al.* 2012) to better understand the mechanisms underlying local community assembly, as well as distribution and diversity patterns at broader scales (Jarzyna and Jetz 2016; Brum *et al.* 2017). Additionally, approaches that directly consider the effect of species on ecosystems, such as functional diversity, have been developed (Tilman *et al.* 1997; Gómez-Ortiz and Moreno 2017). Likewise, new strategies to evaluate the evolutionary relationships of species through their phylogeny have been proposed (Webb *et al.* 2002). These new approaches for assessing diversity, such as functional and phylogenetic diversity, aim for a comprehensive quantification of biodiversity (Rosenzweig 1995; Cadotte *et al.* 2011; Rattis *et al.* 2018). However, few studies have assessed diversity using these approaches simultaneously (Weinstein *et al.* 2014; Stevens and Gavilanez 2015; Brum *et al.* 2017).

Studies on mammals and the multiple dimensions of biodiversity seek to understand the processes involving these vertebrates within ecosystems. Several of these studies consider characteristics such as body size, relating them to the functions provided by mammals within their natural habitats (Smith and Lyons 2011). Safi *et al.* (2011) suggested that phylogenetic diversity and species richness increase in relation to mean annual temperature, while functional diversity decreases along with a higher seasonality. González-Maya *et al.* (2016) reported that functional diversity in mammal communities within the Neotropics decreases with the degradation of ecosystems and the loss of threatened species. On the other hand, Oliveira *et al.* (2016) found that species richness and functional diversity are decoupled in various regions of the world, and that species richness is closely correlated with environmental conditions while functional diversity depends mainly on non-equilibrium factors, including the evolutionary time to overcome the conserved niche. According to this analysis, species-rich regions (especially the Neotropics) could have many species that may be functionally redundant.

Primates are one of the most seriously threatened animal groups in tropical areas, mainly due to habitat loss, deforestation, and fragmentation (Stevenson 2016; Brum *et al.* 2017; Roncancio *et al.* 2010; Bueno *et al.* 2013; Rattis *et al.* 2018). They play central ecological roles in ecosystems as dispersers, pollinators, predators, and prey. Additionally, they are part of the diet of various native cultures in the region (Cueva 2005; de la Torre 2010; de la Montaña 2013). In Ecuador, primates have been studied in aspects such as conservation status, demography, diversity, diet, distribution, and survival in forest patches under anthropic pressure (Lizcano *et al.* 2016; Cervera *et al.* 2017). Although these studies are an important contribution to the knowledge of primates, they have favored a one-dimensional perspective of diversity (*i. e.*, taxonomic diversity) without considering their evolutionary history and ecological function (Cisneros *et al.* 2014; Brum *et al.* 2017).

The present study focuses on characterizing in multiple dimensions of diversity Ecuadorian primate communities inhabiting different ecosystems of Ecuador, and evaluating the influence of environmental, structural, and spatial factors as possible assembly mechanisms of these communities.

## Materials and methods

The characterization of primate communities of Ecuador was conducted through a systematic survey of literature, using databases such as Scopus, Google Scholar, and ISI Web of Science, using the following keywords (in English and Spanish): "primate community + Ecuador", "primate diversity + Ecuador", "primates + Ecuador". We also reviewed theses and unpublished reports issued between 1989 and 2017. Studies that met our selection criteria were used to ensure data comparability (Table 1). Primate community composition (incidence) for the selected study sites were obtained from the papers. Spatial coordinates were projected in UTMs and later converted to WGS 84. This procedure allowed for the spatial reference to be compatible with the raster files containing altitude data and type of ecosystem (MAE 2013).

Taxonomic diversity was characterized using presence/absence data for each study site. Functional diversity was estimated based on morphological, ecological, and behavioral data of the recorded species based on the information available in PanTHERIA (Jones *et al.* 2009) and All the World's Primates (Rowe and Myers 2016) databases. We included variables related to body weight, body size, home range, and population density, which are related to how individuals interact with each other and the environment (Lefcheck *et al.* 2015). In addition, niche breadth of each species was estimated based on the number of ecosystems they inhabit in Ecuador, which was determined using species range maps and a layer with information on the ecosystems of mainland Ecuador (MAE 2013), usingQGIS version 2.10 (QGIS Development Team2015).

Information regarding primate species diet was obtained from the database published in the database All the World's Primates by Rowe and Myers (2016). We also conducted a thorough literature search regarding diet of each of the species reported. Based on this information, the following functional characteristics were determined:

*Trophic breadth*: Maximum number of food categories used by a species, with 13 being the highest number. For this category we grouped species in three levels: low (between 1 and 4 categories), medium (between 5 and 9 categories), and high (between 10 and 13 categories).

*Percentage of fruit in the diet*: Percentage of fruit in the total food consumed was calculated based on the food records reported in the All the World's Primates database (Rowe and Myers 2016).

**Table 1.** Criteria considered for the study selection regarding primate communities of Ecuador.

| N | Criteria | References |
|---|---|---|
| 1 | Actual sightings, indirect records not considered. | Gavilánez and Stevens 2013 |
| 2 | Study duration (≥21 days). | Buckland *et al.* 2010 |
| 3 | Methodology, 10 km transects considering important areas in each ecosystem, flexibility in ravines and rivers, among others. | Buckland *et al.* 2010 |
| 4 | Works covering 5 % of the study surface. | Gavilánez and Stevens 2013 |
| 5 | Data from long-term studies with available information (presence/absence). | This study |
| 6 | Communities separated from each other by 10 km (avoiding pseudo-replicate samples), considering different ecosystems and biogeographical and anthropogenic barriers. | Ayres and Clutton-Brock 1992;<br>Naka and Brumfield 2018 |
| 7 | Nocturnal monkeys (*Aotus* spp.) excluded due to their different habits. | Gavilánez and Stevens 2013 |

*Trophic guilds*: Trophic guilds used in this study were adapted from those proposed by Benchimol and Peres (2014). Five trophic guilds were defined: 1 = Folivore-facultative frugivore: species that consume leaves and some fruits according to availability; 2 = Frugivore-folivore: species that feed mainly on fruits and leaflets; 3 = Frugivore-insectivore: species that feed mainly on fruits, insects, and sometimes leaflets; 4 = Granivore-frugivore-insectivore: species with a wide food range, mainly seeds, fruits, and insects according to their availability; 5 = Insectivore-frugivore-gummivore: species that mainly consume insects, fruits, bark, and exudates.

We calculated the Gower index using functional characteristics to build a distance matrix. This matrix was used to estimate the functional diversity indexes FD, FDISP, $MPD_{FD}$, and $MNTD_{FD}$, which characterize the diversity and dispersion of species in the functional space (Table 2).

Finally, phylogenetic diversity was characterized using the phylogeny by Kuhn *et al.* (2011), updating the nomenclature to Tirira *et al.* (2020). The phylogenetic diversity indexes PD, PSC, $MPD_{PD}$, and $MNTD_{PD}$ (Table 2) were calculated based on metrics by Webb *et al.* (2002) and Helmus *et al.* (2007).

Similarity between communities was evaluated via cluster analysis, which also served for comparing the diversity between the resulting groups (functional and phylogenetic). Gower distance was used for functional diversity and divergence times, in millions of years, for phylogenetic diversity. This analysis was performed to assess whether different functional and phylogenetic groups of primates could be identified. All analyses were performed in R.

To determine the influence of different assembling mechanisms on the variability of the taxonomic, functional, and phylogenetic structure of primate communities, three groups of predictor variables were defined (environmental/environmental filtering - X1, spatial/ dispersal limitation - X2, and structural/competition - X3). These variables are key to diversity and composition patterns of mammal communities, including Neotropical primates (Plasencia-Vázquez *et al.* 2014; Aguirre *et al.* 2016; Gavilanez and Stevens 2013). Lastly, a variance partitioning analysis was applied (Borcard *et al.* 1992; Legendre and Legendre 1998; Legendre and Gallagher 2001) to discriminate the extent to which the variables contribute to the variation in the taxonomic, functional, and phylogenetic dimensions of primate community structure and whether they do so in isolation or synergy.

Environmental data were obtained from the BioClim database using a 30s (~1 km$^2$) spatial resolution (Hijmans *et al.* 2005) using the coordinates of each locality using QGIS (QGIS Development Team 2015). A principal component analysis (PCA) of 19 bioclimatic variables was performed to obtain a subset of orthogonal axes (Legendre and Legendre 1998). Based on this analysis, six representative environmental variables (that represented more than 90% of variability in environmental data) were selected to evaluate their influence on community structure (Table S4). The influence of spatial processes associated with dispersal limitation (Beaudrot and Marshall 2011) was assessed with a matrix of Euclidean distances between the identified communities. Forest structure elements, particularly canopy height (Oliveira and Scheffers 2019), are variables related to the availability of resources and niches (Gouveia *et al.* 2014), therefore associated with competition. Canopy height data were obtained from the layers created by Simard *et al.* (2011), which resulted from the use of a "LIDAR" device. This information for each community identified was obtained by overlapping the corresponding raster layer.

Statistical analyses were performed in R version 4.1.1 (R Core Team 2017) using the packages Vegan (Oksanen *et al.* 2018), FD (Laliberté *et al.* 2014), picante (Kembel *et al.* 2010), and spatstat (Baddeley and Turner 2005).

## Results

Of the 192 studies reviewed, 46 were conducted in Ecuador, and 14 primate communities that met the established requirements were selected. Four of these communities were distributed in the coastal region and ten in the amazon region. The total number of species recorded was 17, representing 80 % of the diversity of primates in Ecuador. The community with the highest richness was located in the surroundings of the Kiwcha settlements in the northern region of the Yasuní National Park (Amazon region), with 12 species. In contrast, communities with lowest rich-

**Table 2**. Functional and phylogenetic diversity indexes selected for the analysis of primate community structure in Ecuadorian communities.

| | **Index** | **Characteristic** | **Reference** |
|---|---|---|---|
| Functional | FD | Sum of the length of branches of a functional dendrogram built through a cluster analysis. | (Petchey and Gaston 2006) |
| | FDISP | Mean distance of each species to the centroid of the community in the functional trait space. | (Laliberté *et al*. 2014) |
| | $MPD_{FD}$ | Calculates the mean distance per pair that separates taxa based on a matrix of functional distances between species. | (Webb *et al.* 2002) |
| | $MNTD_{FD}$ | Calculates the mean distance of the nearest taxon for each species pair based on a matrix of functional distances. | (Webb *et al.* 2002) |
| Phylogenetic | PD | Calculates the sum of the total phylogenetic branch length for species coexisting in a community. | (Helmus *et al*. 2007; Kuhn *et al*. 2011) |
| | PSC | Measurement of the degree to which coexisting species are related by comparing with the expected variance of a hypothetical trait that evolves neutrally. | (Helmus *et al*. 2007) |
| | $MPD_{PD}$ | Mean phylogenetic distance per pair between all possible pairs of species coexisting in a community. | (Webb *et al*. 2002) |
| | $MNTD_{PD}$ | Mean minimum phylogenetic distance of the nearest taxon for a community. | (Webb *et al*. 2002) |

ness were in western part of Ecuador, near the coast, Jama Coaque and Pacoche, with two species each (Table 3). The 14 communities covered nine ecosystems, three in the coastal region and six in the amazonn (Figure 1).

A marked variation was found in the functional attributes (Table S2). Average weight for the species registered was 3,088.4 ± 2,807.4 g (range: 123.94 to 9,067.9 g). Average size (head and body) was 387.6 ± 118.1 (154.6 to 576.3) mm. Of the recorded species, *Cebus aequatorialis* was found in the largest variety of ecosystems. Furthermore, we observed variations between communities in functional characteristics related to diet. The primate community with the highest number of trophic guilds was Kichwa, with five guilds. The most common guild was granivore-frugivore-insectivore, with 13 species, while the least common was frugivore–insectivore, with two species. Most of the recorded species had a narrow trophic breadth. The "high" trophic breadth was the least represented category, absent in six communities. The community near the Kichwa settlements had the highest number of fruit-eating species in their diet (See Table S3).

Again, the community with the highest diversity in all dimensions was the Kichwa community in the Amazon (Table 4), which showed a wide range of coexisting functional groups and evolutionary lineages. On the other hand, some coastal communities showed low functional diversity values, although functional diversity indexes such as $MPD_{FD}$, were relatively high since the species that compose

Table 3. Geospatial data. richness (S). and composition of primate communities used for the analyses.

| **Community** | **Latitude** | **Longitude** | **Elevation (m)** | **S** | ***Alouatta palliata*** | ***Alouatta seniculus*** | ***Ateles belzebuth*** | ***Ateles fusciceps*** | ***Plecturocebus discolor*** | ***Cheracebus lucifer*** | ***Cebuella pygmaea*** | ***Cebus aequatorialis*** | ***Sapajus macrocephalus*** | ***Cebus capucinus*** | ***Lagothrix lagotricha*** | ***Pithecia napensis*** | ***Pithecia milleri*** | ***Leontocebus lagonotus*** | ***Leontocebus nigricollis*** | ***Leontocebus tripartitus*** | ***Saimiri cassiquiarensis*** | **References** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kichwa | -0.4538 | -76.4406 | 248 | 12 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | Cueva 2005 |
| Cuyabeno | -0.5874 | -75.4706 | 221 | 8 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | de la Torre *et al*. 1995 |
| Kutukú Foothills | -2.585 | -77.7672 | 315 | 7 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | Zapata Ríos *et al*. 2006 |
| Jama Coaque | -0.1158 | -80.1249 | 294 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Whyte 2005 |
| Oglán | -1.3202 | -77.6193 | 477 | 5 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | Carrillo-Bilbao y Martín-Solano 2010 |
| Pacoche | -1.0334 | -80.8333 | 292 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Cervera *et al*. 2015 |
| Payamino | -0.5097 | -77.2796 | 318 | 5 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | Gavilánez-Endara 2013 |
| South Pompeya | -0.7021 | -76.4383 | 250 | 6 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | Pozo 2004 |
| Colonso-Chalupas Reserve | -0.7017 | -77.9691 | 300 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | Álvarez-Solas *et al*. 2016 |
| Cayapas River | 0.9156 | -78.9113 | 111 | 3 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Madden y Albuja 1989 |
| San Miguel River | 0.2778 | -76.3928 | 286 | 6 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | Zapata Ríos 2001 |
| Tesoro Escondido | 0.5419 | -79.1449 | 280 | 3 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Miller *et al*. 2016 |
| Tiputini | -0.6167 | -76.1667 | 246 | 9 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | Blake *et al*. 2010 |
| Station Tiputini | -0.6379 | -76.1497 | 220 | 9 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | Marsh 2004 |

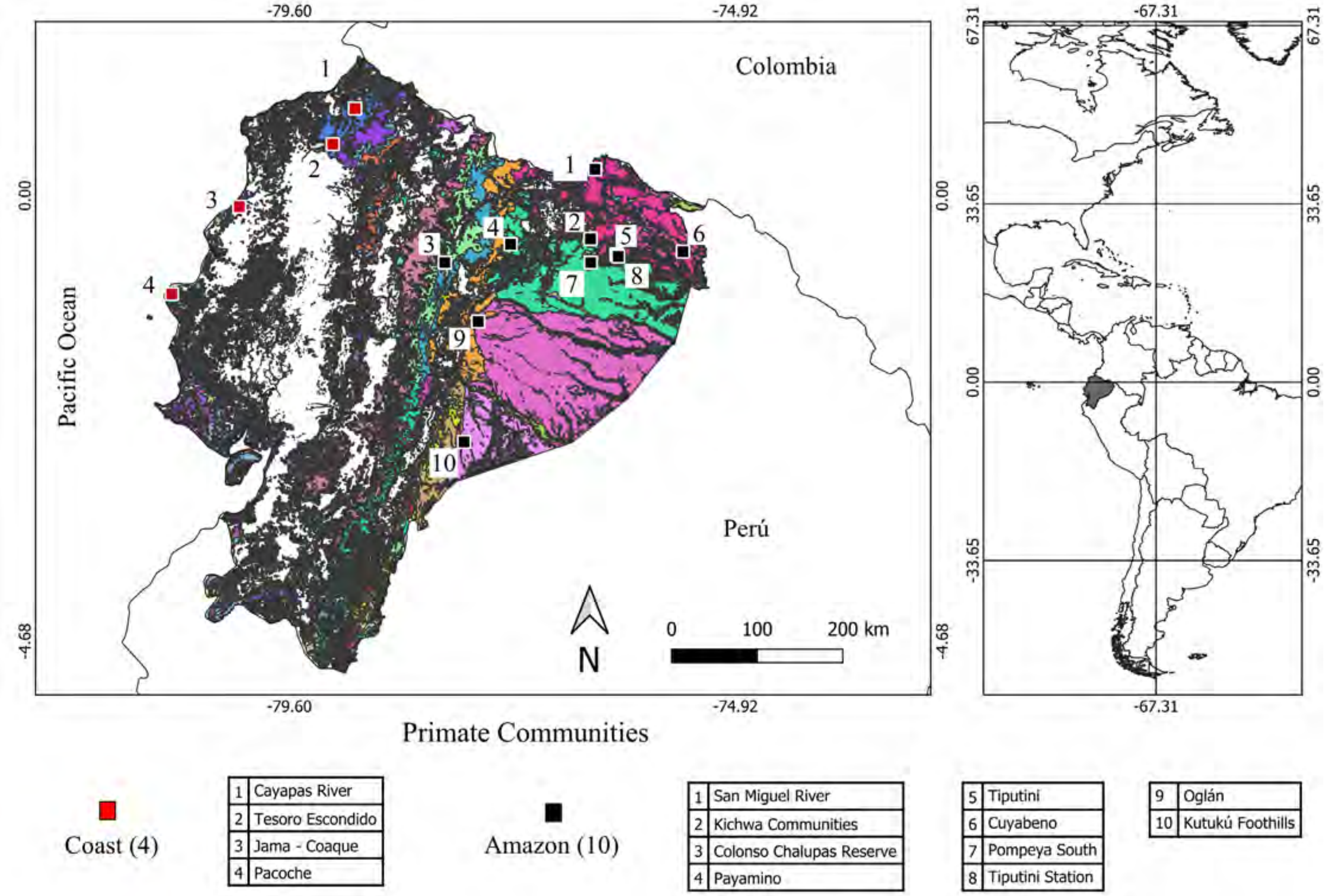

**Figure 1.** Ecuador map indicating the 14 primate communities considered in the study.

them differ functionally. Despite having an intermediate richness, the community of the San Miguel River showed the greatest functional dispersion (FDISP; mean distance of each species to the centroid of the composition), with a high $MPD_{FD}$ value. The primate community in Pompeya Sur had the lowest functional dispersion and was composed of functionally similar species (low $MNTD_{FD}$ values).

The 17 species identified in the 14 communities were grouped into four primate families (Figure 2). The Kichwa community showed the greatest phylogenetic diversity (Table 4), with a PD value of 163.2. The $MPD_{PD}$ index, representing the mean phylogenetic distance between species pairs, was higher for Pacoche and Jama-Coaque communities since the species in them belong to different and phylogenetically distant families ($MPD_{PD} = 40.5$). By contrast, the primate community inhabiting the foothills of Kutukú had the most phylogenetically related species ($MNTD_{PD} = 0.19$). The Phylogenetic Species Clustering (PSC) index indicated that the communities with the phylogenetically closest species were Rio Cayapas and Tesoro Escondido, which are geographically close in the northwest of the country, within the equatorial Chocó.

Five functional groups were identified (Figure 2). Species of the family Atelidae were clustered into two functional groups. *Ateles fusciceps, Alouatta palliata,* and *A. seniculus* were more closely related in terms of body weight, body size, and trophic breadth, while the group of *Ateles belzebuth* and *Lagothrix lagothricha* shared the same trophic breadth and guild. The representatives of the family Pitheciidae formed two functional groups. The species of the genus *Pithecia* were functionally similar to *Cebus aequatorialis* in terms of body weight and body size, and shared almost the same trophic guild. The titi monkeys of the genera *Cheracebus* and *Plecturocebus* were functionally related to *Sapajus macrocephalus* and *Saimiri cassiquiarensis*, sharing the same trophic guild and a similar home range. The species of the family Callithrichidae formed a single functional group with a similar home range, trophic guild, size, and weight. The phylogenetic clustering showed that pitheciids and atelids are the oldest families in the study area.

As for taxonomic diversity, both environmental (X1) and spatial (X2) variables separately explained the highest variation (X1 = 28 % and X2 = 24 %, respectively) in the taxonomic composition of the communities. On the other hand, structural variables (X3) only accounted for 1 % of the variation. Functional diversity, environmental variables (X1), and forest structure (X3) were associated with a

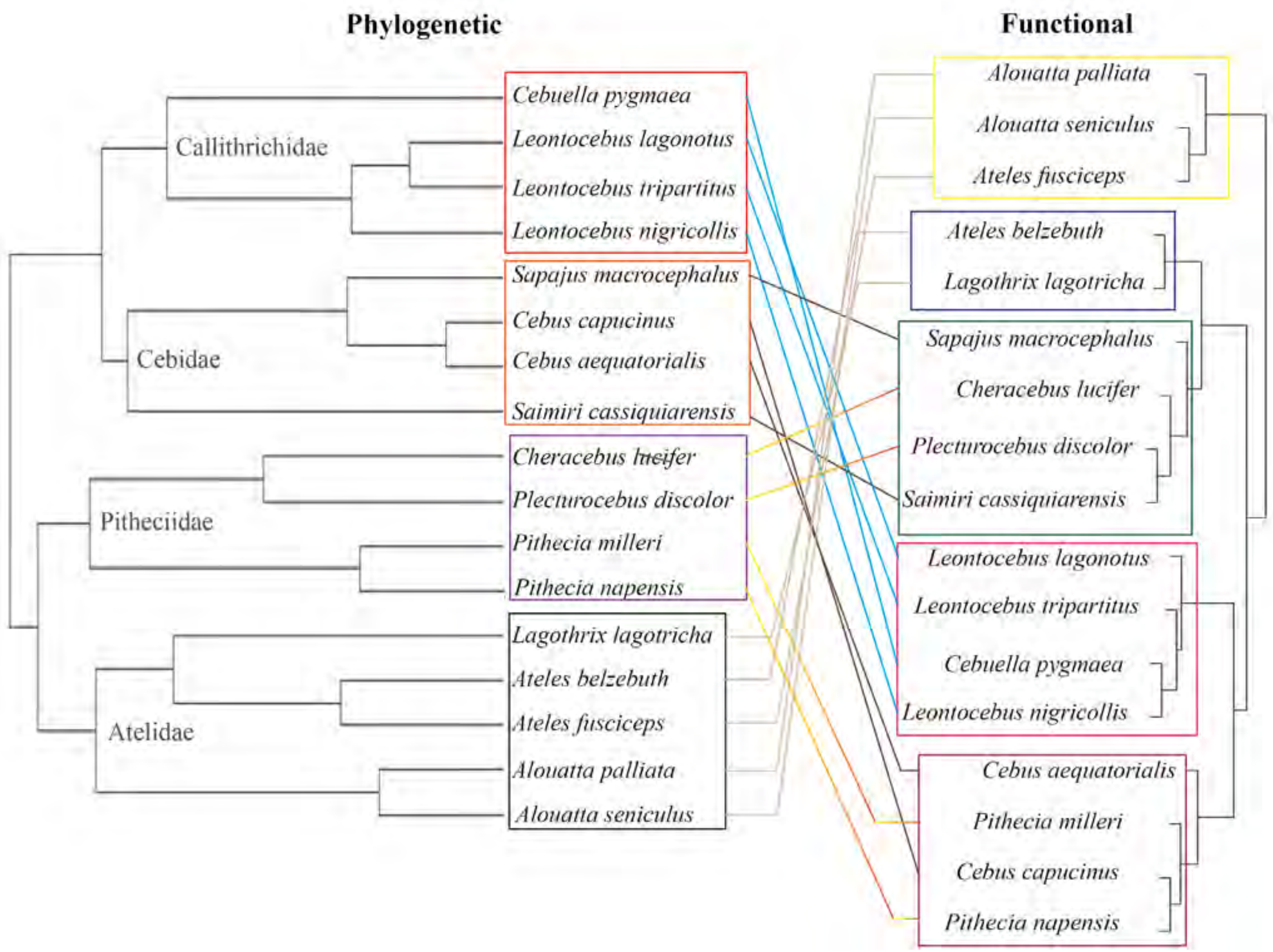

**Figure 2.** Contrast of phylogenetic and functional diversity between groups. Squares mark groups; lines indicate the distribution based on functional traits.

greater variation in the functional diversity of communities (25 %). Finally, the cluster that included the three predictor variables explained 25 % of the variation in phylogenetic diversity (Figure 3).

## Discussion

The taxonomic diversity recorded in the present study is consistent with the one reported by Sampaio *et al.* (2018) in communities of the southern Amazon, Purus state, Brazil, reflecting the high diversity of mammals that characterizes the western Amazon (Voss and Emmons 1996). This great diversity has been related to the large rivers that limit species dispersal (Ayres and Clutton-Brock 1992; Van Roosmalen *et al.* 2002). It has also been reported that the high diversity of primate species in the Amazon region is associated with high fruit production levels (Stevenson 2016; Camaratta *et al.* 2017) and structural complexity that creates microhabitats due to the different orography in the region (Homeier *et al.* 2010).

Communities of the western region show a low diversity (S = 4) and are represented by endemic, and highly threatened species such as *Ateles fusciceps* and *Cebus capucinus*, which inhabit the easternmost section of the tropical Andes hotspot in the Chocó area. These areas, and the primate communities that inhabit them, are subject to environmental, biotic, and anthropic pressures that influence at the local (behavior) and macro (distribution) levels, affecting their composition, diversity patterns, and roles in the ecosystems (Kamilar and Beaudrot 2018; Kaisin *et al.* 2020).

At the functional level, the variety of guilds (*n* = 5) and broad trophic niche of the species were important, mainly in Amazonian communities. Multiple species presented complementary functional traits that are important in the functioning of ecosystems (Pereira-Bengoa *et al.* 2010; Córdova-Tapia and Zambrano 2015). The most common trophic breadth category was low (1 to 4 food types in the diet), indicating that most registered species have a level of specialization in their diet, which can make species sensitive to forest conversion (Cervera *et al.* 2017). On the other hand, species with broad trophic niche (*e. g.*, *Sapajus macrocephalus*) were recorded to include between 10 to 14 food types in their diet. These were common in Amazonian regions where resource availability may be higher. In some cases, when a generalist species becomes locally extinct, its ecological role may be assumed by another species (Galetti *et al.* 1994; Stoner *et al.* 2003; Link *et al.* 2006; Gómez-Posada 2012).

**Table 4.** Functional diversity (FD) and phylogenetic diversity (PD) indexes of the 14 primate communities analyzed in the study.

| | **Fuctional** | | | | **Phylogenetic** | | | |
|---|---|---|---|---|---|---|---|---|
| **Comunidades** | **FD** | **FDISP** | **MPD$_{FD}$** | **MNTD$_{FD}$** | **PD** | **PSC** | **MPD$_{PD}$** | **MNTD$_{PD}$** |
| Kichwa | 3.463 | 0.230 | 0.335 | 0.168 | 163.227 | 0.4268 | 36.554 | 23.252 |
| Cuyabeno | 2.940 | 0.237 | 0.352 | 0.195 | 139.328 | 0.223 | 37.882 | 31.535 |
| Kutukú Foothills | 2.541 | 0.220 | 0.332 | 0.198 | 111.976 | 0.356 | 36.699 | 26.125 |
| Jama Coaque | 1.194 | 0.169 | 0.338 | 0.338 | 40.562 | 0 | 40.562 | 40.562 |
| Oglán | 2.234 | 0.237 | 0.367 | 0.252 | 70.898 | 0.209 | 35.979 | 32.100 |
| Pacoche | 1.194 | 0.169 | 0.338 | 0.338 | 40.562 | 0 | 40.562 | 40.562 |
| Payamino | 2.214 | 0.220 | 0.343 | 0.233 | 91.726 | 0.154 | 37.876 | 34.319 |
| South Pompeya | 1.933 | 0.199 | 0.299 | 0.153 | 103.406 | 0.239 | 36.625 | 30.875 |
| Colonso-Chalupas Reserve | 1.519 | 0.222 | 0.345 | 0.222 | 70.922 | 0.205 | 35.912 | 32.235 |
| Cayapas River | 1.497 | 0.202 | 0.351 | 0.305 | 57.319 | 0.116 | 38.213 | 35.864 |
| San Miguel River | 2.562 | 0.247 | 0.374 | 0.252 | 103.149 | 0.243 | 36.590 | 30.703 |
| Tesoro Escondido | 1.497 | 0.202 | 0.351 | 0.305 | 57.319 | 0.116 | 38.213 | 35.864 |
| Tiputini | 3.082 | 0.235 | 0.347 | 0.168 | 152.906 | 0.252 | 37.785 | 30.342 |
| Station Tiputini | 3.082 | 0.235 | 0.347 | 0.168 | 152.906 | 0.252 | 37.785 | 30.342 |

Coastal communities comprise the same trophic guilds (facultative folivore - frugivore and granivore - frugivore - insectivore), indicating lower interspecific competition levels associated with resource availability. Differences in the diet of coexisting species (howler, capuchin, and spider monkeys) have been attributed to historical competition events that led to divergent dietary choices or foraging techniques (Fleming 1979; Arcos *et al.* 2013; Cervera *et al.* 2015). However, it is worth highlighting that all coastal species include at least a low proportion of fruit in their diet, contributing to the ecological role of this functional trait (seed dispersers) in these ecosystems. Therefore, these species, and their disappearance can have a long-term impact on western tropical ecosystems, which are highly disturbed in Ecuador (Urbina 2010).

Although functional characteristics of species suggest how they interact with each other and with the environment (Cadotte *et al.* 2011; Meachen and Roberts 2014; Gómez–Ortiz and Moreno 2017), it is necessary to analyze the other dimensions of diversity. The comparison between the phylogenetic and functional clustering of primate species in the communities analyzed in this study showed that relationships between species are defined by the way in which they use the resources, creating cohesive functional groups that reflect an important phylogenetic dispersion, as in the case of Cebidae and Pithecidae. However, callitrichids had a conserved trophic niche, because they are very similar in body size, trophic niche breadth, and trophic guild. These species use the same resources, potentially reducing their coexistence; this is confirmed by analyzing the distribution maps of the species (IUCN 2016), which show no overlap. Furthermore, the atelids formed two subgroups with different functional characteristics.

Communities with high taxonomic diversity, such as those in the lower Amazon, showed patterns of phylogenetic overdispersion (high MNTD$_{PD}$ and MPD$_{PD}$ and low PSC values). This illustrates the coexistence of species representative of ancient (pitheciids and atelids) and recent taxa (cebids and callitrichids), as well as a high functional diversity (high FD and FDISP), indicating that the resources available for use by primate species are diverse (Cooper *et al.* 2008; Kamilar and Guidi 2010).

For the phylogenetic dimension, the best predictor of community structure was structural variability associated with strata diversity, which may be related to a high environmental heterogeneity and niche partitioning among different primate species in a community. Structural variation can foster the coexistence of species with similar requirements and functions, contributing to highly diverse communities, such as those reported in the Amazonian region (Arcos *et al.* 2013; Gómez-Ortiz and Moreno 2017).

Kamilar *et al.* (2015) suggested that zones with climatic stability favor a higher speciation rate. This could be reflected in the communities inhabiting the lower Amazon, which show high phylogenetic diversity. By contrast, the structure of communities within the dry seasonal forests of the Coast, where diversity is lower, seems to be governed by processes related to limited dispersal due to the Andes Mountain range barrier (Beaudrot and Marshall 2011). However, these ecosystems may harbor higher endemism in some groups, including vertebrates (Olguín-Monroy *et al.* 2013).

Our results suggest that both deterministic (environment and habitat structure) and stochastic processes (dispersal) play central roles in the structuring of equatorial primate communities (Cadotte *et al.* 2009; Flynn *et al.* 2011). Part of the variation not explained in this study could be addressed by considering interspecific interactions, spatial scale, and seasonality (Belmaker and Jetz 2013; Stevens and Gavilanez 2015; Weinstein *et al.* 2017).

Regardless of other factors, predictions considering the spatial dimension were the most important to explain taxo-

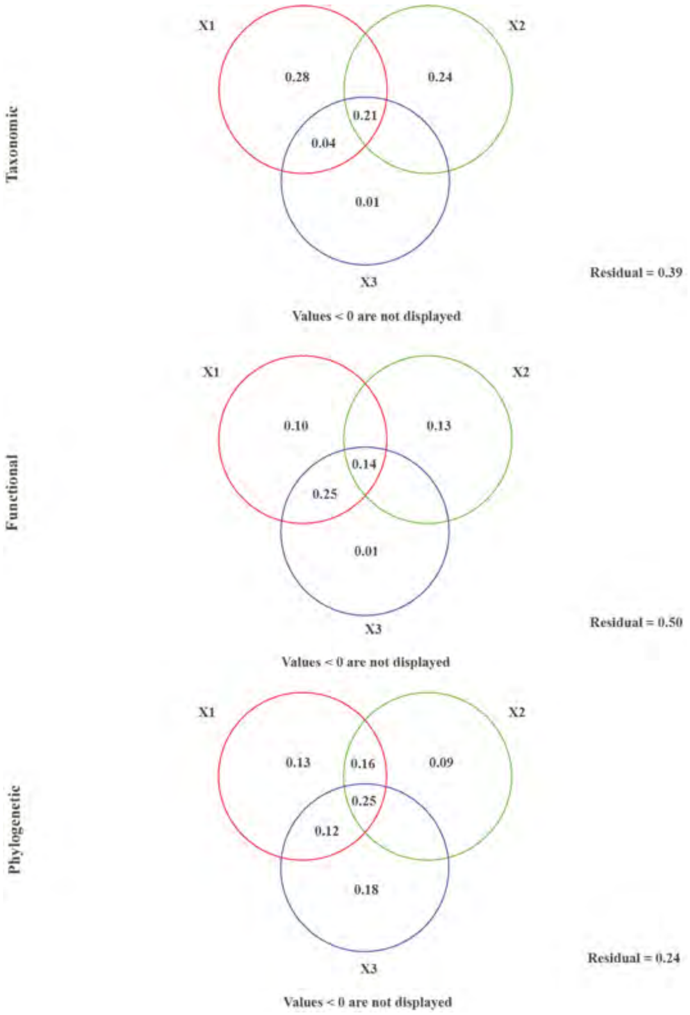

**Figure 3.** Representation of the variance partitioning analysis with a Venn diagram for the three dimensions of biodiversity: X1 = environmental, X2 = spatial (dispersal), X3 = structural (canopy strata).

nomic diversity. These results are supported by Beaudrot and Marshall (2011), who state that dispersal limitation is the primary mechanism in structuring primate communities. Neutral processes (Hubbell 2001) related to spatial factors were important for the taxonomic and phylogenetic dimensions of biodiversity. Our findings show that the distribution of closely related species in communities may be controlled by stochastic factors, such as random speciation, extinction, and ecological drift (Pavoine and Bonsall 2011).

There is an urgent need to understand community diversity patterns and their assembling mechanisms from a perspective encompassing beyond the taxonomic dimension. Our study highlights the complementarity of the information provided by different dimensions of biodiversity. Therefore, diversity should be assessed in a multidimensional way to better understand the mechanisms responsible for the establishment and persistence of communities and their ecological functions in ecosystems. Our findings support the importance of conducting diversity analyses on a spatial scale broader than local communities to make inferences on the ecological processes that influence the assembling and persistence of diversity, particularly in highly diverse communities such as those of Neotropical primates in Ecuador. This study shows that a varied resource availability (structure) could partly define the composition of these communities by reducing competition between species. Finally, our results provide valuable information to develop conservation strategies for Ecuadorian primates, as the roles of spatial processes and environmental and structural variables, and their association with the multiple dimensions of biodiversity, should be considered to set priority areas of conservation in a better way and ensure their maintenance over time. In this way, the environmental issues currently facing these communities and ecosystems can be comprehensively addressed.

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## Supplementary material

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# Population trend and ecology of the most isolated deer in the world, Bawean deer (*Axis kuhlii*): conservation challenges

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The Bawean deer plays a vital role in its small and isolated ecosystem as a herbivore and effective seed disperser, as well as holds cultural importance to the local community. However, the ecology of this Critically Endangered deer is poorly studied. Using random encounter and occupancy modeling based on 29,350 camera trap days between 2017 and 2019, we aimed to provide population estimates, habitat preferences, and behavioral data for this species. The population was 120–277 mature individuals, much less than the number in 1978. The density of Bawean deer could be related to the type of forest and the predation by free-roaming dogs as well as other factors such as the increase of wild pigs on Bawean Island. According to the best occupancy model, the tall and community forests far from human settlements are the most suitable areas for this species. Bawean deer is mainly crepuscular with significant daytime activity. Our results point out free-roaming dogs as a major threat to the native mammal community on Bawean island. We suggest the Bawean deer be listed as Critically Endangered following criteria B1a,b (ii, iii, v) of IUCN. Therefore, effective law enforcement and an adequate conservation strategy, including free-roaming dog control, are required to reduce the impacts of both direct and indirect threats.

El ciervo Bawean juega un papel vital como herbívoro y dispersor efectivo de semillas en su reducido y aislado ecosistema además, tiene importancia cultural para la comunidad local. Sin embargo, la ecología de este ciervo en Peligro Crítico de extinción es poco conocida. El objetivo de este estudio fue estimar el tamaño poblacional, preferencias de hábitat y datos comportamiento para esta especie, utilizando modelos de ocupación y de encuentros aleatorios basados en información de cámaras trampa con un esfuerzo de trampeo de 29,350 días entre 2017 y 2019. El tamaño estimado de la población varió entre 120 y 277 individuos adultos, mucho menos que el número para 1978. La densidad de ciervos de Bawean podrían estar relacionadas con el tipo de bosque y la depredación por perros ferales así como otros factores como el aumento de jabalíes en la isla de Bawean. Según el mejor modelo de ocupación, las áreas más adecuadas para esta especie son los bosques altos y comunitarios alejados de los asentamientos humanos. El ciervo Bawean es principalmente crepuscular pero con una actividad diurna significativa. Nuestros resultados señalan que los perros que se mueven libremente son una gran amenaza para la comunidad de mamíferos nativos en la isla de Bawean. Sugerimos que el ciervo Bawean se clasifique como en Peligro Crítico de extinción siguiendo los criterios B1a,b (ii,iii,v) de la UICN. Se requiere una aplicación efectiva de la ley y una estrategia de conservación adecuada, incluido el control de perros ferales para reducir los impactos de las amenazas directas e indirectas.

***Keywords:*** Activity pattern; *Axis kuhlii*; island; occupancy model; Random Encounter Model.

## Introduction

The biota on islands is particularly prone to extinction (Whittaker *et al.* 2017) and extirpation due to their small population sizes, low genetic diversities, less opportunity to recover by recolonization, higher levels of endemism compared to continents, and susceptibility to stochastic processes. For example, 75 % of land vertebrate extinctions have occurred on islands (Tershy *et al.* 2015). However, islands are also known for their unique biodiversity (Whittaker and Fernández-Palacios 2007) and high levels of endemism (Weigelt *et al.* 2015). The Bawean island, a remnant of a small volcano in the Java Sea (Meijaard 2003), hosts two endemic ungulates, the Bawean deer (*Axis kuhlii*) and the Bawean warty pig (*Sus verrucosus blouchi*) and two endemic raptors, the Bawean serpent eagle (*Spilornis cheela baweanus*) and the spotted wood owl (*Strix seloputo baweana*; Rahman *et al.* 2016; Rahman *et al.* 2017a).

According to the IUCN Red List version 2015.4 (IUCN 2015), deer are one of the most threatened mammal groups (http://www.iucnredlist.org/about/summary-statistics). Of the 53 deer species living in tropical regions, 12 are 'endangered', one is 'critically endangered, and one is extinct (IUCN 2017). The critically endangered (cr) deer species is the Bawean deer (Semiadi *et al.* 2015), which is listed in Appendix I of CITES (CITES 2020), and is one of the

25 priority species legally protected by the Indonesian government (Ministry of Environment and Forestry 2015).

However, the Bawean deer has been little studied in the wild, mainly due to its low densities, remote habitat, and secretive behaviour (Semiadi *et al.* 2015; Rahman *et al.* 2017a). Population trends, ecology, and conservation of the Bawean deer have been reported previously based on limited data (Semiadi *et al.* 2015; Rahman *et al.* 2017a; Rode-Margono *et al.* 2020). However, this information collected during the past decade can be used as a baseline for improving our knowledge of the abundance, distribution, and conservation status of the Bawean deer (Rahman *et al.* 2017a; Rahman *et al.* 2017b), and their prospects for conservation in the country.

Here, we analyze 24 months of intensive camera trapping data to (1) update population estimates and infer population trends, (2) assess seasonal, habitat, and environmental factors influencing recording rates, (3) examine activity patterns, social structure, and reproductive patterns, and (4) provide information on potential threats caused by free-roaming dogs.

## Materials and methods

*Study area.* The Bawean Island is part of the East Java province (Indonesia) and encompasses a total area of 200 km$^2$ (5° 40', - 5° 50' S and 112° 3', - 112° 36' E). The precipitation drives the seasonal climate with the greatest rainfalls occurring between the end of October and April (wet season). The average annual rainfall is approximately 2,500 mm (data from the meteorological station of Sangkapura subdistrict). The study area includes the Bawean Island Nature Reserve and Wildlife Sanctuary (Figure 1), which are characterized by steep topography (with slopes > 60°) and a wide altitudinal gradient (0 to 630 m). The protected areas are divided into five wildlife reserves (38 km$^2$), six nature reserves (7 km$^2$), and three community wildlife reserves (1.6 km$^2$). The island landscape is dominated by a mosaic of tall forests (characterized by *Ficus variegata*, *F. septica*, *Podocarpus rumphii*, and multiple *Eugenia* species, interspersed with dense patches of small trees) on the steep slopes and tops of the higher mountains, pastures, teak forests, community forests, shrublands, human settlements, crops, and fishponds in the lower areas (Nijman 2006; Rahman *et al.*

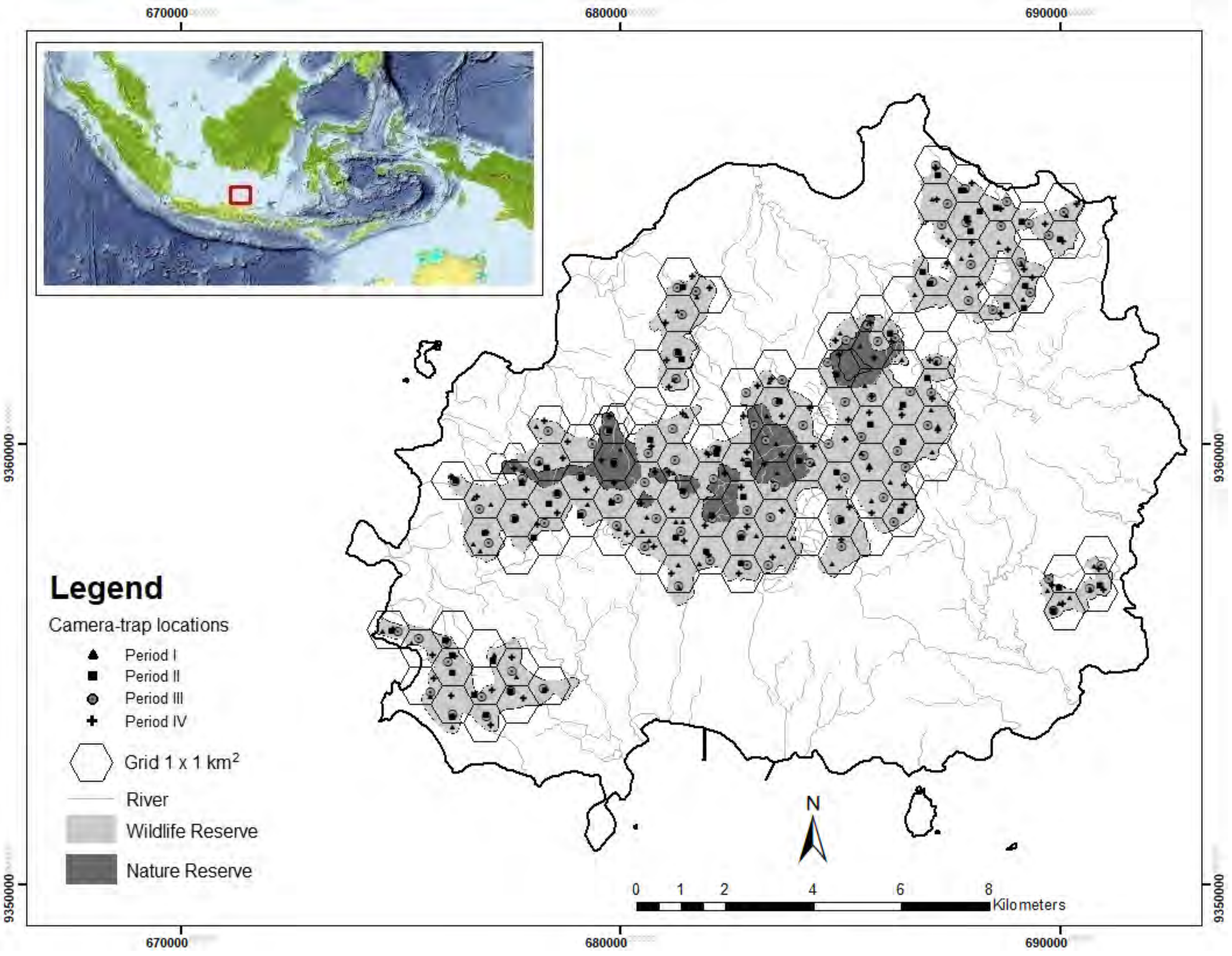

**Figure 1.** Camera trap sampling locations in the Bawean Island Nature Reserve and Wildlife Sanctuary, Indonesia.

2017a). The protected areas are dominated by tall forest (primary or mature secondary forest), teak forest (monoculture of *Tectone grandis* stands with undergrowth dominated by grasses and sparse herbaceous plant and shrub cover), community forest (mixture of cultivated trees such as *Spondias pinnata*, *Artocarpus heterophyllus*, *Tectona grandis*, *Tamarindus indica*, *Bambussa* spp., *Arenga pinnata* and undergrowth dominated by either shrubs or grasses) and shrubland and degraded forest (patches of dense young trees with clear signs of logging and burning, and undergrowth either dominated by grassland and herbaceous plants, or dense shrub cover; Nijman 2006).

*Data collection*. From November 2017 to October 2019, we conducted continuous camera trapping using 30 camera-traps Bushnell Trophy Cam (Model 119877C and HD Max 119576C). Each camera trap was installed in the centre of a regular hexagon grid cell of 1 km by 1 km according to local topography and site accessibility. These cameras were rotated to cover a total of 110 grid cells over two years (Figure 1). Cameras were positioned at 30 - 50 cm above the ground and set to capture both small and large animals throughout the 24 - hour/day, recording sixty-second videos at each trigger, with a 15 seconds resting lapse (Rahman *et al.* 2017a). Records of the same species taken within a 1-hour interval were not considered as independent events (Rovero and Marshall 2009). No camera trap was baited and all videos were stamped with the date and time. Camera traps were visited every 3 – 4 weeks for maintenance, replacement of memory cards or downloading of the videos.

The camera traps were deployed during four periods following the wet and dry seasons: at 76 stations from November 2017 to April 2018 (wet season 1), at 63 stations from May to October 2018 (dry season 1), at 100 stations from November 2018 to April 2019 (wet season 2), and 100 stations from May to October 2019 (dry season 2). Data were managed using CamtrapR software (Niedballa *et al.* 2016). The date and time of all videos were extracted automatically, and the geographical coordinates and habitat type of camera trap installation in the field were converted into digital data in GIS using the ArcMap program.

*Data analysis*. A Chi-squared test was applied to compare the numbers of independent trapping events among seasons. Moreover, we calculated trapping rates (TR) as the ratio between the number of independent trapping events and the sampling effort (measured as the number of days when cameras were active), multiplied by 100 (O'Brien *et al.* 2003). As the number of photographs significantly differed between seasons by Chi-square tests, then we compared the seasonal trapping rate among habitat types in each study site using Kruskal-Wallis tests adjusted for equal numbers and *post hoc* tests for multiple comparisons ($\alpha = 0.05$).

The Bawean deer is a species that cannot be individually identified based on their natural marks therefore, we estimated its density applying the Random Encounter Model (REM; Rowcliffe *et al.* 2008). This method assumes that the population is closed, meaning that there are no births, deaths, immigration, or emigration during the period of estimation (Rowcliffe *et al.* 2008). To fulfill this assumption, the absolute population numbers were calculated for trapping lengths of 60 to 90 days (Rahman *et al.* 2017a; Harmsen *et al.* 2020). We used the following equation to obtain density estimates from camera trap encounter rates (Rowcliffe *et al.* 2008): $gD= Y/t\ \pi/(2+\boldsymbol{\theta})rv$. Where $y/t$ = trapping rate, $\theta$ = angle of detection, $r$ = distance at detection for each camera trap, and $v$ = animal speed movement recorded from videos (Rowcliffe *et al.* 2011). The outcome can then be multiplied by $g$ (mean group size), as the independent unit recorded by the camera is the group rather than the individual (Rowcliffe *et al.* 2008; Zero *et al.* 2013). We assumed these parameter values to be valid throughout the trapping period. The REM used in this study follows Rowcliffe *et al.* (2008); Rowcliffe *et al.* (2011), and Rowcliffe *et al.* (2014), and is described in detail in Rahman *et al.* (2017a). Lastly, the estimated density per km$^2$ was extrapolated to the total size of the Bawean Island protected area to provide an estimate of the population. The error propagation approach by Ku (1966) and Taylor (1997) was used to assess the uncertainty effect of parameter variables on the uncertainty of the density function. As only mature individuals contribute to reproduction, the population size for conservation purposes was corrected by the proportion of adults in groups.

Occupancy models were used to analyze the proportion of area occupied by the Bawean deer. These models were developed considering that the detection/non-detection of species and the environmental conditions may have an influence on the probability of species occupying the area (MacKenzie *et al.* 2006). Fifteen covariates of habitat were used to model the occupancy probability of the Bawean deer. These were distances to the nearest: tall forest edge, community forest, teak forest, shrubland, degraded forest, crop field area, human settlement, protected area border (roughly coinciding with the forest border), water resource, and road (for definitions see Rahman *et al.* 2017a). All distances were calculated in ArcGIS Desktop (Version 10.5.1; Environmental Systems Research Institute, Inc., Redlands, CA). Vegetation productivity was measured as the normalized difference vegetation index (NDVI, cf. Hansen *et al.* 2009). Minimum and maximum daily temperatures and precipitation data were obtained from Sangkapura meteorological station. Lunar illumination was retrieved from the moon calendar (Thomas 1998). We tested the covariates for multicollinearity using Pearson's correlation matrix (STATS package R 3.1.1). We did not include covariates with a correlation > 0.5 in the same candidate model. We grouped camera-trapping data in sampling intervals of seven consecutive days (26 occasions per season; MacKenzie *et al.* 2006).

The outcomes of multi-season occupancy modelling are reported using the R package unmarked (Fiske and Chandler 2011) to evaluate the effect of habitat variables on the proportion of area occupied by the Bawean deer. Incomplete records due to missing covariate values were removed from the model. Models assessed the impact of all previously described site and observation-level covariates on the probability of occupancy, as well as the impact on the probability of detection (MacKenzie *et al.* 2002, 2006; Rahman *et al.* 2019; Rode-Margono *et al.* 2020). All models with a $\Delta$AICc value $< 2$ were competitive (see Rahman *et al.* 2019 for details).

Hereinafter, descriptive statistics were used for analysing behaviour, group size, and group pattern. The R package Activity (Rowcliffe *et al.* 2014) and Oriana circular statistics software (v4, Kovach Computing Services, Anglesey, UK) respectively, were used to estimate the proportion of time spent active and daily activity patterns. Statistical differences among activity levels estimates at sunrise (dawn), sunset (dusk), noon and midnight, were computed with the Wald test. Next, the Chi-square test was used to compare the frequency of observations between day and night, and between sunrise and sunset, whose timing was obtained from the Astronomical Applications Department of the US Naval Observatory.

We used the same camera trap sampling for estimating the daily activity pattern overlap between Bawean deer and free-roaming dogs by applying the statistical methodology developed by Ridout and Linkie (2009), using R version 4.0.2 (R Development Core Team 2020). We used the estimator for the coefficient of overlap as recommended for medium-large sample sizes (Ridout and Linkie 2009). We defined overlap $< 0.5$ as low, 0.5 – 0.75 as moderate and $> 0.75$ as high (Monterroso *et al.* 2014). Due to the overdispersion of data, we used two scales to describe the spatial overlap between species. These were the number of independent contacts per hour per habitat type (correlation type 1: spatial overlap between the two species over the whole study period) and the number of independent contacts per location-month (correlation type 2: spatial overlap for each calendar month). Differences between correlation types 1 and 2 indicated the level of attraction (positive value) or avoidance (negative value) in each calendar month. Based on the results of correlation type 1 and 2, we tested daily activity rhythm in the tall forest using binomial General Linear Models (GLM). Furthermore, we computed Chi-square tests to compare daily and monthly activity and habitat use between species (Batschelet 1981). Lastly, trapping rates from each camera trap (Carbone *et al.* 2001) were used to investigate the spatial overlap between Bawean deer and free-roaming dogs. Trapping rate is a relative index of animal's spatial use and a crude abundance estimate (Carbone *et al.* 2001). We treated each camera trap as an independent spatial point. At each camera trap, the observed TR was correlated between Bawean deer and free-roaming dogs using Spearman's rank correlation coefficient, and spatial overlap between species pairs was assessed using a Pianka index.

## Results

*Trapping rate and population size*. In total, we accumulated 29,350 camera-trap days and 353 independent contacts of Bawean deer. The trapping rate was significantly different between wet and dry seasons, with a lower number of contacts in the wet season than in the dry season (TR wet = 1.03, TR dry = 4.83; $\chi^2 = 14.54$, df = 1, $p < 0.05$). Trapping rate differed among habitat types in both seasons: dry H = 28.16, df = 3, $p < 0.001$; wet H = 19.28, df = 3, $p < 0.001$.

Random encounter modelling based on the four trapping periods yielded variable population estimates for the Bawean deer, ranging from a minimum of 142 individuals in the second wet period to a maximum of 647 in the second dry period (Table 1). Bawean deer were recorded in small family groups (with or without males), pairs, and as single males (Table 2). Fawns and juveniles occurred mainly from March to November. Changes in group structure over the year indicate a reproductive peak in the dry season, partic-

**Table 1.** Camera trapping parameters, adjustment factors, Random Encounter Model output and estimated population size and number of mature individuals of Bawean deer during four camera-trap survey lenghts meeting the assumption of a closed population.

| **Parameters** | **Survey length** | | | |
|---|---|---|---|---|
| | **I (15 Feb.-30 Apr. 2018)** | **II (4 Jul.-3 Oct. 2018)** | **III (1 Nov. 2018-9 Jan. 2019)** | **IV (20 Jun.-12 Oct. 2019)** |
| Truncated operation length (days) | 13 | 5 | 10 | 21 |
| Number of camera traps | 30 | 24 | 28 | 28 |
| **Parameter estimates** | | | | |
| Trapping rate | 0.032 | 0.080 | 0.030 | 0.080 |
| Day range (km/day) | 1.625 | 2.654 | 1.448 | 2.772 |
| Radial distance (km) | 0.008 | 0.008 | 0.008 | 0.008 |
| Angle (radians) | 0.327 | 0.327 | 0.327 | 0.327 |
| **Adjusment factors** | | | | |
| Mean group size | 1.780 | 1.240 | 1.662 | 1.250 |
| Proportion matures | 0.556 | 0.702 | 0.512 | 0.714 |
| **Model output** | | | | |
| Density (individual/km$^2$) | 3.85-7.59 | 4.54-9.23 | 3.04-8.88 | 6.86-13.88 |
| **Adjusted model output** | | | | |
| Estimated population size | 180-354 | 212-430 | 142-414 | 320-647 |
| Estimated number of mature individuals | 100-197 | 149-302 | 73-212 | 228-462 |

ularly in July (Figure 2). Our overall mean estimate across the entire study period was 143-345 mature individuals.

*Patch occupancy*. Of the 110 camera-trap stations placed in both seasons, Bawean deer were detected at 26 in the wet season and 34 in the dry season. The maximum area occupied by Bawean deer was then 11 km$^2$ and 14.4 km$^2$ out of 46.6 km$^2$ total surveyed area in wet and dry seasons respectively (Figure 3a). The model of ψ (settlement); *p* (protected area) for the wet season and ψ (settlement); *p* (.) for the dry season were the most parsimonious (Table 3). The occurrence of Bawean deer was negatively affected by distance to the nearest human settlements in both seasons (Figure 3b). The total fraction of area for the model-averaged occupancy probability is $\psi$ (SE[$\psi$]) = 0.40(0.08) with detection probability (SE [$\hat{p}$]) = 0.12(0.05) and $\psi$ (SE[$\psi$]) = 0.45(0.08) with detection probability (SE[$\hat{p}$]) = 0.20(0.09), respectively in the wet and dry seasons.

*The daily activity of Bawean deer and free-roaming dogs.* Independent contacts of Bawean deer (*n* = 353) indicated an overall activity level of 0.58, but Rayleigh tests of Oriana circular statistics software did not detect significant deviation from a uniform daily activity distribution (z = 1.14, P = 0.633; Figure 4). Nevertheless, according to Wald tests, deer tended to be more active at dusk than dawn (p = 0.025) or midday (p < 0.01), with a mean activity time of 18.21 ± circular SD 8 minutes. There was no significant difference in the number of encounters between day and night (χ2 = 2.482, df = 1, p = 0.189).

We found a high degree of daily activity overlap between Bawean deer and free-roaming dogs (estimated overlap coefficients < 0.55, = 0.74; Figure 5a). Both species had non-overlapping spatial activity in four habitat types in which the activity peak was not different (Figure 5b).

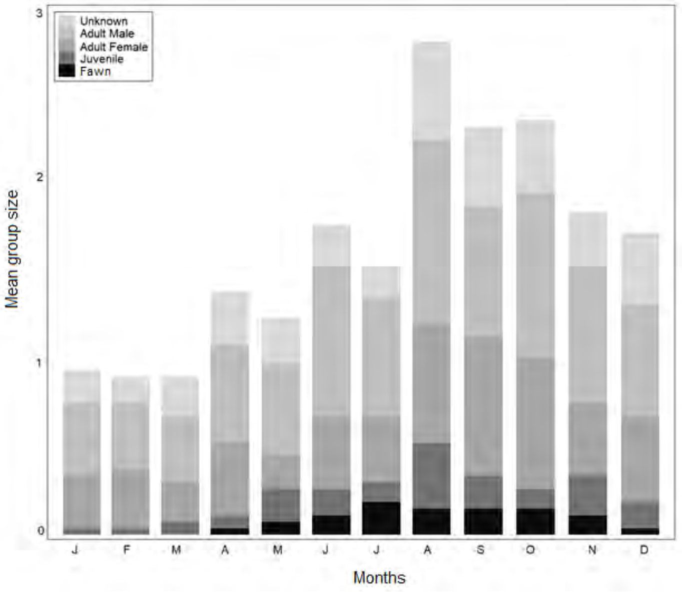

**Figure 2.** Monthly patterns of group size and structure of Bawean deer based on camera-trap records (*n* = 267) in the Bawean Island Nature Reserve and Wildlife Sanctuary, Indonesia.

**Table 2.** Mean group size, mean litter size and group combinations of Bawean deer recorded by camera traps from November 2017 to October 2019 in Bawean Island Nature Reserve and Wildlife Sanctuary.

| Parameters | Value |
|---|---|
| Number of videos | 353 |
| Mean group size ± SD (range, n) | 1.24 ± 0.36 (1-3) |
| Litter size ± SD (range) | 1 ± 0 (1-2) |
| Number of adult females (range) | 0.58 ± 0.51 (0-2) |
| Number of adult males (range) | 0.32 ± 0.49 (0-1) |
| Number of unknown adults (range) | 0.18 ± 0.34 (0-2) |

Tall and community forests, as well as teak forests, were regularly used throughout the year. Whereas in the tall forest, the period of activity during the day increased from March to September and the lowest occurred from December to January (Figure 5c), coinciding with the dry and wet seasons, respectively. The trapping rates of Bawean deer and free-roaming dogs were higher in the community forest, indicating that both species intensively use the same area compared to other areas (Figure 5c). Although both species did not appear at the same time in the same areas. Bawean deer showed a bimodal pattern with equal higher activities at dawn and dusk, and free-roaming dogs displayed high activity in the afternoon and late afternoon (15:00 to 16:00). The model ignored the small activity peak at dawn due to a 95 % confidence interval (Figure 5d). Differences between spatial activities were significant for both species (daily activity: χ2 = 131.33, df = 23, p < 0.001; monthly activity: χ2 = 13.46, df = 10, p < 0.05; habitat use: χ2 = 1.58, df = 3, p < 0.05). In the context of spatial overlap between Bawean deer and free-roaming dogs, there was a positive spatial correlation between the two species (Spearman correlation = 0.28, P < 0.05; Pianka index = 49). Camera traps did not record direct encounters between Bawean deer and free-roaming dogs, but predation of Bawean deer by free-roaming dogs was witnessed at several locations (Figure 6).

## Discussion

Our study provides a robust estimate of Bawean deer density from a large, long-term photographic capture dataset. This monitoring increased detection numbers and improved parameter estimates from previous research by Rahman *et al.* (2017) and Rode-Margono *et al.* (2020), for the most elusive groups within the Bawean deer population. This scope allowed us to address concerns of many previous Bawean deer studies, including small sample sizes, low detection rate, and the limited spatial and temporal extent to provide a complete description of the Bawean deer population in our study area (Rode-Margono *et al.* 2020). Moreover, our approach to spatial and temporal study design may offer useful guidance for future studies of deer and other medium-sized herbivores.

*Density estimates.* Our estimates of Bawean deer density are 7.6 Bawean deer/km$^2$ (including fawns) or 4.4 adults/km$^2$. Similar density estimates have been reported by Rah-

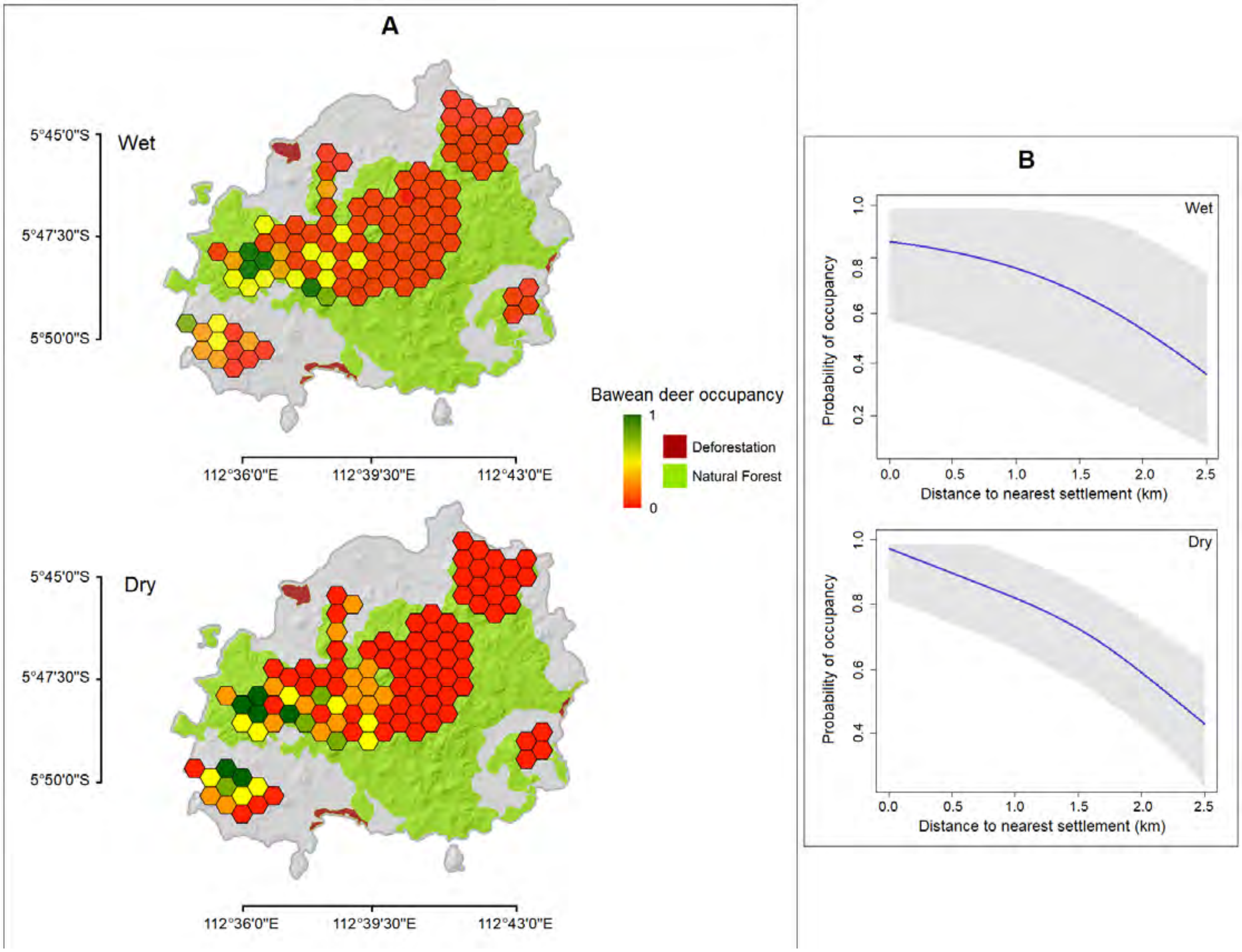

**Figure 3.** (a) Map showing the posterior mean of occupancy probability (ψ) of Bawean deer in wet and dry seasons for each tile of Bawean Island Nature Reserve and Wildlife Sanctuary, Indonesia, and (b) Predicted occupancy and detectability of Bawean deer, in relation to the distance to the nearest human settlement and protected area, respectively.

man *et al.* (2017a), and Rode-Margono *et al.* (2020). They suggest that the Bawean deer population declined from the first survey by Blouch and Atmosoedirdjo (1978) from approximately 400 deer to only 277. This low Bawean deer density most likely results from predation by free-roaming dogs (Rahman *et al.* 2016; Rahman *et al.* 2017a; Rode-Margono *et al.* 2019), but also retaliatory killing incidents following crop field damage, and low forage productivity because of the massive spread of wild pigs (*Sus verrucosus blouchi*) and alien plant species (Blouch and Atmosoedirdjo 1978; Rahman *et al.* 2016; Rahman *et al.* 2017a; Murbani 2018; Rode-Margono *et al.* 2019). Reports indicate that during the past five years' wild pig invasion of local crops has been a major concern for local farmers who requested population control from the local authorities (Semiadi, unpubl data; Nursyamsi pers. comm.).

The rapidly increasing population of wild pigs causes several problems for landowners and livestock, as well as the environment itself (Gürtler *et al.* 2017). In many developing countries, local people in surrounding forests have often endured costly disturbance from wild animals (*e.g.*, wild pigs) in their crop fields causing human-wildlife conflicts (Pandey *et al.* 2016).

Killing pest animals has often led to the accidental killing of other species (Loveridge *et al.* 2017; Rahman *et al.* 2020), hence it is important to investigate the interactions with wild pigs. Although there is no evidence and comprehensive research related to competition between the two species, the diet of wild pigs and the native Bawean deer likely overlap and wild pigs might have a competitive advantage over deer due to their omnivorous diet and aggressive behaviour. Furthermore, on Bawean Island, at least seven invasive alien plant species have been identified *i.e.*, *Ageratum conyzoides*, *Chromolaena odorata*, *Eupatorium inulifolium*, *Lantana camara*, *Imperata cylindrica*, *Stachytarpheta jamaicensis* and *Themeda arguens* (Trimanto *et al.* 2016). The invasion of *Chromolaena odorata* and regrowth of *Tectona grandis* stumps reportedly altered the natural habitat quality of Bawean deer and contributed to the population decrease of Bawean deer (Semiadi *et al.* 2015).

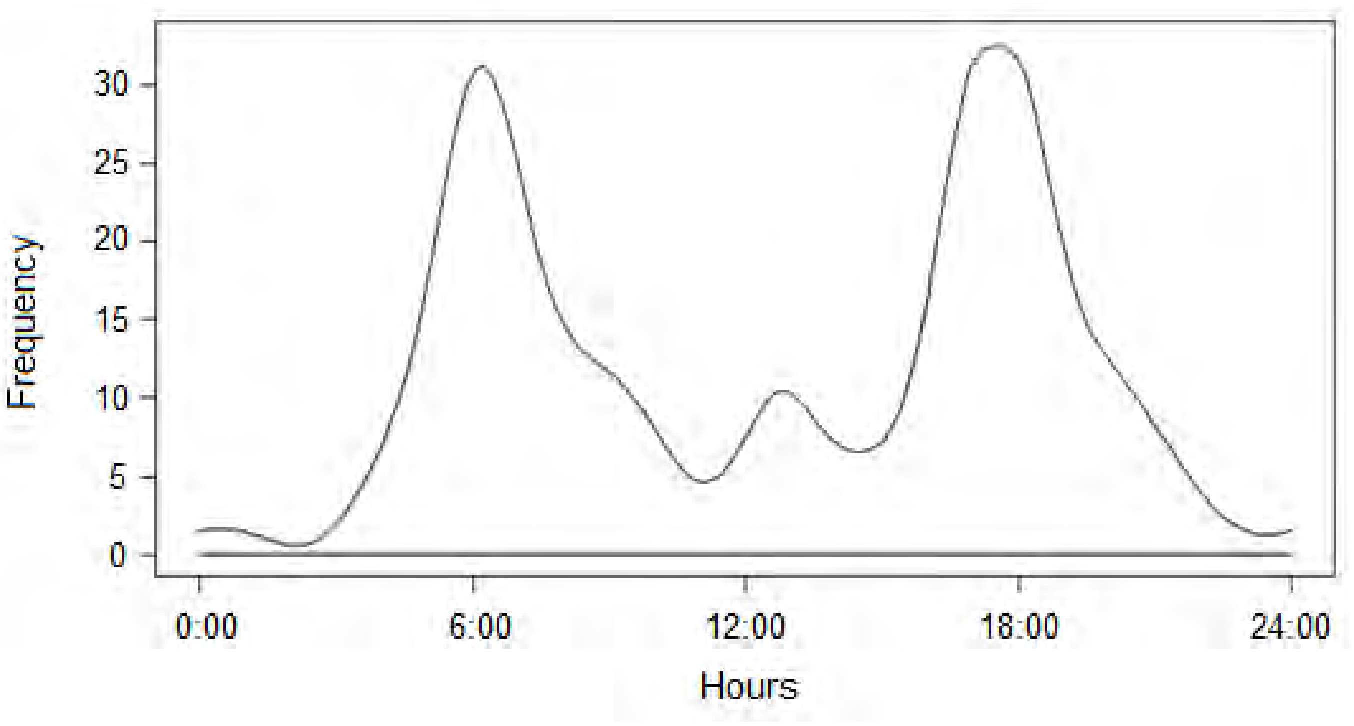

**Figure 4.** Activity plots of Bawean deer in the Bawean Island Nature Reserve and Wildlife Sanctuary, Indonesia, based on camera-trap records (*n* = 267).

Density estimates indicate the existence of a seasonal pattern with the highest values in the dry season. This relates to seasonal changes in spatial activity patterns of Bawean deer in response to resource availability, as suggested by Rahman *et al.* (2017b). These results confirm the variations previously recorded by Rahman *et al.* (2017a) and Rode-Margono *et al.* (2020). Seasonal spatial activity patterns are an important issue for subsequent density estimates aimed at revising the conservation status of the species.

*Landscape occupancy.* Bawean deer occupancy is negatively correlated with distance to the nearest settlement but not with other covariates. Similarly, Rahman *et al.* (2017b) found a negative correlation between camera-trap rate and distance to settlements, together with a preference for tall and community forests near forest edges but not in the inner forest, which is presumably a primary forest. We also recorded a small number of deer in the interior of protected areas. This pattern may be attributable to the existence of a lower diversity of food plant species there than within forest edges (Wirth *et al.* 2008), and to the secretive behaviour of Bawean deer that is difficult to survey in dense habitats (Rahman *et al.* 2016). However, the reasons for a lower abundance or absence in the inner protected area should be further investigated for improving habitat quality as a conservation measure. Moreover, Bawean deer have previously been found also in semi-open cultivated habitats (Semiadi 2004), and this may have been captured in our data by the high probability of occupancy at forest edges. From a conservation perspective, this habitat preference is risky for Bawean deer, as crops are also damaged

**Table 3.** Multi-season occupancy model selection for identifying the main relevant variables of the Bawean deer habitat; roles of covariates in determining probability of occupancy by deer estimated by camera trapping between November 2018 and October 2019, grouped in sampling intervals of 7 consecutive days.

| Model | Number of parameters | AICc | ΔAICc | AIC*w* | Cumulative Weight | Model Likelihood | Cond Psi total average by area |
|---|---|---|---|---|---|---|---|
| Wet season | | | | | | | |
| Ψ (Settlement); *p* (Protected Area) | 4 | 121.15 | 0.00 | 0.40 | 0.40 | 1.00 | 0.528 |
| Ψ (Secondary); *p* (.) | 3 | 124.84 | 3.69 | 0.30 | 0.70 | 0.34 | 0.548 |
| Ψ (Secondary + Cultivated); *p* (.) | 4 | 125.23 | 4.08 | 0.12 | 0.82 | 0.25 | 0.462 |
| Ψ (Primary + NDVI); *p* (.) | 4 | 127.69 | 6.54 | 0.10 | 0.92 | 0.20 | 0.442 |
| Ψ (River + Elevation); *p* (.) | 4 | 128.13 | 6.98 | 0.08 | 1.00 | 0.19 | 0.528 |
| Dry season | | | | | | | |
| Ψ (Settlement); *p* (.) | 3 | 115.33 | 0.00 | 0.58 | 0.58 | 1.00 | 0.106 |
| Ψ (Settlemen + Cultivated); *p* (Protected Area) | 5 | 117.17 | 1.84 | 0.18 | 0.76 | 0.35 | 0.246 |
| Ψ (Settlemen + Cultivated); *p* (Secondary) | 5 | 118.36 | 3.03 | 0.10 | 0.86 | 0.22 | 0.250 |
| Ψ (Settlement); *p* (NDVI) | 4 | 119.31 | 3.98 | 0.10 | 0.96 | 0.20 | 0.069 |
| Ψ (River); *p* (.) | 3 | 122.03 | 6.70 | 0.04 | 1.00 | 0.20 | 0.043 |

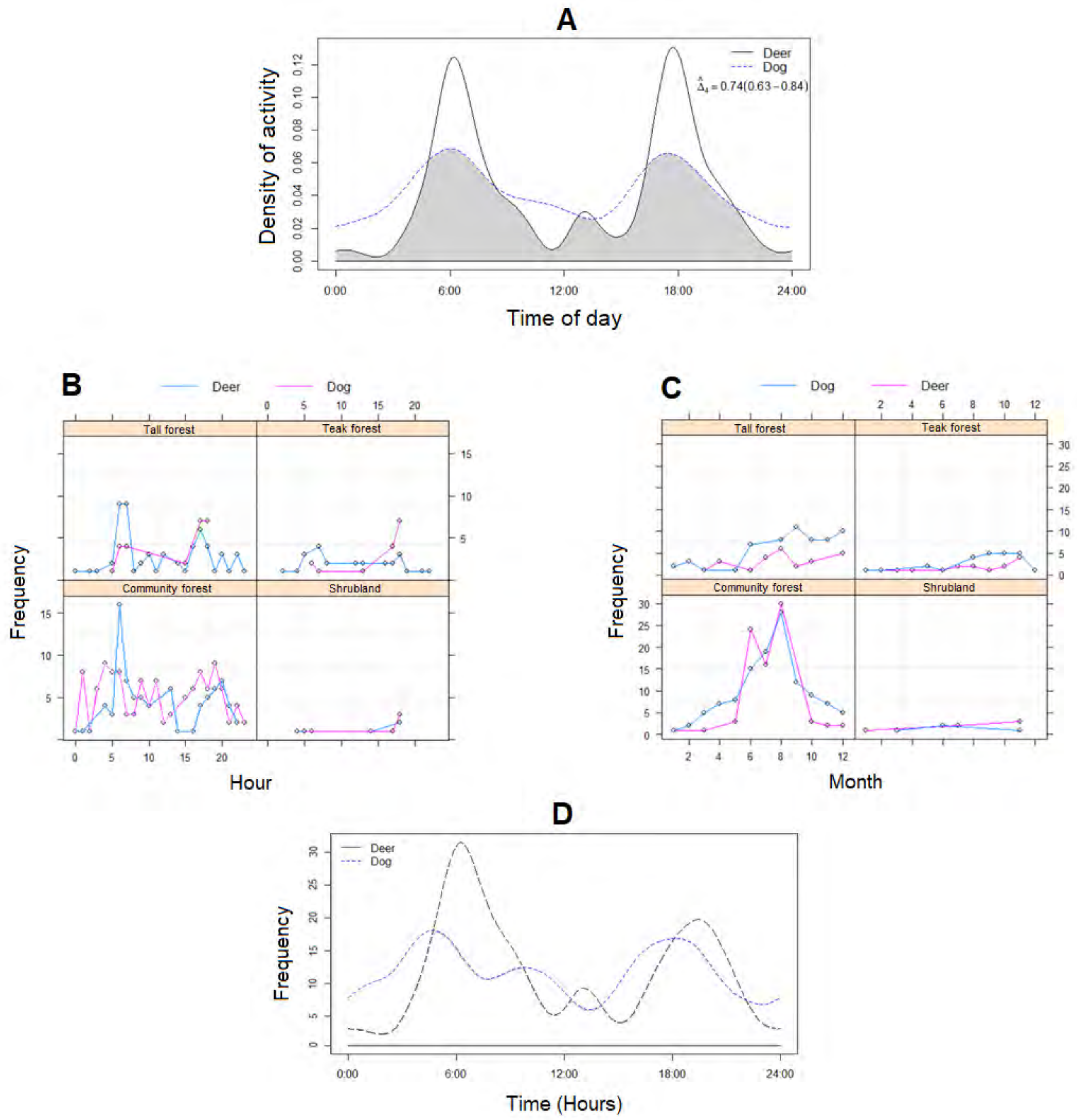

**Figure 5.** Bawean deer and free-roaming dogs in the Bawean Island Nature Reserve and Wildlife Sanctuary, Indonesia, (a) The degree of daily activity pattern overlap, (b) The difference between the number of independent contacts per hour per habitat and (c) per month per habitat, and (d) The level of daily activity rhythm in the tall forest.

by wild pigs instigating non-specific hunting methods (*e. g.* snares), which should only be designated for pest animals (BBKSDA East Java 2009). For example, in 2018, deer deaths were caused by snares in a community forest in the Western Sareden Pudakit area (Mt. Besar).

Our data confirm the importance of protected areas for Bawean deer conservation. As such, they may be a source of dispersing individuals, supporting the persistence of Bawean deer populations in the surrounding areas. Bawean deer distribution on Bawean Island is restricted to extensive low tropical and hill forests remaining, at altitudes between 34 and 320 masl. The areas with verified Bawean deer presence are Gunung Besar and Kumalasa Blocks. High rates of deforestation and habitat fragmentation on Bawean Island have restricted current Bawean deer distribution to mostly protected and/or remote areas on the island. Protected areas and other refuges play a crucial role in maintaining other medium-large herbivore populations in landscapes with large human impacts (Rahman *et al.* 2020; Western *et al.* 2009; Meyer *et al.* 2016).

*Daily activity, group sizes, and group patterns.* Bawean deer showed a similar activity pattern to those reported in other studies (Rode-Margono *et al.* 2020), tending to be crepuscular, with significant day-time activity and some at night. In contrast, Semiadi *et al.* (2015) in their study showed that Bawean deer are primarily nocturnal, active intermittently through the night. The differences may be attributable to various factors. Firstly, previous monitoring of Bawean deer relied solely on the eyes of observers. The ability to collect data on rare or secretive species that are generally difficult to observe directly can lead to significant improvements in understanding the ecological community

**Figure 6.** Bawean deer freshly killed and partially eaten by free-roaming dogs in Bawean Island, Indonesia, from 2014 to 2020 (Source: D.A. Rahman, Nursyamsi).

(Azlan *et al.* 2006). Secondly, hunting has led to high population declines of Bawean deer in the past (Rahman *et al.* 2017b). The activity pattern of ungulates is strongly related to their predators (Prugh *et al.* 2019). With the absence of natural predators and lower hunting activity by humans in the past five years, we surmised whether Bawean deers might be adapted to a more flexible or changing pattern in their behaviour in response to those pressures. The closely related hog deer increased nocturnal activity (Dhungel and O'Gara 1991) as have other deer species (Ikeda *et al.* 2019), because of high hunting activity. Behaviour change provides a window into the animal world that can explain what animals do when they are stressed or threatened and what they prefer and dislike.

The sex ratio was skewed to females (2:1). However, in most studies, more males are recorded than females because males tend to move more and have more extensive home ranges. The camera traps recorded only single adults, pairs, or mothers with infants. This group composition is similar to previous reports (Blouch and Atmosoedirdjo 1978; Semiadi *et al.* 2015; Rode-Margono *et al.* 2020). Our mean group size of 1.24 (range 1–3) is similar to the closely related hog deer, which is reportedly 1.81 ± 0.11 (Sinha *et al.* 2019). Moreover, the reproductive peak in Bawean deer occurs in the middle of the dry season, with no immature individuals observed during the peak of the wet season.

*Potential threat by free-roaming dogs.* This study presents the first results on the activity patterns of both Bawean deer and their potential predators, free-roaming dogs. We report a similar daily activity and use of habitat supporting a temporal and spatial niche overlap which may induce negative effects on the Bawean deer population. The presence of free-roaming dogs can reduce the amount of time spent in foraging activities by Bawean deer and could increase intraspecific competition for space. In the past, dogs were used by local people for hunting activities (Murbani 2018), forcing Bawean deer to increase their activity by night, and to find refuges far from roads and human settlements during the day (Rahman *et al.* 2017b).

We are aware of two Bawean deer killed by dogs in 2014; one female died in the Durin River in 2015; one female from the Mt. Besar Block and one male from the Kumalasa Block were chased by dogs to the residential area in 2016. Two females released near their captive breeding site were killed by dogs in 2017 and one male from Mt. Dedawang in 2020 (Figure 6). Sometimes Bawean deer manage to escape chasing dogs, for example in Mt. Maninjo (Suwari Village) in 2019 and Mt. Gadung (Mt. Besar Block, East Pudakit Village) in 2020. On a larger scale it is well known that the increase of free-roaming dogs, particularly next to protected areas, can generate short-term displacement or extirpation of wild mammal species (Zapata-Ríos and Branch 2016; Doherty *et al.* 2017).

*Conservation issues.* From the 2015 IUCN assessment (Semiadi *et al.* 2015) and the latest study using camera trapping in 2014 (Rahman *et al.* 2017a), our results suggest a significant decline in the area of occupancy and habitat quality. The seasonal population sizes are well below the threshold, including the decline of mature individuals, and the area of occupancy and/or quality habitat is both small and in decline. Therefore, according to IUCN guidelines (IUCN 2012), we suggest the Bawean deer be listed as Critically Endangered following criterion B1a,b (ii,iii,v).

Our data confirm the importance of protected areas for Bawean deer conservation. As such, they may be a source of dispersing individuals, supporting the persistence of Bawean deer populations in the surrounding areas. Protected areas and other refuges play a crucial role in maintaining other medium-sized herbivore populations in landscapes with large human impacts (Western *et al.* 2009; Meyer *et al.* 2016; Rahman *et al.* 2020). The high extent of deforestation and habitat fragmentation on Bawean Island have restricted Bawean deer range to the most protected and/or remote areas on the island. According to the most recent data this range mainly includes low tropical and hill forests between 34 and 320 m asl. of Gunung Besar and Kumalasa Blocks.

Isolation in a small range and population decline continue to be major concerns for Bawean deer conservation (Rahman *et al.* 2017a, Rahman and Mardiastuti 2021). Conserving wide-ranging medium-sized ungulates relies on the protection of population sources and dispersal opportunities from sink populations through connected habitat (Pan *et al.* 2014). Consequently, effective habitat protection is one of the essential actions to improve Bawean deer survival. Moreover, connectivity among forest fragments sheltering Bawean deer should be improved by establishing more community reserves and implementing sustainable land-use practices either as forest management, crop and livestock production where farming has already been established in the community forest. Improving connectivity is a key strategy for the survival of the species and for gene flow among the population. Consequently, effective habitat protection is one of the essential actions to improve Bawean deer survival. Besides, favour mechanisms to reduce conflicts with humans and alien fauna and flora are crucial. Strong law enforcement is needed for halting destructive practices such as poaching, uncontrolled logging, and overgrazing in protected areas, and reducing the misdirected retaliatory killing associated with wild pig control in farmland, with dogs often misdirected and targetting deer. Management of the dog population, which began intensively in mid-2018 through preventing the birth of unwanted of puppies, poisoning, and regulated pig hunting, must be reinforced. The poor practices related to rearing dogs by people who live around the forest and protected areas on Bawean Island show a lack of responsible ownership and are leading to ubiquitous presence of dogs negatively affecting Bawean deer (Murbarani 2018). Community approach programs such as training related to dog-keeping practice and responsible ownership on Bawean Island are necessary to reduce the impacts of free-roaming dogs on Bawean deer, including also to ensure animal welfare and prevention of zoonotic disease through regular vaccination of dogs.

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# Establishing the availability of the mammalian genus name *Antillomys* and species name *Antillomys rayi* (Rodentia, Cricetidae)

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The rodent genus name *Antillomys* and the species name *Antillomys rayi* (Cricetidae: Sigmodontinae) are unavailable, given that the publication where they originally appear did not satisfy the requirements of the International Code of Zoological Nomenclature (ICZN). The names were formally described in the supplementary information (electronic online text) in Word format, and the work itself did not contain evidence that it was registered in ZooBank, as per Article 8.5.3 (amended) of the ICZN. In this note we establish the availability of the names *Antillomys* and *Antillomys rayi*, by fulfilling ICZN's requirements.

El nombre del género *Antillomys* y el nombre de la especie *Antillomys rayi* (Cricetidae: Sigmodontinae) no están disponibles, dado que la publicación donde apareció originalmente no cumplió todos los requisitos del Código Internacional de Nomenclatura Zoológica (ICZN). Los nombres fueran formalmente descriptos en la información complementaria (texto electrónico) en formato Word, y la obra en sí no contenía evidencia de que estuviera registrada en ZooBank, según el Artículo 8.5.3 (modificado) del ICZN. En esta nota, establecemos la disponibilidad de los nombres *Antillomys* y *Antillomys rayi*, cumpliendo con los requisitos de la ICZN.

***Keywords:*** Caribbean Region; nomenclature; Oryzomyini; Sigmodontinae; taxonomy.

## Introduction

*Antillomys rayi* was described by Brace *et al.* (2015) in a phylogenetic study of extinct oryzomyine rodents from the Caribbean. This genus and species were formally described in the supplementary information (electronic online text) of the paper, and although the taxonomic acts were registered in the Official Register of Zoological Nomenclature (ZooBank), the work itself did not contain evidence that such registration had occurred, as per Article 8.5.3 (amended) of the International Code of Zoological Nomenclature (ICZN 1999, 2012). In addition, the supplementary information of Brace *et al.* (2015) was in a Microsoft Word© document file, which cannot be considered a format with fixed content and layout as per Article 8.1.3.2 (amended; ICZN 1999, 2012).

Thus, despite having ZooBank's Life Science Identifiers (LSIDs), the names *Antillomys* and *Antillomys rayi* are not available and some authorities do not recognize these taxa. For instance, the Mammal Diversity Database (MDD 2022) states that "the species was described in a supplemental word file from the original description publication, which makes the species and genus name unavailable for nomenclatural purposes and it needs to be described correctly".

The objective of this note is to comply with the provisions of the International Code of Zoological Nomenclature to make the names *Antillomys* and *Antillomys rayi* available. Therefore, we provide here the complementary information for the new genus and species described by Brace *et al.* (2015). This published work and the nomenclatural act it contains have been registered in ZooBank. The LSID for this publication is: urn:lsid:zoobank.org:pub:84469DB9-3BA3-466C-A107-42046A0CEF56. The year of availability of *Antillomys* n. gen. and *Antillomys rayi* n. sp. is 2023 and must be referred as such in future works.

Taxonomy
Rodentia Bowditch, 1821
Muroidea Illiger, 1811
Cricetidae Fischer, 1817
Sigmodontinae Wagner, 1843
Oryzomyini Vorontzov, 1959

*Antillomys* n. gen.

LSID: urn:lsid:zoobank.org:act:499EF449-A4F2-48A0-949D-527EEE717BC8.

Type species: *Antillomys rayi* sp. nov. (Figure 1)

Etymology: After the Antilles.

Diagnosis: Differs from other Antillean oryzomyines in the following combination of characters: nasal bones with blunt posterior margins, extending posteriorly approximately at same level as lacrimal bones; lacrimals with maxillary and frontal sutures of similar lengths; interorbital region symmetrically constricted, frontal with squared (angular) relief of dorsal and lateral facies and without supraorbital ridges; incisive foramina very small, not extending posteriorly between M1 alveoli, teardrop-shaped; palate with one small posterolateral palatal pit at each side of mesopterygoid fossa; mesopterygoid fossa extending anteriorly between molar rows; M1 anterocone divided by anteromedian flexus; M2 protoflexus absent; anterolophid absent on m2-3; M1 without accessory labial root (four roots total); m1-3 with two roots.

## *Antillomys rayi* n. sp.

LSID: urn:lsid:zoobank.org:act:81F61159-AFC5-4FB9-98D8-4B6E93E5E2AE.

**Figure 1.** *Antillomys rayi* craniodental material. a-c, partial skull (holotype, UF A.98.2): a, dorsal view; b, ventral view; c, lateral view. d, left premaxilla (UF A.98 series), lateral view. e, h, left dentary (UF A.98.20): e, internal view; h, external view. f, left maxillary (UF A.98 series), occlusal view. g, i, left dentary (UF A.98.23): g, occlusal view; i, external view. Scale bar = 5 mm.

Holotype: Partial skull, Florida Museum of Natural History (University of Florida), Zooarchaeology Collection A.98.2 (Figure 1a-c).

Type locality: Indian Creek (AD 900-1100 archaeological site), Antigua Island, Antigua and Barbuda (17° 00' 36" N / 61° 44' 48" W).

Etymology: After Clayton Ray, paleontologist who first identified the distinctiveness of *Antillomys*.

Diagnosis: Differs from other sigmodontine rodents in the following combination of features: very large size, as large or larger than any extant sigmodontine; stout and wide rostrum; dual articulation of lacrimal with maxillary and frontal; symmetrically constricted anterior interorbital region without supraorbital crests; incisive foramen very short and teardrop-shaped; short bony palate (mesopterygoid fossa extends anteriorly between M3); capsular process present in mandibular ramus; M1 with divided anterocone, well developed mesoloph, and anterior protocone-paracone crista (Figure 2); M2 without protoflexus, and mesoflexus with single internal fossette; M3 with developed mesoloph, small posteroloph, and hypoflexus persistent after moderate wear; m1 with enclosed anteromedian fossettids but lacking anteromedian flexid, ectolophid or ectostylid; mesolophid and mesostylid present, connected to entoconid by lingual cingulum; M1-3 with anterolabial cingula; M1 with four roots; M2-M3 with three roots; m1-3 with two roots.

Holotype measurements: length of molar series (occlusal) = 9.32 mm; length of incisive foramina = 5.13 mm; length of diastema = 13.70 mm; breadth of zygomatic plate = 6.53 mm; minimum interorbital width = 7.44 mm.

Distribution: Recorded from 10 Holocene or undated late Quaternary fossil and zooarchaeological assemblages on the islands of Antigua (Indian Creek, Mill Reef; Ray 1962; Brace *et al.* 2015), Barbuda (Two Feet Bay Cave II, "Pleistocene Cave"; Ray 1962; Brace *et al.* 2015), Guadeloupe (Roseau, Pointe-des-Châteaux I, Grotte des Bambous; Goedert *et al.* 2020), and Marie Galante (Blanchard 2, Anse Talisronde, Folle-Anse; Brace *et al.* 2015; Goedert *et al.* 2020) in the eastern Caribbean (Figure 3).

Other examined material: Named specimen repositories: NHM, Natural History Museum (London), Paleontology Collection; UF, Florida Museum of Natural History (University of Florida), Zooarchaeology Collection; G, Musée Edgar Clerc, Le Moule, Guadeloupe. Antigua, Indian Creek: UF Zooarch. A36 (maxillary), UF Zooarch. A47 (maxillary), UF Zooarch. A98 series (44 dentaries, maxillaries and premaxillaries), UF Zooarch. A.98.5 (dentary); Barbuda, Pleistocene Cave: NHM Paleo. M26901 (several dentaries and maxillaries); Two Feet Bay Cave II: NHM Paleo. M20210 (dentary); UF Zooarch., uncatalogued skull; Guadeloupe, Grotte des Bambous and Roseau: G-30 (dentary), G-34 (maxillary), G-35 (maxillary) (Figure 2), G-16 (dentary), G-01 (dentary), G-series (31 dentaries and maxillaries), G-36 (maxillary);

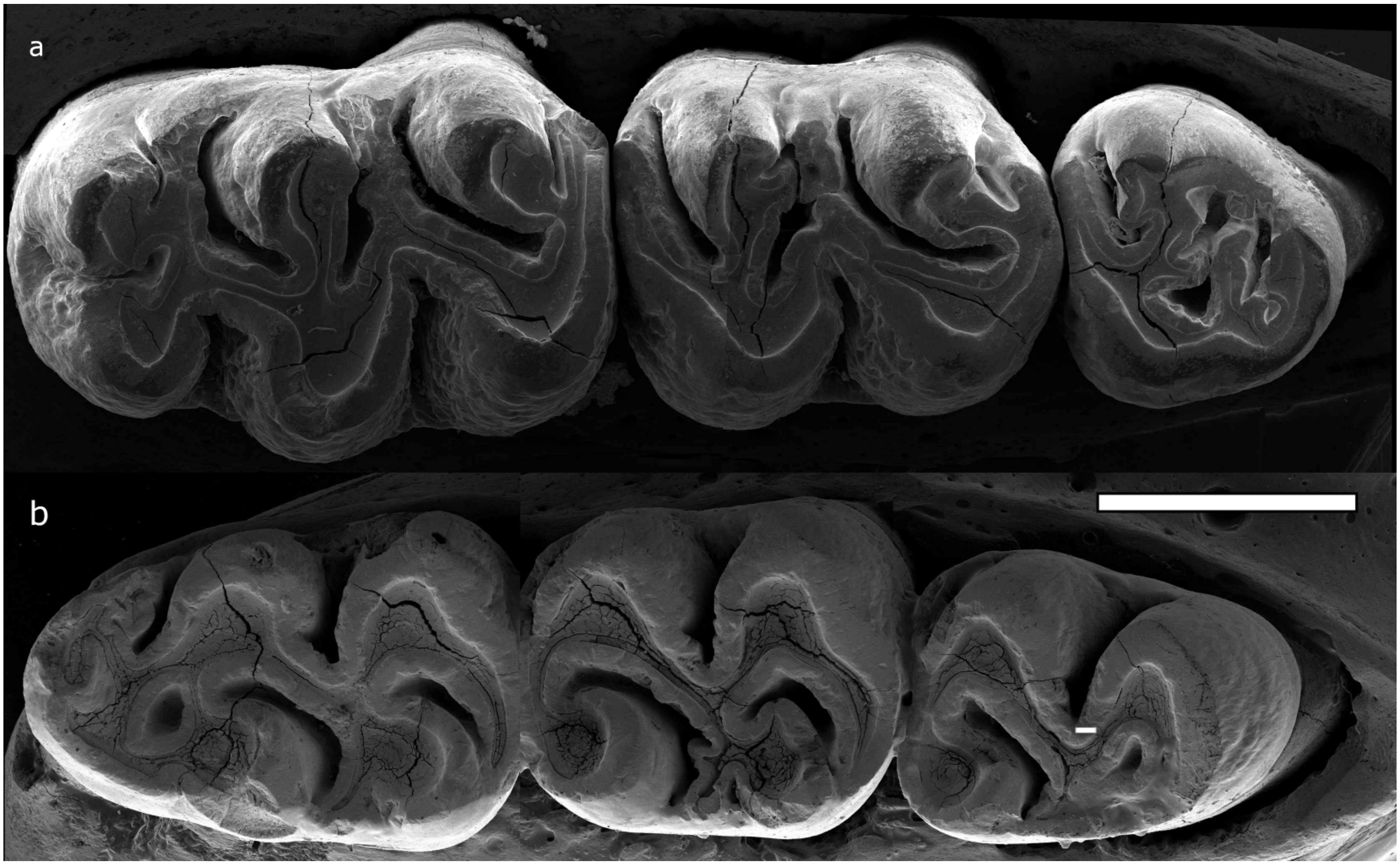

**Figure 2.** Molar series of *Antillomys rayi*. a, upper molar dentition (Musée Edgar Clerc G-35); b, lower molar dentition (UF A.98 series). Scale bar = 2 mm.

Marie Galante, Anse Talisronde, Pits 1 and 2: UF Zooarch. series (several dentaries, maxillaries and humeri; Figure 1d-i). Other than several specimens from Barbuda, all this material is late Holocene (pre-Columbian, >500 ya) in age.

Description: Skull large and robust, with stout and wide rostrum flanked by deep zygomatic notches; interorbital region symmetrically constricted (hourglass shaped), without supraorbital ridges; braincase squared, with very subtle temporal crests. Nasal bones with blunt posterior margins, extending posteriorly approximately at the same level as the lacrimal bones; premaxillaries extending at about same level as nasal; lacrimals with maxillary and frontal sutures of similar lengths. Interorbital region hourglass shaped, frontal with squared (angular) relief of dorsal and lateral facies, without supraorbital ridges. Parietals with broad lateral expansions, a large portion dipping below the temporal ridge posteriorly. The zygomatic plate lacks an anterodorsal spinous process, and its posterior margin lies level to the alveolus of M1. Incisive foramina very small, not extending posteriorly between M1 alveoli, teardrop-shaped. The palatal bridge lacks deep furrows or median ridges, and bony palate is small, with mesopterygoid fossa extending anteriorly between molar rows; palate with one small posterolateral palatal pit at each side of mesopterygoid fossa. The posterior portion of all preserved skulls is broken, and thus most information regarding the basicranium is not available. Mental foramen situated at lateral surface of mandible body; capsular process of lower incisor present, ranging from reduced to well developed (polymorphic). Masseteric ridges can form a single open chevron or be conjoined anteriorly (polymorphic); anterior edge of ridges ventral to m1. Incisors ungrooved and without anterolateral bevel.

Molars bunodont; M1 without accessory labial root (four roots total), M2 and M3 with three roots each; lower molars with two roots each. Labial cingula closing labial flexi present; incipient lophodonty, flexi of opposite sides interpenetrate planes.

M1 anterocone well developed (equal in length and width to protocone-paracone), and divided by anteromedian flexus. Anteroloph reaching labial margin, separated from anterocone by short anteroflexus, which can disappear with slight wear. Protostyle absent; protoflexus broad and deep, with large, gently squared apex. Paraflexus transversely oriented from labial wall, deflected posteriorly close to crown midline and extended along entire length of paracone. Mesoloph well developed; mesoflexus long, transverse, reaching midline of tooth. Paracone connected by enamel bridge to anterior moiety of protocone (prepro-

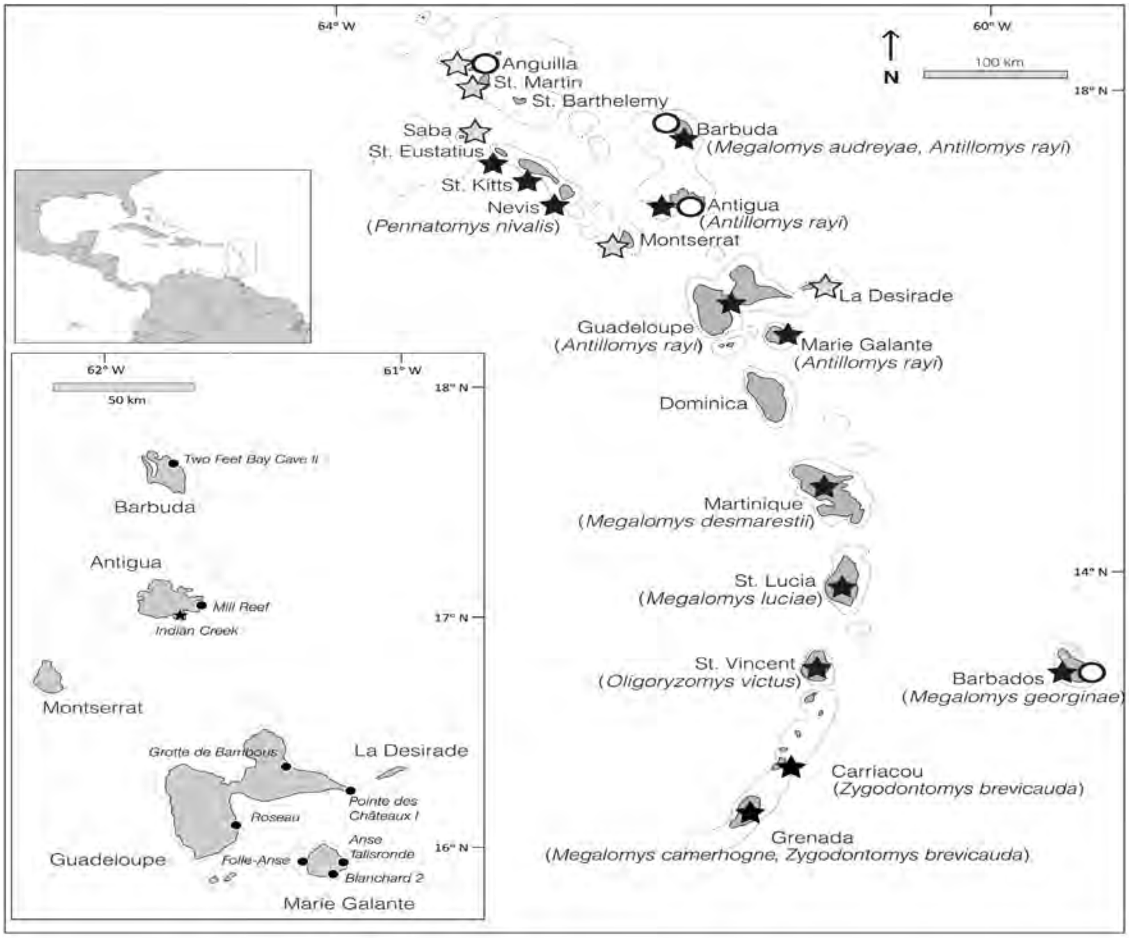

**Figure 3.** Map of the Lesser Antilles, showing distribution of extinct oryzomyines in archaeological sites (stars) and late Quaternary paleontological sites (open circles). Grey stars indicate islands with archaeological sites containing undescribed oryzomyine material. Modern-day sea level and 200 m isobath are both indicated. Lower inset, map of the islands of the northern Lesser Antilles, showing locations of archaeological sites from which *Antillomys rayi* material has been collected; type locality indicated by star.

tocrista); median mure (prehypocrista) connected to posterior moiety of protocone (postprotocrista). Hypoflexus slightly deeper than protoflexus. Metaflexus deep, crescentic, extending over 50 % distance across crown and almost reaching hypoflexus. Posteroflexus small, transverse notch at posterior margin of metacone. Posteroloph discernible on worn teeth.

Second upper molar protoflexus absent; a small indention anterior to protocone might be present. Mesoflexus present as single internal fossette; paracone without accessory loph. Paraflexus slightly posterolinguad, extending 50 % distance across crown. Hypoflexus very deep, sometimes with slightly rounded, expanded apex, and anteroposteriorly shorter than on M1. Metaflexus crescentic, deep and broad, extending well over 50 % distance across crown. Posteroflexus very small and faint, apparently apically bifurcated.

Third upper molar with developed mesoloph and small posteroloph (discernible from metacone by internal fossette). Hypoflexus present, small but persistent after moderate wear. Paraflexus broad and deep on unworn teeth, becoming greatly reduced by wear; can form separate small internal fold adjacent to apex. Mesoflexus large, transverse; can become isolated as an island. Paracone transverse, anteroposteriorly short or triangular; almost isolated by paraflexus and mesoflexus.

Anteroconid well developed, connected to protoconid by paracristid; anteromedian flexid of m1 absent or vestigial, but large anteromedian fossettid apparent in unworn teeth; anterolabial cingulum of m1 present; ectolophid and ectostylid absent; mesolophid present, well developed on m1 and m2 but sometimes joined to entoconid. Anterolabial cingulum present but anterolophid absent on m2 and m3. Posteroflexid of m3 present, well developed.

Comparisons: The only oryzomyine taxon formally described from within the geographic range of *A. rayi* is "*Megalomys*" *audreyae*, known only from a poorly preserved dentary and incisor from "Pleistocene cave-breccia" (specific locality and stratigraphic context unknown) on Barbuda (Hopwood 1926; see also Turvey et al. 2012 for further details). Although this taxon is based on very limited material, it displays several morphological and morphometric characteristics that distinguish it from *A. rayi*. While *A. rayi* specimens always show a capsular process of the lower incisor alveolus, the only available dentary of *M. audreyae* (NHM Paleo. M7406) does not show any evidence of this process. In addition, the available *M. audreyae* dentary possesses an alveolus for an additional rootlet in the lingual position of m1, whereas no *A. rayi* specimens have such an additional rootlet. The alveolar length of the mandibular toothrow of *M. audreyae* (8.30 mm) is much smaller than that shown by any specimens of *A. rayi* (9.24–10.32 mm, mean = 9.72 mm; *n* = 40, including specimens from Antigua, Barbuda, and Guadeloupe); this difference is statistically significant in a one-sample t-test ($t = 29.8$, $p < 0.001$). Additional paleontological research on Barbuda is necessary to further evaluate the phylogenetic status of *M. audreyae*, and the stratigraphic relationship between material assigned to *M. audreyae* and *A. rayi*.

*Antillomys* differs from its sister taxon *Hylaeamys* (see Brace *et al.* 2015; but see Mistretta *et al.* (2021) for alternative placement within Clade 2 of Oryzomyini; Figure 4) in several cranial and dental characters: the interorbital region of *Hylaeamys* is slightly anteriorly convergent with weakly developed supraorbital ridges, while in *Antillomys* the interorbital region is hourglass-shaped without any raised ridge or beads; in *Hylaeamys* the parietals are restricted to the dorsal surface of the braincase, while in *Antillomys* the parietals are expanded onto the lateral surface of the braincase; the mesopterygoid fossa of *Hylaeamys* does not extend anteriorly between the maxillary bones, while in *Antillomys* the mesopterygoid extends between the molar tooth rows; the posterolateral palatal pits in *Hylaeamys* are conspicuous large perforations, while in *Antillomys* the pits are small foramina; and the capsular process is absent in *Hylaeamys*, but present in *Antillomys*. Dentally, the anterocone of M1 is undivided in *Hylaeamys* and divided into labial and lingual conules by an anteromedian flexus in *Antillomys*; the paracone is connected to the protocone by a posterior enamel bridge in *Hylaeamys*, but by an anterior bridge in *Antillomys*; a protoflexus is present on M2 and a posteroloph is present on M3 in specimens of *Hylaeamys*, but consistently absent in *Antillomys*; and ectolophids and ectostylids are present in *Hylaeamys* but not in *Antillomys*.

Remarks: Oryzomyine material from Barbuda was referred to as "*Ekbletomys hypenemus*" by Ray (1962), but this name is not available as it was only reported in an unpublished PhD thesis. Additional material from Guadeloupe and Marie Galante was reported by Goedert *et al.* (2020). See Jones (1985) and Rouse and Morse (1999) for further details on the type locality.

Three characters are variable within the sampled material of *Antillomys*: size of capsular process of the lower incisor alveolus; shape of anterior connection of the masseteric ridges; and presence of a supratrochlear foramen in the humerus. Although examined material of *A. rayi* displays some morphological variation, no consistent morphological differences are observed between *Antillomys* populations on the Antigua–Barbuda or Guadeloupe banks, and our assignment of *Antillomys* material from Guadeloupe and Marie Galante to *A. rayi* is based on the close morphological similarity shown to material from Antigua and Barbuda.

The Caribbean remains a priority area for the study of mammalian diversity and extinction dynamics, and further systematic research is needed to understand the oryzomyine radiation in this region (Figure 4). Our description of *A. rayi* from Antigua, Barbuda, Guadeloupe, and Marie Galante confirms the overall impact of the anthropogenic extinction event in the Caribbean during the late Holocene

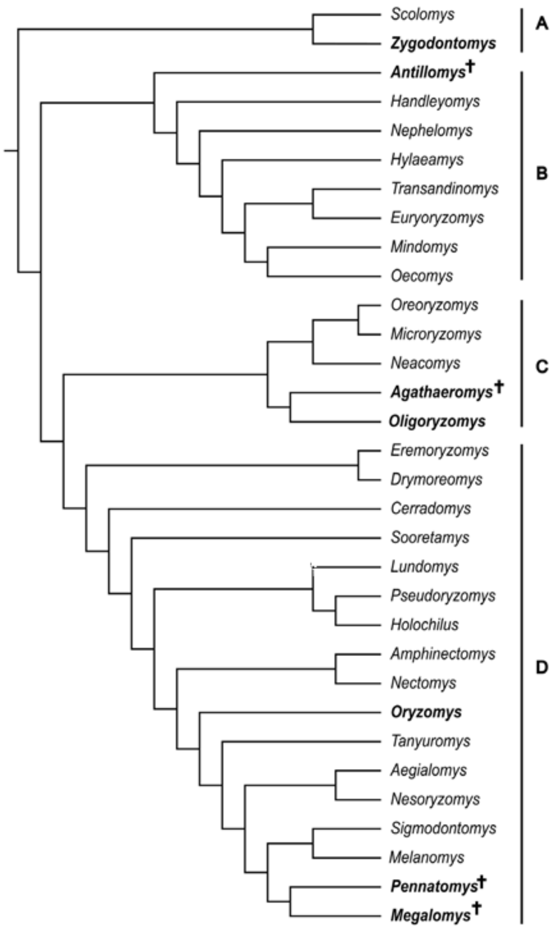

**Figure 4.** Phylogenetic relationships of oryzomyines based on maximum likelihood (ML) analysis of morphology, mtDNA (cytochrome b, 12S) and nuclear (ADH1, IRBP) sequence data (after Mistretta *et al*. 2021: fig. 4). Genera with Caribbean insular representatives are highlighted in bold. Vertical bars on right-hand side of figure indicate taxon membership in clades A–D (see Weksler 2006). See Mistretta *et al*. (2021:437-438) for methodological details.

(Cooke *et al.* 2017) and highlights the need for further work to document and describe the undescribed rice rat material known from zooarchaeological sites on other Lesser Antillean islands, including Anguilla, Montserrat, Saba, and St. Martin (Mistretta *et al.* 2021).

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# Systematics, morphometrics, and distribution of *Eptesicus fuscus miradorensis*, with notes on baculum morphology and natural history

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The brown bat *Eptesicus fuscus* (Chiroptera: Vespertilionidae) is a widely distributed species with up to 11 subspecies ranging from North America, some Caribbean islands, and Central and northern South America. Within the species, *Eptesicus fuscus miradorensis* occurs from North America to South America being the only subspecies that occurs in the continental area of the Neotropical region and might be considered a full species. Also, it has been suggested that *E. f. miradorensis* shows a clinal morphologic variation from the northernmost populations of Central America toward South America. We evaluated the systematic position of *E. f. miradorensis* using genetic samples from Central and South America. In addition, we assessed the morphometric variations of *E. f. miradorensis* using 14 external and cranial measurements of specimens distributed along America. To evaluate the clinal variation and interspecific changes through its distribution, we assigned three groups considering the localities of origin i) North (México), ii) Center (Guatemala-Panamá), and iii) South (Colombia-Venezuela) using multivariate analyzes. We also compiled the localities of the revised specimens and these from databases to determine the environmental factors that potentially constrain the distribution of the taxon. We suggest that *E. f. miradorensis* should be elevated to the species level based on genetic comparisons. Additionally, we did not find sexual dimorphism or size variation associated with its distribution. The species is distributed from México to South America (Colombia, Venezuela, and Ecuador) in elevations that average over 1,000 m, showing a strong association with high mountain ecosystems. This taxon increases to 12 the number of species of bats of the subgenus *Eptesicus* in South America.

El murciélago pardo *Eptesicus fuscus* (Chiroptera: Vespertilionidae) es una especie ampliamente distribuida con hasta 11 subespecies que se extienden por Norteamérica, algunas islas del Caribe, Centroamérica y el norte de Suramérica. Dentro de la especie, *Eptesicus fuscus miradorensis* se distribuye desde Norteamérica hasta Suramérica siendo la única subespecie que habita en el área continental de la región neotropical, y podría ser considerada como especie completa. Además, se ha sugerido que *E. f. miradorensis* presenta una variación clinal desde las poblaciones más septentrionales de Centro América hacia Suramérica. Evaluamos la posición sistemática de *E. f. miradorensis* utilizando muestras genéticas de Centro y Suramérica. Además, evaluamos las variaciones morfométricas de *E. f. miradorensis* utilizando 14 medidas externas y craneales de ejemplares distribuidos a lo largo de América. Para evaluar la variación clinal y los cambios interespecíficos a través de su distribución consideramos tres grupos según las localidades de origen i) Norte (México), ii) Centro (Guatemala-Panamá) y iii) Sur (Colombia-Venezuela) utilizando análisis multivariados. También recopilamos las localidades de los especímenes revisados, así como de bases de datos para determinar las variables que potencialmente limitan la distribución del taxón. Basándonos en las comparaciones genéticas sugerimos que *E. f. miradorensis* debe ser elevado a nivel de especie. Además, no encontramos dimorfismo sexual ni variación de tamaño asociados a su distribución. La especie se distribuye desde México hasta Suramérica (Colombia, Venezuela y Ecuador) en elevaciones que superan en promedio los 1000 m, mostrando una fuerte asociación con ecosistemas de alta montaña. Este taxón aumenta a 12 el número de especies de murciélagos del subgénero *Eptesicus* en Sudamérica.

**Keywords:** America; phylogenetics; species level; taxonomy; variation.

## Introduction

*Eptesicus fuscus* is a medium-sized bat that exhibits sexual dimorphism, with females being slightly larger than males (Kurta and Baker 1990). This species is widely distributed from North America to northern South America and some Caribbean islands (Kurta and Baker 1990). This is the largest neotropical *Eptesicus*, and currently, there are 11 subspecies recognized with parapatric or allopatric distributions (Figure 1), which are differentiated based mainly on size and fur coloration (Burnett 1983a; Hoffman and Genoways 2008). However, in North America, subspecific delimitation of *E. fuscus* is problematic due to multiple contact zones among the subspecies (Hoffman and Genoways 2008).

*Eptesicus fuscus miradorensis* is the only documented subspecies of *E. fuscus* in the continental Neotropics (Davis and Gardner 2008). This subspecies is found in eastern and southern México throughout high elevations in Central America (Davis and Gardner 2008; Turmelle *et al.* 2011) till the Andes of Colombia, Venezuela (Davis and Gardner 2008), and southernmost records in Ecuador (Lönnberg 1921; Arguero and Albuja 2012). The subspecies occurs in an elevational range from 900 to 3,100 m, but mostly over 1,500 m (Davis and Gardner 2008; Solari *et al.* 2013); however, the climatic factors that potentially constrain the distribution of the species to high elevations have not been assessed.

Despite being historically and currently considered a Neotropical subspecies of *E. fuscus* (Moratelli *et al.* 2019; Burgin *et al.* 2020), mitochondrial DNA analyses suggested that samples of specimens from México and Venezuela attributable to *E. f. miradorensis* were significantly differentiated from most other populations of *E. fuscus* (Turmelle *et al.* 2011). Similarly, *E. f. miradorensis* specimens have been recovered as a monophyletic group (Yi and Latch 2022a) or as a weakly diverged lineage based on ultra-conserved elements - UCE (Yi and Latch 2022b).

Along its range, *E. f. miradorensis* can be differentiated from other Neotropical congenerics by its larger size (forearm length > 48 mm; greatest length of the skull generally > 19 mm, and maxillary toothrow length 7 mm or longer; Davis and Gardner 2008), and its strong association with high elevations. Morphometric analyses of the wing and skull characters showed that males and females of *E. f. miradorensis* were correctly classified using discriminant functions in 90 % of cases compared with other subspecies of *E. fuscus* (Burnett 1983a). In addition, a clinal increase in wing and skull size from México to Honduras has been suggested, with a slight reversal in this trend in populations from Costa Rica/Panama (Burnett 1983a). In theory, forearm size decreases, but skull size increases into South America. However, this needed to be adequately tested due to the small sample size from South America included in the analyses of Burnett (1983a).

In general, and despite its wide distribution in the Neotropics, little is known about this taxon, and it is considered a relatively rare bat (Davis and Gardner 2008). We aim to assess the validity of *E. f. miradorensis* as a distinct species using mitochondrial genetic information and contribute to the knowledge of the species by presenting novel information and morphological data from specimens captured in the field and deposited in museum collections. We also update the geographic distribution of *E. f. miradorensis* along its range based on historical and new records. We predict its potential distribution to estimate the areas where the species might be found and explore environmental factors that influence its association with mountain ecosystems.

## Materials and methods

*Molecular analyses and systematics.* To evaluate the taxonomic status of *E. f. miradorensis*, we used four sequences of Cytochrome b (Cytb-b) and three sequences of the mitochondrial gene Cytochrome Oxidase I (COI) of *E. fuscus miradorensis* from Yi and Latch (2022b) kindly provided by the first author and one sequence of Cyt-b from Colombia amplified by us in Ramírez-Chaves *et al.* (2021). The sequences comprise different localities along the complete distribution of the subspecies. To complete our dataset, we gathered sequences of the two genes of additional subspecies of *E. fuscus* from GenBank, including six sequences of Cyt-b and 134 sequences of COI. The list of the used sequences is shown in the Appendix 1.

We aligned all the sequences of each gene using the default parameters of the Clustal W algorithm in BioEdit 7.2.6 software (Hall 1999). We assessed the best-fit evolutionary model per gene using ModelFinder (Kalyaanamoorthy *et al.* 2017) implemented in IQ-TREE software (Nguyen *et al.* 2015) specifying the TPM2uf+I+G4 model for Cyt-b gene and HKY+G4 for COI gene. Then we conducted a maximum likelihood analysis with IQ-TREE (Nguyen *et al.* 2015) using 20,000 replicates to find the best tree. We used nonparametric SH-aLRT and ultrafast-bootstrap (UFBoot; Hoang *et al.* 2018) values as the branch support measure. We conducted Bayesian analysis in MrBayes 3.2.6 (Ronquist *et al.* 2012) and ran two independent replicates of the Metropolis coupled chain Monte Carlo analysis for 10,000,000 generations with trees sampled every 1,000 generations. Convergence was inspected in the program Tracer 1.6 (Rambaut *et al.* 2014) by plotting likelihood values per generation. We discarded 25 % of the samples in each run as burn-in and combined the remaining samples to estimate tree topology, the mean likelihood, and posterior probabilities. Finally, we used the program MEGA 7 (Kumar *et al.* 2015) to calculate average uncorrected p-distances with partial deletion, allowing less than 5 % of gaps, missing data, and ambiguous bases; resulting in two new matrices of 516 bp for COI and 649 bp for Cyt-b for distance calculations. The percentage of genetic divergence was computed as genetic distance x 100.

To clarify the systematics of *E. f. miradorensis,* we provided a list of synonyms and examined the holotype of *Eptesicus fuscus pelliceus* (BMNH 98.7.1.28), considered a junior synonym of *E. f. miradorensis*.

*Morphology and morphometry.* To obtain morphometric and morphological data of *E. f. miradorensis*, we reviewed specimens of the genus *Eptesicus* deposited in the following institutions: Australia: The Queensland Museum (QM); United States: the American Museum of Natural History (AMNH); United Kingdom: the British Museum of Natural History (BMNH); Colombia: the Colección Zoológica, Universidad del Tolima (CZUT), Instituto Alexander von Humboldt (IAvH); Instituto de Ciencias Naturales, Universidad Nacional de Colombia (ICN); Museo de Historia Natural Universidad de La Salle (MLS); Museo de Historia Natural Universidad del Cauca (MHNUC); Museo de Historia Natural de la Universidad de Caldas (MHN-UCa); Museo de la Universidad de Antioquia (MUA, currently Colección Teriológica Universidad de Antioquia CTUA), and Universidad del Valle (UV); México: Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México (CNMA). The list of revised specimens is presented in the Supplementary material.

Of each specimen, 14 skull and external measurements were taken using digital calipers to the nearest 0.1 mm: hind foot length (HF), forearm length (FA), third metacarpal (IIIMT), fourth metacarpal (IVMT), fifth metacarpal (VMT), the greatest length of the skull excluding incisors (GLS), condyle-basal length (CBL), zygomatic breadth (ZB), mastoid breadth (BM), braincase breadth (BBC), breadth across postorbital constriction (POC), length of maxillary toothrow (CM3), breadth across molars (M3M3), and length of the mandible (ML). Total body length (TL), tail length (TV), ear length (EAR), and weight (W) were taken from the labels.

We illustrated the skeleton anatomy of *E. miradorensis* based on micro–Computed Tomography CT-scans of one specimen from México (QM JM 6365) and the direct inspection of three skeletons of specimens from Colombia (MHN-UCa 2022, 3204, 3208). We also described the baculum of one adult specimen from México (QM JM 6365) using CT-scans and obtained three measurements: baculum length, base width, and shaft width. We also compared the bacular morphology with information available in the literature for *Eptesicus* taxa (Hamilton 1949; Brown *et al.* 1971).

All the specimens were identified following the characters proposed in Davis and Gardner (2008): large size (forearm length > 48 mm or longer; greatest length of the skull generally > 19 mm, and maxillary toothrow length 7 mm or longer). We present descriptive statistics of the skull and external measurements for males and females separately for each country. Due to the number of available specimens, sexual dimorphism in wing and skull characters of populations from South America (Colombia-Venezuela) and México was explored using a Mann-Whitney U-test. We also performed a Principal component analysis (PCA) of combined male and female specimens to explore the morphological variation gap and to test the possible directions of the variance associated with wing and skull size from México south to Central and South America. For these analyses, we used the statistical software PAST version 4 (Hammer *et al.* 2001).

*Distribution.* To update the distribution, we obtained geographic information of the specimens reviewed and included records from other sources such as literature (Davis and Gardner 2008; Turmelle *et al.* 2011; Arguero and Albuja 2012) and online databases such as the Global Biodiversity Information Facility (GBIF). The list of the used records is presented in the Supplementary material. We reviewed and curated the database by applying verification procedures and removing duplicate records, records without complete geographical information (geographic region, latitude, longitude, locality, voucher number), or taxonomic uncertainty (Chapman 2005). Spatial filtering of at least a 10 km distance was applied to reduce spatial bias and auto-correlation (Boria *et al.* 2014) using the R package spThin (Aiello-Lammens *et al.* 2015; 1,000 repetitions). With these records, we also performed a Species Distribution Model (SDM) using Maxent version 3.4.0 (Phillips *et al.* 2017) to create a prediction of suitability across the study area (Phillips *et al.* 2006, 2017; Phillips and Dudík 2008) and to explore barriers that may be constrained the distributional potential of the species. We followed the proposal of Soberón (2010) to define our study area by selecting ecoregions (Olson *et al.* 2001) in which the species have been documented. We added a 10 km buffer around the ecoregions polygon to ensure that Maxent selects the bioclimatic data from 'background' pixels from a region in which known records are more likely to form a representative sample of the climatic conditions suitable for the species and within which absences are meaningful (Barve *et al.* 2011). Initially, we considered 55 environmental predictors, of which 19 represent annual trends (*e. g.*, mean annual temperature, annual precipitation), seasonality (*e. g.*, annual range in temperature and precipitation), and extreme or limiting environmental factors (*e. g.*, temperature of the coldest and warmest month, and precipitation of the wet and dry quarters), two monthly precipitation (mm), 12 solar radiation (kJ m-2 day-1), and 12 wind speed (m s-1) at 30 s (~ 1 km$^2$) resolution from WorldClim Version2 (Fick and Hijmans 2017). These variables reflect the information of temperature, rainfall, heat and light, and other radiation given off by the sun and air moving. We consider these climatic predictors because some impose physiological constraints on bats (Velazco *et al.* 2018). We remove highly correlated and redundant variables considering a VIF < 10 (Variance Inflation Factor; Montgomery and Peck 1992). We used ENMeval 2.0 package (Kass *et al.* 2021) to automate the model analysis for the species considering three feature class combinations (lq, lqh, h) and five regularization multipliers settings (one by one to 5). Candidate model performance was evaluated based on the significance of the partial receiver operating characteristic (partial ROC), AUC difference (low value), and omission rate at ten percentiles (OR 10p; Cobos *et al.* 2019; Kass *et al.* 2021). The Cloglog output format was used to describe the environmental suitability of the species (Phillips and Dudík 2008), and it was reclassified to obtain a binary map using the 10-percentile threshold value. We evaluated the correlation between altitude and suitability using a Pearson test to assess if the species has some altitudinal preferences. Finally, we represented the latitude, longitude, suitability values, and altitude in a plot using the ggplot 2.0 package (Wickham 2020) to show the spatial tendencies of the altitude and suitability. The statistical analysis was performed in the R program (R Core Team 2022).

## Results

*Molecular analyses and systematics.* The alignments of both genes were unequivocal and without internal stop codons. The Cyt-b alignment consisted of 1,016 bp, of which 190 bp were parsimony-informative sites, and 678 were invariable sites. The COI alignment comprised 657 bp, 151 bp were parsimony-informative sites, and 441 were invariable sites.

The trees of the two mitochondrial genes (Cyt-b and COI) recovered *E. f. miradorensis* as a monophyletic group, including sequences from a comprehensive geographic coverage of the species. The COI gene recovered *E. f. miradorensis* as a monophyletic clade, including sequences from Colombia, México, and Venezuela, with strong support in the Bayesian analysis (PP = 1) and non-parametric branch support of the maximum likelihood analysis (SH-aLRT = 85) but not supported in ultrafast bootstrap (UFBoot = 89). This clade is sister to a well-supported clade of *E. fuscus pallidus* from southeast USA (PP = 1; SH-aLRT = 93; UFBoot = 96), forming a pair of strongly supported sister clades (PP = 1; SH-aLRT = 99; UFBoot = 95). Other subspecies such as the nominal *E. f. fuscus* from central and west Canada and the west USA, and *E. f. hispaniolae* from the Dominican Republic, formed an unresolved group with *E. guadeloupensis*. Finally, *E. f. bernardinus* from western Canada and the north-western USA appeared as a sister group to the other subspecies of *E. fuscus* included and *E. guadeloupensis* (Figure 1). Similarly, the Cyt-b tree recovered *E. f. miradorensis* as a monophyletic clade with high support in Bayesian inferences (PP = 1) and non-parametric branch support of the maximum likelihood analysis (SH-aLRT = 97) but not supported in ultrafast bootstrap (UFBoot = 91); additionally, its early spit from the remain taxa (*Eptesicus guadeloupensis*, *E. f. fuscus*, and *E. f. bernardinus*) is not well supported (PP = 0.8, SH-aLRT = 66, UFBoot = 62; Figure 1).

The genetic distances are considerably greater comparing *E. f. miradorensis* with other subspecies of *E. fuscus*. For the COI gene, the genetic distances varied between 3.75 % (*E. f. miradorensis* and *E. f. pallidus*) and 10.27 % (*E. f. miradorensis* and *E. f. hispaniolae*), while the distance within sequences of *E. f. miradorensis* was 0.90 %. For the Cyt-b gene, the genetic distances varied between 6.82 % (*E. f. miradorensis* and *E. f. bernardinus*) and 9.81 % (*E. f. miradorensis* and *E. guadeloupensis*), while the distance within sequences of *E. f. miradorensis* was 2.67 % (Table 1).

*Taxonomic remarks.* Based on the information that recovered *E. f. miradorensis* as monophyletic groups in two mitochondrial markers, and considering the high genetic distances compared with other subspecies that are over the values proposed in the genetic concept of species (> 3 %, Bradley and Baker 2001) we suggest that *E. f. miradorensis* should be elevated to species level:

Systematics
Order Chiroptera
Family Vespertilionidae
Genus *Eptesicus* Rafinesque, 1820

*Eptesicus miradorensis* (H. Allen, 1866)

*S*[*cotophilus*]. *miradorensis* H. Allen, 1866:287; type locality "Mirador," Veracruz, México.

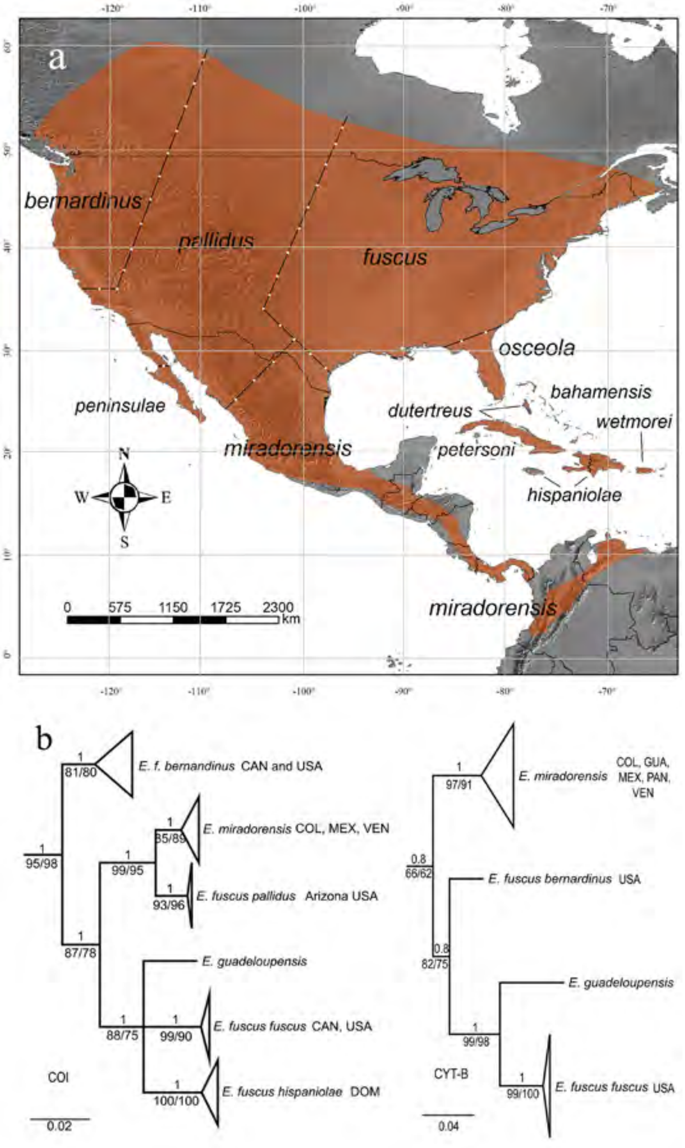

**Figure 1.** a. Map of the distribution of the currently recognized subspecies of *Eptesicus fuscus*. b. Bayesian gene trees of Cyt-b and COI of the *E. fuscus* complex. Upper values of branches show the posterior probability of the Bayesian inference. Values below the branches indicate the maximum likelihood inference's nonparametric (SH-aLRT) and ultrafast (UFBoot) bootstrap values. Country abbreviations. COL: Colombia, CAN: Canada, DOM: Dominican Republic, GUA: Guatemala, PAN: Panama, USA: United States, VEN: Venezuela.

[*Vespertilio* (*Eptesicus*) *fuscus*] *miradorensis*: Trouessart, 1904:77; name combination.

*Eptesicus fuscus miradorensis*: Miller, 1912:62; name combination.

*Eptesicus fuscus pelliceus* O. Thomas, 1920b:361; type locality "La Culata," Merida, Venezuela.

*E*[*ptesicus*]. *s*[*erotinus*]. *miradorensis*: Koopman, 1994:120; name combination

*Eptesicus brasiliensis*: Niceforo Maria [2004]:225; in part.

LSID: urn:lsid:zoobank.org:act:39C7676F-8E25-4F4C-9B92-491AB516E427.

*Holotype:* Not designated. The description was based on a fluid-preserved female (National Museum of Natural History - USNM 5411) collected by Dr. C. Sartorius in Mirador, Veracruz, México (Davis 1966). According to Lyon and Osgood (1909), the specimen could not be located.

*Emended diagnosis: Eptesicus miradorensis* is the largest species of the genus with continental distribution in America (forearm: 48.54 to 51.13 mm). The skull is robust (greatest length of the skull: 19.79 to 20.59 mm; condyle-canine length: 17.7 to 18.47 mm) and presents well developed sagittal and lambdoidal crests, with a triangular appearance in caudal view. The dorsal fur is long (9.0 to 11.0 mm), lustrous, smooth, and shiny, with a brown color. The dorsal coloration is brown to golden yellow, dark to light, and bright, with two bands, the lower band being darker. The ventral coloration is lighter than the dorsal, with two bands, the lower band being darker. Both ventral coloration and dorsal have a mottled appearance. The wing and uropatagium membranes are dark. The ears are large (mean 17.5 mm) with the same coloring of the membranes. The tragus is elongated, reaching about 40 % of the length of the ears. The face is bare, and the skull is not flared and domed (Figure 2).

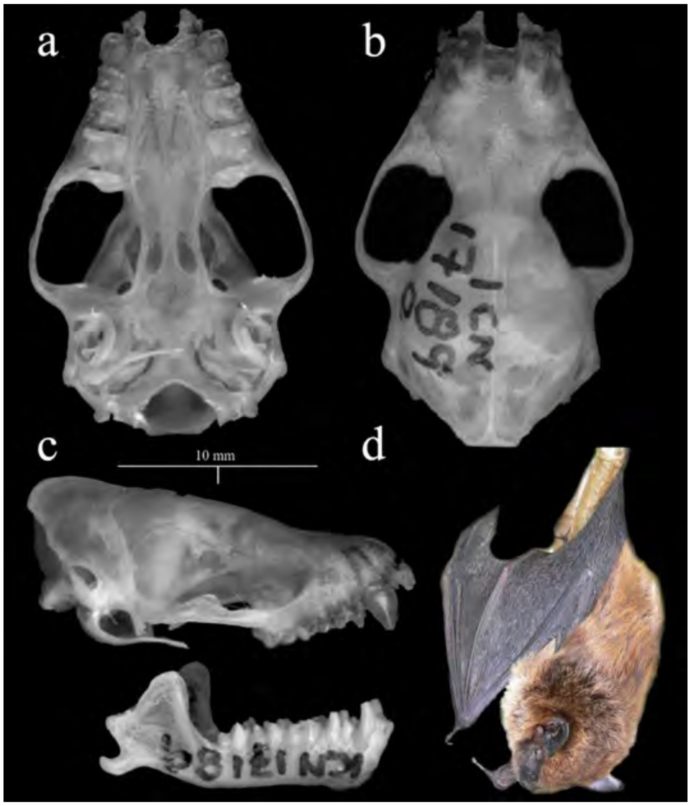

**Figure 2.** Details of the skull (ICN 17189; female from Department of Santander, Colombia) of *E. miradorensis.* a. Ventral view. b. Dorsal view. c. Lateral view. d. Alive specimen from Serranía del Perijá, Colombia (ICN uncatalogued) shows long brownish hair and a dark, naked face.

*Skeleton and baculum morphology.* All specimens analyzed have seven cervical vertebrae, 11 thoracic, six lumbar, the sacral vertebrae fused into a single bone (the sacrum), and eight caudal vertebrae. The number of ribs is 10 (Figure 3). The baculum of one specimen from México (QM JM 6365) is small (length 0.83 mm, base width 0.54 mm, shaft width 0.34 mm), slightly convex dorsally, and slightly concave ventrally (Figure 3). The baculum morphology of *E. fuscus* from North America (without specific locality but likely from the USA; see Hamilton, 1949) is similar in size (0.8 mm length), and general morphology to the one observed in *E. miradorensis* described here.

**Table 1.** Uncorrected p-distances in % for the Cyt-b (above diagonal) and COI (below diagonal) genes. Bold values in the diagonal represent the distances within taxa where the first values are from the Cyt-b gene and the second values correspond to the COI gene.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1. *E. guadeloupensis* | **NA/NA** | 9.81 | 6.66 | | 7.86 | |
| 2. *E. miradorensis* | 8.66 | **2.67/0.90** | 6.83 | | 6.82 | |
| 3. *E. f. fuscus* | 6.39 | 9.21 | **0.39/0.30** | | 6.82 | |
| 4. *E. f. pallidus* | 8.27 | 3.75 | 9.05 | **NA/0.26** | | |
| 5. *E. f. bernardinus* | 8.29 | 7.32 | 7.09 | 7.92 | **NA/0.63** | |
| 6. *E. f. hispaniolae* | 6.59 | 10.27 | 6.93 | 9.24 | 8.32 | **NA/0.78** |

*Assessment of morphometric variation of* E. miradorensis. We reviewed 85 specimens from Colombia, Guatemala, México, Panamá, and Venezuela. All individuals match the diagnostic characters of *E. miradorensis*: large size (FA 48.0 to 54.0 mm, GLS ~ 18.5 to 21.2 mm, weight 10 to 19.6 g), and long brownish hair (8 to 12 mm). Dorsal fur presents lighter tips. Ventral hair coloration is lighter than dorsal coloration. The membranes, ear, and rostrum are dark. The rostrum is naked (Figure 2).

Specimens from the northern distribution range (México) are similar in size to those from South America (Table 2). Colombia-Venezuela and Mexican populations are similar in two wing measurements (FA, IIIMT) between sexes; however, females have larger VMT (Mann - Whitney *U* - test, Z = - 2.031, p = 0.0423, *n* = 9) in South American populations, and IVMT (Z = - 1.979, p = 0.04783, *n* = 16) in Mexican populations. Similarly, both populations found no significant differences in the skull measurements (GLS, CBL, POC, BBC, BM, ZB, M3M3, CM3, ML).

The PCA analysis showed no clear distinction between North/Central and South American populations (Figure 4),

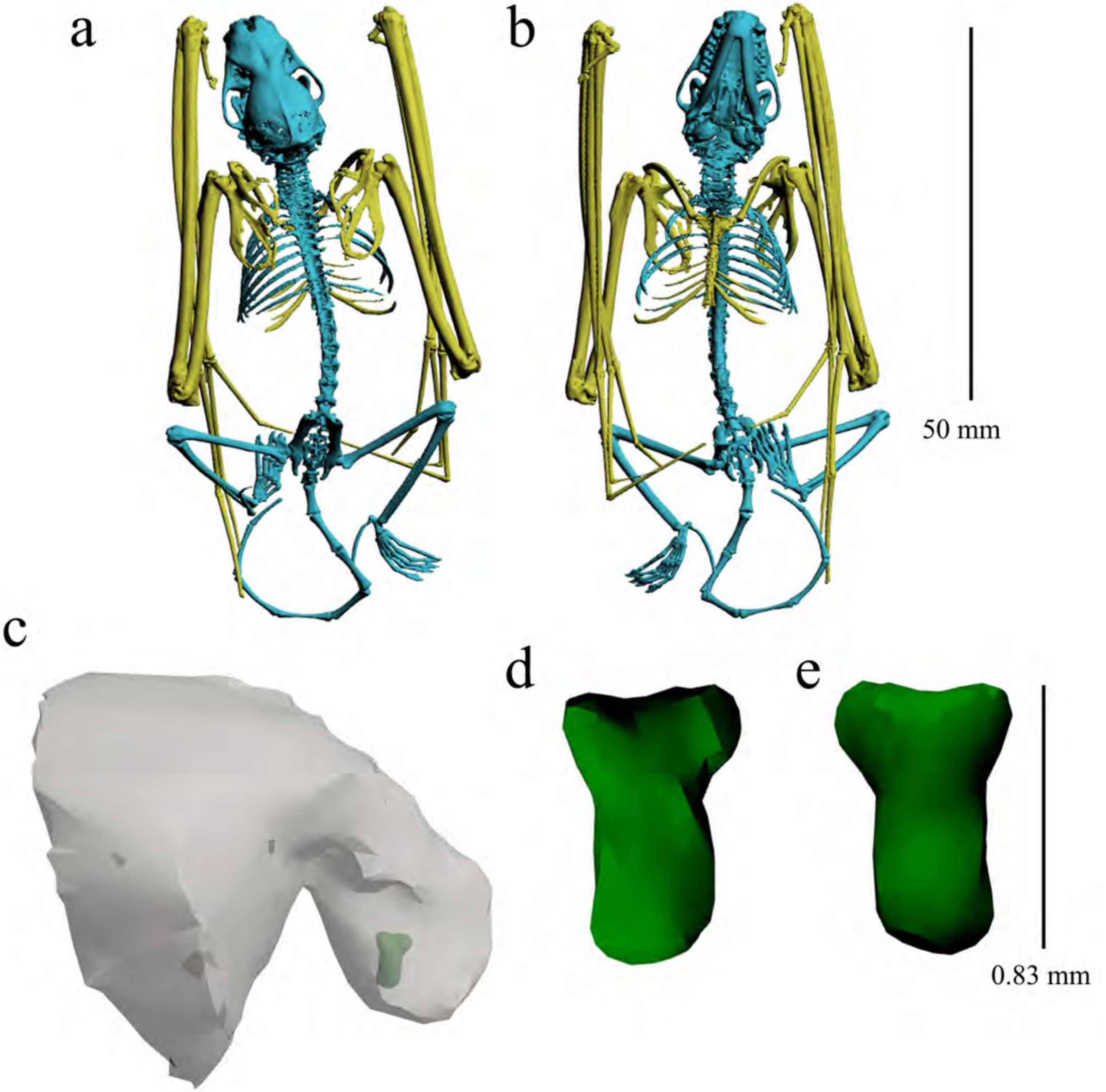

**Figure 3.** Details of the skeleton (a-b) and baculum (c-e) of *Eptesicus miradorensis* (QM JM 6365) from México. a. Dorsal view. b. Ventral view. c. Baculum shape and position (green) embedded in the penis (grey structure). d: Dorsal view of the baculum. e. Ventral view of the baculum.

with specimens from México and Colombia overlapping the first component's negative and positive axis. Specimens from Central America (Guatemala-Panamá) were more centrally distributed along the PC1. The PCA of wing measurements shows that the first principal component accounted for most of the wing size variation (88 %, eigenvalue > 1; *n* = 30). For the cranial characters, the first two PC accounted for the largest variation (76.04 %: PC1: 49.42, PC2: 26.62, eigenvalues < 1; *n* = 18). The variables that contributed most strongly to PC1 were GLS (0.6847) and ML (0.4946).

*Distribution.* We compiled 964 records from 1,098 localities in México, Costa Rica, Guatemala, Honduras, Panamá, Colombia, Venezuela, and Ecuador (Figure 5). The localities cover an altitudinal range between 35 and 4,035 m, with an average greater than 1,951 m (Table 3). The number of localities for each country and the elevation intervals are presented in Table 3. The evaluation metrics showed a model with acceptable complexity (Features classes: H, hinge; regularization multiplier: 3; AUCtrain: 0.87; AUC difference = 5 %, Partial ROC: 0; Omission rate at 10P: 0.1; Number of parameters: 55). Seventeen predictors have a low correlation according with VIF, however, five of them had low impact on the Maxent model, and thus 12 variables were used (Bio2: Mean Diurnal Range, Bio3: Isothermality,

**Table 2.** External and cranial measurements (Meas) of *E. miradorensis* along its distribution range. Values indicate: Mean (interval) n.

| | South America | | | | Central and North America | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Colombia-Venezuela | | Ecuador (from literature) | | Panama | | Guatemala | México | |
| Meas | Male | Female | Male | Female | Male | Female | Female | Male | Female |
| TL | 114.10 (101.9-129.5) 11 | 106.88 (103-130) 11 | 111 | 121 (120-122) 2 | - | - | 114.33 (109-119) 3 | 113.31 (96-125) 17 | 118.53 (110-128) 15 |
| TV | 48.93 (43.0-53.4) 11 | 49.69 (43.0-59.2) 11 | 51 | 53 (56-50) 2 | - | - | 49.33 (47-52) 3 | 42.74 (40.0-44.4) 17 | 48.17 (40-55) 12 |
| EAR | 16.48 (12.8-21.0) 12 | 14.33 (10.8-19.0) 11 | 18 | 22.5 (21-24) 2 | - | - | 19.5 (19-20) 3 | 14.93 (13-16.81) 17 | 17.62 (11-20) 16 |
| W | 13.68 (10-17) 7 | 13.33 (13.5-17.0) 6 | 14.5 | 16 (15-17) 2 | - | - | 17.8 (16.3-19.6) 3 | 15.93 (14-18) 10 | 17.14 (12-21.5) 10 |
| FA | 49.90 (48.02-51.38) 14 | 50.56 (47.65-53.53) 13 | 50.2 (49.4-51) 2 | 52.9 (52.0-53.8) 2 | 50.76 | 49.51 | 50.27 (49.42-50.99) 3 | 48.76 (46.12-52.97) 17 | 50 (47.07-53.04) 16 |
| IIIMT | 46.02 (43.69-48.57) 14 | 47.19 (44.90-50.87) 12 | 48.3 (47.6-49) 2 | 51.25 (50.8-51.7) 2 | 48.25 | 47.35 | 46.86 (46.08-47.9) 3 | 46.25 (43.04-50.83) 16 | 47.68 (44.02-49.93) 16 |
| IVMT | 45.24 (43.00-48.63) 14 | 46.13 (43.95-48.74) 12 | - | - | 46.27 | 46.41 | 46.18 (45.82-46.59) 3 | 45.37 (42.33-48.12) 15 | 46.93 (43.29-48.89) 16 |
| VMT | 43.34 (40.90-46.59) 14 | 44.77 (42.62-48.27) 12 | - | - | 43.44 | 44.2 | 43.77 (43.33-44.51) 3 | 43.88 (39.70-48.11) 15 | 44.77 (42.10-47.12) 15 |
| HF | 11.43 (10.03-12.17) 14 | 11.83 (10.77-13.31) 13 | 9 | 10.5 (10-11) 2 | 10.82 | 11.44 | 10.78 (10.09-11.53) 3 | 10.78 (8-13) 17 | 10.37 (7-12) 16 |
| GLS | 19.14 (18.55-20.02) 9 | 19.34 (18.70-20.12) 11 | 19.85 (19.5-20.2) 2 | 20.90 (20.90-20.90) 2 | 19.49 | 19.84 | 19.85 (19.61-20.09)3 | 19.34 (17.63-21.28) 17 | 18.22 (18.42-20.17) 15 |
| CBL | 18.16 (17.01-18.98) 10 | 18.12 (17.60-18.80) 11 | - | - | 17.92 | 18.3 | 18.21 (17.77-18.20) 3 | 18.48 (17.92-19.42) 17 | 18.42 (16.93-19.4) 15 |
| POC | 4.28 (4.02-4.47) 11 | 4.37 (4.12-4.63) 13 | 4.3 | - | 4.1 | 4.16 | 4.17 (3.93-4.43) 3 | 4.36 (3.77-4.78) 17 | 4.26 (3.94-4.60) 15 |
| BBC | 8.86 (8.65-9.18) 11 | 8.81 (8.62-9.00) 11 | - | - | 8.42 | 8.67 | 8.48 (8.20-8.73) 3 | 8.73 (8.20-9.52) 17 | 8.73 (8.22-9.24) 15 |
| BM | 10.04 (9.58-10.22) 11 | 10.15 (9.82-10.46) 11 | - | - | 9.67 | 9.9 | 10.09 (9.98-10.22) 3 | 10.05 (9.63-10.73) 17 | 10.21 (9.68-10.75) 15 |
| ZB | 13.00 (12.54-13.23) 8 | 13.10 (12.63-13.77) 10 | 13 | - | 12.82 | 12.91 | 12.95 (12.75-13.25) 3 | 13.05 (12.17-14.14) 17 | 13.06 (12.47-13.51) 15 |
| M3-M3 | 8.19 (7.46-8.83) 11 | 8.24 (7.76-8.93) 13 | | - | 8.46 | 8.35 | 8.23 (8.21-8.25) 3 | 8.21 (7.72-8.77) 17 | 8.17 (7.67-8.47) 15 |
| CM3 | 7.34 (7.06-7.68) 11 | 7.36 (7.11-7.62) 13 | 7.5 (7.3-7.7) 2 | 7.65 (7.60-7.70) 2 | 7.56 | 7.57 | 7.53 (7.23-7.78) 3 | 7.38 (6.92-7.91) 17 | 7.32 (6.89-7.62) 15 |
| ML | 15.19 (14.54-16.03) 11 | 15.18 (14.53-15.83) 12 | - | - | 14.8 | 14.89 | 15.11 (14.77-15.51) 3 | 14.49 (13.38-16.10) 17 | 14.65 (14.12-15.39) 14 |

Bio8: Mean Temperature of Wettest Quarter, Bio15: Precipitation Seasonality, Bio19: Precipitation of Coldest Quarter, Prec05: Precipitation May, Prec07: Precipitation July, Prec09: Precipitation September, Prec12: Precipitation December, Srad03: Solar Radiation March, Srad09: Solar Radiation September, Wind07: Wind Speed July). The maps of suitability and distribution (Figure 5) showed that *E. miradorensis* has a potential distribution along the Andes in Ecuador to northern México. The higher suitability values are present in México, Guatemala, north Colombia, and Venezuela. The elevation and species suitability were positively correlated (r = 0.64, p-value < 0.001), indicating low suitability values in the northern (México) and southern (Ecuador; Supplementary material). In the Mexican Transition Zone and Colombia Andes, the species had higher suitability values at medium and high altitudes (~2000 m MSL; Supplementary material), while in the records located at low altitudes such as in northern México, the species had low suitability. Interestingly, maps (Figure 5; Supplementary material) showed that lowlands such as the Isthmus of Tehuantepec or the Nicaraguan depression and some Ecuadorian localities where the species has been recorded have inadequate climatic characteristics.

## Discussion

Neotropical *Eptesicus* are among the least studied bats, perhaps due to their complex systematics and taxonomy. Despite that, in the last 20 years, at least four new species of the genus have been named in South America (Miranda *et al.* 2006; Sánchez *et al.* 2019; Acosta *et al.* 2021; Ramírez-Chaves *et al.* 2021), and the presence of cryptic diversity has been highlighted (Turmelle *et al.* 2011; Ramírez-Chaves *et al.* 2021). The lack of genetic data for *E. miradorensis* in previous phylogenetic analyses or species descriptions (Giménez *et al.* 2019; Sánchez *et al.* 2019) limited the assessment of the morphological and phylogenetic comparisons within the *E. fuscus*' subspecies and Neotropical *Eptesicus* in general. We highlight the importance of using different approaches to understand the real diversity and environmental ranges of cryptic taxa in *Eptesicus*, including assessing the specific status of the subspecies of *E. fuscus* and species groups such as *E. chiriquinus*, *E. furinalis*, and *E. brasiliensis*.

*Systematics*. Allen (1866) named *Scotophilus miradorensis* (= *E. miradorensis*) as a distinct species based mainly on the lustrous yellowish-brown color with a lighter base from specimens from Mirador, Veracruz, México. Thomas (1920) described *Eptesicus fuscus pelliceus*, supported by an adult female (BMNH 98.7.1.28) collected in "La Culata" near Mérida, Venezuela. In the description given by Thomas (1920), he mentioned that this subspecies is "very similar" to *E. f. miradorensis*. Cabrera (1957) and Davis (1966) synonymized both subspecies with the name *E. fuscus miradorensis* having priority. After revising the holotype of *E. fuscus pelliceus*, and specimens captured in the Serranía del Perijá near the type locality of *E. fuscus pelliceus,* we agree with the conclusions of Cabrera (1957) and Davis (1966).

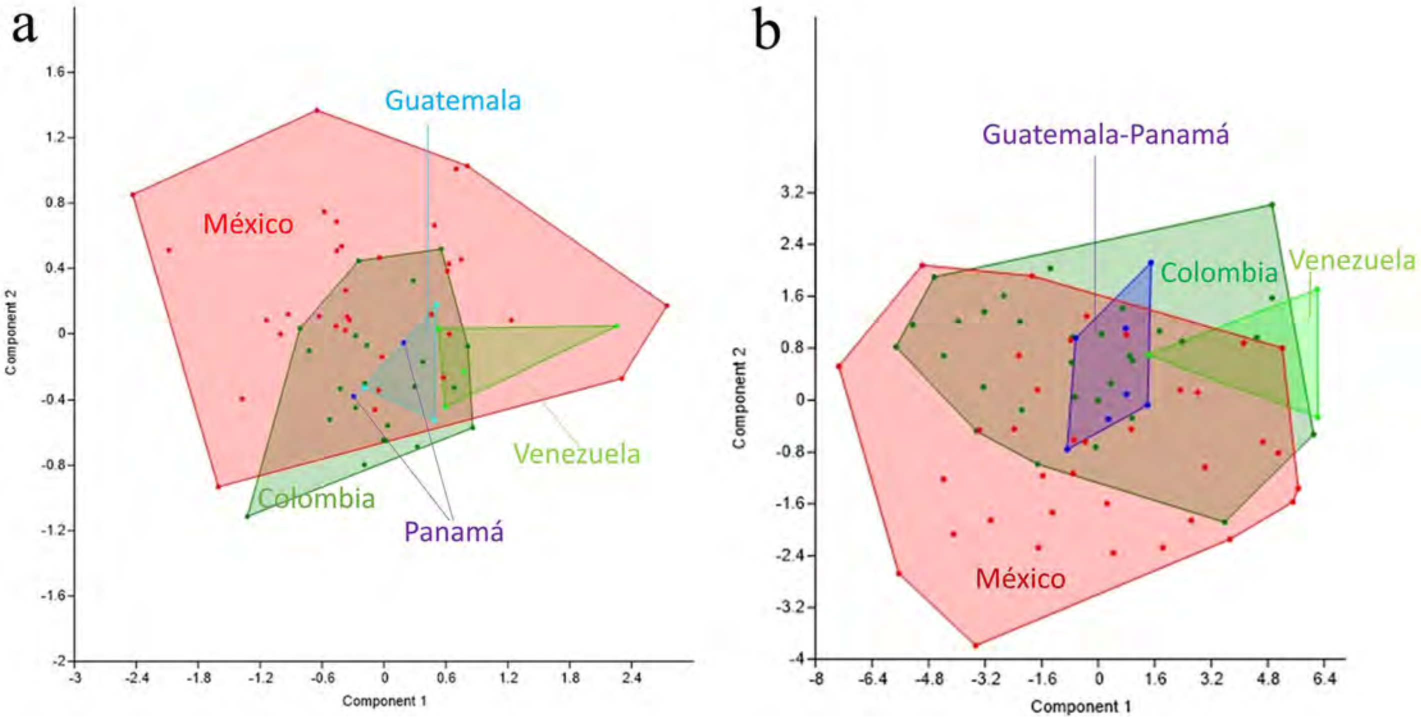

**Figure 4.** PCA plots of (a) cranial and (b) external measurements of *Eptesicus miradorensis* from different localities along its distribution range.

Despite the abundance of *E. fuscus* in North America, only some works have assessed the taxonomic status of its subspecies (*e. g., E. hispaniolae*). Recent works of Yi and Latch (2021a, b) considered *E. f. miradorensis* as part of the same clade as *E. f. bernardinus, E. f. pallidus*, and *E. f. peninsulae* sustaining that subspecies are a product of climatic changes and insolation, these phenomena caused population divergence without speciation with secondary gene flow (Yi and Latch 2022a). However, we support *E. f. miradorensis* as valid species for several reasons. Firstly, some of the taxonomic conclusions of Yi and Latch (2022b) are biased for the putative identification of specimen's tags and long branch attractions; they showed old taxonomic names (*e. g., Histiotus macrotus laephotis* = *Histiotus laephotis*) and multiple paraphyletic clades for several species, some of them demonstrated corresponding to valid species (*e. g., E. diminutus* [Venezuela] = *E. orinocensis*; Ramírez-Chaves *et al.* 2021). Secondly, all continental sequences from México to Venezuela represent a monophyletic linage in the tree presented by Yi and Latch (2021b); these sequences putatively form the *E. miradorensis* clade that differs from the clades corresponding to *E. f. bernardinus*, *E. f. pallidus*, *E. f. fuscus*, and the Caribbean *E. "fuscus"* (see Yi and Latch 2022b).

Finally, secondary gene flow could not be exclusive to sub-specific populations. For instance, Yi and Latch (2022b) recognize that the Caribbean populations of *E. fuscus* correspond to a valid species. Notwithstanding, they also considered the same Caribbean populations as subspecies (Yi and Latch 2022a). The phylogenetic position of the Caribbean clade in nuclear and mitochondrial analyses is nested between the east and the west clades of *E. fuscus*, showing that the *E. fuscus* is paraphyletic. Considering the Caribbean population as one (or various) specific entities reinforces the hypothesis that *E. fuscus* comprises several species with gene flow, including *E. miradorensis*.

*Morphology and morphometry.* We did not detect sexual dimorphism in the forearm length in South American and Mexican populations despite previous claims that females are slightly larger than males (Burnett 1983a, Kurta and Baker 1990). Similarly, we failed to find a clear pattern differentiation in the forearm and skull size between North/Central American and South American populations, even though it was suggested that wing and skull size increase from México to Honduras and decrease in Costa Rican/Panama populations, while wing size decrease and skull size increase towards South America (Burnett 1983a). Previous conclusions might be biased by sample size and the inclusion of specimens from several subspecies. Since subspecies such as *E. f. dutertreus* from Cuba exhibit a high degree of intra-sexual variation and are dimorphic in skull traits (Ari-

**Table 3.** Number of localities collected for *E. miradorensis* along its distribution range by country and reporting the elevation interval (Min: minimum, Max: maximum).

| | | **Elevation (m MSL)** | |
|---|---|---|---|
| **Countries** | **# Records** | **Min** | **Max** |
| Colombia | 42 | 608 | 4,160 |
| Costa Rica | 17 | 1,083 | 1,598 |
| Ecuador | 2 | 2,415 | 2,609 |
| Guatemala | 15 | 1,653 | 1,946 |
| Honduras | 2 | 964 | 1,009 |
| México | 992 | 35 | 4,035 |
| Panama | 2 | 1,062 | 1,102 |
| Venezuela | 26 | 878 | 4,105 |

osa Olea and Mancina 2018), a larger sample should exist be evaluated to explore sexual dimorphism in *E. miradorensis* and the intermediate size of Central American populations.

The information on the baculum morphology of Neotropical *Eptesicus* and other Neotropical vespertilionids is scarce (Brown *et al.* 1971). Hamilton (1949) described the baculum morphology of *E. fuscus* from North America, which is similar in size (0.8 mm length) and general morphology to the one observed in *E. miradorensis* described here. The slight differences between the specimen illustrated here (QM JM 6365) and *E. fuscus* (in Hamilton 1949) are the usually sharp and well-marked distal and proximal points in *E. fuscus* (more rounded in QM JM 6365). Additionally, there are major differences between the baculum of *E. fuscus* and *E. miradorensis* compared with the small-sized *E. furinalis*. The baculum of *E. furinalis* from Nicaragua (Brown *et al.* 1971) is very large (0.9 mm length, 0.6 mm base width) contrasted with *E. fuscus* and *E. miradorensis* (0.8 mm length, 0.5 mm base width) considering the differences in body size among these species. The bacular morphology has been used as a taxonomic trait in several vespertilionid genera in other continents (*e. g.*, Taylor *et al.* 2018; Srinivasulu *et al.* 2019), and often allows differentiation among cryptic taxa (Herdina *et al.* 2014). However, these characters have seldom been used in the taxonomy of neotropical *Eptesicus*. Therefore, variation in this structure should be tested for differentiation in Neotropical *Eptesicus* and other American vespertilionids.

*Distribution.* Although recent records have corroborated the current presence of *E. miradorensis* (as *E. fuscus*) in Ecuador (Arguero and Albuja 2012), one overlooked historical record from this country (Lönnberg 1921) constitutes the southernmost record of this species. Lönnberg (1921) recorded a female specimen (as *E. fuscus pelliceus*) collected in 1917 above Quito (3,352 m elevation) deposited in the collections of the Naturhistoriska riksmuseet (NRM), Stockholm. Lönnberg (1921) mentioned that the specimen closely resembled *E. f. miradorensis* and *E. f. pelliceus* and was assigned to the latter because the fur is basally blackish. The specimen was described as having long and fluffy hair (9 mm), large FA (51.9 mm), and GLS (19.5 mm). Lönnberg (1921) match the diagnostic characters of *E. miradorensis*. The specimen reported by Lönnberg (1921) was not found in one visit to the NRM collections and is apparently lost (Daniela Kalthoff 2014, comm, pers.); however, the remaining information associated with the specimen cast no doubts on its identification. Closer localities from Colombia to the Ecuadorian records are Farallones, Department of Valle del Cauca (Western Andes; Alberico 1994), and the Macizo Colombiano (Central and Western Andes; Ramírez-Chaves 2008; Ramírez-Chaves *et al.* 2010), in the Department of Cauca, not Medellín, Colombia as stated by Arguero and Albuja (2012).

It is essential to highlight the strong association of *E. miradorensis* to mountain ecosystems, especially in elevations over 1,500 m in México and Colombia (Davis and Gardner 2008, Ramírez-Chaves 2008). Elevation and additional environmental variables such as elevation, precipitation, and slope have been suggested to play an important role in the distribution of this species (Pérez 2011). In addition, elevation explains significant amounts of morphological differentiation in *E. fuscus* (including *E. miradorensis* as subspecies of *E. fuscus*; Burnett 1983b). The results of the Maxent model indicated that specific environmental variables could limit the regional distribution of the species (Precipitation, Solar Radiation, Wind Speed, altitude, and latitude). It is proposed that biotic interactions, such as exposure to physical stress limit distribution at lower latitudes, but, in contrast, abiotic factors such as predation and interspecific competition restricts the distribution at higher latitudes (Brown *et al.* 1996). Results of the MaxEnt that showed low suitability values in northern México and Ecuador would indicate that the distribution boundaries of *E. miradorensis* are related to the effects of the latitude. Furthermore, the southern limits of the species in South America remain

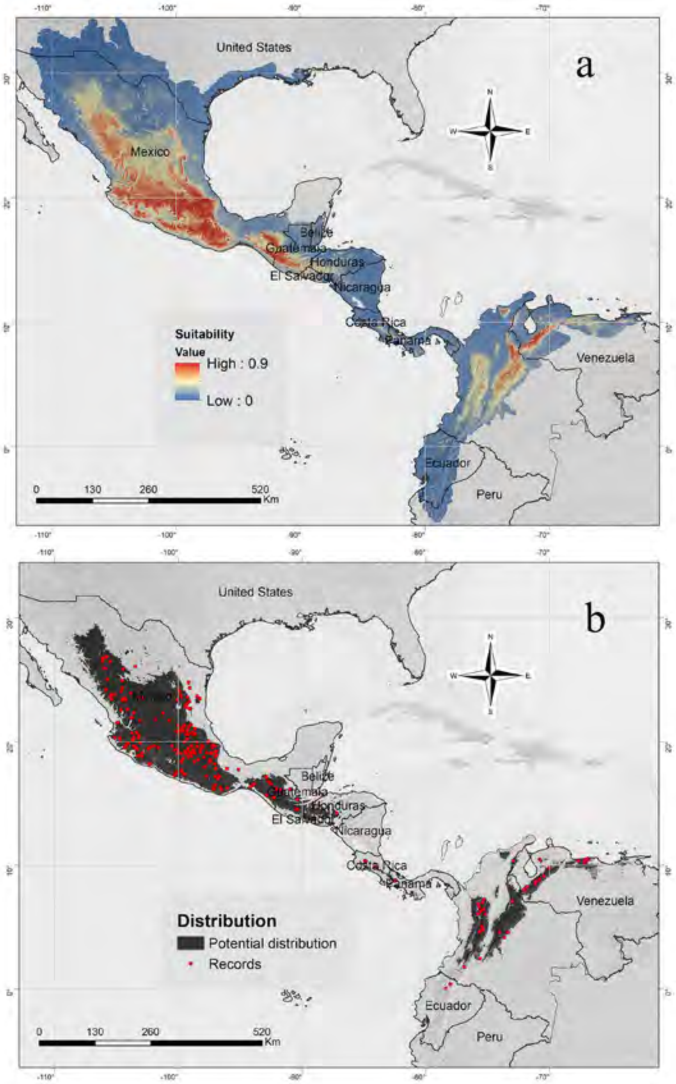

**Figure 5.** a. Suitability map of *Eptesicus miradorensis* in America using the Maxent algorithm. The higher suitability values are present in México, Guatemala, north Colombia, and Venezuela. b. Binary distribution map of *Eptesicus miradorensis* using the 10-percentile threshold value. Red points represent the species records.

unclear, and the role of the Amotape Huancabamba Zone as a possible barrier needs to be assessed, as suggested for other taxa (Quintana *et al.* 2017). Although in South America *E. miradorensis* inhabits predominantly Andean forests at medium to high elevations, some records below the 1,000 m of altitude in Colombia captured by us come from dry forests within forested areas adjacent to dry shrubs. In these dry forests, the species was reported lactating in January 2016. According to recent captures in urban and peri-urban areas in Central Cordillera, the species supports some ecosystem transformation. Specimens have been captured leaving from house roofs in which other species such as *Molossus molossus* inhabit.

With the elevation of *E. miradorensis* to the species level, currently, there are 12 recognized species of short-eared Neotropical *Eptesicus* (Sánchez *et al.* 2019; Acosta *et al.* 2021; Ramírez-Chaves *et al.* 2021). In addition, the systematics of *Eptesicus* remains controversial because some authors (*e. g.*, Giménez *et al.* 2019) include the long-eared bats of the genus *Histiotus* as a subgenus of *Eptesicus.* Therefore, the information we provided here can be helpful for additional integrative analyses at a continental scale. If *Histiotus* is considered part of *Eptesicus*, the richness of this genus could reach more than 37 species globally, being one of the family's most diverse genera of insectivore bats Vespertilionidae after *Myotis*, *Murina*, and *Pipistrellus* (Moratelli *et al.* 2019; Burgin *et al.* 2020).

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## Appendix 1

List of sequences used in this study indicating their locality, museum voucher or citation, and GenBank accession numbers of *Cyt-b* and **COI** genes.

*Eptesicus fuscus bernardinus (n = 23)* CANADA: British Columbia: Nadin-Davis *et al.* (2017) **KY203308**; Nadin-Davis *et al.* (2017) **KY203321** ND; Nadin-Davis *et al.* (2017) **KY203323** ND; Nadin-Davis *et al.* (2017) **KY203330** ND; USA: California: MVZ 148681 *AF376835* ND; Streicker *et. al.* (2010) **GU723062**; Streicker *et. al.* (2010) **GU723063**; Streicker *et. al.* (2010) **GU723064**; Streicker *et. al.* (2010) **GU723065**; Streicker *et. al.* (2010) **GU723066**; Streicker *et. al.* (2010) **GU723067**; Streicker *et. al.* (2010) **GU723068**; Washington: Streicker *et. al.* (2010) **GU723069**; Streicker *et. al.* (2010) **GU723070**; Streicker *et. al.* (2010) **GU723071**; Streicker *et. al.* (2010) **GU723072**; Streicker *et. al.* (2010) **GU723073**; Streicker *et. al.* (2010) **GU723074**; Streicker *et. al.* (2010) **GU723075**; Streicker *et. al.* (2010) **GU723076**; Streicker *et. al.* (2010) **GU723077**; Streicker *et. al.* (2010) **GU723078**; Streicker *et. al.* (2010) **GU723079**.

*Eptesicus fuscus fuscus (n = 110)* CANADA: Alberta: No data **JF498651**; No data **JF498652**; No data **JF498653**; No data **JF498654**; Nadin-Davis *et al.* (2017) **KY203196**; Nadin-Davis *et al.* (2017) **KY203233**; Nadin-Davis *et al.* (2017) **KY203291**; Ontario: No data **JF498595**; No data **JF498596**; No data **JF498597**; No data **JF498598**; No data **JF498600**; No data **JF498601**; No data **JF498602**; No data **JF498603**; No data **JF498604**; No data **JF498605**; No data **JF498606**; No data **JF498607**; No data **JF498608**; No data **JF498609**; No data **JF498610**; No data **JF498611**; No data **JF498612**; No data **JF498613**; No data **JF498614**; No data **JF498615**; No data **JF498617**; No data **JF498618**; No data **JF498619**; No data **JF498620**; No data **JF498621**; No data **JF498622**; No data **JF498623**; No data **JF498623**; No data **JF498624**; No data **JF498625**; No data **JF498626**; No data **JF498627**; No data **JF498628**; No data **JF498629**; No data **JF498630**; No data **JF498631**; No data **JF498632**; No data **JF498633**; No data **JF498634**; No data **JF498635**; No data **JF498636**; No data **JF498637**; No data **JF498638**; No data **JF498639**; No data **JF498640**; No data **JF498641**; No data **JF498642**; No data **JF498643**; No data **JF498644**; No data **JF498645**; No data **JF498646**; No data **JF498647**; No data **JF498648**; No data **JF498649**; No data **JF498650**; Nadin-Davis *et al.* (2017) **KY203226**; Nadin-Davis *et al.* (2017) **KY203228**; Nadin-Davis *et al.* (2017) **KY203240**; Nadin-Davis *et al.* (2017) **KY203304**; No data **MG423513**; Saskatoon: Nadin-Davis et al. (2017) **KY203225**. USA: Georgia: Streicker *et. al.* (2010) **GU723031**; Streicker *et. al.* (2010) **GU723032**; Indiana: Streicker *et. al.* (2010) **GU723024**; Streicker *et. al.* (2010) **GU723025**; Streicker *et. al.* (2010) **GU723026**; Streicker *et. al.* (2010) **GU723027**; Streicker *et. al.* (2010) **GU723028**; Streicker *et. al.* (2010) **GU723029**; Streicker *et. al.* (2010) **GU723030**; Iowa: Streicker *et. al.* (2010) **GU723020**; Streicker *et. al.* (2010) **GU723021;** Streicker *et. al.* (2010) **GU723022;** Streicker *et. al.* (2010) **GU723023**; Massachusetts No data *EU786867*; Platt *et al.* (2018) *MF143474*; FMNH214994 *MF038479*; Michigan: Streicker *et. al.* (2010) **GU723033**; Streicker *et. al.* (2010) **GU723034**; Streicker *et. al.* (2010) **GU723035**; Streicker *et. al.* (2010) **GU723036**; Streicker *et. al.* (2010) **GU723037**; Streicker *et. al.* (2010) **GU723038**; Streicker *et. al.* (2010) **GU723039**; Streicker *et. al.* (2010) **GU723040;** Streicker *et. al.* (2010) **GU723041**; Streicker *et. al.* (2010) **GU723042**; Streicker *et. al.* (2010) **GU723043**; Streicker *et. al.* (2010) **GU723044**; Streicker *et. al.* (2010) **GU723045**; New Jersey: Streicker *et. al.* (2010) **GU723046**; Streicker *et. al.* (2010) **GU723047**; Texas: No data *EU786866*; Virginia: Streicker *et. al.* (2010) **GU723048**; Streicker *et. al.* (2010) **GU723049**; Streicker *et. al.* (2010) **GU723050**; Streicker *et. al.* (2010) **GU723051**; Streicker *et. al.* (2010) **GU723052**; Streicker *et. al.* (2010) **GU723053**; Streicker *et. al.* (2010) **GU723054**; Streicker *et. al.* (2010) **GU723055**; Streicker *et. al.* (2010) **GU723056**; Streicker *et. al.* (2010) **GU723057**; Streicker *et. al.* (2010) **GU723058**.

*Eptesicus fuscus hispaniolae (n = 2)* DOMINICAN REPUBLIC: ROM125305 **KX355027**; ROM125343 **KX355028**.

*Eptesicus fuscus pallidus (n = 3)* USA: Arizona: Streicker *et. al.* (2010) **GU723059**; Streicker *et. al.* (2010) **GU723060**; Streicker *et. al.* (2010) **GU723061**.

*Eptesicus guadeloupensis (n = 2)* GUADELOUPE: Hassanin et al. (2017) *MF038480*; Hassanin et al. (2017) **MF038579.**

*Eptesicus miradorensis (n = 6)* COLOMBIA: Caldas: MHN-UCa2022 *MW926796*; Cundinamarca: FMNH49154 **OP157114**; GUATEMALA: Chimaltenango: AMNH74304 *OP157111*; MÉXICO: Oaxaca: AMNH190167 *OP157112* **OP137059**; PANAMA: Chiriquí: USNM541106 *OP157110*; VENEZUELA: Distrito Federal: AMNH143003 *OP157113* **OP137060**.

*Myotis riparius (n = 2)* ECUADOR: TTU 102811 *JX130571*; Orellana: ROM103988 **JQ601604.**

*Eptesicus bottae (n = 1)* IRAN: Bisotun: No data *EU786802*.

*Eptesicus seroroninus (n = 1)* LAOS: Houaphan: No data **HM540267**.

*Eptesicus furinalis (n = 2)* ARGENTINA: Santa Fe, MG-ZV-M 175 *MT262849*; MÉXICO: Quintana Roo: ROM33850 **JF447246.**